ESCCA
European Society
for Clinical Cell Analysis

ESCCA 2017

Cytometry at the crossroads of cultures

THESSALONIKI ● GREECE

24 - 27 SEPTEMBER 2017

ABSTRACTS

This year, the European Society for Clinical Cell Analysis (ESCCA) organised its annual Conference over 4 days in Thessaloniki, Greece.

Two workshops (one dedicated to CLL MRD and one to cytometry analysis tools) with a limited number of participants as well as ESCCA certification exams took place before the conference. The meeting included three Education Programmes from which the participants had to choose in advance: (i) Advanced Hemato-Oncology; (ii) Clinical Diagnostic Cytometry and (iii) Advanced Immunology. A group of experts in these fields coordinated, presented, and discussed these topics with the attendees.

The organisation of the Conference was similar to that of previous years, that is, invited oral presentations and selected peer-reviewed abstracts. In addition joint sessions with UK-NEQAS for Leucocyte Immunophenotyping, the Hellenic Cytometry Society and the Hellenic Society of Hematoly were organised. Most of the invited abstracts and selected abstracts presented as oral abstract presentation or poster during the Conference are published in this issue of *Clinical Cytometry*.

On behalf of the Organising and Scientific Committees,

Katherina Psarra
Chair of the Organizing and Scientific Committee

TABLE OF CONTENTS

Disclaimer
ESCCA and Global Conference Support, its Board members, officers, agents and employees (hereafter referred to as: the organisers) accept no liability for personal injuries or loss, of any nature whatsoever, or for loss or damage to property either during or as a result of the ESCCA 2017 Conference.
Delegates have been advised to arrange adequate travel, health, and other insurance before they leave their home country. Participation is completely at the participant's own risk. The organisers will not be liable and will not compensate for any damage incurred to participants during the meeting. The organisers are hereby released from all claims.

PLENARY PRESENTATIONS

PLENARY SESSION 1: KEYNOTE LECTURES

PLE-01-01

2016 WHO Update: Acute Leukemia

Michael Borowitz
Johns Hopkins Medical Institutions, BALTIMORE, U.S.A.

Introduction: The WHO Classification has become the standard language used to diagnose and treat hematopoietic neoplasms. Since the last formal update in 2008 there have major advances in the molecular understanding of these tumors.
Results and Discussion: Rather than a new classification framework, the 2016 WHO Classification instead integrates new clinical and biologic data into the previous classification, while also including some new entities. The complex mutational spectrum of acute leukemia has presented a classification challenge. For the most part, entities in the classification are mutually exclusive, while many newly-discovered mutations overlap defined entities and thus are not directly included. In AML, mutations of NPM1, and biallelic mutations of CEPBA are distinct entities within the broader category of AML with recurrent genetic abnormalities; they have excellent prognosis even in the face of MDS-related changes. AML with mutated RUNX1 is included as a provisional entity, although only if no MDS or other abnormalities are noted because the relative importance of this mutation in these settings is not determined. Other changes in AML classification in 2016 include variants of familial AML, and clarification of criteria for diagnosing erythroleukemia. In ALL, the most important new entity has been termed "BCR-ABL-like", so-called because its gene expression signature closely resembles that of ALL with BCR-ABL-1 translocation. Unlike other entities, this cannot be defined by a single molecular lesion; cases have in common rearrangements of receptor tyrosine kinases or other cytokine receptor molecules including among others ABL1/2, PDGFRB, CSF1R, JAK2, EPOR and CRLF2. Many have activating mutations of other kinases. These account for a large subset of B ALL, up to 25% in some adult cohorts. Interest in these leukemias is driven in by their poor prognosis, and by the possibility that they might respond to targeted therapy; to date this has only been demonstrated for patients with ABL-class fusions including PDGFRB and CSF1R. Also new is the recognition of the unique pediatric B-ALL with intrachromosomal amplification of chromosome 21, included because of its unique biology and requirement for intensive therapy. Flow cytometry unfortunately plays little role in identifying most new entities, although CRLF2 translocations are invariably associated with upregulated expression easily detected by flow. However, one other new entity, early T precursor ALL, is defined specifically by its immunophenotype as a T cell lacking surface CD3, CD8 or high levels of CD5 and also coexpressing myeloid or stem cell markers.

PLE-01-02

2016: WHO update: mature lymphoid malignancies

Andy Rawstron
HMDS, St. James's Institute of Oncology, Leeds Teaching Hospitals NHS Trust, LEEDS, United Kingdom

The 2016 revision of the World Health Organization classification of lymphoid neoplasms aims to refine the diagnostic criteria, particularly of lesions at the very early stages of lymphomagenesis, and to detail the molecular landscape of lymphoid neoplasms. Many of the revisions have limited direct impact on the clinical flow cytometry laboratory but affect multi-disciplinary diagnosis. Hairy Cell Leukaemia is closely associated with the BRAF V600E mutation, while mutations in MAP2K1 are frequent in HCL-variant and HCL that use IGHV4-34 and correlation between molecular and phenotypic findings are required for diagnosis. The MYD88 L265P mutation has been recognised as a driver mutation in the majority of lymphoplasmacytic lymphoma (LPL) and Waldenström macroglobulinemia (WM), and there is recognition that IgM MGUS is typically related to LPL/WM while IgG/A MGUS are more closely related to plasma cell myeloma and it may be helpful to focus flow cytometry investigations accordingly. The 2016 criteria now require the distinction of diffuse large B-cell lymphoma (DLBL) into germinal-centre B-cell-like (GCB) and activated B-cell-like (ABC) molecular "subgroups", currently based on gene expression profiling or immunohistochemistry algorithms although it may be feasible to develop flow-cytometry based approaches to facilitate the cell of origin (COO) classification. Several key changes in the updated WHO classification address the issue of limited clonal expansions of B cells or T cells

that have a low clinical risk. The Monoclonal B-cell lymphocytosis (MBL) category has been revised to distinguish cases with very low levels of abnormal B-cells that appear to have virtually no risk of progressing to CLL. In situ mantle cell and follicular lymphoma have been reclassified to in situ mantle cell and follicular neoplasia to reflect the low probability of disease progression and paediatric -type follicular lymphoma has been distinguished as a localized clonal proliferation with excellent prognosis and a conservative therapeutic approach may be sufficient. There are now approximately one hundred mature B/T-cell neoplasms and the revised classification require further consolidates the need for correlation between molecular, histological and immunophenotypic features for accurate diagnosis.

PLENARY SESSION 2: ESCCA INFORMATICS PROJECTS

PLE-02-01

Health-RI: a next generation of research infrastructure to improve translation from biology to clinic

Mariska Bierkens, Gerrit Meijer
Netherlands Cancer Institute, AMSTERDAM, Netherlands

Traditionally, researchers tend to capture, process, and analyse their data in local isolation for (at least) the duration of the project. Issues encountered during research are repeatedly solved, or experiments repeated, due to lack of awareness of existing solutions to the witnessed problem, or lack of knowledge that the data has already been collected elsewhere. In short, solutions and data generated in earlier research are hardly accessible due to fragmentation of efforts, systems, and tools, even within the location where the research is performed. This impedes integration and reuse of existing data (for example alteration patterns of molecular markers in correlation with clinical outcomes) and hinders implementation of translational research findings in the clinical setting. More generally, it impedes the transition towards personalised medicine & health, in which disease prevention and provision of healthcare will be customised to the individual person, thereby improving population health as well as quality of life for patients with (chronic) diseases.
High-quality research and personalised medicine & health require collaboration between researchers and sharing of data and solutions, requiring a new generation of research infrastructure in which data and solutions are made 'Findable, Accessible, Interoperable and Reusable' (FAIR). Therefore, several Dutch research organisations have joined forces in the Health-Research Infrastructure (Health-RI) initiative which offers expertise and services from pre-existing research infrastructure projects as combined expertise and services under one unifying name. Services offered by Health-RI include consultancy-type support (e.g. ethical and legal services for setting up a biobank), and expertise and infrastructure solutions for data management (e.g. applications to accommodate data catalogues and for data-integration, visit https://www.health-ri.org/services for more information).
Making use of the Health-RI service portfolio is a great way for researchers to work towards their own FAIR data management. As a result of using these services, research questions may be answered faster, results may be validated quicker and insight necessary to improve our understanding of how persons could prevent disease or how they could benefit from personalised treatment will be obtained quicker, accelerating the timeframe in which patients may benefit from research findings.

PLE-02-02

Daedalus: an ESCCA project to elevate the impact of cytomics on translational research

Iannis Drakos, Nicolas Derian, Katherina Psarra
ESCCA, PARIS, France

Introduction: The ESCCA project to advance cytome (flow and mass cytometry) in translational research and precision medicine, Daedalus, is a multidisciplinary effort to utilize cellular markers and enhance the benefits of cross-analyses between clinical, biological and OMIC data. The main strength of the Daedalus approach is derived from the observation that cellular characteristics affect and being affected by various factors among the collective of the heterogeneous data.
Methods: As a case-study, we chose a widely used open-source next-generation information system (NextGen IS): i2b2 tranSMART. Our selection was motivated by the system design, which focuses on users with biomedical background, the wide spectrum of supported data (e.g. genome, transcriptome, exome, SNPs, comparative genomic hybridization, proteome, metabolome, and epigenome), the system's popularity and its adoption by some of the most credible academic and industrial institutions. As a NextGen IS, our case-study is capable of

reading beyond the data values and also understand their properties and the relationships between them. To be able to integrate cytome data into the system, we developed a standardized and automated pipeline going from unprocessed FCS files up to the system's ontologies.
Results: The inclusion of cytome in a NextGen IS significantly increased the biomarker discovery options not only within the domain itself, but also for other clinical, biological and OMIC domains. The Daedalus approach is benefitting cytometry on multiple levels, first by providing advanced options for biomarker discovery within cytometry; second by significantly improving biomarker discovery in general; and third, as a result of the first and second point, by increasing the significance of cytometry in translational research and precision medicine.

PLE-02-03

Working with ESCCABase
Claudio Ortolani
University of Urbino, URBINO, Italy

ESCCABase is an instrument consisting in a database of cytometric files freely consultable by operators entitled to do it.
The nature of these cytometric files is various, covering all the technical, clinical and experimental aspects of Flow Cytometry. Files are being chosen because of their diagnostic and/or methodological relevance, and also because the presence of accurately identified and well described artefacts and errors.
ESCCABase is expected to produce a series of results.
The first result is the constitution of an online decision support. According to this activity, every operator entitled to access the database will be able to compare her or his results with the ones obtained from the analysis of analogous cases recorded in ESCCABase. This function is somehow like the one supported by an Atlas, which provides the consultant with a reference standard. Of course, the comparison between files may be carried out not only with files obtained from diagnostic procedures, but also with files obtained from experiments, like evaluation of apoptosis, cell proliferation, Calcium mobilization, and so on.
The second result is the constitution of a corpus of approved and catalogued material to be used in several teaching and training activities. This "official" material could be adopted in frontal lectures, distance learning activities, exams for accreditation in Cytometry, and external quality assessment (EQA).
The third result is the creation of a network among operators willing to contribute to the growth of the project by submitting their files for uploading. These files will be taken in consideration by the ESCCABase Committee, evaluated, and uploaded if suitable. As a by-effect, this could lead to the creation of special cytometric registers reserved to rare diseases (such as Primary Immunodeficiencies), able to document frequency, phenotype and other features of the selected disease. Moreover, the preferential uploading of specific analytical protocols could indirectly contribute to standardization, harmonization, and global quality of the procedures.
Finally, the fourth result is the creation of a shared archive of diagnosis-driven data-sets, which, when integrated with non-cytometric data, could be fed to data mining algorithms in order to highlight the existence of unexpected patterns and associations.

PLENARY SESSION 3: INTRAOPERATIVE DELINEATION OF GLIOMA MARGINS. THE ROLE OF FLOW CYTOMETRY

PLE-03-01

Neurosurgical management of gliomas-current concepts
Spyridon Voulgaris
University Hospital Of Ioannina, IOANNINA, Greece

Gliomas are the most frequent primary intracranial tumor, representing 27% of all tumors and 80% of malignant brain tumors. Even though relatively rare, they are associated with significant mortality and morbidity. Glioblastoma, constitute 45% of all gliomas and has a poor outcome, with a 5-year survival of 3%. Because of their infiltrative nature there is no surgical cure. Cytoreductive surgery remains important to high-quality patient care and for the molecular characterization of the disease. Gross total excision is associated with better survival compared to subtotal resection and biopsy. Apart from the extent of tumor resection, patient's age and Karnofsky performance scale score at presentation are additional factors of prognostic significance. By incorporating advanced surgical techniques with tumor's molecular signature and targeted radiotherapy and chemotherapy better outcome can be achieved.

PLE-03-02

Intraoperative flow cytometry

Georgios Markopoulos
University of Ioannina, IOANNINA, Greece

Introduction: Cancer is among the leading causes of human mortality. Cancer cells acquire during tumor development specific characteristics, collectively defined as the hallmarks of cancer, which are not present in their normal counterparts. Among the hallmarks of cancer are self-sufficiency in growth signals, insensitivity to anti-growth signals and limitless replicative potential that lead to cell cycle progression and increased cell prolirefation. An additional enabling characteristic of cancer is genomic instability and mutation that may lead to chromosome imbalance and aneyploidy.
Surgical removal of tumors can be a potential cure. The challenge of a tumor removal operation is to define tumor margins and not to excessively remove healthy tissue. This need is more necessary in tissues such as the brain, where removal of tumor is among the most effective treatments against gliomas, while the removal of healthy tissue may lead to several health issues. Due to that, a lot of research is focused on methods for intraoperative delineation of brain tumor margins.
Based on the nature of cancer, there is a significant difference between cancer and normal cells in their DNA content and/or the percentage of dividing cells. Flow cytometry is a gold standard method for analysis of cell cycle and has the advantage of being operator independent, with high accuracy and reproducibility. Due to that, DNA content and cell cycle distribution analysis has been one of the first applications of flow cytometry. However, technical difficulties and the substantial time required for sample preparation has hindered its intraoperative use.
Results and Discussion: Our research team has developed a rapid method for cell cycle analysis using flow cytometry during brain tumor surgery, as a means of defining tumor margins intraoperatively. We represent a methodology on the use of flow cytometry to discriminate tumor from healthy tissue.
During glioblastoma resection we have found significant differences in G0/G1, S-phase and G2/M fraction between tumor core and perilesional tissue samples, making possible the identification of tumor margins. The method showed 100% specificity and sensitivity in pediatric brain tumors, in a series of 68 tumor samples, where G0/G1 phase and mitosis fraction was sufficient to discriminate between malignant from normal tissue. We found that neoplastic lesions had less than 89% G0/G1 phase and more than 2% mitosis fraction, compared to normal tissue.
Collectively, intraoperative flow cytometry, is a promising methodology to define tumor margins and to increase the efficiency of a potential cure for cancer.

PLE-03-03

Intraoperative MRI and 5-ALA

George Alexiou
University Hospital of Ioannina, IOANNINA, Greece

To date, intraoperative MRI and 5-aminolevulinic acid (5-ALA) are both used for the intraoperative identification of gliomas margins, since frozen sections suffer from significant delay from acquisition to interpretation and suboptimal accuracy due to freezing artifacts in several samples. Intraoperative MRI has been proven to increase the resection during glioma surgery. However, it is time consuming and of high cost, and available in only few institutes. The use of 5-ALA leads to intracellular accumulation of fluorescent porphyrins in malignant glioma cells. Intraoperative, the use of violet-blue illumination permits tumor identification by the surgeon. This approach has been demonstrated to increase progression free survival. However, several limitations exist such as decreased fluorescence at tumor margins and in low grade gliomas. Thus, alternative techniques that will enable the differentiation between neoplastic and normal tissue are needed.

PLE-03-04

Intraoperative mass spectrometry of glioma metabolites

Babar Vaqas, Kevin O'Neill, Zoltan Takats
Imperial College London, LONDON, United Kingdom

Background: Mass spectrometry offers a novel dimension-rich modality to characterize brain tumors and guide surgery in real-time. It seamlessly integrates with traditional biopolar forceps to offer a level of tissue analysis never available before to the surgeon. We evaluated the use of the iKnife in 50 patients undergoing craniotomy for brain tumors.

Methods: A single centre prospective observational study was designed involving a consecutive series of 50 patients undergoing craniotomy and resection of gliomas. A neuronavigation system was used to register iKnife readings. Precise intraoperative readings from different tumor zones were taken and compared to matched core biopsy samples verified by routine histopathology. Ex-vivo tissue samples from these cases were also analysed.
Results: Multivariate statistics including PCA/LDA analysis was used to analyse the mass spectra obtained and compare these to the histological data. The system correctly identified normal versus tumor tissue in ex-vivo samples, this was then used to inform the in-vivo sample analysis. The system was able to correctly characterise intrinsic low grade and high grade gliomas. IDH mutation status was identifiable using the iKnife indicating metabolome level analysis is possible.
Conclusions: Intraoperative mass spectrometry represents a system which can accurately and rapidly identify tissue being operated on and easily integrates with the current setup in all operating rooms. The information is collected during routine use of bipolar forceps raising the possibility of controlling tumor resection using the real-time molecular data. Further multicentre studies are required to investigate patient-patient and machine-machine variability.

PLENARY SESSION 4: CHECK POINT INHIBITORS IN CANCER IMMUNOTHERAPY

PLE-04-01

Immune checkpoint blockade in lymphoma

Carmelo Carlo-Stella
Humanitas University, ROZZANO (MILANO), Italy

Introduction: Recent advances in the field of immunology have generated novel immune-based strategies and improved the utility of existing therapeutic options such as allogeneic hematopoietic stem cell transplantation. These new approaches offer vast clinical promise to impact a wide array of diseases, including acute myeloid leukemia, acute lymphoid leukemia, Hodgkin and non-Hodgkin lymphoma and myeloma as well as non malignant hematologic disorders. The most exciting emerging therapies are T-cell checkpoint inhibitors which nonspecifically amplify immune responses and therefore have significant off-target events.
Results and Discussion: The recent availability of T-cell checkpoint inhibitors (anti-PD-1 and anti-PD-L1) has not only provided unprecedented efficacy data in Hodgkin lymphoma (HL) but has also provided valuable therapeutic options to transform patient care in a variety of other hematological cancers. In HL these results are likely related to the genetic dependence of HL on the PD-1 pathway through 9p24 amplification and PD-L1/PD-L2 overexpression. Despite early results with PD-1 blockade hint at a potential paradigm shift in cHL, there are several emerging issues. In particular, criteria for response assessment and determinants of resistance to checkpoint blockade therapy (CBT) are challenging issues. Developing translational strategies based on high-throughput approaches for studying the genome, epigenome and transcriptome in order to analyze disease eradication and identify treatment-emergent mutations associated with resistance to CBT will help in providing sustainable therapeutic decisions.

PLE-04-02

The PD-1 immune checkpoint on human NK cells in normal and pathological condition

Silvia Pesce
University of Genova, GENOVA, Italy

Introduction: PD-1 is an immunologic checkpoint that limits immune responses by delivering potent inhibitory signals to T cells on interaction with specific ligands expressed on tumor/virus-infected cells, thus contributing to immune escape mechanisms. Therapeutic PD-1 blockade has been shown to mediate tumor eradication with impressive clinical results. Little is known about the expression/function of PD-1 on human natural killer (NK) cells. Here we sought to clarify whether human NK cells can express PD-1 and analyze their phenotypic/functional features.
Methods: We performed multiparametric cytofluorimetric analysis of PD-1^{+} NK cells derived from healthy donors and from patients affected by seropapillary ovarian carcinoma. Moreover we performed their functional characterization using degranulation, cytokine production and proliferation assays.

Results: We provide unequivocal evidence that PD-1 is highly expressed (PD-1^{bright}) on a NK cell subset detectable in the peripheral blood of approximately one fourth of healthy subjects. These donors are always serologically positive for human cytomegalovirus. PD-1 is expressed by $CD56^{dim}$ but not by $CD56^{bright}$ NK cells and is confined to fully mature NK cells characterized by the $NKG2A^{-}KIR^{+}CD57^{+}$ phenotype. Proportions of PD-1^{bright} NK cells were higher in the ascites of a cohort of ovarian-carcinoma patients suggesting their possible induction/expansion in tumor environments. Functional analysis revealed a reduced proliferative capability in response to cytokines, low degranulation and impaired cytokine production upon interaction with tumor targets.
Conclusions: We have identified and characterized a novel subpopulation of human NK cells expressing high levels of PD-1. These cells have the phenotypic characteristics of fully mature NK cells and are increased in ovarian-carcinoma patients. They display low proliferative responses and impaired anti-tumor activity that can be partially restored by antibody-mediated disruption of PD-1/PD-L interaction. On the basis of these results innovative immunotherapeutic treatment could be designed based on the combined blockade of different inhibitory NK receptors.

PLENARY SESSION 5: BEST POSTER ABSTRACT PRESENTATIONS

PLE-05-01 (poster # 55)

Leukemic stem cells detection in PB by flow cytometry: a simple and rapid new diagnostic tool for Chronic Myeloid Leukemia

Santina Sirianni[1], Elisabetta Abruzzese[2], Alessandra Iurlo[3], Anna Sicuranza[1], Sara Galimberti[4], Luana Schiattone[1], Antonella Gozzini[5], Patrizia Pregno[6], Giovanni Caocci[7], Marzia Defina[1], Ilaria Ferrigno[1], Veronica Candi[1], Monica Bocchia[1], Donatella Raspadori[1]
[1]Ematologia AOUS, SIENA, Italy
[2]Hematology S.Eugenio Hospital, ROMA, Italy
[3]Fondazione IRCCS Ca' Granda Ospedale Maggiore, MILANO, Italy
[4]Dept. Clinical and Experimental Medicine, PISA, Italy
[5]Hematology, University of Firenze, FIRENZE, Italy
[6]A.O. Città della salute e della Scienza di Torino, TORINO, Italy
[7]Ematologia Ospedale Binaghi, CAGLIARI, Italy

Introduction: Diagnosis of Chronic Myeloid Leukemia (CML) implies documenting in bone marrow (BM) or in peripheral blood (PB) Philadelphia (Ph) chromosome by cytogenetics and BCR-ABL1 fusion by FISH or RT-PCR. Lately, a specific co-expression of dipeptidylpeptidaseIV (CD26) within the CD34+/CD38–/Lin– stem cell fraction appeared a robust biomarker for identifying CML LSCs in BM. We recently demonstrated that CD34+/CD38-/CD26+ LSCs can be easily identified by flow-cytometry also in PB during TKI therapy.
Methods: We here investigated accuracy and specificity of CD34+/CD38-/CD26+ assessment in PB as a new diagnostic tool in 134 pts with clinical suspicion of CML. All pts were evaluated for PB CD26+LSCs, cytogenetics, FISH and/or BCR-ABL1 RT-PCR analysis; in 62/134 pts CD26+LSCs were tested also in BM. We used a flow-cytometry 4-color staining procedure. $2.0x10^6$ leucocytes were incubated with CD45V500 (c.2D1), CD34FITC (c.581), CD38APC (c.HIT2), CD26 (c.M-A261) and negative controls (BD Pharmigen). Acquisition and analysis of at least 1.0×10^6 CD45+ cells were done by FACSCanto II with DIVA8 software (BD, Biosciences).
Results: In 104/134 pts we showed CD34+/CD38-/CD26+ LSCs in PB and in all of them CML was confirmed by cytogenetics, FISH and RT-PCR analysis. Median value of circulating PB CD26/µL was 15,49 (range 0,12-698) and a positive correlation with leukocyte count ($p < 0.01$) was found. All CD26+ PB-BM matched pairs (57/62) showed superimposable results in terms of absolute number of CD26+LSCs/µL (18,28 and 18,38 respectively) while the percentage of CD26+ cells within the CD34+/CD38- fraction appeared lower in BM than in PB samples (median 28,18 and 36,86; range 0,55-77,14 and 5,59-98,57 respectively). In 30/134 (22.3%) PB samples and in 5/62 BM samples CD26+ LSCs were not detected and no one was found Ph or BCR-ABL1 positive. Pts with CD26 neg PB/BM samples were subsequently diagnosed as Idiopathic Myelofibrosis, Myelodysplastic/Myeloproliferative disorders benign neutrophilia and Ph+ acute lymphoblastic leukemia.
Conclusions: Flow-cytometry evaluation of PB CD34+/CD38-/CD26+ LSCs is a feasible, very rapid and highly specific alternative/complementary diagnostic tool for CML. To validate these data in a larger cohort of patients we are developing a pre-titrated lyophilized antibody mixture (lyotube, BD Biosciences) to maximize sensitivity and to optimize standardization and working time, with the further aim to monitor stem cells minimal residual disease in CML patients.

PLE-05-02 (poster # 33)

Probabilistic models for automated flow cytometric analysis of minimal residual disease in chronic lymphocytic leukemia

Konstantia Kotta[1], Dimitrios Kalatzis[2], Ilias Kalamaras[2], Vassiliki Douka[3], Andy Rawstron[4], Achilles Anagnostopoulos[3], Dimitrios Tzovaras[2], Kostas Stamatopoulos[1]
[1]INAB, CERTH, THESSALONIKI, Greece
[2]ITI, CERTH, THESSALONIKI, Greece
[3]Hematology Department and HCT Unit, „G. Papanikolaou„ Hospital, THESSALONIKI, Greece
[4]HMDS, St. James's Institute of Oncology, Leeds Teaching Hospitals NHS Trust, LEEDS, United Kingdom

Introduction: Flow cytometry has an established role for the detection of minimal residual disease (MRD) in haematologic malignancies. In chronic lymphocytic leukemia (CLL), MRD monitoring is only recommended for clinical trials and post allogeneic hematopoietic cell transplantation. However, MRD assessment may soon be incorporated in routine clinical practice thanks both to the advent of novel agents/regimens and the considerable evidence that the levels of MRD can be a reliable predictor of outcome. Hence, it is essential to develop methods for improving all aspects of the procedure, including *in silico* methods for the accurate interpretation of the findings.
Methods: A Gaussian Mixture Model (GMM) has been employed for density estimation of the observed data log-likelihood. The model is trained using mini-batch gradient descent with adaptive learning rate schemes. To this end, flow cytometric FCS files of anonymized patient data from 8 CLL patients assessed for MRD have been utilized. The FCS files have been previously analysed and classified, as MRD positive (+) or MRD negative (-) by flow cytometry experts. We used 4 sets of FCS files per case (MRD (+)/MRD (-), total n=8 datasets), where we made a decision for each cell measurement and counted the number of pathological cell populations to provide a diagnosis. By estimating the *a posteriori* probabilities of the latent variables (sample quality), we derived the cluster assignments for positive and negative measurements.
Results: Our model was able to correctly classify 75% (6 out of 8) of the analysed files. Differences between manual and automatic cluster determination ranged between 0.03-0.06 %. Slight discrepancies and overestimations show that the methodology exhibits increased sensitivity to MRD positive cell population detection. Further analyses and more samples are necessary to discriminate between higher sensitivity versus false positive results.
Conclusions: A statistical model has been developed for flow cytometric CLL MRD data analysis that minimises subjectivity by estimating cluster assignments as a function of the data. It allows the detection of CLL MRD positive samples with a sensitivity of up to 10^{-6}. While further refinements and tests will be conducted in the future, to the best of our knowledge this is the first general-purpose method providing a gating-free, robust and reliable framework for statistical MRD analysis. It is also flexible enough that it could be adapted to different panels allowing safe MRD determination even by less experienced users.

PLE-05-03 (poster # 56)

CD73, an ecto-5'-nucleotidase, is a commonly overexpressed aberrant marker in Acute Leukaemias

Mahima Sanyal, Pg Subramanian, Gaurav Chatterjee, Dilshad Dhaliwal, Ganesh Kumar Viswanathan, Sitaram Ghogale, Nilesh Deshpande, Yajamanam Badrinath, Ashok Kumar, Sumeet Gujral, Prashant Tembhare
Tata Memorial Hospital, MUMBAI, India

Introduction: CD73 is a membrane bound glycosyl-phosphatidylinositol linked protein. It is an ecto-5'-nucleotidase that catalyses dephosphorylation of nucleoside monophosphates. Over-expression of CD73 has been described in many solid cancers and is a potential target molecule for immunotherapy. Data on CD73-expression in hematolymphoid neoplasms is limited to few B-cell acute lymphoblastic leukaemia (B-ALL) studies with small cohort. However, expression-pattern of CD73 in acute myeloid leukaemia (AML) and T-cell acute lymphoblastic leukaemia (T-ALL) is unknown. Hence, we evaluated the expression-pattern of CD73 in T-ALL and AML as well as a large cohort of B-ALL as an additional aberrant-marker.
Materials and methods: Expression-pattern of CD73 (PECF594; Clone-AD2) was evaluated in 458 cases of acute leukaemia using 10-color flow cytometric immunophenotyping (FCI) on Navios flow-cytometer. Data was analysed using Kaluza-V1.3-software. Mean fluorescent intensity (MFI) of CD73 was determined as geometric mean (GM) on normal myeloblasts, leukaemic-blasts, monocytes, and B/T lymphocytes. CD73-positivity was defined as >10% positive blasts.
Results: A total of 458 cases were studied which included 250 B-ALL, 65 T-ALL and 143 AML. M:F ratio was 1.7 and median age was 17.1 years (range, 1-61 years). Median (range) MFI of CD73 on monocytes, negative lymphocytes (internal negative control), positive subset of B & T lymphocytes (positive control) was 0.395 (0.04-2.18), 0.235 (0.05-1.98), 7.535 (1.4-19.65) and 6.295 (1.97-16.16) respectively. CD73 was positive in 81.6% of BALL, 30.3% T-ALL,

and 33.3% AML samples. Median percentage and range of CD73-positive blasts in B-ALL, T-ALL and AML were 39.9% (0.06%-99.9%), 2.3% (0.07-63.6%) and 3.7% (0.01-99.7%). Median (Range) of MFI of CD73-expression in positive (>10%-blasts) blasts of B-ALL, T-ALL and AML were respectively 6.36 (1.4-54.6), 2.5 (0.09-41) and 3.1 (0.15-23.7). Median (range) MFI of CD73-expression in normal myeloblasts [from B-ALL minimal residual disease (MRD) samples] and hematogones were 0.59 (0.52-0.65) and 0.3 (0.12-0.45) respectively. CD73-MFI was significantly higher in AML-blasts (P<0.001) and B-blasts (<0.001). Among T-ALLs, CD73 expression had strong association with early-precursor-T-ALL, ETPALL (CD73 positivity in 10/14 cases of ETPALL compared to 10/51 cases of non-ETPALL, P=0.005)

Conclusion: CD73 is most commonly expressed in B-ALL followed by AML and T-ALL. Among T–ALLs, CD73 expression is significantly associated with ETPALL. CD73 is not expressed in normal myeloblasts and B-cell-precursors. Hence, it is a valuable marker in the diagnosis and MRD monitoring in acute leukaemia. This data also shows that anti-CD73 immunotherapy has potential scope in the treatment of acute leukaemia, especially B-ALL and ETPALL.

PLE-05-04 (poster # 57)

Identification of Minimal Disseminated Disease in T-Cell Acute Lymphoblastic Lymphoma by Flowcytometric Immunophenotyping

Ganesh Kumar Viswanathan, Prashant Tembhare, Nikhil Patkar, Sumeet Gujral, Gaurav Chatterjee, Dilshad Dhaliwal, Y Badrinath, Sitaram Ghogale, Nilesh Deshpane, Mahima Sanyal, Manisha Suthar, Shripad Banavalli, Gaurav Narula, Manju Sengar, Bhausaheb Bagal, Navin Khattry, Subramanian Papagudi Ganesan
Tata Memorial Centre, MUMBAI, India

Introduction: T-lymphoblastic lymphoma (T-LBL) with minimal disseminated disease (MDD) is defined as extra-medullary T-LBL with <25% morphologically identifiable blasts in the peripheral blood (PB) and/or bone marrow (BM) but with the presence of abnormal T-lymphoblasts in BM detected by flowcytometric immunophenotyping (FCM-IPT). Published literature of this rare subgroup is sparse.

Aim: This study aims at identifying MDD in cases of T-LBL with <25% blasts in PB and BM using 8-10 colour FCM-IPT and study the clinical and immunophenotypic features.

Methods: A retrospective analysis of 3 year data in 40 children of T-LBL (diagnosed on mediastinal and/or lymph node biopsy) with predominantly lymphomatous presentation and <25% blasts in PB and BM was done. Clinical and laboratory parameters were analysed with FCM-IPT data. FCM-IPT of BM aspirate was performed on a 3-laser-10-color flowcytometer and analysed using Kaluza® software. A minimum of 1,00,000 events were acquired and MDD was quantified.

Results: Mean age was 10.2 years (range:2-18 years). M:F ratio was 2.1:1. Mean (range) of hemoglobin, WBC count and platelet count were 12.7g/dl (9.4-16), 11.7 x 10^9/L (5.2-30.3) and 411 x 10^9/L (142-875) respectively. None showed morphologically unequivocally identifiable blasts in PB. LDH was raised in the majority [mean 612U/L, N< 190U/L; range (166-1450)]. CSF examination was negative in all cases indicating that it is unlikely to have CNS involvement in patients of T-LBL with <25% blasts in PB and BM.

MDD was seen in 14 cases (35%) and ranged from 0.007% to 18.5% (mean:1.4%; median:1.2%). Mean (range) morphologically identifiable bone marrow blast/hematogone count in the group without MDD was 2.7% (1-4%) and in the group with MDD was 3.7% (0-15%). Seven cases of T-LBL with MDD showed <5% blasts in BM indicating sensitivity and necessity of FCM-IPT. PET-CT did not show increased FDG uptake in BM in any case with MDD.

Conclusion: MDD is present in one-third of cases (35%) of T-LBL with <25% blasts in PB and BM. This underlines the importance of FCM-IPT in cases with <25% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as (1) limited published data are available, (2) these show inferior event free survival in T-LBL with MDD as compared to patients without MDD and (3) there is a need for post-induction BM examination for residual disease evaluation in MDD positive cases and intensification of therapy if positive.

PLE-05-05 (poster # 58)

Multicenter study of the antibody VS38c, at diagnosis, MRD and patients undergoing Daratumumab treatment for Multiple Myeloma (MM)

Ricardo Morilla[1], Timothy Farren[2], Daniel Payne[3], Robert Podovei[1], David Bloxham[4], Ulrika Johansson[5], Alan Dunlop[6], Ruth de Tute[7], Alison Morilla[1]
[1]Royal Marsden Hospital, SUTTON SURREY, United Kingdom
[2]The Royal London Hospital, LONDON, United Kingdom
[3]Leicester Royal Infirmary UHL NHS Trust, LEICESTER, United Kingdom
[4]Addenbrooke's Hospital, CAMBRIDGE, United Kingdom
[5]University Hospitals Bristol, BRISTOL, United Kingdom
[6]King's College Hospital, LONDON, United Kingdom
[7]St James Hospital, LEEDS, United Kingdom

Introduction: The VS38c antibody recognizes the 63-kDa reversibly palmitoylated transmembrane protein p63 present in all plasma cells (PC). The purpose of this study was:
To assess whether the VS38c expression in PC was comparable with that seen with the membrane CD38 antibody.
To determine if VS38c could identify PC in patients on the novel Daratumumab (anti-CD38) therapy.**Methods:** Seven participating UK laboratories tested VS38c-FITC in patients with multiple myeloma (MM) at diagnosis, and following therapy for MRD, including those treated with Daratumumab. All laboratories applied their own multicolour (MFC) panels, staining and analysis protocols. All panels contained the membrane antibodies: CD45/CD19/CD56/CD38/CD138/CD117 and intracytoplasmic Kappa/Lambda in one tube and another with identical membrane antibodies and intracytoplasmic VS38c FITC. The general method was to stain for membrane antibodies followed by fixation, permeabilization and finally incubation with VS38c. Acquisition was performed on both Navios (Beckman Coulter) and BD FACSCanto II (Becton Dickinson) platforms. Routine analysis of PC was performed according to local protocols with their respective data analysis software.
Results: A total of 74 BM samples from MM patients were tested, comprising of 35 diagnostic, 32 MRD and 7 MRD treated with Daratumumab. Two normal BM were included as controls. When comparing CD38 and VS38c expression on the diagnostic samples, there was a strong correlation between the two antibodies (R^2=0.9752, p<0.01), where the total PCs ranged between 0.5% and 33% of all TNCs. For MRD cases (excluding Daratumumab), again a strong correlation was observed between the two antibodies (R^2=0.9819, p<0.01), where PCs ranged between 0.01 to 2% of all TNCs. When assessing patients on Daratumumab, there was no correlation between CD38 expression or VS38c, as CD38 appeared downregulated or lost (R^2=0.1789, p=NS). 71% of Daratumumab cases were membrane CD38 negative, however the VS38c antibody detected residual PC in all cases. Despite the small number of cases, there was a significant difference observed between CD38 and VS38c (p=0.03). The expression of CD38 versus VS38c, were similar to the total percentage of PC in the other two treated patients.
Conclusions: The percentages of PC expressing CD38 and VS38c were comparable in MM patients at diagnosis and MRD following standard treatment. However, Daratumumab treated patients demonstrated a loss of membrane CD38, but retained the VS38c transmembrane expression. In summary, VS38c could be preferentially used in routine MFC MM panels both for diagnostic samples, and those receiving standard myeloma therapy or novel anti-CD38 therapeutic agents.

PLE-05-06 (poster # 59)

CD44 is highly expressed in adult B-cell precursor Acute Lymphoblastic Leukemia and is a useful minimal residual disease monitoring marker

Pearl Rodrigues, Rohitkumar Kori, Pg Subramanian, Gaurav Chatterjee, Dilshad Dhaliwal, Sitaram Ghogale, Nilesh Deshpande, Yajamanam Badrinath, Ashok Kumar, Dhanlaxmi Shetty, Ganesh Kumar Viswanathan, Sumeet Gujral, Prashant Tembhare
Tata Memorial Hospital, MUMBAI, India

Introduction: CD44, a surface glycoprotein, is a cancer stem cell (CSC) marker that regulates their self-renewal, tumor initiation, metastasis, and chemoradioresistance. It plays vital role in pathogenesis of acute myeloid leukemia and chronic lymphocytic leukemia. Anti-CD44-monoclonal antibody (RG7356) holds great promise in the treatment of these neoplasms. Recently, we published its expression-pattern and role in MRD detection in childhood B-cell precursor acute lymphoblastic leukemia (BCPALL) but till date there is no data in adult-BCPALL. Hence, we studied the expression pattern of CD44 and its role in the MRD detection in the adult-BCPALL.
Methods: We studied the CD44 (FITC, clone-G44.26, BD) expression in leukemic-blasts of 170 adult BCPALL samples using 10-color flow-cytometric immunophenotyping on Navios flow-cytometer. Data-analysis was performed using Kaluza-V1.3-software. CD44-expression was considered as positive with ≥20% CD44-positivity.

Mean fluorescent intensity (MFI) and coefficient-of-variation of immunofluorescence (CV-IF) of CD44 was measured on logical scale of bivariate dot-plot. Cytogenetic studies were performed using FISH and conventional methods.
Results: Median age of 170 BCPALL patients studied was 26 years (range 15 – 66 years; M:F – 2.3). CD44 was positive in 86% adult BCPALL. Mean & median (range) of MFI and percentages of CD44 on blasts at diagnosis were 18.89, 17.1, (1.42-69.4) and 81.9, 97.8. (0.02-99.99) respectively. Mean & median (range) of the MFI of CD44 in early-B-cell precursors (CD34+) and late-B-cell precursors (CD34-) were 0.32, 0.29 (0.25-0.39) and 0.31, 0.33 (0.22-0.52). Abnormal over-expression of CD44 was statistically significant with p-value <0.001 using Mann Whitney-U-test. Mean, median & SD of CV of immunofluorescence of CD44 in leukemic-blasts were 79, 74 & 29 demonstrating its relatively homogenous-expression. Cytogenetics was available in 160/170 (94.1%) samples and cytogenetic abnormalities were seen in 85/160 (53%) of cases (BCR-ABL1, 29.37; TCF3/PBX1, 6.25%; MLL-gene rearrangement, 3.75%, hyperdiploidy 7.5% and hypodiploidy 0.6%). CD44 was not associated with any underlying cytogenetics. Of 170 cases, post-induction (day-29) MRD was available in 74 cases and positive in 42/74 (56.8%) cases. In MRD-positive samples, CD44 was over-expressed in in 38/42 (90.4%) samples. Mean, median & SD of MFI and percentages of CD44 in MRD+ blasts were 23, 20.24, 15.7 and 72.4, 86.72, 36 respectively.
Conclusion: CD44 is highly expressed in adult-BCPALL and is a very useful MRD monitoring marker in adults. Hence, anti-CD44-monoclonal antibody(RG7356) therapy may have a potential scope in the treatment of adult-BCPALL.

PLENARY SESSION 6: PRELEUKEMIC STATES

PLE-06-01

Leukemic stem cell embryonic antigens: utility for minimal residual disease follow-up

Lydia Campos Catafal, Tiphanie Picot, Carmen Mariana Aanei, Pascale Flandrin-Gresta, Denis Guyotat
CHU Saint Etienne- Hopital Nord, SAINT ETIENNE CEDEX2, France

Acute Myeloid Leukemia is driven by leukemic stem cells which can be identified by cross lineage expression or arrest of differentiation compared to normal hematopoietic stem cell. Embryonic stem cells exhibit characteristics of self-renewal, arrest of differentiation and pluripotency controlled by specific transcription factors or receptors. Thus, it may be hypothesized that alterations in the expression of one or more of these proteins could contribute to transformation of normal cells into malignant somatic cells carrying tumor-forming capacities The purpose of our study was to evaluate the expression of “embryonic antigens” (OCT4, NANOG, SOX2, SSEA1 and SSEA3) in hematopoietic stem cell subsets ($CD34^+CD38^-$ and $CD34^+CD38^+$) from normal bone marrows and in samples from acute myeloid leukemia patients. We observed an up-regulation of the transcription factors OCT4 and SOX2 in leukemic cells as compared to normal cells. Conversely, SSEA1 protein was down-regulated in leukemic cells. The expression of OCT4, SOX2, SSEA3 was higher in $CD34^+CD38^-$ than in $CD34^+CD38^+$ subsets in leukemic cells. There was no correlation with biological characteristics of the leukemias. We evaluated the prognostic value of markers expression in 69 patients who received an intensive treatment. The rate of complete remission was not influenced by the level of expression of markers. Overall survival was significantly better for patients with high SOX2 levels that was unexpected because of the inverse correlation with favourable genetic subtypes. In this study, we show for the first time, that embryonic antigens usually found in embryonic stem cells and germ cell tumours, which contribute to the maintenance of this embryonic state, are detected on the surface of or within leukemic cells. The role of OCT4, NANOG and SOX2 needs to be investigated in this context, in order to know whether they are directly implicated in oncogenesis, or are only a consequence of the abnormal differentiation pattern observed in AML. Whatever their role, they may represent an interesting marker for the identification of residual leukemic cells and assessment of treatments.

PLE-06-02

Clonal B Cell Lymphocytosis Of Marginal Zone Origin

Kostas Stamatopoulos
Institute of Applied Biosciences, THESSALONIKI, Greece

According to the WHO 2008 Classification, the term monoclonal B lymphocytosis (MBL) encompasses cases with lymphocytosis persisting for more than 3 months in individuals with no evidence suggestive of a distinct B lymphoproliferative disorder. Immunophenotypic findings further subclassify MBL into the following three

categories: (i) MBL with a “CLL-like phenotype” (CD5 and CD23+ve cases, Matutes score 3-5); representing 75% of all MBL cases (ii) MBL with an “atypical CLL phenotype” (CD5+ve, CD23-ve $CD20^{bright}$, Matutes score 3) and (iii) MBL “non-CLL phenotype” (CD5-ve cases, Matutes score <3).
Atypical and non-CLL phenotype MBL cases, especially the later, can pose a biological, diagnostic and clinical challenge. After excluding the possibility of leukemic presentation of distinct lymphoma entities (e.g. mantle cell or follicular lymphoma), most non-CLL phenotype MBL cases will be found display morphologic and/or immunophenotypic features suggestive of marginal zone (MZ) derivation, however not falling into a distinct MZ lymphoma of the WHO 2008 classification, at least at presentation. For this reason, we argued that these cases could represent a provisional entity within the spectrum of clonal B lymphoproliferations of MZ origin for which we proposed the term Clonal B Cell Lymphocytosis of Marginal zone origin (CBL-MZ).
Although most published data supports an ontogenetic relationship of CBL-MZ with marginal-zone (MZ) lymphomas of the spleen, larger cohorts and more comprehensive evaluation of relevant cases are imperative in order to draw definitive conclusions regarding the underlying biology and clinical implications. Nonetheless, based on the available information, most CBL-MZ cases follow a rather indolent clinical course and only a small subset of cases progress to a well known lymphoma, in most cases a MZ lymphoma of the spleen. From a practical perspective, comprehensive evaluation of CBL-MZ should include detailed immunophenotyping, cytogenetic (mostly FISH) analysis, $MYD88^{L265P}$ mutation screening and, perhaps, immunogenetic analysis. Imaging should be perormed at diagnosis, however, the role and diagnostic value of bone marrow biopsy at diagnosis remain debatable. Finally, treatment initiation should be considered only in cases with evidence of progression.

PLE-06-03

Oligoclonality in AML

Jacqueline Cloos, Diana Hanekamp, Jeroen Jwm Janssen, Gert Ossenkoppele, Gertjan Jl Kaspers, Gerritjan J Schuurhuis
VU University Medical Center, AMSTERDAM, Netherlands

Introduction: The majority of acute myeloid leukemia (AML) patients achieve a complete remission with current chemotherapy. However, recurrence of the disease results in a significant treatment failure rate. Therefore, prevention of relapse is the best option to further improve outcome of AML patients.
Methods: The molecular and cellular factors that are implicated in the development of relapse are best studied in samples of AML patients with relapse and in particular in those for which samples are available both at diagnosis and relapse. Samples are commonly investigated on known recurrent genetic aberrations in the bulk of leukemia cells but additional valuable data come from studying subpopulations with different immunophenotypic characteristics as defined by flow cytometry.
Results: AML shows highly instable genetics between diagnosis and relapse. In particular, FLT3-ITD is highly variable, while WT1 is often shown to be gained and seldom lost, and NPM1 mutation is highly stable between diagnosis and relapse. This information was key in the decision to add NPM1 mutation status to flow cytometry based minimal residual disease measurements in NPM1 positive patients. Moreover, patients with pronounced changes in mutations and gene expression profiles between diagnosis and relapse were the fastest to relapse. Most importantly, the relapse of leukemia is often caused by very minor subpopulations within the leukemic stem cell compartment that pre-exist prior to treatment and expand after therapy to cause relapse. This was substantiated by next generation sequencing, which could prove the heterogeneity of AML subpopulations and clonal selection during therapy. This clonal and immunophenotypic evolution is relevant for current minimal residual disease measurements including the leukemia stem cell load, since upcoming subpopulations with aberrant phenotypes, not identified at diagnosis, do emerge. Whether these upcoming subpopulations consist of the relapse initiating cells is currently under investigation.
Conclusion: Based on these data it was recommended that molecular diagnostic data acquired at relapse should be used for risk-group stratification of relapsed AML patients. The assessment of the characteristics of relapsed AML are key to interfere in the disease progression and guide the choice of therapy for the individual patient either to prevent relapse or to offer opportunities for novel treatment strategies in relapsed AML. A sophisticated personalized and targeted approach should therefore not only aim to target the right pathways in an individual patient, but also the right cells and at the right time, early during initial treatment.

PLENARY SESSION 7: STANDARDISATION VS. HARMONISATION

PLE-07-01

ESCCA Harmonization Projects

Andy Rawstron
[5]HMDS, St. James's Institute of Oncology, Leeds Teaching Hospitals NHS Trust, LEEDS, United Kingdom

There are multiple different national and international groups aiming to standardise aspects of flow cytometry, often focussed on the most advanced analytical approaches. ESCCA recognises that the members are situated in economically diverse locations and that consensus approaches for clinical diagnostic cytometry should be available to resource-limited laboratories where possible while identifying recommended markers to improve consistency in better-resourced settings. The ESCCA harmonisation group identifies relevant topics by survey of the members to determine the most pressing requirements for harmonisation and validation. In co-ordination with the ICCS the topics of Sezary disease / mycosis fungoides, hairy cell leukaemia, and chronic myelomonocytic leukaemia have been highlighted for harmonisation. In collaboration with the ERIC group, the diagnosis and monitoring of CLL has been previously harmonised and approaches for prospective validation with identification of suitable antibodies is ongoing. Member surveys demonstrate an approximately equal need for fully standardised vs. more flexible assays. Approaches for designing clinical diagnostic assays which meet a required specification but have sufficient potential for responding to clinical developments will be discussed.

PLE-07-02

B-ALL EuroFlow

Vincent van der Velden
Erasmus MC, ROTTERDAM, Netherlands

Most current treatment protocols for B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) include minimal residual disease (MRD) measurements. The current gold standard is based on PCR analysis of rearranged antigen receptor genes, which is applicable in virtually all patients, reaches sensitivies down to 10^{-5} and is subjet of a yearly quality assurance program (EuroMRD). Although flow cytometry (FCM) can be used for MRD detection as well, studies so far indicate that the specificity and sensitivity of FCM-MRD diagnostics is inferior to PCR-based MRD diagnostics. Furthermore, to improve FCM-based MRD diagnostics, more objective and efficient discrimination of BCP-ALL cells from normal BCP cells and improved sample preparation procedures for acquisition of larger numbers of cells are a prerequisite.
Within EuroFlow, we designed a fully-standardized 8-color antibody panel and laboratory procedure to measure MRD in BCP-ALL patients with a sensitivity of $\leq 10^{-5}$. Leukocyte markers and the corresponding antibodies and fluorochromes were selected based on their contribution in separating BCP-ALL cells from normal/regenerating BCP cells in multidimensional principal component analyses. After five multicenter design-test-evaluate-redesign phases (only possible due to the standardized approach) with a total of 319 BCP-ALL patients at diagnosis, two 8-color antibody tubes were selected, which allowed separation between normal and malignant BCP cells in 99% of studied patients. These two tubes were tested with a new erythrocyte bulk-lysis protocol allowing acquisition of high cell numbers in 377 bone marrow follow-up samples of 178 BCP-ALL patients. Comparison with RQ-PCR-based MRD data showed a clear positive relation between the percentage concordant cases and the number of cells acquired. For those samples with >4 million cells acquired, concordant results were obtained in 93% of samples. Most discordances were clarified upon high-throughput sequencing of antigen-receptor rearrangements and blinded multicenter re-analysis of flowcytometric data, resulting in an unprecedented concordance of 98% (97% for samples with MRD <0.01%).
Our data show that the fully-standardized EuroFlow BCP-ALL MRD strategy is applicable in >98% of patients with sensitivities at least similar to RQ-PCR ($\leq 10^{-5}$), if sufficient cells ($>4x10^6$, preferably more) are evaluated. This fully-automated approach will also allow automated database-guided expert-supervised analysis of data, thereby facilitating standardized (and thus more objective and reproducible) interpretation of MRD data.

PLE-07-03

B-ALL IBFM
Michael Dworzak
St. Anna Children's Hospital, VIENNA, Austria
This abstract could not be included in view of an upcoming publication.

PLENARY SESSION 8: ESCCA AWARDS SESSION

Nominees for the ESCCA Best Oral Award, in alphabetical order of the presenting author:

PAR-05-03

Association between the expression of factor XIII-A in lymphoblasts and the 'B-other' cytogenetic group in children with acute lymphoblastic leukemia
Zsuzsanna Hevessy, Bettina Kárai, Eszter Szánthó, László Csáthy, Aniko Ujfalusi, Katalin Gyurina, István Szegedi, János Kappelmayer, Csongor Kiss
University of Debrecen, Faculty of Medicine, DEBRECEN, Hungary

Introduction: Previously we confirmed that the expression of subunit A of blood coagulation factor XIII (FXIII-A) was a sensitive intracellular marker for the classification of acute myeloid leukemias (AML). Moreover, the lack of expression of FXIII-A in acute promyelocytic leukemia was associated with an unfavorable outcome. Recently we identified B-cell lineage leukemic lymphoblasts as a new expression site for FXIII-A. On the basis of FXIII-A expression, various subgroups of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) can be identified. Recently several studies have focused on the examination of 'B-other' ALL. Approximately 50% of 'B-other' ALL cases were classified as *BCR-ABL1*-like ALL with unfavorable outcome. *IKZF1* deletion, which is a secondary abnormality, also often occurs in 'B-other' ALL. We aimed to investigate retrospectively FXIII-A expression as a possible prognostic marker and its association with other prognostic factors in childhood BCP-ALL among patients treated according to the BFM ALL-IC 2002 protocol.
Methods: Fifty-five children with BCP-ALL were included in the study. Bone marrow samples were obtained by aspiration and the presence of FXIII-A was detected by flow cytometry. G-banding and fluorescent in situ hybridization was performed according to standard procedures.
Results: Of the 55 common ALL patients enrolled in the study, 18 exhibited FXIII-A-negative lymphoblasts and 37 showed FXIII-A positivity. The 10-year event-free survival (EFS) and overall survival (OS) rate of FXIII-A-positive and FXIII-A-negative patients showed significant differences (EFS: 84% vs. 61%, respectively; p=0.031; OS: 89% vs. 61%; p=0.008). Of all the parameters examined, there was correspondence only between FXIII-A expression and 'B-other' genetic subgroup. Multivariate Cox regression analysis of FXIII-subtype and genetic group or 'B-other' subgroup identified FXIII-A negative characteristic as an independent predictor for poor outcome in BCP-ALL (EFS: HR: 3.6, 95% Cl: 1.2-11.3, p=0.027; OS: HR: 5.6, 95% Cl: 1.5-20.12, p=0.009). In the FXIII-A positive cases reduction of blast percentage was significantly higher in the FXIII-A-positive blast subclone than in the FXIII-A-negative one in day 8 peripheral blood (p=0.031) and both in day 15 (p=0.0003) and in day 33 (p<0.0001) bone marrow aspirate samples.
Conclusion: We found an excellent correlation between long-term survival and the FXIII-A-positive phenotype of lymphoblasts, which proves that FXIII-A expression character in lymphoblast is not only a useful LAIP but also a powerful prognostic factor in childhood BCP-ALL. In addition, FXIII-A expression is associated with the 'B-other' characteristics, therefore, FXIII-A can help to identify those cases that may require further detailed genetic examination using expensive methods.

PAR-05-04

Minimal residual disease and log -reduction explain the superior value of double autologous stem cell transplant in multiple myeloma

Giovanni Rossi[1], Antonietta Falcone[1], Maria Marta Minervini[1], Giovanni Pio de Cillis[1], Chiara de Waure[2], Leuconoe Grazia Sisti[2], Daniela Valente[1], Vincenzo Chiello[1], Potito Rosario Scalzulli[1], Angelo Michele Carella[1], Nicola Cascavilla[1]
[1]IRCCS „Casa Sollievo della sofferenza„, SAN GIOVANNI ROTONDO (FG), Italy
[2]Catholic University of Sacred Heart, ROME, Italy

Introduction: Previous studies have demonstrated that the identification of minimal residual disease (MRD) by flow cytometry (FC) at day 100 following autologous stem cell transplant (ASCT) predicts both progression free survival (PFS) and overall survival (OS) in younger patients with multiple myeloma (MM). Anyway, median PFS after single ASCT is 27 and 16 monthes within MRD negative and MRD positive patients. These results, not exhaustive for younger patients, suggested that further treatments are desirable after single transplant. Although it was clinically demonstrated that double ASCT effected superior RFS and EFS rate, biological explanations of these findings were not given and single ASCT remain the treatment of choice in MM. Thus, we firstly investigated the role of MRD and Log-reduction of plasma cells (PCs) in quantifying the degree of tumor reduction after any ASCT. Secondly, we defined the most predictive time point by monitoring MRD and Log-reduction.
Methods: Bone marrow samples from 30 patients who underwent double ASCT were assessed by FC at different time points: post induction (MRD1 and LOG1), post first- (MRD2 and LOG2) and post- second (MRD3 and LOG3)ASCT. MRD (>0.01%) was evaluated by a six-color FC. Log –reduction was calculated as a logaritmic ratio between the PCs at presentation and PCs at each time of assessment.
Results: A significant difference was evidenced among the three time points from ANOVA test for both LOG-reduction ($p<0.001$) and MRD ($p=0.005$). In particular, LOG3 was significantly greater than LOG2 ($p<0.001$) and LOG1($p<0.001$). Similarly, MRD achieved after double ASCT was deeper than MRD achieved after single ASCT ($p=0.005$) and after induction($p<0.001$). Then, frequency of MRD positive patients after double ASCT was significantly lower than that found after the first ASCT (n= 15 vs n= 23,$p=0.008$) and after induction (n=15 vs n=27, $p=0.004$). When the survival analysis was considered, a significant reduction of PFS was observed in patients belonging to an unfavorable cytogenetics risk group ($p<0.001$) and patients showing a MRD over 0.01% (34.1 vs 17.6 mths, $p=0.031$) as well as a Log-reduction lower than 2.57 ($p=0.01$) after double ASCT . Results of MRD and LOG –reduction post double ASCT were confirmed at multivariate analysis ($p=0.004$ and $p=0.01$, respectively).
Conclusions: Our results by FC sustained the double ASCT as the goal treatment strategy in MM because of a deeper reduction of PCs, a higher frequency of MRD positive patients and a longer PFS compared to single ASCT.

PAR-04-05

Co-expression of CD25/CD34/CD123 is a highly predictive indicator of FLT3-ITD mutations in acute myeloid leukemia

Manisha Suthar, Gaurav Chatterjee, Yajamanam Badrinath, Nikesh Kunder, Shruti Chaudhary, Swapnali Joshi, Sitaram Ghogale, Nikhil Patkar, Pg Subramanian, Sumeet Gujral, Prashant Tembhare
Tata Memorial Centre, NAVI MUMBAI, India

Introduction: Fms-like tyrosine kinase receptor internal tandem duplications (FLT3-ITD) mutation is a well-established prognostic factor in acute myeloid leukemia (AML). FLT3-ITD mutation screening by PCR is not sensitive enough to identify small clones which may be responsible for relapse. Flow-cytometry (FC) is a powerful technique that identifies small blast clones and also effectively indicates concurrent genetic lesions. Recently, CD25 is shown to be a poor prognostic marker in the intermediate-risk AML (IR-AML) and also shown to be associated with FLT3-ITD mutations. However, predictive value of CD25 alone in detection of FLT3-ITD positive AML is not very high. We evaluated the frequency of CD25 expression in AML and predictive value of co-expression of CD25/CD34/CD123 for FLT3-ITD mutation in AML.
Methods & Materials: We studied the expression-pattern of CD25 (PE-CF594, clone-B1.49.9), CD34(APC, clone-581), CD123(BV421, clone-9F5) in 201 AML patients using 10-color FC on Navios Cytometer and data was analyzed using Kaluza-v1.3 software. Positive expression of CD25, CD34 and CD123 were defined with cut off >20% of leukemic-blasts. For FLT3-ITD mutations, the genomic DNA was extracted and the FLT3 region was amplified using PCR. Fragment length analysis was done by capillary electrophoresis and identification of additional peaks was done if present. Cytogenetic studies were performed by conventional method and FISH. The study was conducted in Tata Memorial Centre and approved by institutional ethical committee.
Results: CD25 was found to be positive in 36 (17.9%) cases with expression-pattern of strong, weak, subset and variable expression in 27.8%, 16.7%, 38.9% and 16.7% samples. Molecular studies revealed FLT3-ITD mutations

were also found in 32 (15.9%) cases. Sensitivity and specificity of CD25 expression alone in prediction of FLT3-ITD mutation was 40.62% (95% CI, 23.70% to 59.36%) and 86.39 % (95% CI, 80.28% to 91.17%) respectively. The positive and negative likelihood ratio was 2.9 and 0.7 respectively. However, sensitivity and specificity of co-expression of CD25/CD34/CD123 in identifying AML with FLT3-ITD mutation was 56.3% (95% CI, 0.32-0.8) and 97.6% (95% CI, 0.8-0.94) respectively. On Chi-square-test analysis this association was found to be statistically significant with $p<0.001$. The positive likelihood ratio and negative likelihood ratio of co-expression of CD25/CD34/CD123 in identifying AML with FLT3-ITD mutation was 4.43 and 0.48 respectively.
Conclusion: Co-expression of CD25/CD34/CD123 is a highly specific immunophenotypic indicator in determination of AML with FLT3-ITD mutations. It can be considered as surrogate marker for FLT3-ITD mutation in AML.

PLENARY SESSION 10: IMMUNOTHERAPY IN HEAMATOLOGICAL MALIGNANCIES

PLE-10-01

The era of engineered T cells for immunotherapy

Christos Georgiadis, Roland mr Preece, Lauren dr Nickolay, Aniekan dr Etuk, Waseem prof Qasim
UCL, GOS Institute of Child Health, LONDON, United Kingdom

Introduction: Gene-edited T cells are reaching clinical phase testing as new reagents including TALENs and CRISPR/Cas9 nucleases have emerged allowing for efficient and highly specific cell engineering. Significant reports of high remission rates, in particular using chimeric antigen receptors (CARs) against CD19 in B cell malignancies using lentiviral and gamma-retroviral gene-addition technology have already emerged. Editing of T cells however, is providing compelling evidence supporting the potential refinement of current autologous cellular therapies, which tend to be bespoke interventions that require extensive expertise and infrastructure. The ability to use banked CAR T cells, generated from non-HLA matched donors in an 'off-the-shelf' manner forms an attractive prospect that has driven attempts to overcome HLA-barriers, both in terms of allo-reactivity from infused cells and host-mediated rejection. Initial therapeutic applications have included the production of such universal T cells expressing CARs against leukaemia antigens CD19. These approaches have delivered CAR genes using integrating vectors and transiently expressed DNA-targeting nucleases to disrupt loci such as the T cell receptor alpha constant chain (TRAC). In the absence of coupling, transgene expression and editing effects are unlinked, and yields are variable and the resulting T cells populations heterogeneous.
Results and discussion: We report a novel self-inactivating lentiviral vector platform that couples CAR expression with clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 effects through a hybrid terminal-TRAC guide element incorporated into the ΔU3 3' long terminal repeat. Duplication of pol III-sgRNA following reverse transcription magnifies expression, and subsequent electroporation mediated delivery of Cas9 mRNA mediates transient DNA cleavage. The 'terminal' CRISPR configuration, used in combination with downstream depletion of residual T-cell receptor (TCR) expressing cells, resulted in highly homogenous populations where terminal-TRAC CAR cells were >96% CAR+ and >99% TCR-. Molecular signatures identified by whole genome sequencing confirmed on-target scission, with *in silico* predicted off-target sites found to be intact. *In vitro* cytotoxicity studies confirmed functional integrity of the cells and *in vivo* anti-leukaemic effects were corroborated in a humanised immunodeficient mouse model. Configurations of 'terminal' have now been designed to permit multiplexing of the CRISPR effects, demonstrated with the auxiliary knock-out of Beta-2-microglobulin (B2M) bestowing protection to the engineered T cells. We demonstrate that the platform is readily scalable for early phase evaluation in relapsed refractory malignancies, and anticipate a wide range of other applications requiring ex-vivo gene editing.

PLE-10-02

The prospects of cellular immunotherapy for lymphoma

Anastasios Karadimitris
Imperial College London, LONDON, United Kingdom

Cellular immunotherapy for blood cancers, including for lymphomas has entered a new era with the advent of the chimaeric antigen receptor (CAR) technology. CARs are engineered as fusion molecules in which ectodomains, usually comprising antibody-derived, single chain variable fragments, engage with high affinity with a surface antigen on the target cell while the CAR endodomain comprising CD3z and co-stimulatory molecule-derived

modules relay activation, proliferative and persistence signals to the CAR-engineered immune cells, usually T cells. CAR-T cells specific for CD19 (CAR19T), after rigorous pre-clinical testing, are now in clinical development and have received FDA approval. Early clinical results indicate considerable efficacy with up to 50% complete remission rates in patients with relapsed/chemo-refractory B cell lymphomas. Similarly, highly encouraging results have been reported in multiple myeloma with CAR T cell immunotherapy targeting the plasma cell-specific antigen BCMA.
While the proof-of-concept and the clinical potential of CAR immunotherapy are being established, efforts are focused on optimisation of this novel therapeutic approach. Amongst the different facets of CAR technology optimisation, the type of immune cell(s) best suited for CAR immunotherapy has not been established. In this respect, I will discuss pre-clinical data in relation to the potential and promise of invariant NKT cells, a rare but powerful subset of T cells with regulatory and effector functions, as a platform for developing CAR-based immunotherapy for a variety of blood cancers including lymphoma.

PLE-10-03

Adoptive T cell immunotherapy for the treatment of post-allogeneic hematopoietic cell transplantation infections

Evangelia Yannaki
GEORGE PAPANICOLAOU HOSPITAL, THESSALONIKI, Greece

Opportunistic infections represent leading causes of morbidity and mortality in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), hence limiting its broader application and success. Conventional treatment of opportunistic infections with pharmacological agents (anti-viral or/and anti-fungal drugs) is costly, often ineffective or associated with substantial toxicities while it can also lead to the growth of resistance. Moreover, some of the deadliest viral infections post-transplant (HHV-6, ADV) are lacking specific drug treatment. Due to the failure and the toxicities associated with conventional therapies of opportunistic infections, both the human and the financial cost for the national economies is enormous.
Adoptive immunotherapy using virus-specific T-cells (VSTs) arose over the last years, as an appealing alternative strategy for the treatment of post-transplant infectious complications. Early efforts provided the proof of principle that seropositive donor-derived virus-specific T-cells (VSTs) targeting cytomegalovirus (CMV) or Ebstein-Barr virus (EBV) restore virus-specific immunity and effectively control viral infections. Subsequent studies focused on how to simplify the time- and labor-intensive manufacturing process and extend the number of targeted viruses. Indeed, recently “user-friendly” protocols have been developed and the administration of donor’s antigen-specific T cells simultaneously targeting multiple viruses to treat post-transplant viral infections has provided highly promising clinical results (response rates 70-90%). Given the current rapidity of production, the durable responses by one-time treatment and the excellent safety profile, it is expected that VSTs as a cellular product will be highly cost-effective over conventional pharmacotherapy. Current challenges to be addressed remain the development of VSTs from virus-naïve donors or the optimization of third-party VSTs towards “off the shelf” approaches.

ORAL ABSTRACT PRESENTATIONS

PARALLEL SESSION 1: IMMUNOLOGICAL CELL POPULATIONS

PAR-01-01

The different facets of myeloid-derived suppressor cells in cancer patients

Susanna Mandruzzato
University of Padova, PADOVA, Italy

Introduction: Myeloid-derived suppressor cells (MDSCs) are a major population of regulatory cells that are expanded in different types of cancer and capable to impair anti-tumour innate and adaptive immune responses. It is well known that MDSC have complex phenotypes that are not restricted to a single defined myeloid cell population, but rather a plastic phenotype responsive to tumour-derived soluble factors. There is an increasing interest for monitoring circulating MDSCs in cancer patients, since circulating levels of MDSCs have been correlated to tumor burden and overall survival in different types of cancers and in some cases they demonstrated a prognostic role for the outcome of therapy. In this respect, they might represent a promising biomarker also for immunotherapy response. For this reason, monitoring MDSC expansion is of clinical importance, and flow cytometry is the technique of choice for their identification. However, because of their heterogeneous composition, accurate phenotyping of these cells requires a multicolor approach, and there are divergences in their phenotypic definition.
Results and discussion: Over the years, our laboratory focused on the identification of MDSCs in cancer patients and studied genomic, phenotypical and functional aspects of such cells. We also demonstrated that MDSCs can be expanded in vitro from the bone marrow of healthy donors in the presence of cytokines combination and evaluated the role of different subsets of MDSC as biomarkers of response or toxicity in melanoma patients following ipilimumab treatment. Finally, in collaboration with the Cancer Immunoguiding Program (CIP) of the Association of Cancer Immunotherapy (CIMT), this laboratory is coordinating a proficiency panel program that aims at harmonizing MDSC phenotyping.

PAR-01-02

Pitfalls in automated gating of Lymphocyte Populations in HIV patients

Maria Arroz
CHLO, Hospital S. Francisco Xavier, LISBON, Portugal

Introduction: The evaluation of peripheral blood T, B and NK lymphocyte populations is the most routinely performed application in clinical flow cytometry. In individuals infected with human immunodeficiency virus (HIV), the accurate enumeration of absolute CD4+ T lymphocytes is an essential parameter for monitoring and reducing the risk of disease progression. The expression of the antigens used to define such populations is so uniform that it allows automated software analysis at the end of sample acquisition. Nevertheless it is mandatory to look at all the dot plots displayed before the validation of the results. In the vast majority of cases no corrective action is needed but in some situations measures need to be taken.
Results and Discussion: The correct definition of the lymphocyte gate using CD45 vs. side scatter (SSC) must be ensured and manual correction applied whenever necessary. Endogenous interference results from substances not naturally found in the patient's specimen such as hyperbilirubinemia and cryoglobulins, may require a different methodology in order to minimize those interferences and sometimes, as a consequence, single platform can no longer be used and a dual platform is required.
Under-expression of CD45, uncommon dim expression of CD19 or existence of a chronic lymphoproliferative syndrome are other causes of incorrect automated analysis. The scope of this presentation is to highlight such cases and how to troubleshoot them before asking for another specimen, which will most probably present with the same problem. Any abnormal subpopulation identified should be further characterized by the use of a more comprehensive panel. The results should be documented and correlated to the clinical context of the patient.

PAR-01-03

Monitoring of circulating Plasmablasts in IgG4 Related Disease

Raffaella Milani, Emanuel Della-Torre, Marco Lanzillotta, Barbara Migliavacca, Gabriele Torriani, Luca Santoleri, Fabio Ciceri
IRCCS San Raffaele Scientific Institute, MILANO, Italy

Introduction: IgG4-Related Disease (IgG4-RD) is a systemic fibro-inflammatory condition characterized by a perturbation of the B cell compartment. Circulating plasmablasts, the precursors of tissue resident antibody secreting plasma cells, are considered one of the best currently available biomarker of IgG4-RD activity, because they are elevated in patients with untreated IgG4-RD, decline with disease remission, and re-emerge during disease flare, as opposed to IgG4 count, which as been shown to be a less reliable one.
Methods: Between September 2014 and June 2017 we analyzed 107 samples from 20 patients with active IgG4-RD. Plasmablasts were measured in all patients prior to any treatment and after immunosuppressive therapy. The immunophenotype evaluation was performed on EDTA whole blood samples, using a lyse-no-wash technique (ammonium chloride) and a panel of directly conjugated antibodies (CD3FITC-CD56PE-CD4ECD-CD138PC5.5-CD27PC7-CD20APC-CD19A700-CD38A750-CD8PB-CD45KO, Beckman-Coulter). Ten-color flow cytometry was performed using a Navios cytometer (Beckman Coulter) and Navios software. The single platform method was used to determine absolute counts, employing fluorospheres (Flow-Count™ Fluorospheres Beckman-Coulter). An immunological gating strategy utilizing CD45 versus side scatter was used to select the populations of granulocytes ($CD45^{int}SSC^{high}$), lymphocytes ($CD45^{bright}SSC^{low}$) and monocytes ($CD45^{bright}SSC^{int}$). The analysis of lymphocyte subpopulations was performed on lymphocyte population gate, using quadrant dot plot statistics. The absolute plasmablast count per microliter was measured by gating for CD19+CD20-CD38+^{bright}CD27+. The maximum number of total events to be collected was 1 milion or an acquisition time of 600 seconds. Plasmablast populations identified as previous described, from patients of interest, were prepared in order to obtain a final suspension medium concentration of 1x10e6-1x10e7 cells/ml and were sorted by MoFlo™ XDP High Speed Cell Sorter (Beckman Coulter) and directly recovered on microscope slides. Slides, stained according to the May-Grünwald-Giemsa (MGG) technique, were evaluated by light microscope for morphology.
Results: The median value of circulating plasmablasts at baseline was 2270 cells/mL (range, 130-40840), compared to normal circulating level of 0-650 cells/mL. For patients who responded to therapy the median value dropped to 300 cells/mL (range, 0 – 3610), with consensual response of clinical and radiological parameters (IgG4-RD responder index and CT /PET/MRI). Morphology evaluation of sorted cells showed that circulating plasmablasts can not be distinguished from normal lymphocytes, taking into account the changes related to the procedure.
Conclusions: The single platform absolute count of circulating plasmablasts is a useful and accurate tool for diagnosis and monitoring IgG4-RD and offers clinicians a quick evaluation without further discomfort for patients.

PAR-01-04

Predictive/prognostic value of myeloid-derived suppressor cells and regulatory T cell subsets in untreated non-small cell lung cancer patients

Eleni-Kyriaki Vetsika[1], Despoina Aggouraki[1], Afroditi Katsarou[1], Marianthi Gkioulmpasani[1], Filippos Koinis[1], Vassilis Georgoulias[1], Athanassios Kotsakis[2]
[1]University of Crete, Laboratory of Translational Oncology, HERAKLION, CRETE, Greece
[2]University Hospital of Crete, Dept of Medical Oncology, HERAKLION, CRETE, Greece

Introduction: Circulating myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) represent a heterogeneous population of cells with immunosuppressive properties. Using flow cytometry, the frequency and functionality of different MDSCs and Tregs subtypes and their association with the clinical outcome were investigated in non-small cell lung cancer patients (NSCLC) patients.
Methods: Peripheral blood from 156 chemotherapy-naive NSCLC patients, stage III/IV, was collected prior to 1st line chemotherapy. 31 normal, age-matched donors (ND), were used as controls. Multi-colour flow cytometry was used to assess MDSCs subtypes (monocytic and granulocytic) and $CD4^+$ Treg subsets (naive, effector and terminal effector). Phenotypic characterization of MDSCs subpopulations was determined in strictly immature myeloid cells. The patients' clinical outcome, [progression free survival (PFS) and overall survival (OS)], was compared according to the frequency of MDSCs and Tregs subtypes (high vs low expression, as defined by their percentage above the 90% percentile of ND).

Results: Two monocytic [$CD15^-$M-MDSC: $CD14^+CD15^-CD11b^+CD33^+HLA\text{-}DR^-Lin^-$ and $CD15^+$M-MDSC: $CD14^+CD15^+CD11b^+CD33^+HLA\text{-}DR^-Lin^-$] and a granulocytic [G-MDSC: $CD14^-CD15^+CD11b^+CD33^+HLA\text{-}DR^-Lin^-$] subpopulation of MDSCs were significantly elevated ($p<0.001$) compared to ND, expressing inducible nitric oxide synthase and reactive oxygen species, respectively. In addition, MDSCs, co-cultured with $CD3^+$ T cells, were able to significantly suppress IFN-γ secretion by T cells. Increased percentages of M-MDSCs were associated with worse response to treatment ($p=0.02$) and patients with normal levels of $CD15^+$M-MDSC had longer OS and PFS compared to those with high levels ($p=0.008$ and $p=0.005$, respectively). Multivariate analysis revealed that increased percentages of $CD15^+$M-MDSC were independently associated with decreased PFS and OS.
All $CD4^+$ Treg subsets exhibited highly suppressive activity by TGF-β and IL-10 production. The percentages of naive Treg ($CD3^+CD4^+CD25^{high}CD127^{-/low}CD152^-FoxP3^{low}CD45RO^-$) were found elevated in NSCLC patients compared to ND and were associated with poor clinical outcome, whereas the percentage of terminal effector Treg ($CD3^+CD4^+CD25^{high}CD127^-CD152^+FoxP3^+CD45RO^+$) was lower compared to ND and higher levels were correlated with improved clinical response. No difference in the frequency of effector Treg $CD3^+CD4^+CD25^{high}CD127^{-/low}CD152^+FoxP3^{low}CD45RO^+$ was found between ND and NSCLC patients and no association with response to treatment. At baseline, normal levels of naive and effector Treg were associated with longer OS compared to high levels, while the high frequency of the terminal effector Treg was correlated with PFS and OS.
Conclusions: The data provide evidence that the increased frequency of M-MDSC subpopulations and particular $CD4^+$ Treg subtypes are associated with an unfavorable clinical outcome in patients with advanced/metastatic NSCLC. Depletion or blocking may be a beneficial therapeutic strategy.

PARALLEL SESSION 2: NEW CAPABILITIES FOR FLOW CYTOMETRY

PAR-02-02

Principles of acoustic focusing cytometry and its application to rare population analysis

Michael Ward[1], Jolene Bradford[1], Laura Garcia Rico[2], Jordi dr Petriz[2]
[1]ThermoFisher Scientific, EUGENE, U.S.A.
[2]Josep Carreras Leukaemia Research Institute, BARCELONA, Spain

Introduction: Acoustic focusing flow cytometry uses ultrasonic standing waves to focus cells in a flowing sample to the center of an acoustically resonant capillary. Current commercial instruments combine both acoustic and hydrodynamic focusing by aligning cells in the capillary just prior to injection into a sheath manifold. This combination allows high precision measurements at volumetric sample input rates up to an order of magnitude higher than for instruments using hydrodynamic focus alone. This can be exploited to alter protocols and decrease times for rare cell analysis, particularly in combination with no lyse no wash assays and other protocols using sample dilution. The acoustic field also has an orientation effect that can increase precision of scatter measurements for non–spherical cells like erythrocytes.
Methods: The theory and implementation of acoustic focusing technology in flow cytometers are described. Benefits and limitations of the wide dynamic range of volumetric sample throughput it enables are discussed in the context of no lyse no wash protocols designed for small volume precious samples and live cell phenotypic and functional analysis.
Results: The acoustic capillary used, focuses cells into a single line, according to the cells' intrinsic properties relative to the media in which they are suspended. The acoustic focusing cytometer is more aptly named an "acoustic assisted flow cytometer" since this line of focused cells is injected into the flow cytometer's sheath manifold, where the sample is further focused hydrodynamically. This combination provides precise single particle alignment in interrogating lasers, even with large hydrodynamic sample cores at sample flow rates of 1000 µL/min, but the instrument can also inject sample at rates as low as 12.5 µL/min, where the sample cores are as small as in conventional instruments. Example protocols for combined phenotypic and functional assessment of indicators for live primitive stem cells in hematopoietic pathologies are demonstrated. Additionally, the acoustic orientation effect is shown for reagent free assessment of erythrocyte cell pathologies.
Conclusions: Extended flexibility of sample flow rates allows rethinking of conventional protocols, skipping centrifugation and lysis steps and allowing dilutions that would otherwise make analysis times intolerably long. An increasing clinical interest in analyzing live cells and their responses to drugs or other stimuli with as little sample manipulation as possible, makes acoustic focusing an important tool for discovery. Combining minimal sample prep with the acoustic orientation effect also shows promise as a method for analysis of erythrocyte health.

PAR-02-03

Detection of circulating cell-derived microparticles across different cardiovascular risk groups

Eugenia Gkaliagkousi[1], Barbara Nikolaidou[1], Efi Yiannaki[2], Eleni Gavriilaki[1], Antoni Lazaridis[1], Anastasios Vamvakis[1], Areti Triantafyllou[1], Dimitra Markala[2], Stella Douma[1]
[1]Aristotle University of Thessaloniki, THESSALONIKI, Greece
[2]Theageneion Hospital, THESSALONIKI, Greece

Introduction: Cardiovascular disease (CVD) remains the leading cause of death in developed countries. Despite advances in the field, there is still a quest for novel mediators that may help us better understand its pathophysiology, prevention and therapy. Therefore, the role of cell-derived microparticles is under investigation in patients with cardiovascular risk factors or diseases. We aimed to detect circulating microparticles derived from endothelial cells (EMPs), erythrocytes (RMPs) and platelets (PMPs) in different cardiovascular risk groups.
Methods: We studied consecutive patients with cardiovascular risk or disease and healthy controls. We recorded patient cardiovascular status and demographics. Comparisons between groups were performed with one-way ANOVA and Bonferroni's correction in the Statistical Package for Social Sciences (SPSS) 20 for Windows. Logarithmic transformation was performed for skewed variables.
Microparticles were detected in platelet poor plasma using a standardized protocol by flow cytometry (CyFlow Cube, Sysmex Partec). Gating was defined by fluorescent Megamix beads and fluorochrome coupled antibodies (CD 235a, CD41, CD144, CD105) were used for microparticles phenotyping in annexin-positive events.
Results: We studied 130 subjects: 27 healthy, 26 newly diagnosed with diabetes mellitus (DM), 49 newly diagnosed with essential hypertension (UH), 17 with myocardial infarction (MI) and 11 with stable coronary artery disease (CAD). All types of cell derived microparticles differed significantly among different disease groups ($p<0.001$). In particular, myocardial infarction patients presented with significantly higher values compared to the other groups: PMPS 1567±1358 ($p<0.001$), EMPS 1031±960 ($p<0.001$), RMPS: 295±222 /μl ($p<0.001$). Cardiovascular risk factors (age and comorbidities) were also significantly increased in patients with myocardial infarction. Regarding the other groups, patients with stable CAD presented no significant difference in EMPs (513±427/μl) and RMPs (133±240/μl) compared to myocardial infarction patients, but presented significantly decreased PMPs (467±310/μl, $p<0.001$). Microparticles levels followed decreasing in patients with newly diagnosed diabetes mellitus, untreated hypertension and controls.
Conclusions: Our study has shown that circulating microparticles are detectable in a reproducible way across different cardiovascular groups and increase according to cardiovascular risk. Our findings suggest that microparticles may be a useful novel marker in these patients and provide more insight into the atherosclerotic and thrombotic process in future studies.

PAR-02-04

Application of a new analysis strategy including recent multidimensional software to hematological multiparametric flow cytometry data

Marie C Bene[1], Francis dr Lacombe[2], Benoît Dupont[3], Nicolas dr Lechevalier[2], Jean-Philippe dr Vial[2], Thomas dr Matthes[4], Nicolas Arraud[4]
[1]Nantes University Hospital, NANTES, France
[2]Bordeaux Univeristy Hospital, PESSAC, France
[3]Beckman Coulter France, VILLEPINTE, France
[4]Hôpitaux Universitaires de Genève, GENEVE, Switzerland

Introduction: The development of mass cytometry, using up to 40 concomitant labels for a given sample, has led to the generation of large databases. In order to properly analyze these extensive files, new software solutions have been devised for mathematical reduction of multidimensional spaces and novel ways to visualize data have developed. Such tools have however still seldom been used for multiparameter analysis of conventional multiparameter flow cytometry data and then mostly in immunological applications.
Methods: Here we report on the feasibility of such an approach and on the conditions to respect to obtain proper results in hematological disease at diagnosis (D) and follow up (FU). FCS files, especially when data comparison from different samples is sought for, must be acquired with harmonized flow cytometry parameters, namely photomultipliers voltage, antibodies and fluorochromes. A second step of normalization and verification/application of compensations, involving biexponential/logical adjustments is then required. Various solutions, including R software and Bioconduct or CRAN packages can subsequently be used to visualize the results.

Results: Such strategies were applied to the concomitant analysis of (D) and (FU) bone marrow samples from patients with Acute Myeloblastic Leukemia (AML) together with a reference merged population of 23 normal bone marrows (NBM). Graphical representations were compared using principal component analysis, t-SNE and FlowSOM algorithms of i)a global file of all three types of samples and ii) each type of sample separately. Both t-SNE and FlowSOM provided a clear identification of normal and abnormal subsets. Separate representations of the three types of samples - (D), (FU) and (NBM)- allowed for both a rapid visualization and accurate quantification of normal and abnormal cell subpopulations. The FlowSOM solution further identified and visually characterized specific subsets both within the diagnostic clone and normal hematopoietic differentiation. Interestingly, FCS files generated by R software could be integrated into Kaluza software and then analyzed with classical analysis tools, further allowing to draw gates around t-SNE clusters or FlowSOM nodes and to backgate on classical biparametric representations of antibody combinations as well as on the classical CD45/SSC bone marrow cartography. The refined and automated results obtained from such recent software and newly available analysis methods could therefore be confirmed by classical manual tools of FCM analysis.
Conclusion: These versatile new tools open the way for a new era of classical flow cytometry analysis in hematological malignancies.

PAR-02-05

Utility of CD148, CD180 & CD200 in the differential diagnosis of B-cell non-Hodgkin lymphoma involving peripheral blood and bone marrow

Prashant Tembhare, Nilesh Deshpande, Neha Jodhawat, Gaurav Chatterjee, Sitaram Ghogale, Yajamanam Badrinath, Dilshad Dhaliwal, Ganesh Kumar Viswanathan, Sridhar Epari, Tanuja Shet, Sumeet Gujral, Pg Subramanian
Tata Memorial Centre, NAVI MUMBAI, India

Introduction: Flow cytometry is widely used in the diagnosis and classification of B-cell non-Hodgkin lymphoma (B-NHL). B-NHL like classical chronic lymphocytic leukemia (CLL), and hairy cell leukemia (HCL) are well-defined by flow cytometry; however, due to lack of specific markers it is challenging to classify other B-NHL like marginal zone lymphoma (MZL), atypical CLL (aCLL), mantle cell lymphoma (MCL), and lymphoplasmacytic lymphoma (LPL), especially in the absence of tissue diagnosis. In this study, we evaluated the utility of the combination of CD148, CD180 & CD200 in the differentia diagnosis of B-NHL involving peripheral blood (PB) and bone marrow (BM).
Methods: We studied the expression of CD148 (clone, REA204), CD180 (clone, MHR73.11) & CD200 (clone, B-ly6) in mature B-NHL using 10-color immunophenotyping on Cytoflex flow-cytometer. Mean florescent intensity (MFI) of these markers was determined as geometric mean and data-analysis were performed using Kaluza-v1.3 software. This study was conducted in Tata Memorial Centre and approved by the institutional ethical committee.
Results: 103 samples from 79 (30 PB & 49 BM) B-NHL patients (age, 24-85 years; M:F-2.24) and 24 BM control samples (uninvolved staging BM; age 17-68 years) were studied. 79 B-NHL included 21-CLL, 6-aCLL, 10-diffuse large B-cell lymphoma (DLBCL), 14-follicular cell lymphoma (FCL), 15-MCL, 9-MZL, 2-Burkitt lymphoma (BL), 1-hairy cell leukemia (HCL) and 1-LPL. Median (range) of MFI of CD148/CD180/CD200 were respectively as follow: in normal B-cells-3.86(0.38-63.67)/91.6(12.19-190)/10.9(1.04-111.5), CLL-3.05(0.12-10.88)/5.0(0.13-46.17)/44.13(10.55-117), aCLL-7.89(1.81-10.86)/ 35.72(5.57-63.4)/12.4(4.97-43.08), DLBCL-5.34(1.27-16.2)/28.26(4.64-98.86)/2.95(0.41-29.9), FCL-4.0(1.41-26.62)/23.83(5.2-78)/2.65(0.59-33.8), MCL-16.25(1.66-165.3)/11.58(0.47-86.6)/1.0(0.17-16.63), MZL-4.38(1.33-59.63)/64.3(18.57-138.7)/6.72(1.03-16.21), BL-7.1(2.79-11.42)/77.45(13.57-141.3)/1.63(0.7-2.57), HCL-0.73/91.5/51.6 and LPL-27.7/6.4/21.95. Expression of CD148 was highest in MCL (p<0.02), CD180 was highest in normal B-cells & MZL (p<0.01), and CD200 was highest in CLL (p<0.01). Combination of bright CD148 with heterogenous/dim/negative CD180/CD200 expression diagnosed MCL correctly in 67%(10/15) (sensitivity-67%/specificity-97%, p<0.001) and addition of CD5(+) & CD10(-) expression improved the sensitivity to 100%. Similarly, the combination of bright CD180 with heterogenous/dim/negative CD148/CD200 expression diagnosed MZL correctly in 67%(6/9) (sensitivity-67%/specificity-84.3%, p=0.002) and addition of CD5(-) & CD10(-) expression improved the specificity to 96%. Combination of bright CD200 with dim/negative CD148/CD180 expression diagnosed CLL in 76.2%(16/21) (sensitivity-89%, specificity-92%, p<0.001). In contrast to CLL, aCLL showed over-expression of CD148 and/or CD180 and under-expression of CD200 (p<0.01).
Conclusion: Over-expression of CD148 was significantly associated with MCL and over-expression of CD180 with MZL. The combination of these new markers- CD148, CD180 and CD200 along-with standard markers- CD5/CD10 are highly useful in the differential diagnosis of low-grade B-NHL involving peripheral blood and bone marrow.

PARALLEL SESSION 3: UK NEQAS LI FOCUS SESSION

PAR-03-01

Technical and analytical error in Clinical Flow Cytometry

Liam Whitby (Sheffield, UK)

Under ISO 15189:2012 section 4.9 laboratories performing clinical testing are responsible for identifying and managing non conformities in any aspect of their testing systems, including the pre-examination, examination and post-examination stages.

UK NEQAS LI routinely monitors the examination stage of laboratories by means of stabilised external quality assessment(EQA)/proficiency testing(PT) samples. The results obtained from analysis of these samples are then compared to the entire user group to identify any outliers and such laboratories are then notified of any performance issues. However, when performing testing within the laboratory flow cytometry consists of both a practical stage (antibody selection/aliquoting of blood/machine setup/sample acquisition) and an analysis phase (FCS file data analysis). Whilst in some areas of flow cytometry e.g. CD4+ T lymphocyte subset analysis the analysis is automatically performed by custom software packages. In other areas such as CD34+ stem cell enumeration and MRD testing the analysis templates, protocols and gate placements are all performed manually by a scientist.

In order to attempt to identify the analytical error inherent in flow cytometry UK NEQAS LI has issued electronic trials consisting of genuine and artificially created FCS files for CD34+ stem cell enumeration and B-ALL MRD measurement to participating laboratories. These FCS files were to be analysed using normal in-house methodologies and the associated results reported back to UK NEQAS LI.

Several laboratories were identified as making calculation errors in the generation of percentage or absolute count values. Overall the returned data highlighted that the variation of results as measured by CV of the electronic trial results compared to the closest matching traditional EQA/PT sample was lower in all electronic cases with an average reduction in variation of approximately one third.

This highlights that, whilst the inherent measurement uncertainty of any flow cytometric assay can be quantified, the main source of variation is the technical stage although laboratories should ensure all staff are of a suitable competence to minimise intra-laboratory variation due to different scientists analysis the same dataset and review their protocols to ensure correct performance of calculations for absolute counts.

PAR-03-02

Harmonisation of leukaemia reporting: Is it achievable

Alison Whitby (Sheffield, UK)

The testing and reporting of haemato-oncology cases by flow cytometry is an area that has previously been shown to show a large amount of diversity between laboratories. Numerous attempts to harmonize panels and reporting have been made by several international groups e.g. Harmonemia, Euroflow, ICCS. However the uptake and adherence to the guidance documents issued by these groups is limited.

As previously discussed at ESCCA 2016 UK NEQAS LI is currently redesigning the leukaemia immunophenotyping external quality assessment(EQA)/proficiency testing(PT) programme to better adhere to international and national best practice guidelines e.g. Guidelines on the use of multicolour flow cytometry in the diagnosis of haematological neoplasms (*Johansson et al* 2014). The preliminary exercises for the updated programme have highlighted a significant variation in the categorization of antigen expression in terms of absent, weak, moderate or strong with significant differences observed for all antigens tested by laboratories.

As such UK NEQAS LI enrolled a core cohort of 14 laboratories to see if harmonization of leukaemia reporting is possible to achieve. Laboratories were provided with electronic FCS files from a case of CLL and asked to analyse using their in-house procedures and report on the antigen expression for CD45, CD19, CD20, CD10, CD5, CD200, CD43, CD3, Kappa and Lambda in terms of positive/negative and the intensity of staining absent/weak/moderate/strong. Following submission of results the laboratories were provided with a guide on classification of staining expression derived from the AIEOP-BFM consensus guidelines 2016 for flow cytometric immunophenotyping of Pediatric acute lymphoblastic leukemia (*Dworzak et al* 2017) and asked to re-analyse the initial FCS file and report the same antigens using the provided classification system.

Results from both data sets were then compared to see if the use of a guidance document improved the harmonization of reporting between the laboratories.

PAR-03-03

Findings from new programmes

Liam Whitby (Sheffield, UK)

Since the ESCCA meeting in 2016 UK NEQAS LI launched an MRD AML programme and a pre-pilot programme for Haematological Malignancy Bone Marrow Aspirate Assessment (HMBMAA).
A total of 5 AML MRD exercises have been issued at MRD levels from 0.14% to 1.73% of the total nucleated cells (excluding the issue of a normal sample with no MRD in July 2017). The results from these exercises have shown a high degree of concordance between centres in terms of the overall result. Additionally there is evidence of sharing best practice producing a beneficial effect with laboratories associated to the iBFM group consistently demonstrating the lowest inter-laboratory variation of any group of laboratories performing in this programme.
There is no consensus in panel design between centres with over 28 different antigens tested in the most recent trial, although HLADR, CD56, CD45, CD33, CD34, CD13, CD117 are included in panels >50% of the time.
With regards to the pre-pilot programme for Haematological Malignancy Bone Marrow Aspirate Assessment (HMBMAA). This was initially a local and then a regional pre-pilot programme but it is now open to any interested participants. This programme is designed to assess the ability of staff to examine a bone marrow aspirate (provided in the form of a digital image), identify the size of a target population e.g. blasts, recognize up to 5 pre-selected by UK NEQAS LI cells of interest and then to decide on the subsequent course of action with regards to further testing.
This programme has highlighted issues in the identification of the selected target population with lymphoid cell population enumeration having a CV of 19.1% whereas blast cell population enumeration has a CV of 6.7%.
For the identification of specific cells the majority of issues related to the maturation stage of the cell of interest differing from the consensus e.g. myelocyte vs metamyelocyte. However there has been data returned where blast cells were mistaken for lymphocytes or erythroblasts.
Finally a large degree of diversity is seen in the further tests that would be requested with a mixture of respondents requesting flow cytometry, genetic testing or both.
These issues highlight the importance of providing external quality assessment (EQA)/proficiency testing(PT) in these areas and we expect further findings as the programmes expand and issue more exercises.

PARALLEL SESSION 4: HEMATOLOGICAL CELL POPULATIONS

PAR-04-01

Normal and malignant mast cells

Cristina Teodosio[1], Andrea Mayado[2], Maria Jara-Acevedo[3], Laura Sanchez-Muñoz[4], Ivan Alvarez-Twose[4], Andres García-Montero[2], Almudena Matito[4], Carolina Caldas[2], Ana Filipa Henriques[4], Javier Ignacio Muñoz-González[2], Noelia Dasilva-Freire[2], José Ignacio Sanchéz-Gallego[2], Luis Escribano[2], Alberto Orfao[2]
[1]Leiden University Medical Center, LEIDEN, Netherlands
[2]Servicio General de Citometría (NUCLEUS), CIC (IBMCC-CSIC/USAL and IBSAL), SALAMANCA, Spain
[3]DNA Sequencing Service (NUCLEUS), University of Salamanca, IBSAL, SALAMANCA, Spain
[4]Instituto de Estudios de Mastocitosis de Castilla La Mancha (CLMast), TOLEDO, Spain

Introduction: Mast cells (MC) are highly versatile cells that originate from CD34+ hematopoietic precursor cells in the bone marrow (BM) but, unlike other leukocytes, migrate via the blood and begin their final maturation under the influence of the local microenvironment of the tissues where they ultimately reside.
Apart from their role in the pathophysiology of various chronic allergic/inflammatory disorders, malignant/clonal mast cells are classically associated with mastocytosis, particularly with its systemic form. Systemic mastocytosis (SM) is characterized by an abnormal accumulation of clonal mast cells in one or more tissues, frequently involving the skin and the bone marrow. Despite most (>90%) adult patients carry the same *KIT* mutation (D816V mutation), the disease presents with multiple variants, with very distinct clinical and biological features, diverse prognosis and therapeutic requirements.
Results and discussion: The usage of standardized multiparameter flow cytometry has allowed for the correct identification and characterization of MC, as well as the establishment of their normal maturational profiles. The knowledge of these patterns, associated with the study of large groups of patients, has led to the identification of aberrant phenotypes (e.g. CD2 and CD25 expression) used for the diagnosis of the disease, as well as of three

immunophentypic profiles that correlate with the diagnostic, molecular and prognostic stratification of the disease. Furthermore, the study of the immunophenotypic features of pathological MC has allowed for the identification of novel potential therapeutic targets (e.g. CD33, CD52) and, as the therapeutic options for the disease improve, particularly for the most aggressive forms of mastocytosis, to monitor minimal residual disease.

PAR-04-02

Eosinophilia: The role of flow cytometry

Georgios Paterakis
Georgios Gennimatas General Hospital of Athens, ATHENS, Greece

The eosinophilias encompass a broad range of non-hematologic (secondary or reactive) and hematologic (primary, clonal) disorders with potential for end-organ damage. Disease prognosis relies on identifying the subtype of eosinophilia. Recognition of the myeloproliferative and lymphocytic variants of hypereosinophilia (HE) provides an etiologic understanding. They are rare chronic conditions characterized by eosinophils ≥1.5x10^9/L, causing organ damage. Most patients with HE lack etiologic understanding for their HE. Another subcategory of benign undefined eosinophilia includes patients in whom elevated blood eosinophilia is not associated with evidence of organ involvement. Flow cytometry seems to attain an increasing role in the diagnostic algorithm of eosinophilia investigation. Lymphocyte-variant HE (L-HE) is a unique subtype of HE characterized by an immunophenotypically aberrant T-cell population, often with clonal T-cell receptor gene rearrangements associated with excessive Th2 cytokine production, such as interleukin IL-5 and IL-4. L-HE typically affects the skin, lungs, and digestive tract, and many patients have asthma and atopic disease. The abnormal T-cell subpopulations often have aberrant surface phenotypes, often being CD3−CD4+ and less commonly being CD3+CD4−CD8−. Further investigation of the CD3−CD4+ aberrant T-cell sub-populations may reveal high expression of CD5 and loss of CD7. Reference ranges for the T-cell subsets as a percentage of total lymphocytes proposed are CD3-CD4+ 0-2%; CD3+CD4-CD8- 2-15%; CD3+CD4+CD7- 2-10%. Of note, for the "double negative" CD3+CD4-CD8- phenotype, the TCRαβ is considered the pathogenic subtype, and the flow cytometry panel should distinguish between TCRαβ and TCRγδ T lymphocytes. Patients with HE, exhibit clonal TCR rearrangements in 15-20% of cases. T-cell clonality is not equated with a T-cell lymphoma. TCR gene rearrangement analysis may be performed using a multiplex polymerase chain reaction of the junctional sequence of TCR-γ. Interestingly flow cytometry analysis of the Vβ domains of expressed TCRβ chains is increasingly used as an alternative approach to screen abnormal selective Vβ repertoires, that could possibly relate to L-HE. Vβ mAbs are used in multi-color combinations with CD3, CD4 and CD8 to further define the TCRVβ frequencies of normal T4 and T4 lymphocytes as well as abnormal subpopulations expressing Vβ. Reference ranges for Vβ domains have been assessed in several control studies. Frequencies above 20% are consistently abnormal. In conclusion, HE is an indication to perform flow cytometry in blood and marrow not only to diagnose hematologic malignancies related to HE but also to seek aberrant T cell subpopulations in relation to the L-HE variant.

PAR-04-03

Characterization of redox signaling sensitivity associated with leukemia disease progression by phospho-specific flow cytometry

Maria Teresa Scupoli[1], Chiara Cavallini[1], Roberto Chignola[1], Ilaria Dando[1], Omar Perbellini[2], Elda Mimiola[1], Ornella Lovato[1], Carlo Laudanna[1], Massimo Donadelli[1]
[1]University of Verona, VERONA, Italy
[2]S. Bortolo Hospital, VICENZA, Italy

Introduction: B-cell receptor (BCR) signaling is a key determinant of variable clinical behavior and a target for therapeutic interventions in chronic lymphocytic leukemia (CLL). Endogenously produced H_2O_2 is thought to fine-tune the BCR signaling by reversibly inhibiting phosphatases. However, relatively little is known about how CLL cells sense and respond to such redox cues. In this study, we used phospho-specific flow cytometry to characterize redox sensitivity of BCR signaling in CLL patients.
Methods: Phosphorylation levels of SYK, ERK1/2, p38, NF-κB p65, and JNK, were analyzed at the single-cell level in 42 CLL cell samples using phospho-specific flow cytometry. Phosphorylation was measured in the basal condition and following H_2O_2 stimulation. mRNA expression level of *catalase*, *copper-zinc superoxide dismutase* (*CuZnSOD*) and *manganese superoxide dismutase* (*MnSOD*) was quantified by qRT-PCR. Circulating B cells from healthy individuals were analyzed as controls.

Results: H_2O_2 induced a significant increase in phosphorylation of BCR signaling proteins, which is higher and more heterogeneous in CLL than normal B cells. Phosphorylation response of SYK was significantly higher in the patient subsets defined by the mutated *IGHV* status (M-CLL) (*P*=0.032), ZAP70 (*P*=0.020) and CD38 (*P*=0.005) expression. Phosphorylation response of ERK1/2 was significantly higher in patients expressing ZAP70 (*P*=0.040). To assess the impact of redox signaling sensitivity on disease progression, measured as time to first treatment (TTFT), we examined time-to-event modeling utilizing signaling data and currently used prognostic parameters. Kaplan-Meier curves showed statistically significant slower progression (longer TTFT) in patients with higher pSYK (*P*=0.003) and pERK1/2 (*P*=0.012). To test the hypothesis that redox signaling hypersensitivity could be a decreased antioxidant cellular capability, we analyzed major cellular antioxidant systems in CLL cells. We showed that samples exhibiting signaling hypersensitivity to H_2O_2 expressed lower levels of *catalase* (*P*=0.009), which converts hydrogen peroxide to water and molecular oxygen.
Conclusions: This study shows that sensitivity of BCR signaling proteins to redox cues, is of clinical relevance in CLL as redox signaling hypersensitivity is associated with a lesser aggressive behavior of the disease. Redox hypersensitivity of BCR signaling proteins is linked to a lower antioxidant capability of cancer cells. The key advance of this study is defining redox signaling hypersensitivity and lower antioxidant capability as intrinsic characteristics of CLL with favorable parameters and an indolent clinical course. Overall, these results show that phospho-specific flow cytometry enables to capture biologically and clinically relevant information of pathologic cells.

PAR-04-04

Accumulation of classical monocytes defines a subgroup of MDS that frequently evolve into CMML

Orianne Wagner-Ballon[1], Dorothée Selimoglu-Buet[2], Bouchra Badaoui[1], Emmanuel dr Benayoun[1], Andrea dr Toma[1], Pierre prof. Fenaux[3], Bruno prof. Quesnel[4], Gabriel dr Etienne[5], Thorsten prof. Braun[6], Nassera dr Abermil[7], Margot Morabito[2], Nathalie dr Droin[2], Eric Solary[2]
[1]Hopitaux universitaires Henri Mondor, CRÉTEIL, France
[2]Institut Gustave Roussy, VILLEJUIF, France
[3]APHP, Hôpital Saint-Louis, PARIS, France
[4]Centre Hospitalier Régional Universitaire de Lille, LILLE, France
[5]Institut Bergonié, BORDEAUX, France
[6]APHP, Hôpital Avicenne, BOBIGNY, France
[7]APHP, Hôpital Saint-Antoine, PARIS, France

Introduction: Accumulation of classical monocytes MO1 ($CD14^{++}CD16^{-}$) ≥94% of total peripheral blood monocytes analyzed by flow cytometry is a specific and sensitive tool that distinguishes chronic myelomonocytic leukemia (CMML) from reactive monocytosis. A relative monocytosis, defined as a percentage of peripheral blood monocytes ≥10%, has been described in a subgroup of myelodysplastic syndromes (MDS) likely to evolve into genuine CMML. Here, we compare the monocyte subset repartition in CMML and in MDS.
Methods: Patients with a diagnosis of CMML (n=158) or MDS (n=84) according to the 2008 WHO classification were included between January 2015 and March 2017. These patients were reclassified in light of the 2016 updated-WHO criteria and were tested for flow cytometry monocyte subset assay.
Results: Of the 158 patients with CMML according to 2008 WHO criteria, 152 (96%) fulfilled the 2016 WHO criteria that include both a persistent peripheral blood monocytosis ≥ 1 $x10^9$/L and monocytes accounting for ≥10% of the white blood cell differential count. These patients were subdivided into CMML-0 (62), CMML-1 (66) and CMML-2 (24). MO1 accumulation was observed in 141 patients, notably in 100% of CMML-2. Among the 11 CMML patients with MO1 percentage <94%, five cases (4 CMML-1 and 1 CMML-0) presented an inflammatory state associated with an excess of intermediate $CD14^{++}CD16^{+}$ monocytes, thus masking the MO1 accumulation. A MDS had been initially diagnosed in 15 of the 152 studied CMML and 11 of them already showed marrow monocytosis ≥5% at diagnosis.
Besides, we prospectively analyzed monocyte subset repartition in the blood of 84 MDS patients at diagnosis. MO1 accumulation ≥94% was detected in 29 of them (35%). Compared to other MDS, these "CMML-like" MDS displayed a higher WBC number (6.1±3.9 vs 4.4±2.0$x10^9$/L; p=.05), a higher absolute monocyte count (0.6±0.2 vs 0.4±0.2$x10^9$/L; p<.05), and a higher fraction of monocytes in the bone marrow (5.8±3.8 vs 3.7±3.5$x10^9$/L; p<.05). Follow-up of 44 MDS patients showed that a monocyte count ≥1$x10^9$/L with a monocyte fraction ≥10% of WBC was detected significantly more often in "CMML-like" MDS than in other MDS patients (P<.01). In less than 1 year, 7 out of 16 MDS patients with a "CMML-like" phenotype at diagnosis evolved into overt CMML.
Conclusions: Altogether, we suggest that "CMML-like" MDS could be an entity that is likely to evolve into genuine CMML and that a MO1 accumulation ≥94% should be considered to be included as a major criterion for both CMML and CMML-like MDS diagnosis.

PAR-04-05

Co-expression of CD25/CD34/CD123 is a highly predictive indicator of FLT3-ITD mutations in acute myeloid leukemia

Manisha Suthar, Gaurav Chatterjee, Yajamanam Badrinath, Nikesh Kunder, Shruti Chaudhary, Swapnali Joshi, Sitaram Ghogale, Nikhil Patkar, Pg Subramanian, Sumeet Gujral, Prashant Tembhare
Tata Memorial Centre, NAVI MUMBAI, India

Introduction: Fms-like tyrosine kinase receptor internal tandem duplications (FLT3-ITD) mutation is a well-established prognostic factor in acute myeloid leukemia (AML). FLT3-ITD mutation screening by PCR is not sensitive enough to identify small clones which may be responsible for relapse. Flow-cytometry (FC) is a powerful technique that identifies small blast clones and also effectively indicates concurrent genetic lesions. Recently, CD25 is shown to be a poor prognostic marker in the intermediate-risk AML (IR-AML) and also shown to be associated with FLT3-ITD mutations. However, predictive value of CD25 alone in detection of FLT3-ITD positive AML is not very high. We evaluated the frequency of CD25 expression in AML and predictive value of co-expression of CD25/CD34/CD123 for FLT3-ITD mutation in AML.
Methods & Materials: We studied the expression-pattern of CD25 (PE-CF594, clone-B1.49.9), CD34(APC, clone-581), CD123(BV421, clone-9F5) in 201 AML patients using 10-color FC on Navios Cytometer and data was analyzed using Kaluza-v1.3 software. Positive expression of CD25, CD34 and CD123 were defined with cut off >20% of leukemic-blasts. For FLT3-ITD mutations, the genomic DNA was extracted and the FLT3 region was amplified using PCR. Fragment length analysis was done by capillary electrophoresis and identification of additional peaks was done if present. Cytogenetic studies were performed by conventional method and FISH. The study was conducted in Tata Memorial Centre and approved by institutional ethical committee.
Results: CD25 was found to be positive in 36 (17.9%) cases with expression-pattern of strong, weak, subset and variable expression in 27.8%, 16.7%, 38.9% and 16.7% samples. Molecular studies revealed FLT3-ITD mutations were also found in 32 (15.9%) cases. Sensitivity and specificity of CD25 expression alone in prediction of FLT3-ITD mutation was 40.62% (95% CI, 23.70% to 59.36%) and 86.39 % (95% CI, 80.28% to 91.17%) respectively. The positive and negative likelihood ratio was 2.9 and 0.7 respectively. However, sensitivity and specificity of co-expression of CD25/CD34/CD123 in identifying AML with FLT3-ITD mutation was 56.3% (95% CI, 0.32-0.8) and 97.6% (95% CI, 0.8-0.94) respectively. On Chi-square-test analysis this association was found to be statistically significant with $p<0.001$. The positive likelihood ratio and negative likelihood ratio of co-expression of CD25/CD34/CD123 in identifying AML with FLT3-ITD mutation was 4.43 and 0.48 respectively.
Conclusion: Co-expression of CD25/CD34/CD123 is a highly specific immunophenotypic indicator in determination of AML with FLT3-ITD mutations. It can be considered as surrogate marker for FLT3-ITD mutation in AML.

PARALLEL SESSION 5: FLOW CYTOMETRY IN CLINICAL TRIALS

PAR-05-01 & PAR-05-02

Challenges of flow Cytometry in Global Clinical studies

Christèle Gonneau
Covance, MEYRIN, GENEVA, Switzerland

The recent increase in the use of flow cytometry in global clinical trials can in large part be attributed to the requirements for immune monitoring in the evaluation of novel immunotherapies for cancer, autoimmunity and chronic viral diseases. The generation of high quality flow cytometry for multicenter, longitudinal trials requires robust pre-analytical, analytical and post-analytical processes as well as cross-site standardization.
In this presentation, we will review the current best practices for multi-center standardization by providing overviews of assay validation and global operationalization processes that ensure the integrity and the quality of the data generated: from specimen collection, transportation, assay performance monitoring, staff training to data analysis.
Challenges of instrument to instrument standardization will also be addressed. We will first describe the performance of the current cross standardization process of our 22 instruments globally. We will then present a case study of a novel approach to instrument standardization in which specific fluorochrome bound beads are used in order to improve the comparability between instruments.
Overall, the presentation will review the scientific and operational processes that are key for conducting multi-center clinical trials and for generating flow cytometry data that is compliant to global quality standards.

PAR-05-03

Association between the expression of factor XIII-A in lymphoblasts and the 'B-other' cytogenetic group in children with acute lymphoblastic leukemia

Zsuzsanna Hevessy, Bettina Kárai, Eszter Szánthó, László Csáthy, Aniko Ujfalusi, Katalin Gyurina, István Szegedi, János Kappelmayer, Csongor Kiss
University of Debrecen, Faculty of Medicine, DEBRECEN, Hungary

Introduction: Previously we confirmed that the expression of subunit A of blood coagulation factor XIII (FXIII-A) was a sensitive intracellular marker for the classification of acute myeloid leukemias (AML). Moreover, the lack of expression of FXIII-A in acute promyelocytic leukemia was associated with an unfavorable outcome. Recently we identified B-cell lineage leukemic lymphoblasts as a new expression site for FXIII-A. On the basis of FXIII-A expression, various subgroups of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) can be identified. Recently several studies have focused on the examination of 'B-other' ALL. Approximately 50% of 'B-other' ALL cases were classified as *BCR-ABL1*-like ALL with unfavorable outcome. *IKZF1* deletion, which is a secondary abnormality, also often occurs in 'B-other' ALL. We aimed to investigate retrospectively FXIII-A expression as a possible prognostic marker and its association with other prognostic factors in childhood BCP-ALL among patients treated according to the BFM ALL-IC 2002 protocol.
Methods: Fifty-five children with BCP-ALL were included in the study. Bone marrow samples were obtained by aspiration and the presence of FXIII-A was detected by flow cytometry. G-banding and fluorescent in situ hybridization was performed according to standard procedures.
Results: Of the 55 common ALL patients enrolled in the study, 18 exhibited FXIII-A-negative lymphoblasts and 37 showed FXIII-A positivity. The 10-year event-free survival (EFS) and overall survival (OS) rate of FXIII-A-positive and FXIII-A-negative patients showed significant differences (EFS: 84% vs. 61%, respectively; p=0.031; OS: 89% vs. 61%; p=0.008). Of all the parameters examined, there was correspondence only between FXIII-A expression and 'B-other' genetic subgroup. Multivariate Cox regression analysis of FXIII-subtype and genetic group or 'B-other' subgroup identified FXIII-A negative characteristic as an independent predictor for poor outcome in BCP-ALL (EFS: HR: 3.6, 95% Cl: 1.2-11.3, p=0.027; OS: HR: 5.6, 95% Cl: 1.5-20.12, p=0.009). In the FXIII-A positive cases reduction of blast percentage was significantly higher in the FXIII-A-positive blast subclone than in the FXIII-A-negative one in day 8 peripheral blood (p=0.031) and both in day 15 (p=0.0003) and in day 33 (p<0.0001) bone marrow aspirate samples.
Conclusion: We found an excellent correlation between long-term survival and the FXIII-A-positive phenotype of lymphoblasts, which proves that FXIII-A expression character in lymphoblast is not only a useful LAIP but also a powerful prognostic factor in childhood BCP-ALL. In addition, FXIII-A expression is associated with the 'B-other' characteristics, therefore, FXIII-A can help to identify those cases that may require further detailed genetic examination using expensive methods.

PAR-05-04

Minimal residual disease and log -reduction explain the superior value of double autologous stem cell transplant in multiple myeloma

Giovanni Rossi[1], Antonietta Falcone[1], Maria Marta Minervini[1], Giovanni Pio de Cillis[1], Chiara de Waure[2], Leuconoe Grazia Sisti[2], Daniela Valente[1], Vincenzo Chiello[1], Potito Rosario Scalzulli[1], Angelo Michele Carella[1], Nicola Cascavilla[1]
[1]IRCCS „Casa Sollievo della sofferenza„, SAN GIOVANNI ROTONDO (FG), Italy
[2]Catholic University of Sacred Heart, ROME, Italy

Introduction: Previous studies have demonstrated that the identification of minimal residual disease (MRD) by flow cytometry (FC) at day 100 following autologous stem cell transplant (ASCT) predicts both progression free survival (PFS) and overall survival (OS) in younger patients with multiple myeloma (MM). Anyway, median PFS after single ASCT is 27 and 16 monthes within MRD negative and MRD positive patients. These results, not exhaustive for younger patients, suggested that further treatments are desirable after single transplant. Although it was clinically demonstrated that double ASCT effected superior RFS and EFS rate, biological explanations of these findings were not given and single ASCT remain the treatment of choice in MM. Thus, we firstly investigated the role of MRD and Log-reduction of plasma cells (PCs) in quantifying the degree of tumor reduction after any ASCT. Secondly, we defined the most predictive time point by monitoring MRD and Log-reduction.
Methods: Bone marrow samples from 30 patients who underwent double ASCT were assessed by FC at different time points: post induction (MRD1 and LOG1), post first- (MRD2 and LOG2) and post- second (MRD3 and LOG3)ASCT. MRD (>0.01%) was evaluated by a six-color FC. Log –reduction was calculated as a logaritmic ratio between the PCs at presentation and PCs at each time of assessment.

Results: A significant difference was evidenced among the three time points from ANOVA test for both LOG-reduction ($p<0.001$) and MRD ($p=0.005$). In particular, LOG3 was significantly greater than LOG2 ($p<0.001$) and LOG1($p<0.001$). Similarly, MRD achieved after double ASCT was deeper than MRD achieved after single ASCT ($p=0.005$) and after induction($p<0.001$). Then, frequency of MRD positive patients after double ASCT was significantly lower than that found after the first ASCT (n= 15 vs n= 23,$p=0.008$) and after induction (n=15 vs n=27, $p=0.004$). When the survival analysis was considered, a significant reduction of PFS was observed in patients belonging to an unfavorable cytogenetics risk group ($p<0.001$) and patients showing a MRD over 0.01% (34.1 vs 17.6 mths, $p=0.031$) as well as a Log-reduction lower than 2.57 ($p=0.01$) after double ASCT . Results of MRD and LOG –reduction post double ASCT were confirmed at multivariate analysis ($p=0.004$ and $p=0.01$, respectively).
Conclusions: Our results by FC sustained the double ASCT as the goal treatment strategy in MM because of a deeper reduction of PCs, a higher frequency of MRD positive patients and a longer PFS compared to single ASCT.

PAR-05-05

Prognostic value of flow cytometric bone marrow investigation in neuroblastoma patients

Alexander Popov[1], Alexander Druy[1], Egor Shorikov[2], Tatiana Verzhbitskaya[3], Grigory Tsaur[3], Alexander Solodovnikov[3], Larisa Fechina[3]
[1]National Research Center for Pediatric Hematology, Oncology and Immunology, MOSCOW, Russian Federation
[2]PET-technology, EKATERINBURG, Russian Federation
[3]Regional Children Hospital / Research Institute of Medical Cell Technologies, EKATERINBURG, Russian Federation

Introduction: Bone marrow (BM) micrometastases detection in children with neuroblastoma (NB) is crucial for correct patients staging and risk group stratification. Flow cytometry (FC) is widely available, fast and easy-to perform approach for finding NB cells among normal BM hematopoietic cells. Aim of the study was to investigate prognostic significance of flow cytometric tumor cells' detection in BM of children with NB at the time of diagnosis.
Methods: 51 patients (24 boys and 27 girls) aged from 6 days to 15 years (median age 1 year 3 months) with NB were included in the study. BM samples at the time of diagnosis were obtained from 1-5 aspiration sites per patient (median 3 samples per patient). 4-5-color FC was applied for CD45(-)CD56(+)CD81(+)GD2(+)CD9(+)-cells evaluation.
Results: NB cells were detected in BM by FC more frequently comparing to conventional cytomorphology (49.0% and 29.4% patients respectively, $p=0.043$). Patients with NB cells detected in BM by FC had significantly worse event-free survival, overall survival and progression-free survival (28.0±9.0%, 35.8±10.7% and 34.3±10.4% respectively) in comparison to children with negative result of immunophenotyping (83.5±7.6%, 87.7±6.7% *и* 86.8±7.1% respectively, $p<0.001$ in all cases). BM involvement detection by FC maintained its prognostic significance in following patients groups distinguished by other stratification criteria: patients without *MYCN* amplification, patients without BM lesion as assessed by cytomorphology, patients younger and older than 1 year, patients with localized tumor (stages I-III) as well as with stage IV. In multivariate analysis immunophenotyping proved to be an independent prognostic factor when analyzed jointly with other risk factors such as age, disease stage and *MYCN* amplification. In 42 patients BM involvement was also studied by RQ-PCR for *PHOX2B* and *TH* genes expression. 31of them (73.8%) had BM disease detected by both FC and RQ-PCR. 8 patients (19.1%) were positive by FC only, 3(7.1%) – only by RQ-PCR. Positivity of BM by FC led to dramatic decreasing of EFS in this subgroup also: 0.30(0.10) vs. 0.84(0.09), $p<0.001$, while deterioration of EFS in *PHOX2B/TH* expression was not significant: 0.40(0.13) vs. 0.69(0.09), $p=0.061$. Within groups of patients divided by RQ-PCR positivity, presence of BM disease by FC retained prognostic significance ($p=0.027$ in RQ-PCR-positive and $p=0.022$ in RQ-PCR-negative cases).
Conclusions: Thus flow cytometric BM involvement detection has very strong prognostic impact even stronger than RQ-PCR. It could be used in combination with other parameters for the treatment strategy choice in patients with NB.

PARALLEL SESSION 6: QUALITY CONTROL AND DATA ANALYSIS

PAR-06-01

QUALITY CONTROL AND DATA ANALYSIS: Flow cytometry

Iannis Drakos
ESCCA, PARIS, France

Description: Flow Cytometry is a mature technique with history that spans over almost 100 years of research and development. Today it constitutes a robust method for data production and as a result it is included in numerous clinical and research processes.
The high standards of quality control and the extensive options for data visualization allow a cytometry professional to gain a comprehensive insight of the analyzed sample, but also to identify possible deficiencies during the sample preparation, the equipment settings and the data curation.
The same high QC standards of cytometry, originating from the long history of the technology and its involvement in clinical prognosis and diagnosis, may also cause complications in the involvement of cytometry in multidisciplinary approaches or strictly research protocols.
During this session we will try to briefly describe how QC can affect data analysis in both ways.

PAR-06-02

QUALITY CONTROL AND DATA ANALYSIS - Molecular Biologie

Nicolas Derian
UPMC, POISSY, France

Description: Today, Omics are essential in biomedical field as they allow the use of the system biology approach. This approach focuses on how individual's biological systems (transcriptome, proteome, metabolome…) are affected by a specific biological status but also how they are affecting each other.
This approach implies the need of producing a large amount of data, with next-generation sequencing for example, and dedicated technologies were developped for this purpose. A vast collection of quality control and normlization methods were developped in parallel. These methods are different enough to lead to different biological findings.
In this session we will see that the normalization of OMICs data is not straightforward and methods to bypass this problem.

PAR-06-03

TERS -application of a new tool for standardizing functional T-cell assays

Florian Kern[1], Pavlo Holenya[2], Maren Eckey[2], Tatjana Teck[2], Nicole Bidmon[3], Kim Beilstein[3], Tana Omokoko[3], Petra Simon[3], Cecile Gouttefangeas[4], Marij Schoenemakers-Welters[5], Sjoerd van der Burg[5], Ugur Sahin[3], Holger Wenschuh[2]
[1]Brighton and Sussex Medical School, BRIGHTON, United Kingdom
[2]JPT Peptide Technologies, BERLIN, Germany
[3]Biontech, MAINZ, Germany
[4]University of Tübingen, TÜBINGEN, Germany
[5]Leiden University Medical Centre, LEIDEN, Netherlands

Introduction: T-cell assays such as Elispot and ICS are complex and show significant coefficients of variation. These may result from sample acquisition, storage, and shipment, as well as processing. The analysis of these tests is subjective and an additional source of variability. The lack of external standards for functional T-cell assays makes it hard to control their day-to-day performance in clinical monitoring studies and limits their use in multi-center settings.
Methods: T-cell receptor engineered reference samples (TERS) are a recent development that is now commercially available. This new type of assay standard can be produced from PBMC using linear RNA and be adjusted with respect to the frequency of specific TCR-bearing T-cells required. TERS kept frozen deliver stable signals over time and can be used in connection with stimulation based assays (Elispot, ICS) as well as MHC-multimer staining. Here we tested several of our new commercially available batches of TERS in regards to monitoring intra and inter-assay variation.

Results: Our results with the now commercially available TERS kits confirm the usefulness of these robust new standards. We demonstrate how using TERS to generate Levey-Jennings Charts will be useful for monitoring daily assay performance during monitoring studies. Longer studies will need to show if Westgard rules can or should be applied for quality control purposes.
Conclusions: TERS can be integrated in routine quality control procedures for T-cell assays in analogy to high and low controls used for most clinical assays. They can be produced as and when required. This allows users to accept or reject assay runs based on an objective control and provides a new level of quality control for functional T-cell assays over time.

PAR-06-04

Evaluation of CD319 (SLAMF7) as a novel gating marker for plasma cells in flow cytometric immunophenotyping of multiple myeloma

Sitaram Ghogale, P. G. Subramanian, Keziah Cherian, Gaurav Chatterjee, Nilesh Deshpande, Badrinath Yajamanam, Ashok Kumar, Ganesh Kumar, Prashant Tembhare
Tata Memorial Centre, NAVI MUMBAI, India

Background: Traditionally, plasma cells (PCs) are identified using strong CD38 and CD138 expression in flow-cytometric Immunophenotyping (FCI). However, variable loss of CD138 and decreased-expression of CD38 is well-known in clonal-PCs. The recent approval of anti-CD38-monoclonal antibody (Daratumumab) therapy is shown to cause down-regulation of CD38 expression. Hence, traditional markers may not be adequate for FCI of PCs. Recently, CD319 (SLAMF7) is claimed to be useful for FCI-PC gating but in a small cohort of samples and clinical-trial settings. However, its utility in routine laboratory practice for plasma cell gating is still not established. We investigated the value of CD319 as gating marker in FCI of MM in routine laboratory practice.
Methods: We analyzed expression-pattern of CD319 (PE, clone-CRACC) in PCs from bone marrow (BM) of 80 newly-diagnosed MM and 15 control samples (uninvolved staging BM). FCI characterization was performed on Navios flow-cytometer and data-analysis was performed using Kaluza-v1.3-software. Expression-levels of CD319 in PCs against remaining hematopoietic cells (HCs) was determined as the ratio of mean fluorescent intensity (MFI) of CD319 in PCs to MFI of HCs (denoted as "MFI-R"). The pattern of expression (homogenous/heterogenous) was determined as coefficient-of-variation of immunofluorescence (CV-IF). Statistical analysis was performed using SPSS-v16.
Results: Median (range) of total PCs/viable cells (TPCs) in MM and control samples were 3.85% (0.12%-85%) and 1.83% (0.3%-6.53%). Median & standard deviation (SD) of MFI-R of CD319 in abnormal PCs (aPCs) were 13.85 & 8.03 and normal PCs (nPCs) were 18.3 & 6.8 respectively. This data revealed that CD319 was strongly expressed in PCs than rest of HCs. Expression-levels of CD319 was relatively lower in aPCs compared to nPCs (p=0.005). It was positive in 100% of PCs in all control-samples and 95% (76/80) MM-samples. In 4-MM samples, it was negative in 3%, 10%, 15% & 84% of total aPCs. Median (SD) CV-IF of CD319 in PCs was 55.98 (7.8) and that of CD138 and CD38 was 96.57 (56) and 64.15 (19.2). Thus, CD319 and CD38 showed homogenous-expression but CD138 showed significantly heterogenous-expression. The correlation between TPC gated with CD319vsCD45 gating-strategy and CD138vsCD38vsCD45 gating-strategy was found to be very high with Pearson-correlation test (r=0.94, p<0.001).
Conclusion: CD319 shows bright and homogenous expression in PCs, whereas CD138 is a highly heterogenous marker with almost one-third of cases with weak-expression. Hence, CD319 is a useful gating-marker in flow-cytometric diagnosis and monitoring of MM, especially with up-coming Daratumumab-based therapeutic protocols.

PAR-06-05

AML MRD by multiparameter flow cytometry using LAIP and LSC: Methodological aspects in multicentric study of the French AML Intergroup

Adriana Plesa[1], Florent Dumezy[2], Francois Vergez[3], Carmen Aanei[4], Isabelle Arnoux[5], Christine Arnoulet[6], Valerie Bardet[6], Elsa Bera[7], Lydia Campos Guyotat[8], Claude Capron[9], Nicolas Chapuis[10], Agnes Charpentier[11], Edouard Cornet[12], Jean Feuillard[13], Franck Genevieve[14], Estelle Guerin[13], Julien Guy[15], Veronique Harrivel[16], Marie Christine Jacob[17], Hélène Lapillonne[18], Magali Le-Garf Tavernier[19], Remi Letestu[20], Camille Lours[21], Anne Catherine Lhoumeau[6], Stephanie Mathis[9], Tiphanie Picot[8], Anna Raimbault[19], Victoria Raggueneau[22], Tatiana Raskivalova[17], Mikael Roussel[23], Veroniuqe Saada[24], Veroniuqe Salaun[12], Richard Veyrat Masson[25], Orianne Wagner-Ballon[26], Jaja Zhu[9], Xavier Thomas[27], Karine Celli-Lebras[28], Christian Recher[29], Hervé Dombret[28], Claude Preudhomme[2], Christophe Roumier[2]
[1]Laboratory of Hematology, PIERRE BENITE, France
[2]Laboratory of Hematology and Flow cytometry, CHU-Lille, LILLE, France

[3]Laboratory of Hematology and Flow cytometry, CHU-Toulouse, TOULOUSE, France
[4]Laboratory of Hematology and Flow cytometry,CHU-Saint Etienne, SAINT ETIENNE, France
[5]Laboratory of Hematology and Flow cytometry, CHU-Marseille, MARSEILLE, France
[6]Laboratory of Hematology and Flow cytometry, IPC-Marseille, MARSEILLE, France
[7]Laboratory of Hematology and Flow cytometry, CHU-Rouen, ROUEN, France
[8]Laboratory of Hematology and Flow cytometry, CHU-Saint Etienne, SAINT ETIENNE, France
[9]Laboratory of Hematology and Flow cytometry, Saint Cloud Hospital, AP-HP, Paris, PARIS, France
[10]Laboratory of Hematology and Flow cytometry, Cochin Hospital, AP-HP, Paris, PARIS, France
[11]Laboratory of Hematology and Flow cytometry, Lille St Paul Hospital, LILLE, France
[12]Laboratory of Hematology and Flow cytometry, CHU-Caen, CAEN, France
[13]Laboratory of Hematology and Flow cytometry, CHU-Limoges, LIMOGES, France
[14]Laboratory of Hematology and Flow cytometry, CHU-Angers, ANGERS, France
[15]Laboratory of Hematology and Flow cytometry, CHU-Dijon, DIJON, France
[16]Laboratory of Hematology and Flow cytometry, CHU-Amiens, AMIENS, France
[17]Laboratory of Hematology and Flow cytometry, CHU-Grenoble, GRENOBLE, France
[18]Laboratory of Hematology and Flow cytometry, Trousseau Hospital, AP-HP, Paris, PARIS, France
[19]Laboratory of Hematology and Flow cytometry, Pitie Salpetriere Hospital, AP-HP, PARIS, France
[20]Laboratory of Hematology and Flow cytometry, Avicene Hospital, AP-HP, Paris, PARIS, France
[21]Laboratory of Hematology and Flow cytometry, Lyon-Sud Hospital, HCL-CHU Lyon, LYON, France
[22]Laboratory of Hematology and Flow cytometry, Versailles Hospital, AP-HP, Paris, PARIS-VERSAILLES, France
[23]Laboratory of Hematology and Flow cytometry, CHU-Rennes, RENNES, France
[24]Laboratory of Hematology and Flow cytometry, IGR, AP-HP, PARIS, France
[25]Laboratory of Hematology and Flow cytometry, CHU-Clermont Ferrand, CLERMANT FERRAND, France
[26]Laboratory of Hematology and Flow cytometry, Creteil Hospital, AP-HP, PARIS, France
[27]Department of Hematology Lyon-Sud Hospital, HCL-CHU Lyon, France, LYON, France
[28]Department of Hematology, Saint Louis Hospital, AP-HP, Paris, France, PARIS, France
[29]Department of Hematology, CHU-Toulouse, France, TOULOUSE, France

Introduction: MRD monitoring is now mandatory for treatment response evaluation in AML clinical trials (ELN-2017 guidelines). In this setting, beside to molecular biology methods, multiparameter flow cytometry assay represents the most reliable approach. The aim of this study was to implement MRD flow in 25 haematology laboratories participating in the French AML Intergroup.
Methods: We designed a 2-tube panel to identify the pattern of LAIP (Leukaemia Aberrant Immunophenotype) and the LSC (Leukemia Stem Cells) in CD34+CD38- cell compartment. A CD34/CD38/CD45/CD117 " backbone " was used in both tubes, completed by CD7/CD56/CD13/CD33/CD19/CD90/Mix (CD97+CLL1+TIM3)/CD123 and CD45RA. The panel was implemented on Becton Dickinson or Beckman Coulter cytometers. Harmonisation of the sensitivity between platforms was performed using Rainbows calibration beads in all laboratories (13 BD and 12 BC). Data were analysed using the DIVA/INFINICYT and KALUZA software respectively, based on similar analytical strategies.
Results: To detect any bias between the platforms, we tested 10 EDTA fresh regenerative marrow samples in parallel at 2 different platforms (one CANTO and one NAVIOS). After "bulk" lysis technique (NH4Cl) cells were immunostained before washing. 500,000 cells were acquired in each tube. Merged data were used to evaluate the resolution for targeted population using KALUZA or INFINICYT. Specific rare-events populations (nHSC…) were evaluated by both platforms and the locally collective data were analysed. The results did not reveal any bias induced by the platforms or the softwares in terms of pattern distribution and intensity of signal. Then, standardisation of the data analysis strategy obtained in the participating canters was evaluated using a virtual quality control (CQA) by sharing FCS3.0 data files from one healthy donor and one from AML patient. About 90% of the locally analysed data from 13 centers were included in an interval centralized on the average of the series mean +/- 25%. Individual training was proposed to the operators which answers some outliers data, and the new virtual CQA will be propose to evaluate the reduction of discrepancies between the centers.
Conclusions: This methodological validation protocol is a mandatory step to consider the use of MRD flow in AML clinical trials. Choosing a multicentric approach could be challenging, but our first results are promising and showed the feasibility of this concept when: (i) a straight harmonisation of the instruments sensitivity and samples preparation are established, and (ii) training and systematic education among the analytical operators are regularly performed.

POSTER PRESENTATIONS - SUMMARY

Below an overview of the **poster titles and presenting authors** (as specified upon submission of the abstract) in the order of the poster (board) numbers.
The abstracts can be found after this overview.

001
High-sensitivity 5-, 6-, and 7-C assays for PNH WBC that can be performed on both Canto II and Navios platforms
Robert Sutherland
University Health Network/Toronto General Hospitla, TORONTO, Canada

002
Paroxysmal Nocturnal Hemoglobinuria Clone in Pediatric Aplastic Anemia Cases- A Single Centre Study of FLAER based screening from North India
Khaliqur Rahman
Sanjay Gandhi Post Graduate Institute of Medical Sciences, LUCKNOW, India

003
High sensitivity of the Hematoflow™ solution for chronic myelomonocytic leukemia screening
Orianne Wagner-Ballon
Hopitaux universitaires Henri Mondor, CRÉTEIL, France

004
Time dependent stability of light scatter and fluorescence characteristics of PNH cells
Iuri Marinov
Institute of Hematology and Blood Transfusion, PRAGUE, Czech Republic

005
Diagnosis of B-cell lymphoproliferative disorders: application of flow cytometry to clinical practice
Vasiliki Douka
G. Papanicolaou Hospital, THESSALONIKI, Greece

006
CD56 and CD16 monocyte subpopulations do not overlap in most myeloid neoplasms. Classical monocytes >94% are not specific to CMM
Georgios Paterakis
Georgios Gennimatas General Hospital of Athens, ATHENS, Greece

007
Detection of cell subsets with aberrant phenotype in pediatric normal or regenerating bone marrow samples
Eliana Gkika
Aghia Sophia" Children's Hospital", ATHENS, Greece

008
Evaluation of early (Day 15) treatment response in peripheral blood by flow cytometry in B-cell Acute Lymphoblastic Leukaemia patients
Ketan Ingle
Tata Memorial Hospital, MUMBAI, India

009
Creation of a database of B-cell lymphomas with Infinicyte and comparison of database-guided diagnosis with expertise-guided diagnosis by laboratory physicians
Jan Dirks
University Hospital Basel, BASEL, Switzerland

010
CD56 expression affects blast quantification in bone marrow aspirate and biopsy in myeloid neoplasm
Jan Dirks
University Hospital Basel, BASEL, Switzerland

011
Differences in the hematopoietic profile of multiple myeloma patients in prolonged remission may predict clinical outcome
Ioannis Kostopoulos
National and Kapodistrian University of Athens, ATHENS, Greece

012
Monitoring of small paroxysmal nocturnal hemoglobinuria clone in patients affected by severe aplastic anemia or aplastic anemia
Raffaella Milani
IRCCS San Raffaele Scientific Institute, MILANO, Italy

013
Automated sample preparation for CE-IVD dried screening tube for hematolymphoid malignacies
Massimo Geuna
Laboratory of Immunopathology. AO Ordine Mauriziano, TURIN, Italy

014
MET expression among hematopoietic system is restricted to plasma cells and plasma cell neoplasms
Casanova, Elena
Candiolo Cancer Institute, FPO–IRCCS, Lab. Cancer Stem Cell Research, CANDIOLO, Italy

015
Correlation between the Flow cytometric phenotyping and the Morphological differentiation in dyserythropoiesis
Hans Veenstra
Radboud University Medical Centre, NIJMEGEN, Netherlands

016
A rare case of Early T-cell Precursor Acute Lymphoblastic Leukaemia arising from a BCR-ABL1 negative Myeloproliferative Neoplasm
Timothy Milne
Barts Health NHS Trust, LONDON, United Kingdom

017
Evaluation of expression of proteins involved in active DNA demethylation by indirect multicolor flow cytometry in patients with acute leukemia
Anna Labejszo
Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz, BYDGOSZCZ, Poland

018
Minimal residual disease monitoring in multiple myeloma patients by flow cytometry: a single center experience
Natalya Pronkina
Research Institute of Fundamental and Clinical Immunology, NOVOSIBIRSK, Russian Federation

019
Diagnostic value of CD34+ cell subpopulations flow cytometry analysis in the bone marrow of MDS patients
Evgenia Konsta
Attikon University Hospital, Second Department of Internal Medicine and Research, ATHENS, Greece

020
The Role of Multicolor Flow Cytoemtry in the diagnosis of Sezary Syndrom/ Mycosis Fungoides
Mesude Falay
Ankara Numune Education and Resarch Hospital, ANKARA, Turkey

021
Lymph Node Assessment By Cytology And Multiparametric Flow Cytometry: A Prospective Study Of 176 Samples
Zuriñe Diez Gallarreta
Hospital Universitario Basurto, BILBAO, Spain

022
Flow Cytometry In 105 Consecutive Patients With Cytopenia And Suspeicion Of MDS: Strong Correlation With AML-Evolution And Survival
Zuriñe Diez Gallarreta
Hospital Universitario Basurto, BILBAO, Spain

023
Evaluation of the impact of standardized 8-color flow cytometry protocols (EuroFlow) on the diagnostic accuracy of poorly differentiated acute leukemias
Rafik Terra
Hopital Maisonneuve Rosemont, MONTREAL, Canada

024
Multisite performance evaluation study of the BD OneFlow™ Acute Leukemia Orientation Tube (ALOT)
David Bloxham
Cambridge University Hospitals NHS Foundation Trust, CAMBRIDGE, United Kingdom

025
Multisite Performance Evaluation Study of the BD OneFlow™ B-Cell Chronic Lymphoproliferative Disorder T1 (B-CLPD T1) Panel
Paula Fernandez
Kantonsspital Aarau, AARAU, Switzerland

026
Nonneoplastic lymphocyte subsets of blood and bone marrow in patients with angioimmunoblastic T-cell lymphoma
Valentina Dvirnyk
National Research Center for Hematology, MOSCOW, Russian Federation

027
Isolated blastic plasmacytoid dendritic cell neoplasm relapse in the central nervous system diagnosed by flow cytometry
Eirini Grigoriou
Evagelismos Hospital, ATHENS, Greece

028
Clinical and Biological features of Acute Undifferentiated Leukemia
Ilana Slouzkey
Rambam health care campus, HAIFA, Israel

029
AML manifestation in patient with blastic plasmacytoid dendritic cell neoplasm.
Darya Drokova
National Research Center for Hematology, MOSCOW, Russian Federation

030
Blastoid cells in the bone marrow of a patient with severe aplastic anaemia treated with antithymocyte globulin and eltrombopag
Marta Santiago
Hematology Department, Hospital Universitario y Politécnico La Fe, VALENCIA, Spain

031
Radar analysis shows two different types of erythropoietic maturation patterns in patients with myelodysplastic syndromes
Despoina Violidaki
Lund University Hospital, LUND, Sweden

032
Ca2+ signaling capacity of chronic lymphocytic leukemia B cells is attenuated after in vivo Ibrutinib treatment
Konstantia Kotta
INAB, CERTH, THESSALONIKI, Greece

033 *Best Poster Abstract (also presented in session PLE-05)*
Probabilistic models for automated flow cytometric analysis of minimal residual disease in chronic lymphocytic leukemia
Konstantia Kotta
INAB, CERTH, THESSALONIKI, Greece

034
Multidisciplinary diagnostic and monitoring approach with flow Cytometry and NGS in ALL
Laura Koumas
Karaiskakio Foundation, NICOSIA, Cyprus

035
Determination of vimentin in CLL cells by flow cytometry
Eszter Szánthó
University of Debrecen, Faculty of Medicine, DEBRECEN, Hungary

036
Decrease in CD157 expression in some mature neutrophil populations can complicate the interpretation of PNH evaluation by flow cytometry
Phuong ph Nguyen Vo Thanh
LHUB Porte de Hal, BRUSSELS, Belgium

037
Analysis of Checkpoint Marker Expression on Immune Cells Using a 12-Color Assay on the BD FACSLyric™ Flow Cytometer
Suraj Saksena
BD Biosciences, SAN JOSE, U.S.A.

038
A modular, 12-color flow cytometry panel for the immunophenotyping of healthy and AML subjects on a BD FACSLyric™ flow cytometer
Tri Le
BD Biosciences, SAN JOSE, U.S.A.

039
Accurate enumeration of CD34+ cells with the BDTM Stem Cell Enumeration Kit on the BD FACSLyricTM system
Tri Le
BD Biosciences, SAN JOSE, U.S.A.

040
The impact of preanalytical errors on the identification of myelodysplastic phenotypes by flow cytometry
Bettina Kárai
University of Debrecen, DEBRECEN, Hungary

041
Evaluation of B-clonality in HIV positive patients in different clinical stages of the disease and its association with clinical parameters
Carlos Saavedra
Hospital Universitario Fundación Santa Fe de Bogotá, BOGOTÁ, Colombia

042
Utility of flow cytometry in the detection of tumoral cells in cerebrospinal fluid from patients with acute leukemia
Carlos Saavedra
Hospital Universitario Fundación Santa Fe de Bogotá, BOGOTÁ, Colombia

043
Eight color flow cytometry for monitoring minimal residual disease in Acute Promyelocytic Leukemia (APL): outcome and comparison with PML-RARA detection
Maura rosane Valerio Ikoma
Amaral Carvalho Hospital, BAURU, Brazil

044
Flow cytometric analysis of B-cell subpopulations as an approach for the B-cell lymphomas diagnosis from lymph node samples
Andreja Brozic
Institute of Oncology Ljubljana, LJUBLJANA, Slovenia

045
Which cutt-off value of EMA binding test for Hereditary spherocytosis?
Falay Mesude
Ankara Numune Education and Resarch Hospital, ANKARA, Turkey

046
Aberrant multilineage proliferation of precursor cells driven by monosomy 7 as a secondary malignancy after relapsed acute lymphoblastic leukemia
Lukasz Sedek
Medical University of Silesia in Katowice, ZABRZE, Poland

047
Immunophenotypic characteristics of acute myeloid leukemia with myelodysplasia-related changes - a single institution experience
Nada Kraguljac Kurtovic
Clinical Center of Serbia, Clinic of Hematology, BELGRADE, Serbia

048
Assessment of AML MRD using difference from 'digital' normal
Alan Dunlop
Viapath @ Kings College Hospital, LONDON, United Kingdom

049
Relevance of paroxysmal nocturnal haemoglobinuria clone monitoring in patients with bone marrow failure syndromes
Lourdes Cordón
Hematology Research Group, Instituto de Investigación Sanitaria La Fe, VALENCIA, Spain

050
Optimising gating strategies for residual disease detection in CLL patients receiving novel inhibitor therapies
Richard Leach
Leeds Teaching Hospitals NHS Trust, LEEDS, United Kingdom

051
Study of the effect of several biochemical drugs in myeloproliferative neoplasms cell lines, based on the status of RUNX1/CBF-BETA/P300/HIPK2 complex
Guadalupe Herrera
University of Valencia, VALENCIA, Spain

052
The role of flowcytometry in diagnosis of myelodysplastic syndrome with bone marrow hypoplasia
Judit Beata Kopeczi
Clinical Hematology and BMT Unit, TARGU-MURES, Romania

053
Prospective multicentric evaluation of paroxysmal noctural hemoglobinuria frequency in cases with isolated thrombocytopenia
Alberto Orfao
Cancer Research Centre (IBMCC, CSIC-USAL), University of Salamanca, SALAMANCA, Spain

054
Comparative effects of several drugs in myeloproliferative neoplasms cell lines based on the status of the RUNX1/CBF-BETA/P300/HIPK2 complex
Carlos Lozano-Asensio
Incliva Foundation, VALENCIA, Spain

055 *Best Poster Abstract (also presented in session PLE-05)*
Leukemic stem cells detection in PB by flow cytometry: a simple and rapid new diagnostic tool for Chronic Myeloid Leukemia
Santina Sirianni
Ematologia AOUS, SIENA, Italy

056 *Best Poster Abstract (also presented in session PLE-05)*
CD73, an ecto-5'-nucleotidase, is a commonly overexpressed aberrant marker in Acute Leukaemias
Mahima Sanyal
Tata Memorial Hospital, MUMBAI, India

057 *Best Poster Abstract (also presented in session PLE-05)*
Identification of Minimal Disseminated Disease in T-Cell Acute Lymphoblastic Lymphoma by Flowcytometric Immunophenotyping
Ganesh Kumar Viswanathan
Tata Memorial Centre, MUMBAI, India

058 *Best Poster Abstract (also presented in session PLE-05)*
Multicenter study of the antibody VS38c, at diagnosis, MRD and patients undergoing Daratumumab treatment for Multiple Myeloma (MM)
Ricardo Morilla
Royal Marsden Hospital, SUTTON SURREY, United Kingdom

059 *Best Poster Abstract (also presented in session PLE-05)*
CD44 is highly expressed in adult B-cell precursor Acute Lymphoblastic Leukemia and is a useful minimal residual disease monitoring marker
Pearl Rodrigues
Tata Memorial Hospital, MUMBAI, India

060
Colorectal cancer CTCs detection using two FCM approaches with EpCAM/EGFR and CKs
Aris Spathis
National and Kapodistrian University of Athens, ATHENS, Greece

061
A novel 4-colour panel for the detection of leukocytic populations in bronchoalveolar lavages and correlation with morphological counting
Aris Spathis
National and Kapodistrian University of Athens, ATHENS, Greece

062
Phenotypic detection of endothelial progenitor and circulating endothelial cells and their proliferation capacity with exercise in chronic heart failure patients
Katherina Psarra
Immunology and Histocompatibility Dept, Evangelismos Hospital, ATHENS, Greece

063
Immune Monitoring of Patients Receiving Rituximab Therapy
Michelle Delelys
Massachusetts General Hospital, BOSTON, U.S.A.

064
Selected Lactobacillus strains change H. pylori induced T cell profiles via dendritic cells modulation
Anna Helmin-Basa
Nicolaus Copernicus University Ludwik Rydygier Collegium Medicum in Bydgoszcz, BYDGOSZCZ, Poland

065
Lymphoid sub-populations in the bone marrow of adult and pediatric ITP patients
Nagwa Hassanein
Prince faisal cancer center, BURYDAHA, Saudi Arabia

066
Flow cytometry is a useful tool for identification of Neuroblastoma infiltrating the bone marrow
Nagwa Hassanein
Prince faisal cancer center, BURYDAHA, Saudi Arabia

067
Increased expression of neutrophil and monocyte CD64 in patients with active tuberculosis
Arianna Gatti
Hematology Laboratory and Transfusion Center, LEGNANO (MILANO), Italy

068
Evaluation of Activation and Homing Markers on Regulatory T cells using 12-Color BD FACSLyric™ Flow Cytometer
Suraj Saksena
BD Biosciences, SAN JOSE, U.S.A.

069
Cynomolgus macaca fascicularis as a model for pre-clinical studies with a specific biomarker important in inflammatory diseases
Rita Vicente
Sanofi, MONTPELLIER, France

070
Cytofluorimetric characterization of immune responses induced by a vaccine targeting the cancer stem cell antigen xCT in breast cancer models
Laura Conti
University of Torino, TORINO, Italy

071
Immunophenotypic and functional characterization of monocytes and macrophages derived from human induced pluripotent stem cells (iPSCs)
Silvia Della Bella
University of Milan - Humanitas Clinical and Research Center, ROZZANO (MI), Italy

072
BAFF and sCD23: modulators of the circulating B-cell compartment during pregnancy in atopic women?
Catarina Martins
NOVA Medical School|Faculdade de Ciências Médicas - Universidade Nova de Lisboa, LISBOA, Portugal

073
Evaluation of WC1 yo T cells in young and old water buffaloes (Bubalus bubalis)
Claudio Ortolani
Università di Urbino, URBINO, Italy

074
Evaluation of the immunophenotypic profile in systemic sclerosis patients at different disease stages
Elena Trombetta
IRCCS Policlinico di Milano, MILANO, Italy

075
Successful implementation of a simplified CD8+ degranulation assay for the diagnosis of Familial Hemophagocytic Lymphohistiocytosis syndrome
Marianna Tzanoudaki
Aghia Sophia" Children's Hospital", ATHENS, Greece

076
Antigen specific memory B cell fluctuations induced by the pneumococcal conjugate and plain polysaccharide vaccine in HIV-infected patients
Marianna Tzanoudaki
Dept. of Immunology & Histocompatibility, Aghia Sophia Children's Hospital, ATHENS, Greece

077
Antigen specific T cell subset alterations during treatment for M. tuberculosis infection in children
Eleni Ploumi
Aghia Sophia Children's Hospital, ATHENS, Greece

078
Effects of hyperglicemia on cultured myeloid cells from diabetic patients
Enrica Trevisiol
Università degli Studi di Padova, TREVISO, Italy

079
Single Platform CD34+ Cell Enumeration on the New BD FACSVia™ System
Yang Zeng
BD Life Sciences, SAN JOSE, U.S.A.

080
Evaluation of Instrument QC Tracking Results for the BD FACSVia™ System
Yang Zeng
BD Life Sciences, SAN JOSE, U.S.A.

081
Evaluation of the Long-Term Stability of the BD™ CS&T Setup Workflow on the BD FACSLyric™ System
Yang Zeng
BD Life Sciences, SAN JOSE, U.S.A.

082
Utility of PNH testing involving CD24 versus CD157 as the main GPI linked protein
Timothy Milne
Barts Health NHS Trust, LONDON, United Kingdom

083
In vitro evaluation of a new selective iNOS inhibitor on normal rat astrocytes and C6 rat glioma cells
Marialucia Gallorini
University of G. d'Annunzio, CHIETI, Italy

084
Demonstration of extended open vial stability of CE-IVD conjugated antibodies
Ankitha Channabasappa
Beckman Coulter, BANGALORE, India

085
Assessment and validation of internal acceptance criteria for sample stability for specimens undergoing clinical flow cytometric analysis
Alan Dunlop
Viapath Analytics LLP, LONDON, United Kingdom

086
CD7 expression in a case of chronic lymphocytic leukemia
Valentina Dvirnyk
National Research center for Hematology, MOSCOW, Russian Federation

087
A Real-Time Flow Cytometric Assay of Platelet Activation in Whole Blood of Dolphins (Tursiops truncatus) Using Human-Reacting Monoclonal Antibodies
José-Enrique O'Connor
The University of Valencia, VALENCIA, Spain

088
Flow Cytometric Analysis of Phagocytosis and Oxidative Burst in Animals Using Whole-Blood Assays Designed for Human Diagnostics
José-Enrique O'Connor
The University of Valencia, VALENCIA, Spain

089
Flow cytometry analysis of CD30 expression in acute T-lymphoblastic leukemia/lymphoma patient
Darya Drokova
National Research center for Hematology, MOSCOW, Russian Federation

090
Ten-color and 12-antibodies flow cytometry panel for high sensitivity detection of minimal residual disease in B-lymphoblastic leukemia
Rodolfo patussi Correia
Hospital Israelita Albert Einstein, SAO PAULO, Brazil

091
Channel-free & compensation-free dead cell exclusion: N+1 panel design
Roy Edward
BioStatus Limited, SHEPSHED, United Kingdom

092
Fast and accurate prediction of positive and negative urine cultures by flow cytometry
Bijan Moshaver
University Hospital Geneva, GENEVA, Switzerland

093
Automated flowcytometric identification of disease specific cells by the ECLIPSE algorithm
Leo Koenderman
University Medical Center Utrecht, UTRECHT, Netherlands

094
Investigating the role of CD25, CD69, CD74, CD133 and CD135 in detecting minimal residual disease in childhood acute myeloid leukemia
Dilshad Dhaliwal
Tata Memorial Centre, NAVI MUMBAI, India

095
The detection of coated platelets is a sensitive flow cytometric method to identify dasatanib induced side effects
János Kappelmayer
University of Debrecen, Department of Laboratory Medicine, DEBRECEN, Hungary

096
CD81 negative expression predicts a poor cytogenetic and prognostic risk group in chronic lymphocytic leukemia
Giovanni Rossi
IRCCS Casa Sollievo della sofferenza"", SAN GIOVANNI ROTONDO (FG), Italy

097
Fully automated enumeration of CD34+ hematopoietic stem cells using the MACSQuant Analyzer 10
Stefanie Pflitsch
Miltenyi Biotec, BERGISCH GLADBACH, Germany

098
Quality assurance of flow cytometry assays: Results of three-year semi-annual immune monitoring inter-site comparisons of the TCRab-Haplo2010 trial
Stefanie Biedermann
Miltenyi Biotec GmbH, BERGISCH GLADBACH, Germany

099
Assessment of the degree of inter-centre flow cytometry standardization of Lymphoid Screening Tube
Paula Amat
Hematology Department, Hospital Clínico Universitario, VALENCIA, Spain

100
Dried Leukocytes (Lysed Whole Blood) as Controls in Flow Cytometry
Yanli Liu
BD Biosciences, SAN JOSE, U.S.A.

101
Detection of endogenous alkaline phosphatase activity in intact leukemic cells
Laura G. Rico
Jose Carreras Leukaemia Research Institute, BADALONA (BARCELONA), Spain

102
Acoustophoretic Orientation of Red Blood Cells for Diagnosis of Red Cell Health and Pathology
Laura G. Rico
Jose Carreras Leukaemia Research Institute, BADALONA (BARCELONA), Spain

103
Yellow-green laser next generation-based flow cytometry for CD34+ progenitor cell counting
Laura G. Rico
Jose Carreras Leukaemia Research Institute, BADALONA (BARCELONA), Spain

104
Enhanced cellular discrimination using a novel optical microsystem
Antoine Leblanc-Hotte
Polytechnique Montreal, MONTREAL, Canada

105
Immunophenotypic features of bone marrow tumor cells in Burkitt lymphoma/leukemia: implications for B-lineage acute lymphoblastic leukemia diagnostics
Irina Demina
National Research Center for Pediatric Hematology, Oncology and Immunology, MOSCOW, Russian Federation

106
Immunophenotypic heterogeneity of acute leukemia meeting the early thymic precursor (ETP) criteria
Olga Illarionova
National Research Center for Pediatric Hematology, Oncology and Immunology, MOSCOW, Russian Federation

107
Pre-activated mesenchymal stromal cells prolong allograft survival in a pre-clinical model of corneal transplantation
Kevin Lynch
Regenerative Medicine Institute, GALWAY, Republic of Ireland

108
Thrombosis and Paroxysmal Nocturnal Hemoglobinuria Clones
Jos Pouwels
Treant Zorggroep location Scheper, EMMEN, Netherlands

109
Human bone marrow mesenchymal stem cells expansion and multipotency is strongly influenced by the electroactivity of the culture substrate
Lourdes Cordón
Grupo de Investigación en Hematología, Instituto Investigación Sanitaria La Fe, VALENCIA, Spain

110
Cerebro-Spinal Fluid lymphocyte counts in various neurologic conditions
Ling Yang
National University Hospital, SINGAPORE, Singapore

111
Endoplasmic Reticulum (ER) stress and lysosomal/mitochondrial re-organization induced by lysates of different Campylobacter jejuni strains
Barbara Canonico
University Of Urbino Carlo Bo, URBINO, Italy

112
Preanalytical phase in flow cytometric evaluation of bone marrow
Valentina Dvirnyk
National Research Center for Hematology, MOSCOW, Russian Federation

113
Development and validation of flow cytometry panels: A laboratory experience
Chryso Pierides
Karaiskakio Foundation, NICOSIA, Cyprus

114
Assessment of mesoporous silica nanoparticles toxicity by flow cytometry
Guadalupe Herrera
INCLIVA, VALENCIA, Spain

115
Endothelial Progenitor Cells and Diabetic Peripheral Neuropathy
Nikolitsa Kafasi
Laiko General Hospital, GALATSI, Greece

116
Validation of a Flow cytometry density assay to support anti-CD38 antibody clinical program in Multiple Myeloma patients
Wilfried Passe-Coutrin
Sanofi R&D, ALFORTVILLE, France

117
Detection of Circulating Tumor Cells: Comparison between molecular and cytometric methods
Dimitrios Athanasios Ntanovasilis
Research Genetic Cancer Center S.A., FLORINA, Greece

118

The BD FACSLyric™ System* and Assay Portability for Phenotyping T-, B- and Natural Killer Lymphocytes.

Imelda Omana-Zapata
BD Biosciences, SAN JOSE, California, USA

119

Enumeration of T-, B- and NK- cells in whole blood with the BD FACSLyric™ system*: A multisite study.

Imelda Omana-Zapata
BD Biosciences, SAN JOSE, California, USA

001

High-sensitivity 5-, 6-, and 7-C assays for PNH WBC that can be performed on both Canto II and Navios platforms

D. Robert Sutherland[1], Fernando Ortiz[2], Rakesh Nayyar[2], Amr Rajab[3], Andrea Illingworth[4], Miroslav Benko[5], Iuri Marinov[6]
[1]University Health Network/Toronto General Hospitla, TORONTO, Canada
[2]Cytoquest Corporation, TORONTO, Canada
[3]Lifelabs, TORONTO, Canada
[4]Dahl Chase Laboratories, BANGOR, U.S.A.
[5]ExBio, PRAHA, Czech Republic
[6]Institute of Hematology and Blood Transfusion, PRAGUE, Czech Republic

Introduction: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired Hematopoietic Stem Cell disorder characterized by an inability to make glyco-phosphatidyl-inositol (GPI)-linked cell surface structures. The bacterial lysin Aerolysin binds to the GPI moiety of such structures and the fluorescent version of this toxin (FLAER) is increasingly used to detect GPI-deficient WBCs by flow cytometry. While FLAER-based assays are widely used to detect PNH, FLAER is not available in all countries and is expensive to obtain in some others. To address this issue, a predicate 5-color FLAER/CD157-based assay was compared with a 6-color non-FLAER assay containing CD15FITC, CD157PE, CD24APC, CD14APCH7, CD64PECy7 and CD45PerCPCy5. The results confirmed very good agreement between the FLAER and non-FLAER-based tubes, suggesting that a cost effective simultaneous evaluation of PNH neutrophils and monocytes is possible without FLAER (Marinov et al, Cytometry Part B. ePub Jun 13, 2016).
Methods: Here, we have used a more direct approach with a 7-color assay comprising FLAER, CD157PE, CD15PerCPeFluor710, CD64PECy7, CD24APC, CD14APC-eFluor780 and CD45eFluor450 in a single tube. Using this cocktail of carefully selected conjugates, it is possible to stain a sample once, but acquire/analyze on either Navios and/or Canto II instruments. 6-C (minus CD14) and 5-C (minus CD24 and CD14) versions were also developed and compared with the clinical 5-C assay performed on the FC500/Navios using FLAER, CD157PE, CD64ECD, CD15PC5 and CD45PC7.
Results: With the 7-C assay, CD15-gated PNH neutrophil clone size can be quantified using either FLAER and CD157, FLAER and CD24, or CD157 versus CD24. The CD64-gated PNH monocyte clone size can be quantified using either FLAER and CD157, FLAER and CD14, or CD157 and CD14. Thus, the FLAER-based analysis plots can be directly compared with the non-FLAER plots in each lineage within the same stained sample. Analysis of more than 20 PNH samples has shown that the FLAER-based plots derive virtually identical data to the non-FLAER plot for neutrophils (r2=0.999) and monocytes (r2=0.997) and that closely similar data can be acquired using both Canto II and Navios platforms with 7-, 6- and 5-C versions of the assay. Assessment of several non-PNH samples confirms the extremely low background rate of PNH phenotypes (neutrophils and monocytes) with all 3 approaches.
Conclusions: We have developed and validated ICCS Guidelines-compliant, cross-platform (Navios and Canto II) 5-, 6- and 7-C PNH WBC assays. Data from the 7-C version confirms that non-FLAER and FLAER-based approaches generate the same data for PNH neutrophils/monocytes.

002

Paroxysmal Nocturnal Hemoglobinuria Clone in Pediatric Aplastic Anemia Cases- A Single Centre Study of FLAER based screening from North India

Khaliqur Rahman, Navkirti Mittal, Ruchi Gupta, Tanvi Gupta, Manoj Sarkar, Anshul Gupta, Sanjeev Kumar, Soniya Nityanand
Sanjay Gandhi Post Graduate Institute of Medical Sciences, LUCKNOW, India

Introduction: Fluorescent aerolysin (FLAER) based flow cytometric screening of leucocytes is currently the gold standard to detect the presence of a paroxysmal nocturnal hemoglobinuria (PNH) . There is a paucity of literature related to prevalence of PNH clones in paediatric aplastic anaemia (AA) patients especially from the developed nations.
Methods: We performed a retrospective analysis over a period of 42 months to study the prevalence of PNH clones in paediatric (age less than 18 yrs) AA cases, using FLAER based flow cytometric screening and analysed their clinico-pathological features.
Results: PNH clone was identified in 95 (32.7%) of the 282 patients screened. These were comprised of 38 cases of non severe, 40 cases of severe and 17 cases of very severe anemia. The age range was 4 to 17 yrs (median-14 yrs)

with an M:F ratio of 2.5:1. The median clone sizes (taken as proportion of PNH positive neutrophils) was 2.15% (range: 0.05-93.1%). There were 29(30.6%) patients with a minor (< 1%) clone, 42 (44.2%) with a small (1-10%), 12 (12.6%) with a medium (11-30%) and 12(12.6%) patient with a large (>30%) clone size. Only one patient, who harboured a large clone size showed clinical and laboratory evidence of hemolysis. None of the patient had any evidence of thrombosis. No association was noted between the severity of aplastic anemia and the presence of or the size of PNH clone. None of the patients tested for chromosome breakage analysis (n=42), showed an increased breakage. With the limited follow up data available (n=42), the treatment response to immunosuppressive therapy (IST) was similar to that of PNH negative patients.

Conclusion: There is a high prevalence of PNH clones in paediatric AA patients, which in majority of cases are of small sizes. Even if the clone size is large, it uncommonly gets translated into clinical or laboratory evidence of hemolysis. The use of IST does not show a better outcome as compared to PNH negative cases.

003

High sensitivity of the Hematoflow™ solution for chronic myelomonocytic leukemia screening

Orianne Wagner-Ballon[1], Romain Vazquez[1], Mikael Roussel[2], Bouchra Badaoui[1], Nicolas Freynet[1], Sihem Tarfi[1], Eric Solary[3], Dorothée Selimoglu-Buet[3]

[1]Hopitaux universitaires Henri Mondor, CRÉTEIL, France

[2]Centre Hospitalo-Universitaire Rennes, RENNES, France

[3]Institut Gustave Roussy, VILLEJUIF, France

Introduction: Classical monocytes, defined as $CD14^{++}CD16^{-}$, also called MO1, are easily quantified in peripheral blood by flow cytometry. Accumulation of such classical monocytes MO1 ≥94% can accurately distinguish chronic myelomonocytic leukemia (CMML) from reactive monocytosis with high specificity (94,1%) and high sensitivity (91,9%). HematoFlow™ solution (Beckman-Coulter) provides routinely white blood cell differentials by flow cytometry. Since it also allows CD16-negative monocyte quantification, the HematoFlow™ solution appears as a useful tool to manage monocytosis which remains a common issue in routine laboratories.

Methods: From January 2016 to April 2017, whole blood samples tested for MO1 percentage determination by the reference method were systematically processed with the HematoFlow™ solution, in order to quantify CD16-negative monocyte percentage. Thus, 153 samples were tested for both method in the same time regardless of the absolute monocyte count and even though a precise diagnosis was not available.

Results: The median of age of the patients was 74 years (range: 15 to 99). Complete blood counts presented wide range of both leukocytosis (median: 8.2 $x10^9$/L; range: 1.7 to 44.1) and monocytosis (median: 1.3 $x10^9$/L; range: 0.1 to 13.9). Comparison of the fraction of classical monocytes (MO1) measured by using the reference method and the fraction of CD16-negative monocytes calculated by the HematoFlow™ showed a significant correlation ($r=0.87$, $p<0.0001$).

Interestingly, measurement of the CD16-negative monocyte percentages obtained with HematoFlow™ leant towards a slight overestimation of the genuine classical monocyte percentages obtained by the reference method. Of the 73 cases with MO1 ≥94% by the reference method (i.e. suspected of being diagnosed as CMML), all were found having a CD16-negative monocyte percentage ≥ 94% by the Hematoflow™ method. In other words, the Hematoflow™ solution did not generate any false negative result, indicating a sensitivity of 100%. Conversely, of the 80 patients with a fraction of MO1 <94% by the reference method (i.e. not being diagnosed as a CMML according to this parameter), 22 (27.5%) displayed a percentage of CD16-negative monocytes ≥94%, indicating a specificity of 72.5%.

Importantly, the calculation of the CD16-negative monocyte percentage can be easily computerized and integrated to the middleware without needing an operator intervention.

Conclusions: Altogether, these results indicate that the HematoFlow™ solution provides a useful approximation of the MO1 percentage and can be used as a flag system for monocytosis management and CMML detection. This new application makes the Hematoflow™ solution an exciting tool for screening rapidly and accurately the pathological monocytosis.

004

Time dependent stability of light scatter and fluorescence characteristics of PNH cells

Iuri Marinov[1], Andrea Illingworth[2], Martina Kohoutová[1], Tereza Hulicková[3], Adam Pešek[1], Petra Vojtová[1], D. Robert Sutherland[4]
[1]Institute of Hematology and Blood Transfusion, PRAGUE, Czech Republic
[2]Dahl-Chase Diagnostic Services, BANGOR, MAINE 04401, U.S.A.
[3]VŠCHT, PRAGUE, Czech Republic
[4]University Health Network/Toronto General Hospital, TORONTO, Canada

Introduction: Following published guidelines, peripheral blood for PNH testing should be kept at room temperature after collection and should not be frozen or exposed to direct sun. Processing should follow within 24-48 hours.
Aim: To validate the time dependent stability of light scatter and fluorescence characeristics of PNH cells stored at 4^0C for the purpose of quality assessment programs.
Methods: Peripheral blood from 10 PNH patients was collected and stored for 9 days at 4^0C. Using a validated approach each day a sample was stained and FSC, SSC, % PNH (type III, type II+III) RBCs, Ne and Mo were recorded and analyzed using mean, SD, CV, linear regression, Pearson r^2, Wilcoxon rank test and Bland Altman agreement test.
Results: The CV (%) for FS/SS (10 patients evaluated for 9 consecutive days) ranged from 8.27/10.85 to 22.52/21.64 (median 14.71/16.07), from 15.68/11.79 to 78.53/20.44 (median 23.17/16.52) and from 4.7/3.8 to 10.4/11.32 (median 8.8/6.1) for gated Ne, Mo and RBCs respectively.
The CV for mean percentage target PNH events (10 patients evaluated for 9 consecutive days) ranged from 0.1/0.03 to 6.7/6.5 (median 2.29/1.96), from 0.1/0.07 to 5.09/3.7 (median 1.2/0.86) and from 0.7/0.5 to 7.6/4.05 (median 3.93/2.5) for Type III/Type II+III Ne, Mo and RBCs respectively. The Pearson's correlation coefficient (*r)*, P-value and Bland-Altman mean bias for the comparative analysis of PNH Ne (type II and Type II+III) were: 0.99/0.45/0.03 (day1 vs day2), 0.99/0.21/0.12 (day 1 vs day3), 0.99/0.35/0.07 (day1 vs day4), 0.99/0.33/0.19 (day1 vs day5), 0.99/0.17/0.26 (day 1 vs day6), 0.99/0.27/0.26 (day 1 vs day 7), 0.99/0.15/0.6 (day 1 vs day8), 0.99/0.1/0.6 (day 1 vs day 9), for comparative analysis of PNH Mo (type II and type II+III) were: 0.99/0.84/0.07 (day1 vs day2), 0.99/0.39/0.1 (day 1 vs day3), 0.99/0.22/0.2 (day1 vs day4), 0.99/0.1/0.6 (day1 vs day5), 0.99/0.4/0.3 (day 1 vs day6), 0.99/0.19/0.5 (day 1 vs day 7), 0.99/0.11/0.7 (day 1 vs day8), 0.99/0.27/0.7 (day 1 vs day 9) and for comparative analysis of PNH RBCS (type II and type II+III): 0.99/0.7/0.04 (day1 vs day2), 0.8/0.21/0.07 (day 1 vs day3), 0.99/0.68/0.08 (day1 vs day4), 0.99/0.35/0.2 (day1 vs day5), 0.99/0.32/0.3 (day 1 vs day6), 0.99/0.64/0.2 (day 1 vs day 7), 0.99/0.32/0.3 (day 1 vs day8), 0.99/0.3/0.3 (day 1 vs day 9).
Conclusion: The reported results demonstrate that peripheral blood stored at 4^0C for up to 7 days could be reliably used for the purposes of regional PNH quality assessment programs.

005

Diagnosis of B-cell lymphoproliferative disorders: application of flow cytometry to clinical practice

Vasiliki Douka, Eleni Gavriilaki, Michail Iskas, Varvara Tachynopoulou, Olga Iossif, Rita Avramidou, Maria Papathanasiou, Apostolia Papalexandri, Giorgos Papaioannou, Anastasia Athanasiadou, Niki Stavroyianni, Aliki Tsompanakou, Achilles Anagnostopoulos
G. Papanicolaou Hospital, THESSALONIKI, Greece

Introduction: Recent advances in flow cytometry have improved our ability to phenotype B-cell lymphoproliferative disorders. Despite application of novel markers into clinical practice, phenotypic variability remains a challenge.
Method: We recorded data from samples of patients with B-cell lymphoproliferative disorders sent for flow cytometry analysis from January 2016 – May 2017. We also documented available diagnostics from cytogenetic, molecular and bone marrow histology. Flow cytometry panels were analyzed using our updated 6-colour panel (kappa and lambda immunoglobulin light chain, CD5, CD19, CD20, CD22, CD23, CD25, CD27, CD10, CD11c, CD43, CD200, CD79b, CD38, FMC7, CD103, CD45) in BD FACSCanto II, including ROR1 and CD180 as novel markers.
Results: We studied 163 patient samples. Eighty-six patients were diagnosed with chronic lymphocytic leukemia (CLL), all of them being CD5+/CD23+/CD43+/CD200+. CD180 was positive in 19, whereas ROR1 in 58 out of 59 CLL samples analysed. Regarding cytogenetics, we detected trisomy 12 in 9 CLL patients (Matutes score 4 or 5) with a distinct cytometry panel: CD38high, ROR1 positive and CD11c negative (in 8/9). In CLL patients without trisomy 12, CD38 was positive only in 17/77 and CD11c in 51/77.
Mantle cell lymphoma (MCL) was diagnosed in 5 patients shown as CD5+/CD23-/ CD38++/ ROR1+ /CD200-/11c-/180-/lambda clonality, indicating that ROR1 may not be helpful in differentiating CLL from MCL. In contrast,

CD200 proved to be an important marker in this differential diagnosis.
Positive CD10 was found in 10 non-CLL patients: 2 with follicular lymphoma, 3 with Burkitt, 2 with diffuse large B-cell, 1 with marginal zone lymphoma and 2 with hairy-cell leukemia (HCL). HCL was diagnosed in 5 patients: CD25+/CD11c+/FMC7+/ CD200++/CD79b+ /CD22+/ CD103+.
Among the other lymphoproliferative disorders, 13 patients were diagnosed with splenic marginal zone lymphoma. Although there was no distinct phenotype in these CD5-/CD10-/CD23-/CD43- patients, CD200 was negative in 10/13 and CD180 positive in all but one patients.
In addition, we studied 4 patients diagnosed with Waldenström macroglobulinemia confirmed by bone marrow histology. Three of them carried the MYD88 mutation. In flow cytometry, all patients were CD5-/CD23-/CD11c-/CD10-/CD38+.
Discussion: Our data confirm that distinct flow cytometry phenotypes or combinations of markers indicative of certain B-cell disorders may be useful in clinical diagnostics. However, the role of flow cytometry in clinical diagnosis of these disorders needs to be further investigated.

006

CD56+ and CD16+ monocyte subpopulations do not overlap in most myeloid neoplasms. Classical monocytes >94% are not specific to CMML

Georgios Paterakis[1], Elpiniki Krhtikou-Griva[1], Eleni Goumakou[1], Georgios Androutsos[1], Paraskevi Vasileiou[2]
[1]GEORGIOS GENNIMATAS GENERAL HOSPITAL OF ATHENS, ATHENS, Greece
[2]FLOWDIAGNOSIS FLOW CYTOMETRY LABORATORY, ATHENS, Greece

Introduction: CD56 is known to be expressed in monocytes and blasts of several myeloid malignancies. CD16 expression has defined monocyte subpopulations as classical monocytes (CD14+CD16-), intermediate (CD14+CD16+) and non-classical (CD14-CD16+). They have been studied in several conditions as well as in myeloid and monocytic malignancies. In Chronic Myelomonocytic Leukemia (CMML) classical monocytes (CM) over 94% were found to be a specific and sensitive diagnostic marker (Blood 2015, 125(23):3618). The aim of our study was to assess the expression of both CD16+ and CD56+ antigens in the mature and immature monocytic component of various acute and chronic myeloid neoplasms, to investigate their interrelationship and maturation patterns.
Methods: The monocytic lineage was defined by CD64 and CD14 expression. The granulocytic component was discriminated by CD66+. The expression of CD56 and CD16 antigens were assessed in monocytic lineage of different maturation stages. Blood or marrow samples were studied in patients with CMML in chronic phase (n=4), in transformation of CMML to Acute Myelomonocytic Leukemia (CMML-AML, n=4), Myelodysplastic syndromes, MDS RAEB1 and RAEB2 (n=6), MDS in transformation (MDS-AML,n=2), Chronic Myeloid Leukemia CML n=4 , MDS-MPN n=7 and chronic reactive monocytosis RM n=10.
Results: All RM did not have CD56+ monocytes over 10% of total monocytes while CM>94% were found in 3/10 cases. In CMML CD56+ in 2/4 and CM>94% in 2/4. In CMM-AML CD56+ in 2/3 while CM>94% were not observed. Promonocytes CD56+ 1/3 and monoblasts CD56+ 1/3. All MDS examined had no CD56 expression on monocytes, while CM>94% were found in 3/6. In MDS-AML CD56+ monocytes were noted in 1/2 while CM>94% were not found. In MDS-MPN CD56+ in 6/6 while CM>94% in 1/7. In CML CD56+ monocytes were observed in 3/4 and CM>94% in 3/4 cases. There was observed monocytic population with co-expression CD16+ and CD56+ only in 2/36 cases; in one patient with CMML-AML (44%) and in another with MDS-AML (25%).
Conclusions: CD56+ monocytes>10% of monocytes were found a sensitive but not specific diagnostic marker in CML and MDS-MPN cases. In CMML they were observed with less frequency while in MDS in low frequency. CM>94% were common in CMML in chronic phase but not in CMML-AML transformation. In our study, it did not appear such a specific CMML monocyte marker, because it was observed with increased frequency in RM, CML and MDS. CD56+ and CD16+ co-expression appeared rare and the two lines seemed not to overlap in reactive or neoplastic monopoiesis.

007

Detection of cell subsets with aberrant phenotype in pediatric normal or regenerating bone marrow samples

Eliana Gkika[1], Marianna Tzanoudaki[1], Eleni Ploumi[1], Kaisari Katerina[2], Christina Oikonomopoulou[2], Eftychia Petrakou[2], Maria Theodosaki[2], Virginia Polaki[1], Anastasia Limioti[1], Vasiliki Kitra[2], Maria Kanariou[1]
[1]„Aghia Sophia„ Children's Hospital, ATHENS, Greece
[2]Bone Marrow Transplantation Unit M.V. Vardinogianni-Elpida Pediat. Oncology Unit, ATHENS, Greece

Introduction: MRD detection relies on the aberrant immunophenotypic features of leukemic cells. However, when it comes to lower concentration levels, suspicious populations, with aberrant marker co expression are often detectable. This may lead to a diagnostic dilemma, even more so in cases of phenotypic shift and in the regenerating bone marrow (BM) setting. The present study aimed to investigate the existence of such “aberrant” populations in normal or regenerating BM samples and to estimate their expected concentration level.
Methods: 7 normal BM samples (grafts from Hematopoietic Stem Cell –HSC- Donors), 1 autologous BM graft of a Neuroblastoma patient and 2 follow up BM samples from transplanted Acute Leukemia (AL) patients (1 and 2 years post HSC transplantation) were chosen for study. Median age of the children was 3.4 years (0.4-10.8y). The following antigens were tested with 10 color Flow Cytometry (Navios, Beckman Coulter) in certain combinations: CD3, CD7, CD2, CD16, CD56, CD33, CD117, CD99, CD34, CD19, CD45. FSTOF/FS based doublet discrimination was performed in all samples. FMO analysis and alternative fluorochrome conjugates were used to check the validity of selected aberrant phenotypes. Results were expressed as a percentage of total nucleated cells (TNCs, defined based on SS/FS plot). At least 500,000 (and up to 1,500,000) TNCs were collected.
Results: The following subsets were detected in clusters of >300 events in all samples tested accordingly: (1) CD34+CD7+CD33+ cells at a median concentration of 0.07% (0.04%-0.2%), (2) CD33+CD2+ cells with monocytic CD45/SS characteristics at a median percentage of 0.5% (0.09%-0.8%) and (3) CD33+CD56+ cells with monocytic CD45/SS characteristics at a median percentage of 0.1% (0.06%-0.5%). The above phenotypes were not in any way related to the LAIPs of the 2 studied AL patients. Various additional smaller clusters of >30 cells which were detected at a <10-4 level, were not evaluated.
Conclusions: At the -4log level, various “aberrant” subsets may be detectable, even in normal BM samples of children. This should be taken into consideration, before characterizing suspicious cells as abnormal at this level. Given the particularities of hematopoiesis in children, further evaluation is needed, especially for adult samples.

008

Evaluation of early (Day 15) treatment response in peripheral blood by flow cytometry in B-cell Acute Lymphoblastic Leukaemia patients

Ketan Ingle[1], Prashant Tembhare[2], Gaurav Chatterjee[2], Sumeet Gujral[2], Nikhil Patkar[2], Yajamanam Badrinath[2], Sitaram Ghogale[2], Nilesh Deshpande[2], Ashok Kumar[2], Dhanlaxmi Shetty[3], Pratiksha Devre[2], Rohit Kori[2], Shweta Kedia[2], Shripad Banavali[4], Brijesh Arora[4], Gaurav Narula[4], Papagudi Subramanian[2]
[1]TATA MEMORIAL HOSPITAL, MUMBAI, India
[2]Hematopathology Laboratory, Tata Memorial Centre, MUMBAI, India
[3]Department of Cytogenetics, Tata Memorial Centre, MUMBAI, India
[4]Department of Medical Oncology, Tata Memorial centre, MUMBAI, India

Introduction: Apart from the conventional risk factors such as age, presenting WBC count and cytogenetics, response to treatment is shown to be the most important independent prognostic factor in B-ALL, in particular, post-induction minimal residual disease (MRD) level in the bone marrow (BM). We evaluated peripheral blood (PB) MRD level on day 15 of induction chemotherapy as an early indicator of treatment response and its predictive value for post-induction (Day 35) BM-MRD level.
Methods: After ethics committee approval and obtaining written informed consent and assent, we studied 121 B-ALL patients younger than 15 years, from single institution treated with ICiCLe (Indian Childhood Collaborative Leukaemia Group) protocol in the year 2016. An observational, prospective study was done. After the start of induction phase chemotherapy, day 15 PB and day 35 BM-MRD were detected by flow cytometry. ROC (Receiver Operating Characteristic) curve analysis was done to determine best cut-off value for day 15 MRD, in predicting post-induction BM-MRD.

Results: 65.3% patients were day 15 PB-MRD positive (any positive value), while post-induction BM-MRD positivity was seen in 27.3% (MRD ≥0.01%) and 40.5% (any positive MRD value). A cut-off of ≥0.18% for day 15 PB-MRD (24.8% patients) gave highest specificity (86.4%) and NPV (Negative Predictive Value- 83%), while moderate sensitivity (54.5%) and PPV (Positive Predictive Value- 60%), in predicting post-induction BM-MRD (Odds ratio- 7.6 (3.04-19.0), p <0.0001). Specificity and NPV improved in high-risk subgroups: National Cancer Institute (NCI) criteria- High risk patients (88.4% and 85.1%) and Prednisone Poor Responders (PPR- 84.2% and 100%). These subgroups also had higher sensitivity and PPV: NCI-HR (63.6% and 70%) and PPR (100% and 57%). Specificity and NPV for day 15 PB absolute blast count (as derived from total WBC count), with cut-off ≥0.3 x 10^9/L (25.6% patients), were 84% and 82.2% respectively.
Conclusions: Day 15 PB-MRD (≥0.18%) and day 15 PB Absolute blast count (≥0.3 x 10^9/L) levels have high specificity and Negative Predictive Value in prediction of post-Induction BM-MRD, thereby useful in detection of patients which are likely to be post-induction MRD negative. Day 15 early monitoring of MRD in peripheral blood is a simpler method and is more useful in high risk patients. These conclusions need to be corroborated with long term patient outcome data.

009

Creation of a database of B-cell lymphomas with Infinicyte and comparison of database-guided diagnosis with expertise-guided diagnosis by laboratory physicians

Jan Dirks, Paul Duwe, Dimitrios prof. Tsakiris, Beatrice dr. Drexler
University Hospital Basel, BASEL, Switzerland

Introduction: Immunophenotyping of hematological neoplasia is complex and requires knowledge of physiological and aberrant antigen expression and maturation patterns by the laboratory physicians. Novel flow cytometric software allows "automated population separation" (APS) analysis, integrating all measured parameters. In combination with a database of reference cases, new cases can be compared with and assigned to predefined entities.
In this study we created a database for different B-cell lymphoma entities in the Infinicyte™ software (Cytognos), with the objective to compare database-guided diagnosis with expertise-guided diagnosis by laboratory physicians.
Methods: We retrospectively selected flow cytometric data of cases diagnosed with B-cell lymphoma according to the WHO 2008 criteria at our center between 2010 and 2015. We included a total of 158 cases with the following lymphoma diagnosis in our database: chronic lymphocytic leukemia (CLL, 40 cases), mantle cell lymphone (MCL, 24 cases), hairy cell leukemia (HCL, 20 cases), follicular lymphoma (FL, 25 cases), lymphoplasmacytic lymphoma (LPL, 28 cases), splenic marginal zone lymphoma (sMZL, 21 cases). All cases were analysed using the same antibody panel and settings, allowing data preparation (file merge and calculate data) and database setup according to Infinicyte guidelines.
Results: Data preparation and inclusion of cases into the database was convenient. Clustering of all cases of one versus the other entities showed good discrimination between all entities, except LPL and sMZL, which showed considerable overlap. To the test the power of the database, we compared the database-guided diagnosis with the expertise-guided diagnosis for 20 additional lymphoma cases resulting in a good overall agreement. Furthermore, we retrospectively searched for cases where our initial flow cytometric diagnosis was not confirmed by subsequent histological and molecular results. Our results suggest that a database-guided diagnosis would have prevented overdiagnosis by not assigning such cases to any of the entities in the database.
Conclusion: Our results show that a flow cytometric database of hematological malignancies with the Infinicyte software is convenient and easy to set up, as long as data standardisation between all files is available. We postulate such a database to be useful for training purposes of inexperienced colleaugues, panel development and correct classification of challenging cases (e.g. overlapping phenotypes, aberrant scatter characteristics/ fluorescence intensities or composite hematological malignancies).

010

CD56 expression affects blast quantification in bone marrow aspirate and biopsy in myeloid neoplasm

Jan Dirks, Severin Baerlocher, Maria dr. Martinez, Alexandar prof. Tzankov, Dimitrios prof. Tsakiris, Beatrice dr. Drexler
University Hospital Basel, BASEL, Switzerland

Introduction: CD56 (NCAM1) is a cell surface glycoprotein, which functions as an adhesion molecule on neuronal cells and natural killer cells.
CD56 is aberrantly expressed in myeloid neoplasms including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Several studies have shown CD56 to be an independent negative prognostic marker in MDS and AML.
Considering the nature of CD56 as an adhesion molecule, blast quantification in bone marrow might depend on the technique performed to obtain bone marrow. Therefore, the objective of our study was to investigate the impact of CD56 expression in myeloid neoplasms on bone marrow aspirate versus biopsy.
Methods: We retrospectively analysed 39 patients with CD56 positive and a matched cohort of 39 CD56 negative myeloid neoplasms from 1997 till 2017. We assigned patients according to CD56 expression strength by flow cytometry in three groups (bright, dim and negative) and compared blast frequencies in bone marrow cytology versus histology for each group.
Results: Patients with bright CD56 expression showed higher blast percentages in bone marrow biopsy than aspirate. CD56 expression strength and not expression per se seems to be causative for this, as cases with CD56 dim expressing blasts did not show significant differences. Interestingly cases with bimodal blast populations in bone marrow (CD56 positive and negative population), showed preferential leukemic dissemination of the CD56 negative population into peripheral blood.
Conclusion: Our results indicate that bone marrow aspirate from myeloid neoplasms with CD56 bright expressing blasts systematically underestimates the total blast percentage. These findings have critical diagnostic and clinical consequences, as correct quantification of blasts is crucial to discriminate between MDS with excess blasts and AML. Therefore, bone marrow aspirate should always be combined with trephine biopsy for a reliable result.

011

Differences in the hematopoietic profile of Multiple Myeloma patients in prolonged remission may predict clinical outcome

Ioannis Kostopoulos, Efstathios Kastritis, Paraskevi Micheli, Ourania Tsitsilonis, Meletios-Athanasios Dimopoulos, Evangelos Terpos
National and Kapodistrian University of Athens, ATHENS, Greece

Introduction: Multiple Myeloma (MM) is an incurable haematological malignancy characterized by uncontrolled proliferation of clonal plasma cells in the bone marrow (BM) and excess production of monoclonal protein. Recent advances in the clinical management of MM have achieved deep responses, prolonged progression-free survival and higher survival rates. The presence of Minimal Residual Disease (MRD) in the BM of treated MM patients is an excellent biomarker during the course of the disease, and has been correlated with early relapse and reduced overall survival. Nevertheless, the biologic background leading to relapse remains unknown.
Methods: BM samples from 53 previously treated MM patients in prolonged complete remission (>2 years) by conventional criteria were analyzed for the presence of MRD via Next-Generation Flow Cytometry (NGFC) according to the Euroflow guidelines. Isolated cells from each sample were labeled using two independent multiparameter (8-color) surface and intracellular marker panels, comprising CD19-PEC7, CD27-BV510, CD38-FITC, CD45-PERCP, CD56-PE, CD81-APCC750, CD117-APC, CD138-BV421, CyIgκ-APC and CyIgλ-APCC750. Five million events were recorded per panel with a BD FACSCantoII and data analysis was conducted with Infinicyt software (Cytognos) allowing maximum information recovery from each sample. Using the same markers, we identified the major BM cell subsets, and analyzed the hematopoietic profile of each patient.
Results: Our analysis revealed 30 MRD^- and 23 MRD^+ patients, mostly at the level of 10^{-5}-10^{-6}. The most informative markers were primarily CD19 and CD45 (all MRD^+ cases were $CD19^-CD45^{dim/-}$) and secondarily CD27 (19/23 MRD^+ cases were $CD27^{-/dim}$). From the clinical viewpoint, to date, 5 patients relapsed, 4 of which found MRD+ in our analysis. Important differences in the patterns of the main hematopoietic subpopulations between MRD^+ and MRD^- samples were detected. Qualitatively, the B-cell compartment in MRD^- samples principally comprised naïve B-cells, contrary to MRD^+ samples where pre-B and memory B-cells were more abundant. Quantitatively, MRD^+ samples were characterized by increased activation of the lymphoid lineage and contained higher percentages of T, NK and NK-T cells. The presence of MRD was also correlated with higher percentages of erythroblasts and monocytes, in contrast to MRD^- samples where neutrophils clearly predominated.

Conclusions: Although further analysis of more BM samples from MM patients is warranted to verify our observations, our results suggest significant qualitative and quantitative differences in the BM hematopoietic cell profile between MRD^+ and MRD^- MM patients. This type of analysis could eventually provide a hematopoietic signature offering prognostic information associated with MM patients' outcome.

012

Monitoring of small paroxysmal nocturnal hemoglobinuria clone in patients affected by severe aplastic anemia or aplastic anemia

Raffaella Milani[1], Maria Teresa Lupo-Stanghellini[2], Fabio Giglio[2], Barbara Migliavacca[1], Gabriele Torriani[1], Luca Santoleri[1], Fabio Ciceri[1]
[1]IRCCS San Raffaele Scientific Institute, MILANO, Italy
[2]IRCCS-San Raffaele Scientific Institute, MILANO, Italy

Introduction: Flow cytometry is the gold standard method for screening of paroxysmal nocturnal hemoglobinuria (PNH). The most reliable marker to detect and monitor small granulocyte or monocyte PNH clones is FLAER especially in conditions such as myelodysplastic syndromes or bone marrow failure, when traditional GPI-linked surface marker expression can be significantly altered. More than 50 % of aplastic anemia patients have a PNH clone detectable by highly sensitive assays, although the underlying mechanism is yet to be unveiled.
Methods: Between May, 2011 and May 2017, sixteen patients with severe aplastic anemia (sAA)/ aplastic anemia (AA) provided 67 blood samples for PNH flow cytometry at different time points in the course of their evaluation and treatment. We performed serial quantification of the PNH clone size by multiparameter flow cytometry using a Navios cytometer (Beckman Coulter). The immunophenotype evaluation is performed on EDTA whole blood samples. Granulocytes, monocytes and red blood cells (RBCs) were gated using forward and side scatter as well as lineage-specific markers. The GPI-linked markers FLAER and CD59 were comparatively evaluated. Neutrophils and monocytes were screened with FLAER/CD24/CD14/CD33/CD45 combination, while CD235a was used to delineate RBCs. The sensitivity of detection allowing identification of PNH clone sizes as small as 0.01% for RBCs and 0.03% for granulocytes.
Results: Eight of 16 sAA/AA patients were detected to have PNH clone positivity. All of them were treated with standard immunosuppressive therapy such as antithymocyte globulin and cyclosporine (ATG/CsA) or CsA alone and two non responder patients received allogeneic stem cell transplantation (alloSCT) after myeloablative conditioning. Granulocyte PNH clone was <1% in 4 patients, 1-5% in 3 patients. Lactate dehydrogenase (LDH) values were normal and Direct Antiglobulin Test was negative in all patients. Haptoglobin level was normal in 5. Two patients with PNH clone 1-5% had very low concentration of haptoglobin. These were patients who did not respond to ATG/CsA and received alloSCT. PNH clone sizes were stable during immunosuppressive therapy courses with a median follow up of 85 months (16-180), while PNH clone became undetectable after alloSCT and haptoglobin levels returned to normal values (20 month and 48 month follow up).
Conclusions: Whether the presence of a small PNH clone in the setting of hypocellular marrow failure has clinical significance or predicts outcome is still controversial, an accurate monitoring of PNH clone with high sensitive assays and hemolysis parameters might be useful to clarify the pathophysiology of these complex and rare diseases.

013

Automated sample preparation for CE-IVD dried screening tube for hematolymphoid malignacies

Massimo Geuna, Nathalie Santoro, Margherita Vizzini
Laboratory of Immunopathology. AO Ordine Mauriziano, TURIN, Italy

Introduction: The use of screening multicolor tube for the basic assessment of hematologic neoplasms is becoming the preferred approach for flow cytometry laboratories involved in oncohematology. The principles guiding the choice of reagents for a screening tube are to address clinical indication, to account for all major population present in the sample (mainly lymphoid) and to provide identification of the largest number of neoplastic states. The Clear Lab LS (Lymphoid Screen) reagent is a CE-IVD 10 color-12 reagents dried tube intended for in vitro diagnostic use as a screening panel for hematolymphoid population (and malignancies). The tube can be used with peripheral blood (PB), bone marrow (BM) and lymph node (LN) speciments. Because the tube contains antibodies against kappa and lambda immunoglobulins light chains, a pre-analytical sample (PB and BM) preparation is required (lyse and wash). This procedure requires a careful handling of the samples, is quite tricky and time consuming, and may represent a limitation to a routine employment of the reagent. Here we develop an automated wash-lyse-wash method on a HTA Nizon Plus device (automatic sample preparation workstation) to

prepare samples for ClearLab staining and compare results obtained with the standard two-tube method in use in the laboratory.
Methods: Peripheral blood samples from 20 normal and 10 pathological patients (CLL, CD5-MBL, Rituximab treated for B-cell neoplasm) were prepared according to the manufacturer instruction (manual wash-lysis-wash) or using an automated procedure on the Nizon Plus device. Both samples were then stained using the dried Clear Lab tube containing the following monoclonal antibodies mixture: CD45, CD3, CD19, CD20, CD56, CD34, CD5, CD10, CD4+lambda and CD8+kappa. Samples were then washed once, resuspended and immediately acquired to flow cytometer.
Results: The comparison of the percentages obtained with the two preparation procedures of the main lymphocyte subpopulations (CD3, CD4, CD8, CD19, CD5, CD56), of kappa/lambda distribution in B-cells and B-cell subsets (CD19+CD5+, CD19+CD10+), as well as the percentages of granulocytes, monocytes and lymphocytes, showed no significant differences between the two preparation methods. A greater cell recovery was observed using the manual procedure that, nevertheless, showed a less complete red cell lysis.
Conclusions: The automated method of sample preparation is not different from the manual procedure described in the data sheet of Clear Lab LS and the result obtained show a very high reproducibility of all cell population analyzed in normal and leukemic samples.

014

MET expression among hematopoietic system is restricted to plasma cells and plasma cell neoplasms
Massimo Geuna[1], Elena Casanova[2], Nathalie Santoro[1], Carla Boccaccio[2]
[1]Laboratory of Immunopathology. AO Ordine Mauriziano, TURIN, Italy
[2]Candiolo Cancer Institute, FPO-IRCCS, Lab. Cancer Stem Cell Research, CANDIOLO, Italy

Introduction: The MET oncogene was reported to be expressed by normal plasma cells and myelomatous cells. Furthermore, in acute myeloid leukemia (AML), MET activation was shown to sustain proliferation and survival. Here we investigated MET expression in normal bone marrow cell compartments and in a panel of hematological malignancies.
Methods: Bone marrow (15 normal, 9 AML, 13 B cell chronic lymphoproliferative diseases - BLPD), peripheral blood (7 AML, 28 BLPD) and tissue samples (11 BLPD) were analyzed for MET expression by flow cytometry using the following mixture of monoclonal antibodies: CD38, a-MET, CD20, CD34, CD33, CD10, CD19, CD45. Gating strategy on normal bone marrow allowed analysis of MET on lymphocytes (B and T+NK, Plasma cells, B cell precursors), CD34+ myeloid precursors, granulocytopoietic population and erythroid cells. Similarly, on pathologic samples, neoplastic population was gated on to evaluate MET expression.
Results: Met expression was detectable only in one case of Multiple Myeloma and one case of Diffuse Large B Cell Lymphoma with plasmablastic differentiation, i.e. the only two cases in our series that displayed plasma cellular differentiation. Moreover, MET was detectable in a variable percentage of plasma cells from normal bone marrows. In all other hematological malignancies analyzed, MET was undetectable either in the neoplastic populations or in normal blood and bone marrow cells. In the two positive cases, MET expression was specifically associated with $CD38^{bright}CD45^{low\ neg}$ plasma cells.
Conclusions: MET expression is associated with the advanced stages of normal and malignant B-cell differentiation, but it is never observed in any other blood cell subpopulation, either neoplastic or normal, including mature and stem/progenitor cells. The functional role of MET in plasma cells is currently unknown, but, according to the anti-apoptotic properties of this receptor, could promote resistance to chemotherapy.

015

Correlation between the Flow cytometric phenotyping and the Morphological differentiation in dyserythropoiesis
Hans Veenstra, Marius Mackenzie, Frank Preijers
Radboud University Medical Centre, NIJMEGEN, Netherlands

Myelodysplastic syndrome (MDS) consists of a heterogeneous group of clonal hematopoietic disorders commonly found in the elderly human population. All disorders are characterized by one or more peripheral blood cytopenias. Bone marrow cells display aberrant morphology and maturation resulting in ineffective blood cell production. MDS affects hematopoiesis at the stem cell level, as indicated by cytogenetic abnormalities, molecular mutations, and morphologic and physiologic abnormalities in maturation and differentiation of one or more of the hematopoietic cell lines. MDS may be involved in more than one hematopoietic cell lineage (erythroid, granulocytic, megakaryocytic) depending on the subtype and stage of the disease. In the last decade flow

cytometry plays an increasing role in the diagnostics of MDS by identification of aberrancies in the different cell lineages, especially the myeloid and monocytic lineages. In the high number of published reports the aberrancies in MDS are well characterized; however, more recently the erythroid lineage is under investigation. In our study we investigated the correlation between the flow cytometrical determination and the golden standard, the morphologic differentiation by microscopy.
In our flow cytometrical MDS diagnostics we introduced the score for erythroid aberrancies. By using a MoAb panel consisting of CD45, CD33, CD36, CD71, CD105, CD117 and CD235a for the determination of different maturation stages of the nucleated erythroid cells. By using an adapted gating strategy, 5 parameters could be scored (CD71 median, CD71 CV, CD71 dim population, CD36 median and CD36 CV).
In 180 patients identified with MDS the flow cytometrical erythroid scores were performed and compared with retrospective data of morphological differentiations by microcopy in particularly focusing on dyserythropoiesis. Furthermore, by using cell sorting of the gated populations and their microscopic investigation, we demonstrated that the sorted populations possess the expected morphology of these erythroid cells.
There was a strong correlation in the occurrence and the severity of dyserythropoiesis between erythroid flow scores and morphology (see table). We could establish that flow cytometrical erythroid score ≥3 strongly correlates with dyserythropoiesis.
We conclude that flow cytometric phenotyping is a reliable diagnostic tool to evaluate the erythroid pathway and to determine the dyserythropoiesis in MDS.

016

A rare case of Early T-cell Precursor Acute Lymphoblastic Leukaemia arising from a BCR-ABL1 negative Myeloproliferative Neoplasm

Timothy Milne, Matthew Smith, Sophie Todd, Marianne Grantham, Amy Roe, Shaun Bevan, Heather Oakervee, Tom Butler, Timothy Farren
Barts Health NHS Trust, LONDON, United Kingdom

Introduction: We report a novel case of transformation to ETP ALL in a case of *BCR-ABL1*- MPN, rather than the more typical AML, AUL or MPAL expected in acute transformation of these diseases. Cases of transformation to T-ALL appear unreported. Myeloid antigen positivity in early T lineage is commonly seen, however MPO positivity is required to prove a MPAL phenotype.
Case presentation: A patient 78 years old originally presented with primary proliferative polycythaemia (1972), which subsequently transformed to secondary myelofibrosis in 2012. The disease was shown to be *JAK2*+ and cytogenetic analysis revealed an abnormal karyotype 46,XX,del(20)(q11.2q11.3). In December 2016 the patient transformed to T-ALL with myeloid antigen positivity, early thymic subtype by immunophenotyping. The acute transformation showed marked myeloid antigen positivity (CD13+/CD33+/CD117+), however the MPO was negative. *JAK2* positivity continued, possibly arising from the residual myeloid disease component (as observed in PMF transformation - 2012). However, the karyotype changed to 45,XX,del(5)(q13q31),-7[10]with no evidence of del(20q) . FISH for BCR-ABL1 [t(9;22)] and KMT2A (11q23) were both negative. The trephine showed ongoing reticulin fibrosis. Histological analysis of a clot showed immature lymphoid cells predominating. The patient was treated following UKALL60+ nonintensive protocol, with decreasing demonstrable T-ALL MRD positivity post induction cycles 1 and 2 (1.20% and 0.09% of TNCs respectively). Following consolidation therapy, the outcome marrow demonstrated 0.01% T-ALL MRD positivity, but with the emergence of an increasing myeloid progenitor population (12% of TNCs: CD13+/CD33+/CD117+/MPO+/CD2-/CD7-/cytCD3-/CD19-/CD79a-), with myeloid cytogenetic clonal evolution.
Conclusion: T lineage lymphoid blast transformation from the background of *BCR-ABL1*-MPN is rare. This case showed clear T-cell lineage assignment by WHO (2016), with strong co-expression of cytCD3+/CD2+/CD5+ and CD7+. Such transformation is plausible- ETP ALL is an entity with myeloid, lymphoid and stem-cell features. However myeloid antigen positivity observed at diagnosis, combined with typically myeloid cytogenetics (5q-, -7) raises the question of how to interpret the significance of My-positivity. In the absence of MPO, this disease would typically not be classified as MPAL. However the background of *BCR-ABL1*-MPN coupled with the cytogenetic findings and My-positivity brings this into question. The response to ALL-directed chemotherapy supports the lymphoid characteristics of the transformation. We believe this case demonstrates the importance of following WHO guidance on lineage assessment by immunophenotyping with cytoplasmic antigen assessment being indispensable. Assumptions of acute transformation lineage based on previous disease history may result in an incorrect diagnosis if appropriate testing with an integrated approach is not followed.

017

Evaluation of expression of proteins involved in active DNA demethylation by indirect multicolor flow cytometry in patients with acute leukemia

Anna Labejszo[1], Lidia Gackowska[2], Maciej Gawronski[3], Marta Starczak[3], Anna Helmin-Basa[2], Daniel Gackowski[3]
[1]Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz, BYDGOSZCZ, Poland
[2]Department of Immunology, University in Torun, Collegium Medicum, BYDGOSZCZ, Poland
[3]Department of Clinical Biochemistry, University in Torun, Collegium Medicum, BYDGOSZCZ, Poland

Introduction: DNA of the cells of many cancers (including leukemia) is characterized by global DNA hypomethylation. Many reports suggest the participation of active DNA demethylation in the development acute leukemia. In turn, enzymes involved in this process include: 1) TET (ten-eleven translocation) proteins, which catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine which can be further oxidized to 5-formylcytosine and 5-carboxylcytosine, 2) thymine DNA glycosylase (TDG), involved in base excision repair of oxidized bases and 3) activation-induced cytidine deaminase (AID), which can convert 5-hydroxymethylcytosine to 5-hydroxymethyluracil.
The aim of this study was to develop a multicolor flow cytometry-based method to examine intracellular expression of proteins involved in active DNA demethylation and deamination processes (TET1, TET2, TET3, TDG and AID) in populations of peripheral blood nuclear cells of leukemic patients - before and after treatment.
Methods: Developed/optimized protocol involve blood collection with tubes containing anticoagulant (EDTA) and the cellular surface antigen-stabilizing agent TransFix (Life Technologies, Carlsbad, CA, USA). Stabilized blood samples were stained with direct conjugated antibodies (PE-anti-human CD56/CD16,PerCP-anti-human CD14, APC-H7-anti-human CD19, BV421 anti-human-CD45, V500-anti-human-CD3, PE-Cy7 –anti-human CD34) for identification of blastic cells and peripheral blood nuclear cell populations (lymphocytes, monocytes and granulocytes). Next, the cells were fixed, permeabilized and co-stained by indirect method with anti-human primary antibodies (TET1, TET2, TET3 and TDG) followed by compatible secondary antibodies conjugated with fluorescent dyes (donkey anti-rabbit Alexa Fluor 488 or rabbit anti-goat Alexa Fluor 647). In this step also direct APC-conjugated antibody anti-human AID was added.
Results: Preliminary results showed that each subpopulation of nucleated blood cells was characterized by differential expression of analyzed proteins. The highest intracellular expression of all analysed proteins, calculated as fluorescence intensity fold change over negative control, was observed in monocytes, except for TET2, which was the greatest in granulocytes. After first course of antileukemic treatment significant increase in TET3 and decrease in TET1 expression was observed in all analyzed cell populations.
Conclusions: Presented method may be useful for assess which subpopulations of peripheral blood cells have the greatest influence on the changes of epigenetic modifications profile observed in genomic DNA of leukemic patients.
Acknowledgment
This work was supported by the Polish National Science Centre (grant number UMO-2015/19/B/NZ5/02208).

018

Minimal residual disease monitoring in multiple myeloma patients by flow cytometry: a single center experience

Natalya Pronkina, Svetlana Sizikova, Egor Batorov, Galina Ushakova, Vera Sergeevicheva, Irina Kruchkova
Research Institute of Fundamental and Clinical Immunology, NOVOSIBIRSK, Russian Federation

Introduction: High-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (SCT) is used for the treatment of young MM patients and produces a high rate of complete remissions (CR). Unfortunately, most patients have a recurrences of the disease. This is due to the persistence of residual tumor cells, known as minimal residual disease (MRD), responsible for tumor relapse.
Patients and Methods: BM samples from 51 MM patients,average age - 54 years (36-70 years), who had achieved partial or complete response or were resistant after chemotherapy, including autologous SCT, were evaluated by multiparameter flow cytometry (MFC). Most of the patients were underwent high-dose chemotherapy with autologous SCT (n=42). Re-evaluation of MRD after therapy was managed to hold in 36 patients. Analysis was performed using a FACSCantoII flow cytometer (BD). Whole bone marrow was analysed using combination of surface and intracellular staining: CD38, CD56, CD27, CD117, CD81,CD19, CD45,cytLambda,cytKappa. The sensitivity of our panel MRD 0.01% (i.e. 10^{-4}).
Results: Among patients in CR (n=20) confirmed the absence of MRD in 6 patients, but 14 CR patients were MRD positive. MRD was detected in all patients with PR and resistant disease (n=31). The relative content of abnormal plasma cells in CR patients with MRD positive (n = 14) was significantly lower than that in PR/resistant patients (n =

31): 0.095% (0,026-0,271%) versus 1,3% (0,203 -5,9%), pU = 0,000092. PR patients (n = 8) had a lower relative content of abnormal plasma cells (as expressed tendency), than patients with resistant disease (n = 15): 0,286% (0,177-1,129%) versus 1,48% (0 , 90-8,0%), pU = 0,053. Besides the relative content of abnormal plasma cells in PR/resistant patients (n = 31) correlated with the serum M-gradient concentration (r_s=0,42; p=0,019) and bone marrow plasma cells (r_s=0,54, p=0,0017).
Conclusions: Currently, we can conclude that MFC could be considered as the method of choice for MRD monitoring in MM. If the disease is measured, then, indeed, enough to evaluate only the M-gradient level of serum. If the M-gradient is not defined, it is necessary to assess the number of abnormal plasma cells in the BM and strive for the high-quality responses at the time of transplantation. And also it can help us to regulate duration of maintenance therapy.

019

Diagnostic value of CD34+ cell subpopulations flow cytometry analysis in the bone marrow of MDS patients
Nikolaos Gardikas[1], Myrofora Vikentiou[2], Evgenia Konsta[2], Violetta Kapsimali[2], Katherina Psarra[3], Christos Kontos[2], Eleni-Dikaia Ioannidou[2], Christina Economopoulou[2], Anthi Bouhla[2], Christoforos Roumpakis[2], Nikolaos Papanikolaou[2], Diamantina Vasilatou[2], Sotiris Papageorgiou[2], George Dimitriadis[2], Vasiliki Pappa[2]
[1]Attikon Hospital, ATHENS, Greece
[2]Attikon University Hospital, Second Department of Internal Medicine and Research, ATHENS, Greece
[3]Department of Immunology and Histocompatibility, 'Evangelismos' Hospital, Athens

Introduction: Immunophenotypic profile of Bone Marrow (BM) cells in Myelodysplastic syndromes (MDS) may provide new tools in order to help in the diagnosis and prognosis of MDS. In the present study, a simple method for studying bone marrow CD34+ main populations in different maturation stages in MDS patients has been designed, in order to improve diagnostic process as well as prognostic capabilities of currently available prognostic scoring systems.
Materials and methods: Bone marrow specimens of 47 patients with MDS (36 Low and 11 High Risk according to IPSS-R) and 27 healthy individuals (as control group) were studied using 5-color multiparameter flow cytometry (MFC). In order to define CD34+ subpopulations in the gate of (A) CD34+ CD45weak cells (CD34+ $CD45^{low/dim}SS^{low/dim}$) and B) Neutrophil precursors (NP, CD34+ $CD45^{int/dim}SS^{int/hi}$), appropriate monoclonal antibody combinations were used: CD90/CD133, CD117/TdT, HLADR/CD64 and CD33/MPO, combined with CD34/CD45/7AAD.
Results: All BM specimens groups were compared between them. Important findings concerned mainly comparisons between Low Risk patients and healthy individuals as follows: (a) in CD34+ CD45weak cells *subpopulation,* CD34+ cells percentage (p= 0.041) as well as CD133+CD90- (p=0.002), CD33+MPO- (p=0.009) percentages were found statistically significantly increased and CD117-/TdT+ percentage (p<0.001) decreased. (b) In NP subpopulation, CD33+/MPO- (p<0.001) percentage was found increased. ROC analysis showed that (1) CD34+ CD45weak cells (p=0.002), (2) CD133+/CD90- CD34+ CD45weak cells (p=0.001), (3) CD117-/TdT+ CD34+ CD45weak cells (p<0.001), (4) CD64-/CD36+ CD34+ CD45weak cells (p=0.012), and (5) CD33+/MPO- NP (p<0.001) percentages possessed potential diagnostic value, as they could efficiently distinguish MDS patients from healthy individuals. The best combination scoring system comprised of CD34+ CD45weak cells, CD117-/TdT+ CD34+ CD45weak cells and CD33+/MPO- NP cells, showing 79.3% sensitivity and 81.8% specificity.
Conclusions: In conclusion, discrimination between low risk MDS patients and healthy individuals can be effective using these CD34+ CD45weak cells and NP findings, which is very important as these categories are usually very difficult to be diagnosed. This score can provide auxiliary predictive and diagnostic information in patients with low risk MDS. Future prospects could include the combination of phenotypic and cytogenetic findings in order to increase the sensitivity of this score for the diagnosis and prognosis of MDS subcategories.

020

The Role of Multicolor Flow Cytoemtry in the diagnosis of Sezary Syndrom/ Mycosis Fungoides
Mesude Falay, Pinar Incel, Gul Ece Ulusan, Melike Pekin, Gülsüm Özet
Ankara Numune Education and Resarch Hospital, ANKARA, Turkey

Flow Cytometry (FC) is frequently used on peripheral blood (PB) T cells lymphoma patients with mycosis fungoides (MF) and Sezary Syndrome (SS). However immonophenotypical stability of MF/SS over time is not well charactirized. Though not being a specific marker for SS and MF, it is observed the loss of CD7,the increase in the ratio of CD4/CD8 (>10) and decrease in the expression of CD26. İn general MFC is utilized as a scanning test for such cases. In this study we aimed to determine the role of MFC in the diagnosys of MF/SS. We studied

retrospectively total of 220 MF/SS cases admitted to our Flow cytometry lab between 2010-2017. The fresh peripheral blood samples of the cases were analyzed by multicolor flowcytometry (MFC) with the panel consisting of MoAb's of CD1a, CD2, CD3, CD5, CD7, CD4, CD8nad CD26. Out of 220 cases, 7 were diagnosed previously as SS, 100 as MF, 2 as eritrodermic MF and 3 as parapseuriasys in dermatology clinic. 108 scanned cases were identified as having benning dermatolgical diagnostics. When we investigate 7 SS cases as immunophenotype, we found out that the ratio of CD4/CD8 is high with 28 in only one case and the rest has less than 10 (4.4-6.2) and the CD& expression is low in three cases (%41-50) and negative in one case. Moreover CD26 expression was negative in all cases. The other T lymphoid markers were observed as normal. It was observed further that the avarage CD4/CD8 ratio in the patients with MF was 1.9 and there were no loss of CD26.
With this findings, MFC can be utilized in the diagnosys of SS by showing the loss of CD26 expression and the increase of the ratio of CD4/CD8. However, the loss of CD7 was observed only in one case. While in the MF cases, we didn't observe any immunophenotypical findings. Therefore more standart markers are required fort he diagnosys. We believe it has limited benfit to utlize MFC as a scanning test.

021

Lymph Node Assessment By Cytology And Multiparametric Flow Cytometry: A Prospective Study Of 176 Samples

Zuriñe Diez Gallarreta, Fernando Marco de Lucas, Paloma Isusi Gorbea, Beatriz Blázquez Ríos, Irene Leal Martínez, Clara Alonso Caballero, Bernabé Dávila de las Fuentes
HOSPITAL UNIVERSITARIO BASURTO, BILBAO, Spain

Introduction: Histopathologic study is considered nowadays the technique of choice for the diagnosis of pathology in lymph nodes. However, it has limitations: subjective analysis, limited reproducibility, reduced amount of reagents for determining antigenic expression and considerable consumption of time and human resources. Cytomorphologic examination and multiparametric flow citometry (C-FCM) may solve some of these obstacles: faster diagnosis and multiparametric analysis with qualitative and quantitative characterization of different cell antigens expression. Limitations of this technique are: impossibility to evaluate lymph node architecture and cellular damage during processing. Our aim was to evaluate in our centre the diagnostic value of C-FCM for oncohematologic diseases in lymphoid tissues and its accuracy in subclassifying Non Hodgkin Lymphomas (NHL), apart from indicating in which circumstances this technique was insufficient.
Methods: From 2015 to 2017, we prospectively analyzed by C-FCM 176 lymph tissue biopsy specimens, mainly obtained (76.7% (135/176)) by large needle biopsy of lymph node. Every sample was evaluated by cytomorphology of touch imprints stained with May-Grümwald-Giemsa and by FCM. Likewise, a part of the same sample was evaluated by histopathology. Median age was 60 years (1-91): 94 male (53.4%) and 82 female (46.6%). 54 (31%) had previous lymphoma history. Statistical analysis was performed employing G-STAT program (version 2.0.1, Biometrics Department of GlaxoSmithKline).
Results: C-FMC detected neoplastic disorders in 120 (68.2%) samples: 82 NHL (67.8%), 11 Hodgkin Lymphomas (HL, 9%), 26 malignant non hematologic neoplasms (NHN 21.5%) and 1 Acute Myeloid Leukemia (0.8%). Sensitivity and specificity were 85% and 83%. Positive predictive value (PPV) and negative predictive value (NPV) were 94.2% and 63.6%, respectively. Sensitivity and NPV increased to 93% and 86% when HL, NHN and T-cell / histiocyte-rich-B-cell lymphomas (TCRBL) were excluded from the analysis. 71/82 LNH (86.6%) were defined according to the 2008 World Health Organization (WHO) classification of hematolymphoid neoplasms, with a concordance of 78% with respect to the histopathological study.
Conclusions: C-FCM provides very valuable information regarding diagnosis of pathology in lymph nodes, showing high sensitivity and specificity. C-FCM is especially useful in NHL, allowing their sub-classification according to WHO criteria with a concordance of 78%. Moreover, being a faster technique compared to histopathology, while keeping a high PPV, it makes it easier to take quick therapeutic decisions, which is relevant in aggressive diseases. However, in NHN, HL and TCRBL, the utility of this technique relies on excluding the most usual NHL diagnosis, remaining histopathology the gold standard for diagnosis.

022

Flow Cytometry In 105 Consecutive Patients With Cytopenia And Suspeicion Of MDS: Strong Correlation With AML-Evolution And Survival

Zuriñe Diez Gallarreta, Fernando Marco de Lucas, Paloma Isusi Gorbea, Beatriz Blázquez Ríos, Irene Leal Martínez, Clara Alonso Caballero, Bernabé Dávila de las Fuentes
HOSPITAL UNIVERSITARIO BASURTO, BILBAO, Spain

Introduction: Diagnosis of myelodysplastic syndromes (MDS) remains a challenge, especially in patients with scant displastic morphology features and/or in the absence of cytogenetic changes. Multiparametric flow cytometry (MFC) findings have been recognized as a co-criterion for the diagnosis of MDS and have demonstrated prognostic value in some studies. Nevertheless, it is not fully implemented for the study of MDS in many centers. Our aim was to prospectively assess the value of MFC in the diagnosis of MDS and correlate its findings to clinical outcome.
Methods: We studied bone marrow (BM) samples from 105 consecutive patients submitted to our hospital between January 2013 and Abril 2015 because of cytopenia and MDS suspected. Cytomorphology was evaluated by two morphology experts and a consensus diagnostic of MDS-confirmed, MDS-suspected or MDS-excluded was emitted. MFC was performed applying at least five-colour staining and a numerical score was calculated following criteria defined by Ogata *et al* (Blood. 2006 Aug 1; 108(3):1037-44), with a score >=2 suggesting MDS.
Results: Median age of patients was 73.5 years. Patients presented with anemia in 88 cases (84%), neutropenia in 36 (34%) and thrombopenia in 49 (47%). Cytomorphology was reported as MDS-confirmed (60), MDS-excluded (22) or MDS-suspected (23). MFC-score was MDS-suggestive in 56 cases, MDS-not suggestive (36) and in 13 cases its use was precluded because of morphology findings. Considering cytomorphology as gold standard, and excluding patients with MDS-suspected but not confirmed, MFC-score sensitivity was 77%, especificity 88%, with positive and negative predictive values of 96% and 56% respectively. Furthermore, MFC-score showed significant correlation with single morphologic findings of granulocytic ($p<0.001$), erythroid ($p=0.001$) and megakaryocytic displasia ($p=0.002$), and a trend to significant association with del7q by FISH ($p=0.085$). In patients with MDS-suspected but not confirmed by morphology, MFC score>=2 was significantly associated with poorer overall survival (log-rank $p=0.012$), with all MFC score<2 patients alive after median follow-up of 35 months. There was also a trend to statistical association between MFC findings and overall survival in the whole series of patients (log rank $p=0.053$). Interestingly, there was a striking difference in risk of evolution to AML (log rank=0.013), with 100% patients with MFC score<2 free from this complication.
Conclusion: MFC analysis of BM provides useful information in the diagnosis of MDS which can be specially helpful in patients with inconclusive morphological findings, showing a strong correlation with clinical outcome in terms of risk of evolution to AML and overall survival.

023

Evaluation of the impact of standardized 8-color flow cytometry protocols (EuroFlow) on the diagnostic accuracy of poorly differentiated acute leukemias

Rafik Terra[1], Vincent Éthier[2], Richard Leblanc[1], Giovanni d'Angelo[1], Josée Hébert[1], Lambert Busque[1], Rafik Terra[1]
[1]Hopital Maisonneuve Rosemont, MONTREAL, Canada
[2]Centre Hospitalier Universitaire de Sherbrooke, MONTREAL, Canada

Introduction: Acute leukemia is a heterogeneous group of disease reflecting stem cell of origin, capacity of maturation and commitment to a specific lineage. Leukemic cells may show early or minimal differentiation and some diagnostic entities can easily be confused with others because of their atypical immunophenotype. In particular, it is well established that some stem cell, myeloid or lymphoid antigens can be expressed on poorly differentiated acute leukemia like immature blastic plasmacytoid dendritic cell neoplasm (BPDCN) and early T-cell precursor leukemia (ETP-ALL). These entities associated with a poor prognosis can be misdiagnosed as acute myeloid leukemia with minimal differentiation (AML-M0) if proper immunophenotyping panels are not routinely performed.
Many laboratories still use their own, non-standardized, laboratory developed antibody panels. To evaluate if the use of a standardized approach has the potential to improve the diagnostic accuracy of rare entities like BPDCN and ETP-ALL, we used the standardized EuroFlow™ approach to reanalyze cases of poorly differentiated acute leukemias.
Methods: Fifty cryopreserved bone marrow or peripheral blood samples collected in the last 15 years by the Quebec Leukemia Cell Bank and classified as AML-M0, AML with MDS-related changes or acute leukemia of ambiguous lineage, were reanalyzed using the 8-color immunophenotyping protocols as described by the EuroFlow consortium using the acute leukemia orientation tube (ALOT) and AML-MDS panels. TCL-1, CD303 and CD304 antibodies were added to confirm the presence of plasmacytoid dendritic cells (pDC). Cases showing a

population with a B or T cell phenotype were further characterized using B or T-ALL panels.
Results: Among the 34 first cases reanalyzed until now, 15 (44%) would have had a different diagnosis. Eight samples (24%) showed a previously unrecognized significant monoblastic/monocytic cell population, one sample showed a clear megakaryoblastic population and one sample revealed a previously unrecognized case of ETP-ALL. Seven samples (21%) revealed a population of pDC including one mature case and six immature cases consistent with BPDCN.
Conclusions: Using limited immunophenotyping panels, even with a global characterization including morphology and cytochemistry, we demonstrated that incorrect diagnosis may result. Extensive immunophenotyping using EuroFlow approach refine the diagnosis with cases of ETP-ALL, BPDCN, and cases with significant monocytic component. The use of standardized approach of flow cytometry can lead to more precise diagnosis of poor prognosis leukemia subtypes and has the potential to change the therapeutic approach with a potential improvement of clinical outcome for some of these patients.

024

Multisite performance evaluation study of the BD OneFlow™ Acute Leukemia Orientation Tube (ALOT)
David Bloxham[1], Edwin Sonneveld[2], Sean Rooney[3], Lydia Campos[4], Paula Fernandez[5], Christopher Green[6], Daiva Gladding[6], Ira Racoma[6], Dennis van Hoof[6], Farzad Oreizy[6], Sylveste Uwumarogie[7], Veronica Fraser[6], Linda Wolfe[7], Dennis Tielemans[8], Maryam Saleminik[6], Balaji Balasa[6], Kevin Judge[6]
[1]Cambridge University Hospitals NHS Foundation Trust, CAMBRIDGE, United Kingdom
[2]Dutch Childhood Oncology Group, THE HAGUE, Netherlands
[3]Our Lady's Children's Hospital, Crumlin, DUBLIN, Republic of Ireland
[4]Centre Hospitalier Universitaire, CEDEX 2, France
[5]Kantonsspital Aarau, AARAU,, Switzerland
[6]BD Biosciences, SAN JOSE, CA, U.S.A.
[7]BD Corporate Clinical Development, FRANKLIN LAKES, NJ, U.S.A.
[8]BD Life Sciences, EREMBODEGEM, U.S.A.

Introduction: The BD OneFlow™ solution for the diagnostic screening for acute leukemia incorporates a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow™ Acute Leukemia Orientation Tube (ALOT) is being developed for flow-cytometric immunophenotyping of aberrant immature hematopoietic cell populations with simultaneous determination of lymphoid and non-lymphoid lineages in acute leukemia patient specimens. We investigated equivalency between the investigational BD OneFlow ALOT dried reagent and the comparator EF liquid reagent systems. Results from BD OneFlow ALOT were also compared to clinical truth derived from the composite of patient history and laboratory findings.
Methods: De-identified, remnant peripheral blood, and/or bone marrow patient specimens were enrolled for the study at five sites. Acquisition and analysis were performed on a BD FACSCanto™ II flow cytometer using the BD OneFlow™ ALOT template and BD FACSDiva™ software. Categorization of specimens into non-lymphoid and lymphoid (B or T), overall agreement, negative agreement, and positive agreement were calculated. For qualitative assessment of aberrant cell populations for ALOT markers, overall agreement was calculated. Agreement analysis on matched paired specimens from the same patient was performed. An agreement analysis between BD OneFlow ALOT and clinical truth results was also performed.
Results: For all evaluable specimens, the BD OneFlow ALOT system gave 100% concordance in 93 of 93 specimens (non-lymphoid 49/49 and lymphoid 44/44), with a lower 95% confidence interval (CI) of the overall agreement of 96.8% when compared to the EF system. In addition, the BD OneFlow ALOT system when compared to the EF system, gave 100% (93 of 93 specimens) agreement for the qualitative assessment of CD45 negative/dim populations for ALOT markers with a lower 95% confidence interval (CI) of the overall agreement of 96.8%. Of the 14 matched paired specimens, overall agreement was 100% concordant. Analytical agreement between BD OneFlow ALOT results and the clinical truth demonstrated 100% concordance.
Conclusions: We conclude that the fully standardized and validated BD OneFlow ALOT system showed equivalence with the EF system for the identification of aberrant cell populations and categorization into non-lymphoid and lymphoid lineages and the BD OneFlow ALOT lineage outcome is also highly concordant with the clinical truth.
The BD OneFlow ALOT is CE Marked according to the European In Vitro Diagnostic Medical Device Directive 98/79/EC.
Disclaimer: The BD OneFlow ALOT is not available for sale in the USA.
23-19943-00

025

Multisite Performance Evaluation Study of the BD OneFlow™ B-Cell Chronic Lymphoproliferative Disorder T1 (B-CLPD T1) Panel

David Bloxham[1], Paula Fernandez[2], David Bloxham[1], Lydia Campos[3], Christopher Green[4], Daiva Gladding[4], Ira Racoma[4], Dennis van Hoof[4], Farzad Oreizy[4], Sylveste Uwumarogie[5], Veronica Fraser[4], Linda Wolfe[5], Dennis Tielemans[6], Balaji Balasa[4], Kevin Judge[4]
[1]Cambridge University Hospitals NHS Foundation Trust, CAMBRIDGE, United Kingdom
[2]Kantonsspital Aarau, AARAU,, Switzerland
[3]Centre Hospitalier Universitaire, CEDEX 2, France
[4]BD Biosciences, SAN JOSE, CA, U.S.A.
[5]BD Corporate Clinical Development, FRANKLIN LAKES, NJ, U.S.A.
[6]BD Life Sciences, EREMBODEGEM, U.S.A.

Introduction: The BD OneFlow™ solution for B-cell chronic lymphoproliferative diseases (B-CLPDs) incorporates a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow™ B-CLPD T1 is being developed to work in conjunction with the BD OneFlow™ Lymphoid Screening Tube (LST) for the immunophenotyping of B cells and distinguishing chronic lymphocytic leukemia (CLL) from other B-CLPDs. The objective of this study was to demonstrate equivalency between the investigational BD OneFlow (LST and B-CLPD T1) dried reagent and the comparator EF liquid reagent systems. Results from BD OneFlow (LST and B-CLPD T1) system were also compared to clinical truth derived from the composite of patient history and laboratory findings.
Methods: De-identified remnant peripheral blood and bone marrow patient specimens were enrolled for the study at four sites. Acquisition and analysis were performed on a BD FACSCanto™ II flow cytometer using BD OneFlow™ LST and B-CLPD T1 templates in BD FACSDiva™ software. Categorization of samples into CLL (typical) or other B-CLPDs, overall agreement, negative agreement, and positive agreement were calculated. For qualitative categorization of the aberrant cell populations, overall agreement was calculated. An agreement analysis between BD OneFlow system (LST and B-CLPD T1) and clinical truth results was also performed.
Results: Compared to the EF system, the BD OneFlow system gave 100% concordance (CLL 54/54 and B-CLPDs 47/47) with a lower 95% confidence interval (CI) of the overall agreement of 97.4%. The BD OneFlow system when compared to the EF system, gave 100% agreement for the qualitative assessment of aberrant B cell populations for CD20+, CD200+, and CD23+ subsets and 99.1% agreement for CD79b+ subset. In the agreement analysis between the clinical trial vs clinical truth results, excluding monoclonal B-cell lymphocytosis (MBL) and MBL of undetermined significance specimens, BD OneFlow system gave 94.2% agreement for CLL patients (49/52) and 97.7% agreement for other B-CLPDs (43/44) with an overall agreement of 95.8% (92/96).
Conclusions: We conclude that the fully standardized and validated BD OneFlow (LST and B-CLPD T1) system showed equivalence with the EF system in distinguishing CLL from other B-CLPDs and the BD OneFlow system diagnostic results were highly concordant with clinical truth.
The BD OneFlow LST and BD OneFlow B-CLPD T1 are CE Marked according to the European In Vitro Diagnostic Medical Device Directive 98/79/EC.
Disclaimer: The BD OneFlow LST and the BD OneFlow B-CLPD T1 are not available for sale in the USA.
23-19942-00

026

Nonneoplastic lymphocyte subsets of blood and bone marrow in patients with angioimmunoblastic T-cell lymphoma.

Elena Rybkina, Ekaterina Zakharko, Natalya Chernova, Darya Drokova, Valentina Dvirnyk
National Research Center for Hematology, MOSCOW, Russian Federation

Introduction: Angioimmunoblastic T-cell Lymphoma (AITL) is a rare T-cell lymphoproliferative disorder characterized by proliferation of helper T-cells with involvement of lymph nodes, liver, spleen and bone marrow. The nonmalignant microenvironment plays an important role in the pathogenesis of this disease. Also patients with AITL have got dysregulation of T-cell mediated immunity, it can be due to quantitative and qualitative changes of T-cell subpopulations. Therefore the aim of this study is to analyse the nonneoplastic lymphocyte subsets in patients with AITL.
Methods: From 2015 to the present, the National Research Center for Hematology used 8-color flow cytometry (Becton Dickinson (BD) FACS Canto II; reagents BD: anti-CD3 FITC, anti-CD16 PE, anti-CD56 PE, anti-CD45 PerCP, anti-CD19 Pe-Cy7, anti-CD5 APC, anti-CD8 APC-Cy7, anti-CD7 BV421, anti-CD4 BV510) for immunophenotype diagnostics of 7 peripheral blood and 6 bone marrow samples from 7 previously non treated patients with AITL.

Tumor population was detected in 3 blood samples (1.5, 13.6 and 16.0% of the total number of lymphocytes) and in 2 bone marrow samples (3.6 and 19.5% of the total number of lymphocytes).
Results: The lymphocyte percentage, NK-cells' count, helper and cytotoxic T-cells' counts were decreased in 7 (100%) patients in peripheral blood and in 5 patients (83%) in bone marrow specimens. Nevertheless percentage of NK-cells in blood specimens was increased in 6 patients (from 8.9 to 29.0%, reference interval 0.5-6.0%). In bone marrow it was within normal limits in 5 patients and was decreased in 1 patient. Concerning B-cell population, 6 patients (86%) had severe B-cell deficiency (B-cell , and 1 patient (14%) had an increased percent of polyclonal B-cells. Minimum content of B-lymphocytes and maximum of NK-cells percentage was detected when tumor population was more than 10.0% (in the blood and in the bone marrow equally).
Conclusions: Patients with AITL have modified ratio of some lymphocyte subsets in peripheral blood and bone marrow samples. However all determined data needs to be completed by a followed up research because of the rarity of this disease and a small number of patients.

027

Isolated blastic plasmacytoid dendritic cell neoplasm relapse in the central nervous system diagnosed by flow cytometry

Eirini Grigoriou[1], Katherina Psarra[2], Argyro Daskalaki[3], Sotirios Zachos[3], Alexandra Tsirogianni[2]
[1]Evagelismos Hospital, ATHENS, Greece
[2]Immunology - Histocompatibility Dep, Evagelismos Hospital, ATHENS, Greece
[3]Neurology Clinic, Evagelismos Hospital, ATHENS, Greece

Introduction: Blastic plasmacytoid dendritic cell (BPDC) neoplasm is a rare and clinically aggressive disease with a predilection for skin and high rate of central nervous system (CNS) recurrence either as isolated relapse or in the context of a systemic relapse.
Methods: A 47 year old Caucasian woman was diagnosed with BPDC neoplasm on the basis of skin biopsy and she was treated with six cycles of CHOP (cyclophosphamide, vincristine, doxorubicin, prednisolone). Six years later, she presented with severe headache, nausea, diplopia and right-sided ptosis. Brain contrast CT scan disclosed a 2x1.5cm contrast-enhancing lesion located in the right sinus cavernosus and extending in the suprasellar region. Cytomorphological analysis of cerebrospinal fluid revealed the presence of agranular cells with high nuclear cytoplasmic ratio. Cerebrospinal fluid and bone marrow aspirate specimens were studied by multicolor flow cytometry.
Results: Flow cytometry immunophenotype of cerebrospinal fluid malignant cells was characterized by the absence of CD5, CD7, CD16, CD36, CD64 and cytoplasmic CD3. The cells were positive for CD2, CD4 (weak expression), CD56, CD123 and HLADR. Bone marrow sample examined by flow cytometry showed no infiltration.
Conclusion: CNS involvement should be considered in cases of BPDC neoplasms regardless of clinical manifestations and flow cytometry examination of cerebrospinal fluid samples should consists part of diagnostic and follow up procedure.

028

Clinical and Biological features of Acute Undifferentiated Leukemia

Ilana Slouzkey, Tehila Azoulay, Margrita Filatov, Galit Sarig, Shimrit Ringelstein-Harlev
Rambam health care campus, HAIFA, Israel

Intreduction: Acute undifferentiated leukemias fall under the classification of "acute leukemia of ambiguous lineage", according to the 2016 revision of the world health organization (WHO) for myeloproliferative neoplasms. These leukemias are characterized by absent expression of markers that are specific and diagnostic for either the lymphoid or myeloid lineage. A rare provisional entity termed natural killer cell lymphoblastic leukemia (myeloid/NK leukemia) has been added to this category in 2008. These leukemias express CD56 along with other immature T-associated markers, and lack other lineage specifying markers.
Acute undifferentiated leukemias are rare; therefore, data regarding diagnosis, prognosis and treatment are lacking in the literature. Our goal was to assess clinical and laboratory data from patients diagnosed with this type of leukemia at the Rambam Health Care Campus, focusing on the unique characteristics and response to initial therapy.
Methods: Sixteen patients were diagnosed with undifferentiated leukemia at our institute from January 2016 to July 2017. Parameters considered at diagnosis were initial blood counts, marrow blast cell percentage, specific phenotypic characteristics, molecular mutations and translocations, and cytogenetic abnormalities. Bone marrow response to initial therapy was assessed. The patients were treated with initial intensive chemotherapeutic

regimens or with hypomethylating agent.
Results: Mean percentage of bone marrow blasts was 44%. Immunophenotyping of bone marrow aspirates of all 16 patients were negative for cyMPO. Five (31%) patients were CD56+ /CD7+, 5 (31%) patients CD56+/CD7- and 6 (38%) patients CD56- /CD7-. No recurrent molecular aberrancies were detected, except for one patient who was NPM1+ and FLT3+. No core binding factor (CBF) genetic changes were observed. Cytogenetic abnormalities were observed in 81% of the cohort. Recurrent abnormalities observed were chromosome 7 and complex karyotype. Fifteen (94%) of the patients responded poorly to first line therapy. Six (38%) died within the first year following diagnosis.
Conclusion: Acute undifferentiated leukemias appear to have a poor prognosis. Factors that may be involved include the high frequency of unfavorable cytogenetic changes, the high expression of markers of immaturity, and the low occurrence of "favorable" prognostic molecular changes. It has previously been shown that the expression of CD56 is a marker of a poor prognosis in various hematopoietic neoplasms. Our data suggest that the combination of CD7 and CD56 expression lead to the poorest outcome. Our data support the provisional inclusion of "myeloid/NK" leukemias as a distinct entity in AML, with specific clinical and biological characteristics requiring further designated research.

029

AML manifestation in patient with blastic plasmacytoid dendritic cell neoplasm
Darya Drokova, Elena Rybkina, Ekaterina Zakharko, Mikhail Drokov, Alina Kokhno, Ekaterina Kolosova, Valentina Dvirnyk
National Research Center for Hematology, MOSCOW, Russian Federation

Introduction: blastic plasmacytoid dendritic cell neoplasm is a rare hematological disease. It is included in the AML group according to WHO classification 2008.
Primary skin lesion is appeared in most of cases (80%). But bone marrow (BM), peripheral blood, lymphatic nodes and spleen could also be involved.
The possibility of the incorrect diagnosis is quite high due to the fact that this pathology occurs rarely. The differential diagnosis is carried out with non-Hodgkin's lymphoma and other leukemia with cutaneous manifestations; as well as with skin melanoma and systemic lupus erythematosus.
Methods: an 8 colour flow cytometry (BD FACS Canto II) was used for bone marrow examination.
Results: female, 67 years old, manifestation of the disease was from peripheral lymph nodes lesion with further involvement of the spleen and bone marrow.
Flow cytometry examination of sternal punctate revealed 11% of tumor cells with **CD45+CD10+CD2+CD4+CD5+CD123bright+CD303+HLA-DRbright+**cyCD3-MPO-CD11c-CD14- immunophenotype. Immunohistochemistry investigation of lymph node biopsy showed proliferation of malignant cells with expression of TCL-1+CD4+CD123+ antigens. So the diagnosis of blastic plasmacytoid dendritic cell neoplasm was confirmed. The patient underwent three courses of chemotherapy treatment according to the program: bortezomib, doxorubicin, vincristine, dexamethasone, L-asparaginase. BM follow-up study revealed 3,4% of tumor cells and the therapy was changed to lenalidomide and 5-azacytidine.
However, after 9 months of chemotherapy the scheduled dynamic BM study found 52% of cells with **CD45+CD34+CD117+CD13+CD15+CD33+CD7+CD56+CD123low+HLA-DRlow+**CD303- immunophenotype which corresponds to AML with CD7 and CD56 co-expression. At the same time the residual population of blastic plasmacytoid dendritic cells with **CD4+CD10+CD303+CD123bright+HLA-DRbright+**CD34-CD117-CD56- immunophenotype still had an amaunt of 2,4%.
Conclusion: this case report demonstrates the manifestation of an acute myeloid leukemia in a patient with blastic plasmacytoid dendritic cell neoplasm.

030

Blastoid cells in the bone marrow of a patient with severe aplastic anaemia treated with antithymocyte globulin and eltrombopag

Marta Santiago[1], Leonor Senent[1], Lourdes Cordón[2], Isidro Jarque[1], Samuel Romero[2], Rafael Andreu[1], Carmen Freiría[1], Federico Gomis[1], Empar Mayordomo[3], Pau Montesinos[1], Anabel Regadera[1], Esperanza Such[1], Ana Vicente[1], Ana Villalba[1], Amparo Sempere[1]
[1]Hematology Department, Hospital Universitario y Politécnico La Fe, VALENCIA, Spain
[2]Grupo de Investigación en Hematología, Instituto Investigación Sanitaria La Fe, VALENCIA, Spain
[3]Anatomopathology Department, Hospital Universitario y Politécnico La Fe, VALENCIA, Spain

Introduction: Aplastic anaemia (AA) is an infrequent heterogeneous disease characterized by pancytopenia and hypocellular bone marrow (BM) without fibrosis or neoplastic infiltration. AA and paroxysmal nocturnal hemoglobinuria (PNH) are often associated, with PNH clones in up to 50% of AA patients. For idiopathic AA, allogeneic haematopoietic stem cell transplantation (allo-HSCT) is the therapy of choice in suitable patients. However, when allo-HSCT is not feasible, immunosuppressive therapy (IST) combining antithymocyte globulin (ATG) and cyclosporine is the standard treatment. Recently, the thrombopoietin receptor agonist eltrombopag has been used for refractory patients. We report on a patient diagnosed with severe AA assessed at our centre after failure of two consecutive IST courses and eltrombopag treatment. An increased number of blast-appearing cells were found raising the diagnosis of clonal evolution to acute leukaemia.
Methods: BM samples were evaluated for cytological, immunophenotypic and molecular studies. Multiparameter flow cytometry (MFC) was carried out in a FACSCanto-II (Becton Dickinson, San Jose, CA). BM cells were stained employing Euroflow standardized panels. PNH study was performed according to Sutherland *et al*., 2014. Cell analysis was done using the Infinicyt™ 1.8 software (Cytognos, Salamanca, Spain).
Results: Complete blood count showed haemoglobin 5.8 g/dL, platelets $11x10^9$/L, and neutrophils $0.2x10^9$/L. Cytological and histological BM studies revealed 20% cells expressing PAX5, CD34, TdT, and CD10. MFC in peripheral blood identified 1.9% neutrophil, 0.4% monocyte, and 0.02% erythrocyte PNH clones. Lymphoid cells accounted for 46% of BM total nucleated cellularity (TNC), and 61% of those were B lymphoid cells with a broad maturation range. Within TNC, 17% were identified as normal hematopoietic progenitors CD34+ (92% were B lymphoid cells CD10+, nuTdT+). Myeloid lineage (27% TNC) did not exhibit immunophenotypic alterations. Cytogenetic analysis revealed monosomy of chromosome 7 and molecular tests showed monoclonal T cell receptor and *RUNX1* somatic mutation. The increase of blastoid cells was attributable to the previous treatment. Finally, the patient was diagnosed with unclassified myelodysplastic syndrome according to the WHO classification.
Conclusion: An increase of non-leukemic blastoid cells in BM has been reported after ATG or myeloablative chemotherapy. MFC allows to distinguish normal progenitor B cells from malignant blasts and to demonstrate a benign proliferation. The evolution to clonal diseases is one of the most severe complications in AA, in which treatment received could play a critical role. A global evaluation is necessary to enable an accurate diagnosis and a close surveillance in order to avoid inappropriate therapy.

031

Radar analysis shows two different types of erythropoietic maturation patterns in patients with myelodysplastic syndromes

Despoina Violidaki[1], Olof Axler[1], Katayoon Jafari[2], Kristoffer Navréd[3], Mats Ehinger[1], Anna prof Porwit[1]
[1]Lund University Hospital, LUND, Sweden
[2]Cross Cancer institute, EDMONTON, Canada
[3]Blekinge Hospital, KARLSKRONA, Sweden

Introduction: Anemia, erythropoietic dysplasia and morphological signs of disturbance in erythropoietic maturation in the bone marrow (BM) are seen in most cases of myelodysplastic syndrome (MDS). By flow cytometry, the reported best discriminators between MDS and pathological controls are CD36 CV, CD71 CV, MFI of CD71 and the percentage of $CD117^+$ erythroid cells. In most studies, these markers were evaluated on lysed BM samples, which could lead to underestimating the numbers of erythropoietic precursors. We have developed a single tube panel and radar plot analysis protocol that facilitate adequate estimation of various BM cell compartments and assessment of erythropoietic differentiation.
Methods: 250 000 BM cells were incubated with ERY panel: CD71FITC, CD13PE, CD117ECD, CD105PC7, CD36PB and CD45KO) and 10 ml of 0.5 mM DRAQ5 (10 resp. 15 min in the dark), followed by 1% formaldehyde fixation. Live gate was set on DRAQ5 positive cells. $1x10^5$ DRAQ5+ events were acquired with a Navios flow cytometer and analyzed using Kaluza software. The results were correlated with cytological evaluation of BM smears.
BM samples included 15 normal/reactive BM, 35 BM samples from patients with anemia but no dysplasia, 13 BM

samples with patients under cytostatic treatment but no residual hematological disease, and 40 BM samples from patients with MDS.
Results: The frequency of erythropoietic cells derived from ERY tube showed good correlation with morphological differential count (R^2=0.7759). We could confirm that CD36 and CD71 CV in erythroids were higher in MDS patients than in control groups (p<0.05). In BM with normal erythropoiesis, erythroid cluster was gated as $CD71^+/CD45^-$ (mean 19%, range 3-40). It could be divided by radar analysis in: earliest precursors ($CD117^+CD105^+$, mean 2% of erythropoiesis) followed by $CD117^-/CD105^{++}$ (22%), $CD117^-CD105^{dim}$ (42%), $CD105^-CD36^{++}$ (8%), and $CD105^-CD36^{dim}$ (25%). Using DRAQ5, a fraction of proliferating erythroid precursors was also determined (mean 28%). In most MDS patients, an aberrant cluster of $CD105^-$ erythroids with low expression of CD71 and CD36 was noted. This cluster was not seen in patients with anemia of other origin. In patients showing this cluster, the fractions of early and proliferating erythroids were decreased. In four studied patients with MDS with ring sideroblasts, immature CD105+ erythroids and proliferating erythroids were high and later stages decreased. Most MDS patients showed an increased cluster of CD117+ myeloid blasts.
Conclusion: The developed panel allows adequate assessment of erythroid cell compartment, which could be of diagnostic use in evaluation of erythroid dysplasia.

032

Ca2+ signaling capacity of chronic lymphocytic leukemia B cells is attenuated after in vivo Ibrutinib treatment
Maria Gounari[1], Stavroula Ntoufa[1], Konstantia Kotta[1], Marina Gerousi[1], Nikos Papakonstantinou[1], Andreas Agathangelidis[1], Lydia Scarfò[2], Aliki Xochelli[1], Eleonora Fonte[2], Pamela Ranghetti[2], Marta Coscia[3], Alessandra Tedeschi[4], Niki Stavroyianni[5], Marta Muzio[2], Paolo Ghia[2], Kostas Stamatopoulos[1]
[1]INAB, CERTH, THESSALONIKI, Greece
[2]Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, MILAN, Italy
[3]Divisione di Ematologia dell'Università di Torino, TORINO, Italy
[4]Department of Oncology/Haematology, Niguarda Cancer Center, MILAN, Italy
[5]Hematology Department and HCT Unit, G. Papanicolaou Hospital, THESSALONIKI, Greece

Introduction: In chronic lymphocytic leukemia (CLL), signaling through the B cell receptor (BcR) plays a critical role in disease pathogenesis and inhibitors of kinases that are essential for BcR signal transduction, such as the btk inhibitor Ibrutinib, achieve impressive responses in relapsed and refractory CLL patients. Here, we explored BcR-anergy induction in malignant cells from CLL patients under Ibrutinib by analyzing for the first time the impact of ibrutinib treatment on Ca^{2+} signaling, that plays a pivotal role in B cell activation, both in basal level and after antigen-like triggering.
Methods: The study group included 27 CLL patients who received ibrutinib as monotherapy in 1^{st} (n=2) or subsequent line (n=25) of treatment. CLL cells were isolated by negative selection from peripheral blood samples collected prior to treatment initiation and, thereafter, at fixed sampling times during the first 6 months of treatment. Purified CLL cells were cultured under standard conditions at a concentration of 3×10^6 cells/ml in the presence of specific BCR ligand [goat F(ab') anti-human IgM (20 mg/ml). Basal cytosolic Ca^{2+} and intracellular Ca^{2+} flux after BcR crosslinking were analyzed by flow cytometry using the fluorogenic probe Fluo3AM.
Results: We found significant reduction in basal intracellular Ca^{2+} levels at +1 and +3 months on Ibrutinib treatment compared to the pre-treatment paired samples (p=0.04 and 0.006, respectively; n=27). To confirm that intracellular Ca^{2+} remained depressed also at later timepoints, we evaluated Ca^{2+} levels at the +6 month timepoint in 9 patients with available material and observed a pattern similar to that obtained at +3 months.
We next investigated if Ibrutinib treatment affects the signaling capacity of CLL cells through their BcRs. To this end, we stimulated CLL cells with anti-IgM and measured calcium flux. We found that at +1 and +3 months after ibrutinib initiation, BcR cross-linking resulted in attenuated Ca^{2+} fluxes compared to the pre-treatment paired samples (p=0.0022 and 0.0004 respectively; n=23). In more detail, prior to treatment initiation, 11/22 patients were classified as BCR-responders, however only 3 remained so after 3 months under Ibrutinib. Twenty of 22 cases analyzed for their ability to induce Ca^{2+} flux in response to anti-IgM stimulation exhibited lower responses after Ibrutinib treatment. Furthermore, in 19/20 aforementioned cases the responses at +3 months of treatment reached the minimum responsiveness levels (0-5% responding cells).
Conclusions: CLL cells under ibrutinib treatment display impaired intracellular calcium signaling capacity, reminiscent of B cells anergized through the BcR.

033 (also Best Poster Abstract Presentation)

Probabilistic models for automated flow cytometric analysis of minimal residual disease in chronic lymphocytic leukemia

Konstantia Kotta[1], Dimitrios Kalatzis[2], Ilias Kalamaras[2], Vassiliki Douka[3], Andy Rawstron[4], Achilles Anagnostopoulos[3], Dimitrios Tzovaras[2], Kostas Stamatopoulos[1]
[1]INAB, CERTH, THESSALONIKI, Greece
[2]ITI, CERTH, THESSALONIKI, Greece
[3]Hematology Department and HCT Unit, „G. Papanikolaou„ Hospital, THESSALONIKI, Greece
[4]HMDS, St. James's Institute of Oncology, Leeds Teaching Hospitals NHS Trust, LEEDS, United Kingdom

Introduction: Flow cytometry has an established role for the detection of minimal residual disease (MRD) in haematologic malignancies. In chronic lymphocytic leukemia (CLL), MRD monitoring is only recommended for clinical trials and post allogeneic hematopoietic cell transplantation. However, MRD assessment may soon be incorporated in routine clinical practice thanks both to the advent of novel agents/regimens and the considerable evidence that the levels of MRD can be a reliable predictor of outcome. Hence, it is essential to develop methods for improving all aspects of the procedure, including *in silico* methods for the accurate interpretation of the findings.
Methods: A Gaussian Mixture Model (GMM) has been employed for density estimation of the observed data log-likelihood. The model is trained using mini-batch gradient descent with adaptive learning rate schemes. To this end, flow cytometric FCS files of anonymized patient data from 8 CLL patients assessed for MRD have been utilized. The FCS files have been previously analysed and classified, as MRD positive (+) or MRD negative (-) by flow cytometry experts. We used 4 sets of FCS files per case (MRD (+)/MRD (-), total n=8 datasets), where we made a decision for each cell measurement and counted the number of pathological cell populations to provide a diagnosis. By estimating the *a posteriori* probabilities of the latent variables (sample quality), we derived the cluster assignments for positive and negative measurements.
Results: Our model was able to correctly classify 75% (6 out of 8) of the analysed files. Differences between manual and automatic cluster determination ranged between 0.03-0.06 %. Slight discrepancies and overestimations show that the methodology exhibits increased sensitivity to MRD positive cell population detection. Further analyses and more samples are necessary to discriminate between higher sensitivity versus false positive results.
Conclusions: A statistical model has been developed for flow cytometric CLL MRD data analysis that minimises subjectivity by estimating cluster assignments as a function of the data. It allows the detection of CLL MRD positive samples with a sensitivity of up to 10^{-6}. While further refinements and tests will be conducted in the future, to the best of our knowledge this is the first general-purpose method providing a gating-free, robust and reliable framework for statistical MRD analysis. It is also flexible enough that it could be adapted to different panels allowing safe MRD determination even by less experienced users.

034

Multidisciplinary diagnostic and monitoring approach with flow Cytometry and NGS in ALL

Laura Koumas, Katerina Nicolaou, Chryso Pierides, Andrie Mitsidou, Elena Socratous, Rafaella Gavrielidou, Jason Chi, Paul a. Costeas
Karaiskakio Foundation, NICOSIA, Cyprus

Introduction: Monitoring of minimal residual disease (MRD) has become routine clinical practice in frontline treatment of acute lymphoblastic leukemia (ALL) with added prognostic value for the patient. Multi-parametric flow cytometry is a powerful, fast analytical tool utilized to assess MRD in ALL patients. The purpose of MRD analysis is to assess the adequacy of response, predict an evolving relapse and ultimately guide therapeutic options, acting possibly as a biomarker in disease. It has been the purpose of the clinical laboratory to assess MRD at the highest possible sensitivity and not miss any possible underlying malignant cells.
A challenge to the flow cytometrist with respect to ALL MRD detection is that analysis may often be subjective and require detection and enumeration of leukemic blasts in the context of normal, often regenerative bone marrow with numerous normal lymphoid precursors and sometimes maturation-associated changes and immunophenotypic shifts. In search for higher sensitivity and precision, there has been advancements towards molecular techniques, with the Next Generation Sequencing (NGS) platform rapidly gaining ground in a more objective ALL MRD testing.
We present the experience in our Center, a multidisciplinary approach setting, where a combination of supplementary techniques including flow cytometry and NGS are utilized to provide comprehensive diagnostic information and subsequently MRD monitoring of the patient.

Methods: Multiparametric immunophenotypic analysis was performed using 6-8 color flow cytometry to evaluate B and T cell progenitors in the diagnostic sample and subsequent follow-up samples from different patients. Our NGS analysis approach in assessing ALL MRD detects the majority of IGH and TRG gene rearrangements using a single multiplex master mix for each locus and simultaneously identifies the DNA sequence specific for each clonal gene rearrangement. This assay aids in the detection of initial clonal populations and identifies sequence information required to track those clones in subsequent samples.
Results: We demonstrate herein a correlation of flow cytometry and NGS ALL MRD findings and highlight the challenging cases in result interpretation, where immunophenotypic shifts rendered monitoring of the leukemic population difficult. Alternatively, we discuss cases where NGS results do not appear to match the percentage of malignant population as determined by flow cytometry, suggesting caution in result interpretation.
Conclusions: We conclude that a multidisciplinary laboratory setting is a powerful clinical entity, where a clinical sample can be supported by complementary disciplines in reaching a solid, highly sensitive diagnostic and disease monitoring assessment.

035

Determination of vimentin in CLL cells by flow cytometry
Eszter Szánthó[1], László Szerafin[2], János Kappelmayer[1], Zsuzsanna Hevessy[1]
[1]University of Debrecen, Faculty of Medicine, DEBRECEN, Hungary
[2]Department of Hematology, Jósa András Teaching Hospital, NYÍREGYHÁZA, Hungary

Introduction: Chronic lymphocytic leukemia (CLL) is the most common leukemia type in adulthood. The course and prognosis of the disease is considerably defined by the vulnerability of the lymphocytes. This vulnerability is determined by the structure of the cytoskeleton and is also represented by the amount of smudge cells (Gumprecht shadows) seen in the peripheral blood smear. We examined vimentin, a cytoskeletal protein, in CLL cells in our work.
Methods: Vimentin was measured by flow cytometry after intracellular staining in separated mononuclear cells from CLL patients (along with cells from an age- and sex-matched control group) stored either in physiologic saline (native) or subjected to osmotic stress by distilled water for 60 sec. According to previous treatments the patients were divided in two subgroups (untreated vs. treated). At the time of the examination none of the patients were actually receiving specific treatment. In order to assess normal B and T cells we applied surface staining for CD19 and CD5. For determining vimentin content of the cells the Mean Fluorescence Intensity (MFI) of CLL cells, normal B cells and T cells were recorded.
Results: We observed significant difference (p=0.021) between the vimentin content of the normal B cells in the control group and the pathologic B cells in treated patients after osmotic stress with vimentin content higher in the pathologic cells. Untreated patients showed no significant difference (p=0.68) from the control group. In treated patients the vimentin content was higher in the surviving B cells after osmotic stress than it was observed in the native sample, although it was not statistically significant (p=0.065). Untreated patients showed no significant difference (p=0.684).
Conclusion: The higher vimentin content observed in the treated patients after osmotic stress is in accordance with those results finding higher vimentin counts in malignant cells of several solid tumours. The higher vimentin content is believed to aid the survival of the malignant cells, although the mechanisms responsible for this are not yet fully understood. The need of treatment suggests a more aggressive disease course and this might be promoted by the higher vimentin content in the pathologic B cells.

036

Decrease in CD157 expression in some mature neutrophil populations can complicate the interpretation of PNH evaluation by flow cytometry
Phuong ph Nguyen Vo Thanh, Daniele Casula, Brigitte dr Cantinieaux
LHUB Porte de Hal, BRUSSELS, Belgium

Introduction: All GPI-dependent markers have to be decreased to correctly identify a PNH-Clone. GPI-dependent markers CD157 and FLAER expressions are diminished on immature granulocytes, eosinophils, basophils and immature monocytes but also on PNH-clone [2]. To our knowledge, an isolated decrease in CD157 has never been described on mature neutrophil population of normal patients.
We report here the case of a 28 years old PNH male patient showing an isolated decrease in CD157 expression on neutrophils after allo-Stem Cell Transplantation (SCT) by 5 colors flow cytometry (Navios, BC).
In addition, we observed 4 other cases out of 213 patients tested during 18 months having a totally or partially

decreased CD157 expression on neutrophils. These patients didn't have any known history of PNH and didn't receive SCT.
Methods: PNH testing was carried out according to the recommendations of Sutherland [1] before transplantation and 1 month, 3 months, 4 months and 7 months after allograft.
The screening was based on the following combinations:
WBC: FLAER-Alexa 450, CD157-PE, CD45-ECD, CD15-PECy5, CD64-PECy7.
RBC: CD235-FITC, CD59-PE
Standardization of the fluorescence intensity was performed with Flow Set beads (BC)
Gating strategy consisted on elimination of doublets, eosinophils, immature granulocytes and monocytes based on the FS/SS and fluorescence intensity of the lineage markers.
PNH clones were identified on mature neutrophils and monocytes by association of GPI-dependent markers CD157 and FLAER and on RBC's by CD59.
Results: Before graft, PNH clone III was 84.07%, 71.3% and 10.9% on neutrophils, monocytes and RBCs respectively. Control 6 weeks after the allograft showed a significant decrease in clone III at 5.6%, 2.4% on neutrophils and monocytes. However, we noticed that 92% of the neutrophils had a deeply decreased CD157 expression associated with normal FLAER expression. We controlled this population with CD24 which also showed a normal expression (combination: FLAER-FITC, 157-PE, CD14-ECD, 33-PC5.5, 64-PC7, 24-APC, 15-PB, 45-KO)
The decreased expression of CD157 on neutrophils persisted at 3 and 4 months post-allograft with a progressive increase in CD157 expression which was normalized at 7 months post allograft.
Conclusion: All 5 patients were of African or North-African origin: the decrease and the polymorphism in neutrophils CD157 expression could be explained by the ethnic origin of the patients. In conclusion, CD157 decreased expression on mature neutrophils can be observed and may compromise the interpretation of the cytometric PNH result. Further control samples and PNH testing on healthy patients are requested to confirm this hypothesis.

037

Analysis of Checkpoint Marker Expression on Immune Cells Using a 12-Color Assay on the BD FACSLyric™ Flow Cytometer

Aaron Middlebrook, Le Tri t, Mirko Corselli, Alice Wang, Alan Stall, Ghanekar Smita, Suraj Saksena
BD Biosciences, SAN JOSE, U.S.A.

Introduction: Modulation of the inhibitory pathways that dampen the immune response may represent a major advance in modern cancer treatment. Antibodies that block ligation of immune checkpoint receptors, such as the programmed cell-death protein 1 (PD-1 or CD279), have demonstrated a durable antitumor response with acceptable toxicity in some patients with advanced melanoma. Despite this success, only a subset of patients benefits from immune checkpoint blockade. The clinical impact of immune checkpoint blockade may be increased by careful assessment of checkpoint receptor expression patterns in patients, data that may inform candidate selection or help to monitor clinical efficacy and adverse events in patients being treated with immunomodulatory drugs. Here, we demonstrate the potential of a comprehensive 12-color immune checkpoint panel using the BD FACSLyric™ platform and stimulated peripheral blood mononuclear cells (PBMCs).
Methods: Expression of the immune checkpoint markers CD134 (OX40), CD273 (PD-L2), CD274 (PD-L1), CD279 (PD-1), CD152 (CTLA-4), CD366 (TIM-3), and CD223 (LAG-3) was enumerated on $CD4^+$ and $CD8^+$ T cells as well as NK cells using a 12-color antibody panel and the BD FACSLyric flow cytometer. PBMCs from healthy donors were cultured ex vivo with or without stimulation and immune checkpoint receptor expression was measured.
Results: Following ex vivo stimulation, the data show that PBMCs exhibited robust increases in immune checkpoint marker expression levels that followed specific patterns depending on the cell type. The expression pattern for $CD4^+$ and $CD8^+$ T cells was similar but not identical while the expression pattern for NK cells was distinct from T cells. Our work suggests that the 12-color BD FACSLyric platform may be useful for characterizing and quantifying immune checkpoint receptor expression, and possibly their inhibition during drug discovery.
Conclusions: Multiparameter flow cytometry has been transforming patient diagnosis and disease monitoring for years, e.g. for classification of leukemia and lymphoma disease states. With the recent burst of reports in immuno-oncology research targeting checkpoint markers for therapy, a comprehensive analysis of checkpoint expression patterns in immune cells may further advance this emerging field. We show that the use of an optimized checkpoint marker panel combined with the performance of the BD FACSLyric system enables users to acquire results with a high degree of informational content.

038

A modular, 12-color flow cytometry panel for the immunophenotyping of healthy and AML subjects on a BD FACSLyric™ flow cytometer

Tri Le, Mirko Corselli, Margaret Inokuma, Noel Warner, Alan Stall, Kevin Judge, Suraj Saksena
BD Biosciences, SAN JOSE, U.S.A.

Introduction: Flow cytometric analysis of the immune system is conventionally used for the characterization of hematologic malignancies. Given instrumentation limitations around the number of detectable parameters, researchers often develop multiple panels for the comprehensive characterization of a subject's immune system. This approach increases the workflow and limits the understanding of complex antigen expression patterns. Here, we developed an 8-color backbone panel for identification of major immune cell subsets. The panel was designed such that 4 additional drop-ins for deeper characterization of a hematological disease of interest could be supplemented. We specifically focused on characterization of acute myeloid leukemia (AML) specimens by adding 4 drop-ins for detecting myeloid blasts with aberrant expression of lymphoid markers. This modular, multiparametric (8 + 4 colors) approach enables enumeration and characterization of the major immune subsets, as well as a deeper characterization of AML subtypes within a single sample.
Methods: An 8-color backbone panel was developed using lineage-specific markers for the detection of monocytes, T, B, and NK cells. Four complementary drop-ins enabled the detection of myeloid blasts and aberrant expression of lymphoid markers. Cells from healthy donors or AML subjects were stained and acquired on a 12-color BD FACSLyric™ flow cytometer. BD Trucount™ tubes were used for absolute cell count.
Results: The backbone panel allowed for clear resolution of $CD3^+$ T cells, further refined as $CD4^+$ helper, $CD8^+$ cytotoxic, and $CD56^+CD16^+$ NKT cells. From the CD3- cell population, $CD19^+$ B cells, $CD56^+CD16^+$ NK cells, and three monocyte subsets differentially expressing CD14 and CD16 were identified. The addition of the 4 drop-ins did not significantly alter the resolution of the main subsets and allowed us to investigate for the presence of $CD34^+CD38^-$ myeloid blasts aberrantly expressing CD2 and CD7. The average absolute count and antigen expression pattern for each subset was determined on healthy donors and used as a reference for the analysis of AML subjects.
Conclusion: We showed the advantages of a basic 8-color panel for broad immune cell analysis and enumeration coupled with a modular 4-color drop-in panel for deeper characterization of diseases of interest. Using this approach, we identified several differences between the phenotypes of healthy and AML specimens using a single sample, thus reducing workflow and cost. From a biological standpoint, the ability to simultaneously analyze 12 parameters provides unique insights into the interplay between different antigens in health and disease.

039

Accurate enumeration of CD34+ cells with the BDTM Stem Cell Enumeration Kit on the BD FACSLyricTM system

Alex Fainshtein[1], Edward Joe[1], Lori Apoll[1], Alice Wang[1], Robert Sutherland[2], Tri Le[1]
[1]BD Biosciences, SAN JOSE, U.S.A.
[2]University Health Network, TORONTO, Canada

Introduction: Hematopoietic stem cells (HSCs) are CD34+ and are responsible for engraftment in the bone marrow transplant setting. Enumerating CD34+ HSCs in peripheral blood, apheresis and cord blood samples provides critical information to the transplant physician. The number of viable CD34+ cells present in the peripheral blood after mobilization and/or chemotherapy predicts the yield of CD34+ cells in the apheresis product. Additionally, the number of CD34+ cells collected predicts time to engraftment after transplantation. The infusion of a minimum of two million viable CD34+ cells per kilogram patient weight generally enables rapid (10-12 days to 500 neutrophils/mL) and sustained engraftment in the auto-transplant setting. The International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol for CD34+ cell enumeration is the most widely used flow cytometric method in clinical laboratories. BD Biosciences has developed an algorithm for the BD FACSLyric™ system that closely follows the ISHAGE protocol. This study was performed to demonstrate the accuracy of the new assay algorithm to enumerate CD34+ HSCs.
Methods: 50 samples (including apheresis, mobilized apheresis, cord blood, bone marrow and normal peripheral blood) were acquired on the BD FACSLyric system. Data was analyzed in parallel using three different methods: 1. Manually, using well-established sequential gating criteria by an academic expert; 2. Manually, gated by BD experts; and 3. Automatically, using the new BD FACSLyric™ algorithm. Results for total and viable CD34+ and total and viable CD45+ cell counts derived by the three methods were compared.
Results: The results show excellent concordance between all three methods ($R^2 = 0.99$). Data from the algorithm generated slightly lower CD34+ cell counts than BD experts, who in turn generated slightly lower data than the academic expert. However, the differences were all below 5% for viable and total CD34+ cells, and less than 1% for viable and total CD45+ cells.

Conclusions: Our results demonstrate that the BD™ Stem Cell Enumeration assay on the BD FACSLyric system accurately enumerates CD34+ cells.

040

The impact of preanalytical errors on the identification of myelodysplastic phenotypes by flow cytometry

Bettina Kárai, Zsófia Miltényi, Lajos Gergely, János Kappelmayer, Zsuzsanna Hevessy
University of Debrecen, DEBRECEN, Hungary

Introduction: Myelodysplastic syndrome (MDS) is a heterogeneous hematopoietic neoplasm. Although the diagnosis and prognosis of MDS are based on morphologic and cytogenetic examinations, several studies suggest that flow cytometry (FCM) can also be a useful diagnostic tool by detecting phenotypic alterations compared to normal cells. We were interested in to what extent time-dependent immunophenotype changes—as a phenotypical variable—might influence FCM results, and we also examined how using two different types of anticoagulants may influence these time-dependent immunophenotype changes.
Methods: We examined 24 bone marrow samples collected in EDTA by 8-color staining. Thirty-seven different immunophenotypic variables were recorded in each case for 3 days, mean florescence intensity (MFI) values were recalculated, and results were compared to day-0 values. To examine the impact of the different anticoagulants, two sets of 17 samples collected in EDTA and heparin were analyzed, respectively.
Results: When we compared the initial immunophenotype of samples collected in EDTA and heparin, we detected significant differences in 13 parameters: SSC and intensity of CD45 and CD11b expression on granulocytes ($p=0.003$, $p=0.007$ and $p<0.001$, respectively); MFI of CD11b, CD13 and CD33 on monocytes ($p<0.001$, $p=0.031$ and $p=0.031$, respectively); MFI of CD34 and CD117 on myeloblasts ($p=0.006$ and $p<0.001$); MFI of CD45 on lymphoblasts ($p=0.002$); MFI and rCV of CD71 on erythroblasts ($p=0.001$ and $p=0.042$); and finally, percentage and MFI of CD38 on plasma cells (PCs) ($p=0.005$ and $p=0.002$, respectively).
Only 8 parameters (MFI of CD15 on granulocytes, percentage and MFI of CD45 and CD34 on myeloblasts, percentage of lymphoblasts, percentage and rCV71 of erythroid precursors and percentage of PCs) proved to be stable during delayed sample handling independent of the type of anticoagulant. MFI of CD13 and CD16 on granulocytes, MFI of CD64, CD15, CD13, and CD33 on monocytes and MFI of CD38 on PCs changed similarly regardless of the type of anticoagulant. Several parameters —SSC, MFI of CD45, HLA-DR, CD11b and CD33 on granulocytes, SSC, CD11b, HLA-DR, CD14 and, CD300e on monocytes, MFI of CD45 on lymphocytes — were altered only in the case of the samples collected in EDTA.
Conclusion: Due to delayed sample processing, considerable MDS-related immunohenotype alterations take place not only on myeloid but also on erythroid cells and rare populations, which can cause false interpretation of the results. Therefore well-defined, standardized sample handling and appropriate interpretation of FCM results are essential.

041

Evaluation of B-clonality in HIV positive patients in different clinical stages of the disease and its association with clinical parameters

Sandra Quijano[1], Carolina Celades[1], Sandra Valderrama[2], Sandra Gualtero[2], Catalina Hernandez[2], Carlos Saavedra[3], Martha Romero[3], Marcos López[4], Wendy Nieto[4], Susana Fiorentino[1]
[1]Pontificia Universidad Javeriana, BOGOTÁ, Colombia
[2]Hospital San Ignacio, BOGOTÁ, Colombia
[3]Hospital Universitario Fundación Santa Fe de Bogotá, BOGOTÁ, Colombia
[4]Fundación Cardiovascular de Colombia, BUCARAMANGA, Colombia

Introduction: Lymphoma is one of the main types of AIDS-defining cancer in HIV patients and the leading cause of morbility and mortality associated with HIV, despite the access to antirretroviral therapy. (1) The clinical evolution to lymphomas in these patients is associated with chronic antigenic stimulation, imbalance of proinflammatory cytokines in the microenvironment, loss of immunoregulatory control of oncogenic herpesviruses possibly implied in lymphomagenesis, like Epstein-Barr virus (EBV), and genetic alterations. (2)(3)(4) It is not currently established whether HIV patients have clonal B cell populations in peripheral blood (PB) that could progress to B lymphoma, and its detection might be helpful to monitoring B-lymphomas early onset, thus to offer timely treatment. The objective of this study is to determine the presence of clonal B lymphocyte populations in the PB of HIV patients and its association with clinical and biological variables.

Methods: We studied 238 PB samples of HIV patients in different clinical stages of the Hospital Universitario San Ignacio (Bogotá, Colombia). The immunophenotypic analyses were performed by flow cytometry (FC) following the standardized protocols by the European EuroFlow Consortium (5) for the screening of B cell lymphoproliferative disorders. EBV load was measured by q-PCR. Clinical data were collected from patient histories.
Results: This study describes for the first time that 31,9% of patients (76/238) have some alteration in TBL, MBL and/or PC Kappa/Lambda ratio. Additionally, 8% of the patients (19/238) have some population considered clonal by the altered Kappa/Lambda relationship. However, the absolute values of these populations with clonality or imbalance are, in general, low counts. In addition, in these patients are aberrant immunophenotypes, including overexpression and underexpression of various B-lineage associated antigens. Regarding the associations of the clinical variables, it was found that patients with clonal or imbalanced B populations also present alterations in B-cell and plasma immunophenotype, the majority of them had clinical characteristics associated with advanced stages of disease and progression and higher presence of EBV viral load.
Conclusions: These findings show that chronic HIV-induced activation has important effects on both the amount and the chronic hyperactivation of B-lymphocytes, the amount of PC and the emergence of abnormal TBL. This is the first report of Kappa/Lambda imbalance and clonality in TBL, MBL, and PC in HIV patients. These results can be associated with higher risk of progression to lymphoma, and the hypothesis should have cytogenetic testing confirmation.

042

Utility of flow cytometry in the detection of tumoral cells in cerebrospinal fluid from patients with acute leukemia

Sandra Quijano[1], Ximena Torres[2], Iliana de los Reyes[2], Paula Guzman[1], Martha Vizcaino[2], Carlos Saavedra[3], Martha Romero[3], Alba Campos[2], Niyireth Peñaloza[2], Paula Rodríguez[3], Gina Cuellar[3], Liliana Martín[3], Ana María Uribe[2]
[1]Pontificia Universidad Javeriana, BOGOTÁ, Colombia
[2]Hospital San Ignacio, BOGOTÁ, Colombia
[3]Hospital Universitario Fundación Santa Fe de Bogotá, BOGOTÁ, Colombia

Introduction: Early diagnosis of central nervous system infiltration favors a better classification and follow-up of patients with acute leukemia and relapse risk in central nervous system (CNS). Different groups in flow cytometry (FC) have demonstrated the usefulness of this technique in the detection of lymphoma cells in Cerebrospinal fluid (CSF) compared to the gold standard that is conventional cytology (CC). Objective: To evaluate the neoplastic infiltration in CSF of patients with acute leukemia (AL) by FC and CC and its relationship with different clinical-biological parameters.
Methods: A total of 123 CSF samples from 51 pediatric patients with LA from the Hospital Universitario San Ignacio and Fundación Santa Fe de Bogotá were analyzed. Samples were analyzed simultaneously by FC and CC. Clinical data were collected from patient histories.
Results: Of the 123 samples studied, 21 (17%) showed tumoral infiltration by FC, whereas CC was only positive in 2 samples (1.6%) and suggestive of infiltration in 2 other cases (1.5%). The agreement between the two techniques was very poor (Kappa index <0.2). Clinically the presence of CNS involvement determined by CF was associated in patients with acute B leukemia with higher leukocyte counts (FC+ ẋ=80,000/µL vs FC- ẋ=18,600/µL; *p*=<0.001) and thrombocytopenia (FC+ ẋ=49,000/µL vs FC- ẋ=132,000/µL; *p*=0.07), a higher percentage of cases with extramedullary infiltration, a lower response to chemotherapy (FC+ 22.2%, FC- 4.2%; *p*=0.002) and steroid therapies (blast cell in periphery blood, FC+ ẋ=57239/µL vs FC-:540/µL; *p*=<0.001) and lower total patient survival (deaths n=11 FC+ 37.5%, n= 24 FC- 9.1%). On the other hand, in acute T leukemia CSF infiltration was associated with lower response to day 8 of steroid treatment (blast cell in periphery blood FC+ ẋ=41,730/µL versus FC-: 1,565/µL; *p*=<0.001) and in acute myeloid leukemia an association with greater tumor burden in bone marrow (FC+: 48.3% vs FC-: 21.1%; *p*=<0.046) and peripheral blood (FC+: 40.7% vs FC-: 33.6%; *p*=<0.029), was observed.
Conclusions: In this work we were able to detect more tumor cells in CSF from pediatric patients with LA using FC (17%) with respect to cytology that only detect (1.6%). The detection of this cells using FC showed association with prognostic value variables. These findings suggest that it is highly recommended to include FC in the routine study of CSF samples from pediatric patients with AL.

043

Eight color flow cytometry for monitoring minimal residual disease in Acute Promyelocytic Leukemia (APL): outcome and comparison with PML-RARA detection

Maura rosane Valerio Ikoma, Leandro Lustri, Joana espricego Conti, Marcimara Penitenti, Camila marques Bertolucci, Ederson roberto Mattos, Fernanda leite Souza Franceschi, Iago Colturato, Mair pedro Souza, Vergilio Antonio rensi Colturato
AMARAL CARVALHO HOSPITAL, BAURU, Brazil

Introduction: As PML-RARA is the molecular signature of APL, PCR detection of this fusion gene transcript is highly specific for monitoring treatment response, PCR is the gold standard and recommendable technique for APL minimal residual disease (MRD) evaluation. Even though flow cytometry (FCM) is also an available method for MRD detection in acute myeloid leukemia (AML), it is known its lower sensitivity and specificity compared to PCR. On the other hand, APL phenotype is more homogeneous and stable after treatment than others AML, allowing the use of FCM for MRD.
Aim: to test the efficacy of FCM for MRD detection in APL, compared to PML-RARA PCR results and also considering the patients' clinical outcome.
Patients, material and methods: evaluation of results of 23 APL patients, from 9 to 65 years old, diagnosed by morphological, phenotype and molecular criteria. From February 2012 to April 2017, 68 APL samples were made for MRD evaluation by FCM (flow MRD). In 23 of them PCR was done concomitantly. For FCM it was used one 8 color tube: CD15FITC(MMA)/ CD33PE(P67.6)/ CD34PerCPCy5.5(8G12) / CD117PEcy7.7(104D2)/ CD11bAPC(D12)/CD14APCH7(MϕP9)/HLADRV450(L243)/ CD45V500(2D1), based on a preliminary proposed tube by the General Service of flow cytometry of the University of Salamanca. A lise-wash sample preparation was used. 1 million total events per tube were acquired in a FACSCanto II. PCR results, with sensitivity 1x 10^{-5}, were obtained by medical records review.
Results: 18 of the 23 results were concordant between FCM and PCR, 3 positive (pos) and 15 negative (neg). 5 of the 23 results were discordant: 3 PCR^{pos} and FCM^{neg} and 2 PCR^{neg} and FCM^{pos}. Of the discordant cases with PCR^{pos} and FCM^{neg}: the first sample was hemodiluted, which can decrease the sensitivity of the flow MRD; the second one was from a patient who did not relapse after 2 years of flow MRD evaluation; and the third one from a patient who relapsed 30 days after the MRD tests. The two patients with PCR^{neg} and FCM^{pos}: both relapsed 30 days after the MRD evaluation. Eight patients had no MRD assessment by PCR, they had negative flow MRD (sensitivity <0,001%) and they did not relapse after 14 to 60 months of the last flow MRD evaluation, with a median of 30,5 months follow up.
Conclusion: although these are preliminary results, FCM was applicable for MRD evaluation of APL, with an acceptable sensitivity (86%) and high specificity (100%) in this series of patients.

044

Flow cytometric analysis of B-cell subpopulations as an approach for the B-cell lymphomas diagnosis from lymph node samples

Andreja Brozic[1], Alojz Ihan[2], Veronika Kloboves Prevodnik[1]
[1]Institute of Oncology Ljubljana, LJUBLJANA, Slovenia
[2]Institute of Microbiology and Immunology, LJUBLJANA, Slovenia

Introduction: Different B-cell subpopulations can be defined with immunophenotypic characterization of human B-cells based on different stages of differentiation and maturation. Most published studies are focusing to analyses of B-cell subpopulations in peripheral blood, mostly referred to their role in autoimmune diseases. Only limited studies describing B-cell subpopulations of lymphoid tissue samples have been done. None of them include cases of malignant diseases. The aim of the study was to define immunophenotype and to describe characteristics of B-cell subpopulations according to differentiation stages in reactive lymphocytic proliferations (RLP) and B-cell lymphoma (BCL).
Methods: Our study included 47 fine needle aspiration and 5 surgical excision lymph node biopsies. Subpopulations of B-cells were determined by 6-color flow cytometry, using classification method based on IgD/CD27 staining. Comparison of B-cell subpopulations of RLP and BCL was done. IgD index was calculated as the ratio of mean channel fluorescence in CD19 positive to that of CD19 negative cells. The study was approved by The National Medical Ethics Committee of the Republic of Slovenia (109/02/14).
Results: Among 52 of our samples, 28 (54%) of them had the final diagnosis of BCL and 24 (46%) RLP. Six different mature CD19 positive B-cell subpopulations in RLP samples were found. In all BCL samples at least one of the B-cell subpopulations was lost. In 93% of BCL samples, expression of analyzed antigens on B-cell subpopulations was different than in RLP. Neoplastic cells had immunophenothypical features of one reactive B-cell subpopulation or mixed phenotype. The IgD index in RLP was significantly higher compared to BCL.

Conclusions: Presented study showed that BCL and RLP samples differ in immunophenothypic features of neoplastic cells and in representation of particular B-cell subpopulation. These results enable accurate differentiation between BCL and RLP. Immunophenotypic characterization of B-cells subpopulations using flow cytometric IgD/CD27 classification may be used as an additional diagnostic procedure in BCL diagnosis and provide information which could enable target specific future therapies.

045

Which cutt-off value of EMA binding test for Hereditary spherocytosis ?

Falay Mesude[1], Mehmet Senes[2], Gulece Ulusan[1], Merve Pamukcuoglu[1], Mehmet Ali Ucar[1], Gülsüm Özet[1]
[1]Ankara Numune Education and Resarch Hospital, ANKARA, Turkey
[2]Ankara Education and Resarch Hospital, ANKARA, Turkey

The eosin-5 ' -maleimide (EMA) binding test is a flow cytometric test widely used to detect hereditary spherocytosis (HS). EMA binds to plasma membrane proteins of red blood cells (RBCs), mainly to band 3 protein. The mean fluorescence of EMA-stained RBCs
in HS patients is lower when compared with control RBCs due to the decreased amount of target proteins. EMA binding test lies in the lack of universal reference ranges for normal controls and HS individuals. The results of the EMA test, obtained from different laboratories, can vary significantly and are not comparable with one another due to the different fluorescence scales from different flow cytometer models .
It is important for every laboratory to establish their own reference range for normal samples, determine the optimal cut-off value of MCF ratio for HS diagnosis.
In this study, our target is to establish our laboraties' own optimum reference range. EMA binding test has performed with BD fascanto device using 120 healthy adult, 60 healthy children and 15 Herediter Sferosytose fact and their MFIs has been calculated.
Average MFI 24.326 (22.955-25.459 in child age group, MFI 27.554 (23.444-33.029) in adult age group have been calculated. Cut-off has been calculated 0.934-1.04 for children and 0.850-1.198 for adult age group according to those averages
We think, using diferrent reference ranges among children and adults will be more valid. Our study with healthy children continues. We will have more accurate results in children age group when we reach to 120 samples.

046

Aberrant multilineage proliferation of precursor cells driven by monosomy 7 as a secondary malignancy after relapsed acute lymphoblastic leukemia

Lukasz Sedek[1], Magdalena Twardoch[1], Aneta Pobudejska-Pieniazek[1], Alicja Sonsala[1], Magdalena Pierzyna-Switala[1], Jan Kulis[1], Lukasz Slota[1], Agata Kowalska-Pawlak[2], Joanna Bulsa[1], Tomasz Szczepanski[1]
[1]Medical University of Silesia in Katowice, ZABRZE, Poland
[2]1st Independent Public Clinical Hospital of the Medical University of Silesia, ZABRZE, Poland

Introduction: Relapses in acute lymphoblastic leukemia (ALL) concern 15-20% of patients. In majority of cases, relapses develop from the same cell lineage as at initial diagnosis. In small proportion of cases a secondary hematological malignancy might develop.
Aim: We present an aberrant, multilineage proliferation of precursor cells secondary to relapsed ALL treatment.
Case report and results: The patient was primarily diagnosed at the age of 3 years as B-cell precursor (BCP)-ALL. He was treated for 26 months with frontline chemotherapy according to ALL-IC BFM 2002 protocol. One year after treatment completion (12.2012) he developed a combined relapse of BCP-ALL both in bone marrow and in one testis. He underwent unilateral orchidectomy and subsequently second line chemotherapy and local radiotherapy for the testicular area were introduced. At 1.5 years of the maintenance treatment (06.2014), CBC revealed pancytopenia, bone marrow morphology showed 7.8% of blasts, while flow cytometric analysis of bone marrow did not reveal residual leukemic cells with original phenotype. In contrast, about 6.1% precursor cells of aberrant immunophenotype were detected, assigned as B1: CD19-, CD34+, CD22dim, CD10++, CD20-, TdTpart+, CD123-, CD38+, CD117+, HLADR+. Moreover, 1.0% aberrant plasmacytoid dendritic cell precursors of the following immunophenotype were present: CD19-, CD34dim, CD22-, CD10++, CD20-, TdT-, CD123++, CD38+, CD117-, HLADR+. Two months later, the B1 population was accompanied by 2 other stages of abnormally maturing BCP – B2: CD19+, CD34+, CD22+, CD10+, CD20-, TdT+, CD123-, CD38++, CD117-, HLADR+ and B3: CD19dim, CD34-, CD22+, CD10dim, CD20part+. Moreover, there was a myeloid precursor cell subset, which did not exhibit phenotypic aberrancies (CD19-, CD34+, CD10-, CD20-, TdT-, CD22-, CD38++, CD117+, CD123-, HLADR++,SSChigh). All BCP subsets showed polyclonal pattern of *IGH* gene rearrangements with PCR analysis. Cytogenetic

examination revealed aneuploid karyotype: 45,XY,del(4)(q31?),-7,der(9)[20] and interphase FISH showed monosomy 7 in >80% of cells. This was also confirmed by CytoScan 750K array, which detected a complex karyotype highlighting chromosome 7 monosomy and chromosome 4 loss (del4q21.1-q25; 40Mb) associated with chromosome 14 gain (14q32.33; 200Kb). In contrast, retrospective FISH analysis of blasts performed at first relapse showed 2 copies of chromosome 7. Due to systematic increase of all abnormal precursor cells subsets (top percentage of 27,5%) and hepatosplenomegaly, the patient was subjected to haploidentical stem cell transplantation.

Conclusion: We report an unusual case of therapy-related, aberrant hematopoiesis resulting from stem cell defect associated with monosomy 7. This proliferation was characterized by accumulation of abnormal B-cell-, myeloid- and plasmacytoid dendritic cell precursors.

047

Immunophenotypic characteristics of acute myeloid leukemia with myelodysplasia-related changes - a single institution experience

Nada Kraguljac Kurtovic[1], Vesna Knezevic[1], Nada Suvajdzic-Vukovic[1], Tijana Dragovic Ivancevic[1], Jelica Jovanovic[1], Andrija Bogdanovic[1], Sandra Bizic-Radulovic[1], Irena Djunic[1], Marijana Virijevic[1], Ana Vidovic[1], Biljana Bozic-Nedeljkovic[2], Mirjana Gotic[1]

[1]Clinical Center of Serbia, Clinic of Hematology, BELGRADE, Serbia

[2]Faculty of Biology, University of Belgrade, BELGRADE, Serbia

Introduction: Diagnosis of acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) depends primarily on the presence of specific cytogenetic abnormalities and/or multilineage dysplasia. Little literature exists about immunophenotypic characteristics of bone marrow (bm) populations on diagnosis in AML-MRC.

Methods: We retrospectively analyzed 41 patients (pts) with AML-MRC on diagnosis, selected from the cohort of 281 adult pts with *de novo* AML, admitted at the Clinical Center of Serbia, Clinic of Hematology during the period 2009-2013 year. Diagnosis of AML was established according to the World Health Organization classification. Immunophenotyping was performed by using 4-color panels of monoclonal antibodies and whole bm lyse/wash staining protocol. Acquisition/analysis were performed on BD FACSCalibur (BD CELLQuest ProSoftware), and included populations of blast cells as well as granulocytic and monocytic precursors, gated according to CD45/cellular granularity (CD45/SSC) patterns. Healthy control bm specimens (n=13) were used to define normal ranges and regular antigens (Ags) expression patterns on explored bm nucleated cell (nc) populations.

Results: The frequency of AML-MRC (14.6%) was lower compared to AML, not otherwise specified (48%) and AML with recurrent genetic abnormalities (37.4%). In AML-MRC, median value of *bm blast cell population* ($CD45^{low}/SSC^{low}$) was 50% nc (range, 18-98% nc). Immunophenotypic profile of blast cell population was characterized by expression of hematopoietic progenitor and early myelo-monocytic Ags ($HLA\text{-}DR^{+het}$, $CD34^{+hi}$, $CD117^{+hi,}$ $CD123^{+het}$, $CD135^{+lo}$, $CD38^{+het}$, $CD33^{+het}$, $CD13^{+hi}$, $CD114^{+lo}$, $cMPO^{p+het}$, $cLysozyme^{p+het}$). The frequencies of later myelo-monocytic Ags (CD11b, CD11c, CD64, CD36, CD14, CD163, CD15, CD35) were lower (≤ 50%), as well as aberrantly expressed lymphoid Ags: CD56 (44%), CD7 (37%), and CD22 (32%). Median value of *bm granulocytic precursors* was 24% nc (range, 0.2-65% nc). Hypogranulation, defined according to lower SSC pattern, was detected with frequency of 50%. At least one of different types of maturation asynchrony in Ags expression was disclosed in the population of granulocytic precursors, with higher frequency for Ags: $HLA\text{-}DR^{+}$ (93%), $CD34^{+}$ (86%), $CD45^{+hi}$ (79%), $CD15^{+het}$ (68%), and $cMPO^{p+}$ (62%). CD56 was the most aberrantly expressed Ag (24%). Median value of *bm monocytic precursors* was 7% nc (1-93% nc) and immunophenotypic abnormalities were not studied in details.

Conclusions: Blast cell population in AML-MRC shows immunophenotypic characteristics of early cells of granulocytic lineage in 58% pts, combined granulocytic and monocytic lineages in 27% pts, and only monocytic lineage in 15% pts. Different immunophenotypic abnormalities on the granulocytic precursor population are detected, and together with hypogranularity may suggest diagnosis of AML-MRC.

048

Assessment of AML MRD using difference from 'digital' normal

Alan Dunlop[1], K Sanchez[1], M Bullard[1], Robin dr Ireland[2]
[1]Viapath @ Kings College Hospital, LONDON, United Kingdom
[2]Dept of Haematological Medicine, Kings College Hospital, LONDON, United Kingdom

Introduction: Minimal Residual Disease (MRD) by multiparameter flow cytometry (MFC) in AML is increasingly used to assess response to treatment, traditionally using a Leukemia Associated Immunophenotype (LAIP). However phenotypic changes regularly occur over the course of treatment therefore using a strict LAIP may result in false negatives. To circumvent this a method known as "difference from normal" (DFN) has been successfully used in a number of large multicentre studies. This relies on individual analysts having in-depth knowledge of normal haemopoiesis and are therefore able to asses all populations present determining MRD levels even with phenotype differences from the LAIP or when no LAIP is available. This method is largely subjective and relies on a high level of expertise which is generally confined to a few individuals.
Methods: 25 non-malignant bone marrow samples were studied using our standard 5 tube, 8 colour AML diagnostic/disease monitoring panel. The resulting 125 data files were analysed to identify and create a reference images for the following; CD34+ myeloid progenitors, CD34- myeloid progenitors, promyelocytes, monocytes, CD34+ lymphoid progenitors and mature lymphocytes.
As a pilot study, cases of post-transplant AML or High Risk MDS were analysed using Infinicyt with a standard gating strategy and each population compared to our non-malignant reference images. Analysts with previous experience in leukemia/lymphoma immunophenotyping but no specific experience in AML MRD analysis carried out interpretation over a 3 month period with a detection limit of 0.1%. Retrospective analysis was made with molecular/cytogenetic results.
Results: 38 cases were included where comparative assays were available. Of these cases 71% were classified as MRD negative with 29% MRD positive. MRD negative cases showed 85% correlation with other techniques with the MRD Positive cases correlating in 82%. All discrepancies could be accounted for when sample quality and limit of detection of each assay are taken into consideration with MFC appearing to be the most sensitive technique currently available.
Conclusions: As MRD status is more frequently being used to direct treatment in AML it is critical that clinical laboratories are able to provide a robust service not reliant purely on the expertise of a small number of individuals. This pilot study has shown that by creating in-house databases of "normal" phenotypes which can be applied to all cases, we are able to non-subjectively analyse MRD in AML using DFN. This is of particular use in large referral /BMT centres where presentation phenotype is not always available.

049

Relevance of paroxysmal nocturnal haemoglobinuria clone monitoring in patients with bone marrow failure syndromes

Amparo Sempere[1], Samuel Romero[2], Lourdes Cordón[2], María José Mas-Alamán[1], Andrés Moret[3], Juan Carlos Marín[2], Amparo Bayona[1], Pilar Cuñat[1], Concepción Molina[1], Inmaculada Murcia[1], Anabel Regadera[1], Santiago Bonanad[1], Rafael Andreu[1], Federico Gomis[1], Isidro Jarque[1]
[1]Hematology Department, Hospital Universitario y Politécnico La Fe, VALENCIA, Spain
[2]Hematology Research Group, Instituto de Investigación Sanitaria La Fe, VALENCIA, Spain
[3]Hemostasis and Thrombosis Research Group Instituto Investigación Sanitaria La Fe, VALENCIA, Spain

Introduction: Association between paroxysmal nocturnal haemoglobinuria (PNH) and bone marrow failure syndromes (BMFS), as aplastic anaemia (AA), is clearly established. BMFS are indications of PNH study since small and occasionally expandable PHN clones with a relevant role in the disease course are observed up to 40% of patients with AA at diagnosis. Flow cytometry (FC) is the technique of choice for diagnosis and monitoring of PNH clones. Consensus guidelines recommend the study of PNH in BMFS, especially in AA, at diagnosis and at least yearly monitoring in the follow-up providing that clinical or analytical changes do not occur. The aim of this study was to assess the feasibility of monitoring recommendations of PNH clones and follow-up of patients with AA, employing high-resolution FC.
Methods: We assessed 191 peripheral blood samples for study of PNH clones in our centre from January 2016 to June 2017. Detection of PNH clones was evaluated with a high-resolution technique (Sutherland *et al.*, 2014) and a close follow-up adapted to PNH guidelines was performed. Samples were stained using fluorescent aerolysin (FLAER), CD157-PE, CD45-PerCP, CD64-APC, CD15-HV450, and CD235a-FITC, CD59-PE for the study of leukocytes (neutrophils and monocytes) and erythrocytes, respectively. Cells were acquired in a FACSCanto-II cytometer and analysis was performed using the Infinicyt™ 1.8 software.

Results: A total of 34 patients had the diagnosis of BMFS, 16 (47%) *de novo*, 13 (38%) during an adequate follow-up, and 5 (15%) reevaluated after a lost period. Overall, PNH cells were detected for the first time in 15 cases (44%), 10 of them new BMFS. Concerning the last 5 cases, median age was 36 years (range, 19-67). Median and range levels were: haemoglobin 10.8 g/dL (10.3-13), reticulocytes $0.10x10^9$/L (0.05-0.19), platelets $143x10^9$/L (37-197), leucocytes $4.10x10^9$/L (2.6-5.1) and neutrophils $2.10x10^9$/L (1.4-2.7); creatinine 0.89 mg/dL (0.48-2.06), total bilirubin 1.41 mg/dL (0.99-2.7), and LDH 1138 U/L (220-1372). According to PNH population, neutrophil, monocyte and erythrocyte clones were 11.12% (0.06-99), 49.62% (0.05-98), and 19.83% (<0.01-94), respectively. At PNH re-evaluation, 3/5 cases showed haemolytic activity and evolved to classic PNH 12 years after AA diagnosis. One of these patients started anti-complement therapy. The remaining 2 subjects were diagnosed of subclinical PNH.
Conclusion: A close monitoring of PNH clones, considering clinical and biological changes, is essential for the control of BMFS patients. Both, the use of high-resolution FC and an integrated management allow detection of evolution to PNH.

050

Optimising gating strategies for residual disease detection in CLL patients receiving novel inhibitor therapies
Richard Leach, Ruth de Tute, Andy Rawstron
Leeds Teaching Hospitals NHS Trust, LEEDS, United Kingdom

Introduction: Ibrutinib-based therapies for Chronic Lymphocytic Leukaemia (CLL) can result in reduced expression of CD19 and CD20 on the surface of neoplastic B-cells, with complete loss of CD19 expression in a small proportion of cases. Decreased CD19 expression may impair detection of minimal residual disease (MRD), particularly in patients receiving combination therapy. From a panel of 43 antibodies, we identified HLA-DR and ROR1 as the only markers that were expressed in the vast majority of CLL cases and were not affected by in vivo ibrutinib exposure. CD22 has also been considered informative for MRD in patients receiving anti-CD20 therapeutic antibodies.
Aim: to assess prospectively the use of CD22, HLA-DR and ROR1 as an alternative to CD19 as primary gating markers for MRD detection in patients receiving ibrutinib-based therapy.
Methods: A total of 176 cases (n=176), comprising 76 bone marrow and 100 peripheral blood samples, were assessed using two different gating strategies. Both approaches contained the ERIC required markers for MRD detection (CD5, CD20, CD43, CD79b, CD81) and B-cell gating was performed using light-scatter characteristics with either (i) CD19 alone or (ii) HLA-DR, ROR1 and CD22 in combination.
Results: There was good concordance between the two gating strategies for the number of neoplastic B-cells detected (linearity=0.994, correlation coefficient Pearson R=0.996). Discrepant results (>10% difference between the two gating strategies) were identified in 21% (26/125 cases evaluated). CD22 expression was not informative in this series because normal B-cells were absent during treatment and CD22 expression was substantially decreased on CLL cells during ibrutinib treatment. In 18% (22/125) the combination was able to identify CLL cells that could not be distinguished from the background using CD19 alone due to reduced CLL-cell CD19 expression, while in 11% (14/125) neoplastic B-cells were missed from the HLA-DR/ROR1 gate. This was due to reduced HLA-DR expression in some cases after treatment with novel therapy combinations and/or weak ROR1 expression in some patients before and during treatment.
Conclusions: HLA-DR and ROR1 can facilitate MRD detection in patients receiving inhibitor therapy but there are a relatively small proportion of cases that would benefit from additional gating markers and CD19 remains the most effective primary gating marker for CLL MRD detection with inhibitor-based therapies.

051

Study of the effect of several biochemical drugs in myeloproliferative neoplasms cell lines, based on the status of RUNX1/CBF-BETA/P300/HIPK2 complex
Carlos Lozano Asencio[1], Raimundo Cervera-Vidal[2], Guadalupe Herrera[3], Eva Villamon[4], Joan Climent[2], Juan Carlos Hernandez-Boluda[4]
[1]Health Research Institut. INCLIVA, VALENCIA, Spain
[2]Health Research Institute. INCLIVA, VALENCIA, Spain
[3]University of Valencia, VALENCIA, Spain
[4]Clinical Universitary Hospital, VALENCIA, Spain

Introduction: Myeloproliferative neoplasms (MPNs) are clonal hematological malignancies with an inherent tendency to progress to acute leukemia, after a variable period of time. Although the mechanisms involved in the disease transformation are still unclear, it´s well known that transcription factor RUNX1 (AML1) is frequently deregulated in human leukemia, and recently, it has been described that the activity of RUNX1 together with CBF-b

cofactor is regulated by the proteins p300 and HIPK2. In fact, HIPK2 phosphorylates both RUNX1 and p300, activating the whole transcriptional complex. Therefore, the study of the status of this complex seems to be interesting in order to understand the mechanisms involved in the leukemic transformation.
Our aim is to study the effect of different typical drugs in several NMPs cell lines with differential expression of these genes in normal conditions and compare it with a context where the complex is chemically downregulated.
Methods: For this purpose, we chemically knocked down some genes of the studied complex and then performed different viability, apoptosis and cell cycle assays, to elucidate if the status of these genes could play an important role in the response to the different biochemical drugs tested.
Results: We show here that the inhibition of the complex had a clear effect in the proliferation and survival of the treated cells, and also produced big changes in cell cycle. Moreover, these effects seem to differ from the effects caused by the typical drugs used in NMPs disorders.
Conclusions: Our results indicate that the Hipk2/p300/Runx1/CBFb complex might be involved in the leukemic transformation as shown by proliferation, apoptosis and cell cycle assays when we compare the effect of inhibiting the complex with the effect produced by the typical drugs used for the treatment of the NMPs. These findings could indicate new pathways that are important in leukemogenesis and open new strategies for the study of the myeloproliferative disorders.

052

The role of flowcytometry in diagnosis of Myelodysplastic syndrome with bone marrow hypoplasia
Judit Beata Kopeczi, Istvan Benedek, Eniko Kakucs, Erzsebet Lazar
Clinical Hematology and BMT Unit, TARGU-MURES, Romania

Introduction: Myelodysplastic syndromes (MDS) are a group of biologically and clinically heterogeneous clonal disorders characterized by dysplastic changes, ineffective hematopoiesis, peripheral cytopenia and increased risk of transformation to acute myeloid leukemia. In most cases bone marrow is hypercellular but in some cases can be hypocellular, a condition that overlaps and is difficult to differentiate from aplastic anemia (AA). Dysplastic myeloid cells +/- megacaryocytes or excess of blasts may be difficult to see. $CD34^+$ hematopoietic progenitors are central to the pathogenesis of both disorders; they are the targets of the autoimmune attack in AA and neoplastic transformation in MDS. Cytogenetic findings typical of MDS may be necessary for diagnosis or % of CD34+ cells suggests MDS. Aim of this study was to illustrate the problems in the differentiation between aplastic anemia and hypoplastic MDS.
Materials and methods: A total of 5 patients with hMDS were evaluated. Hematological data were evaluated according to peripheral blood count, bone marrow biopsy and flow cytometry.
The determination of cellularity within the bone marrow as well as the quantitative evaluation of the three cell lineages of hematopoiesis was performed. Hypoplasia of hematopoiesis was defined as hematopoiesis making up less than 30% within the total areas of the bone marrow sections.
Immunophenptyping of bone marrow samples was performed in all cases.
Results: We present 5 cases with hypocellular MDS in which the diagnosis was difficult. All patients were male with age between 24-75 years. The patients were admitted to our hospital with suspected aplastic anemia. They had pancytopenia in the peripheral blood. The bone marrow was hypocellular and the dysplastic myeloid cells +/- megacaryocytes or excess of blasts were difficult to see.
The examination of bone marrow by flowcytometry shown in all 5 cases 12-16% CD34+ blasts suggesting myelodysplastic syndrome. This finding was very important in the differential diagnosis between hypoplastic MDS and aplastic anemia, in which the CD34+ blasts number is low.
The patients were treated with immunosupressive therapy, granulocyte-colony stimulating factor for granulocytopenia; anemia and thrombocytopenia was corrected with red blood cell and platelet transfusion.
Conclusion: Hypoplastic myelodysplasia must be considered in the differential diagnosis of patients who have bone marrow failure. The flow cytometry analysis of the bone marrow is a very useful method to establish the right diagnosis. Identification and characterization of marrow blast cells may serve as an important tool for distinguishing between aplastic anemia and hypoplastic MDS.

053

Prospective multicentric evaluation of Paroxysmal Noctural Hemoglobinuria frequency in cases with isolated thrombocytopenia

Martin Perez-Andres[1], Marta Morado[2], Enrique Colado[3], Maria Soledad Noya[4], Dolores Subirá[5], Cristina Serrano[6], Amparo Sempere[7], Beatriz Alvarez[8], Alex Freire-Sandes[9], Isusi Paloma[10], Vidriales Maria Belen[11], Jose Angel Diaz[12], Alfredo Minguela[13], Teresa Caballero[14], Maria De Las Mercedes Rey[15], Ana Perez-Corral[16], Fernandez-Jimenez Maria Cristina[17], Elena Magro[18], Angelina Lemes[19], Celina Benavente[20], Helena Bañas[21], Matheus Vescovi Gonçalves[22], Alberto Orfao[1]

[1]Cancer Research Centre (IBMCC, CSIC-USAL), University of Salamanca, SALAMANCA, Spain
[2]Serv. Hematologia. Hosp Univ. La Paz., MADRID, Spain
[3]Serv. Hematologia. Hosp Central Asturias, OVIEDO, Spain
[4]Serv Hematologia. Complejo Hosp. Univ. A Coruña., LA CORUÑA, Spain
[5]Serv. Hematologia. Hosp. Univ. Guadalajara, GUADALAJARA, Spain
[6]Serv Hematologia. Fundacion Jimenez Diaz, MADRID, Spain
[7]Serv. Hematologia. Hospital La Fé, VALENCIA, Spain
[8]Lab. Central Comunidad de Madrid., MADRID, Spain
[9]División de Hematología y Citometría de Flujo, Fleury Group,, SAO PAULO, Brazil
[10]Serv. Hematologia. Hosp Basurto., BILBAO, Spain
[11]Serv. Hematologia. Hosp Univ Salamanca, SALAMANCA, Spain
[12]Serv. Hematologia. Hosp Univ. Santiago de Compostela., SANTIAGO COMPOSTELA, Spain
[13]Serv. Inmunologia. Hosp. Virgen de la Arrixaca, MURCIA, Spain
[14]Serv Hematologia. Hosp. Univ. Virgen del Rocio, SEVILLA, Spain
[15]Lab Unificado Donosti. Hosp de Donosti., DONOSTI, Spain
[16]Hosp. Gregorio Marañon,, MADRID, Spain
[17]Serv. Hematologia. Compl. Hosp de Toledo, TOLEDO, Spain
[18]Serv. Hematologia. Hosp. Univ. Principe de Asturias, MADRID, Spain
[19]Serv. Hematologia. Hosp. Univ. Dr. Negrin, LAS PALMAS DE GRAN CANARIA, Spain
[20]Serv Hematologia. Hosp. Clínico San Carlos,, MADRID, Spain
[21]Serv Hematologia. Hosp San Pedro de Alcantar, CACERES, Spain
[22]División de Hematología y Citometría de Flujo, Fleury Group, SAO PAULO, Brazil

Introduction: Isolated thrombocytopenia is often an incidental discovery associated to a large variety of primary and secondary immunological and hematological disorders. Recently, it has been suggested that thrombocytopenia might also be an early sign of paroxysmal nocturnal hemoglobinuria (PNH), but its incidence and clinical presentation remains unknown.

Methods: Information about 36 individuals with isolated thrombocytopenia (platelet count <100 X 10^9/L) in the absence of anemia (hemoglobin level > 110 g/L), and neutropenia (neutrophils count >1,500 cells/uL) was prospectively collected for 5 years. All samples were tested for PNH following consensus flow cytometry diagnostic screening methods. A case was defined to be PNH positive (PNH^+) when glycophosphatidilinositol (GPI)-deficient cells were found in ≥2 different cell lineages (e.g. monocytes and neutrophils) at frequencies >0.01% of all leukocytes (minimum sensitivity across the participating laboratories).

Data collected included laboratory hematological parameters at diagnosis: (red blood cells-RBC, hemoglobin-Hb-, mean corpuscular value -MCV-, platelets, leukocytes-WBC), bilirubin, lactate dehydrogenase (LDH), haptoglobin and creatinine. LDH and haptoglobin values were normalized per center based on the local upper and lower normal reference values (ULN and LLN respectively) at each center.

Results: A PNH^+ result was observed in 6/36 (17%) individuals with isolated thrombocytopenia. The median percentage (and range) of GPI-def cells in PNH^+ cases was of 11% (0.4%-21%) red blood cells, 8% (0.4%-49%) neutrophils, and 8% (0.4%-43%) monocytes, 2/6 cases having classical PNH with >10% GPI-deficient cells. Although hemolysis and thrombosis was observed in 3/36 (8%) and 2/36 (6%) of cases presenting with isolated thrombocytopenia, respectively, none of the PNH^+ had previously presented with these symptoms. Besides this, haptoglobin was significantly decreased in PNH^+ *vs.* PNH^- isolated thrombocytopenias (4±4 vs. 69±63, respectively; p=0.01), all PNH^+ cases showing decreased haptoglobin levels (Hapto/LLN<0.6) *vs.* 39% of the PNH^-. None of the other parameters analyzed showed a statistically significant association with presence of GPI-deficient cells.

Conclusions: Our results suggest that PNH should be screened by flow cytometry in cases with unexplained isolated thrombocytopenia, even in the absence of other cytopenias, and/or other PNH-related symptoms, such as hemolysis and/or thrombosis, particularly when associated with decreased haptoglobin levels.

054

Comparative effects of several drugs in myeloproliferative neoplasms cell lines based on the status of the RUNX1/CBF-BETA/P300/HIPK2 complex

José-Enrique O'Connor[1], Carlos Lozano-Asensio[2], Raimundo Cervera-Vidal[2], Guadalupe Herrera[2], Eva Villamón[3], Juan-Carlos Hernández-Boluda[3], Joan Climent[2]
[1]The University of Valencia, VALENCIA, Spain
[2]Incliva Foundation, VALENCIA, Spain
[3]Clinical University Hospital, VALENCIA, Spain

Introduction: Myeloproliferative neoplasms (MPNs) are clonal hematological malignancies with an inherent tendency to progress to acute leukemia, after a variable period of time. Although the mechanisms involved in the disease transformation are still unclear, it is well known that transcription factor RUNX1 (AML1) is frequently deregulated in human leukemia, and recently, it has been described that the activity of RUNX1 together with CBF-b cofactor is regulated by the proteins p300 and HIPK2. In fact, HIPK2 phosphorylates both RUNX1 and p300, activating the whole transcriptional complex. Therefore, the study of the status of this complex seems to be interesting in order to understand the mechanisms involved in the leukemic transformation. Our aim is to study the effect of different typical drugs in several MPNs cell lines with differential expression of these genes in normal conditions and compare it with a context where the complex is chemically downregulated.
Methods: For this purpose, we chemically knocked down some genes of the studied complex and then performed different viability, apoptosis and cell cycle assays, to elucidate if the status of these genes could play an important role in the response to the different biochemical drugs tested.
Results: Our results demonstrate that inhibition of the complex had a clear effect in the survival of the MPNs cells and in the way how they respond to the drugs.
Conclusions: We show a new approach to these diseases and giving us new strategies to study the molecular changes that occur during leukemic transformation.

055 (also Best Poster Abstract Presentation)

Leukemic stem cells detection in PB by flow cytometry: a simple and rapid new diagnostic tool for Chronic Myeloid Leukemia

Santina Sirianni[1], Elisabetta Abruzzese[2], Alessandra Iurlo[3], Anna Sicuranza[1], Sara Galimberti[4], Luana Schiattone[1], Antonella Gozzini[5], Patrizia Pregno[6], Giovanni Caocci[7], Marzia Defina[1], Ilaria Ferrigno[1], Veronica Candi[1], Monica Bocchia[1], Donatella Raspadori[1]
[1]Ematologia AOUS, SIENA, Italy
[2]Hematology S.Eugenio Hospital, ROMA, Italy
[3]Fondazione IRCCS Ca' Granda Ospedale Maggiore, MILANO, Italy
[4]Dept. Clinical and Experimental Medicine, PISA, Italy
[5]Hematology, University of Firenze, FIRENZE, Italy
[6]A.O. Città della salute e della Scienza di Torino, TORINO, Italy
[7]Ematologia Ospedale Binaghi, CAGLIARI, Italy

Introduction: Diagnosis of Chronic Myeloid Leukemia (CML) implies documenting in bone marrow (BM) or in peripheral blood (PB) Philadelphia (Ph) chromosome by cytogenetics and BCR-ABL1 fusion by FISH or RT-PCR. Lately, a specific co-expression of dipeptidylpeptidaseIV (CD26) within the CD34+/CD38–/Lin– stem cell fraction appeared a robust biomarker for identifying CML LSCs in BM. We recently demonstrated that CD34+/CD38-/CD26+ LSCs can be easily identified by flow-cytometry also in PB during TKI therapy.
Methods: We here investigated accuracy and specificity of CD34+/CD38-/CD26+ assessment in PB as a new diagnostic tool in 134 pts with clinical suspicion of CML. All pts were evaluated for PB CD26+LSCs, cytogenetics, FISH and/or BCR-ABL1 RT-PCR analysis; in 62/134 pts CD26+LSCs were tested also in BM. We used a flow-cytometry 4-color staining procedure. $2.0x10^6$ leucocytes were incubated with CD45V500 (c.2D1), CD34FITC (c.581), CD38APC (c.HIT2), CD26 (c.M-A261) and negative controls (BD Pharmigen). Acquisition and analysis of at least 1.0×10^6 CD45+ cells were done by FACSCanto II with DIVA8 software (BD, Biosciences).
Results: In 104/134 pts we showed CD34+/CD38-/CD26+ LSCs in PB and in all of them CML was confirmed by cytogenetics, FISH and RT-PCR analysis. Median value of circulating PB CD26/µL was 15,49 (range 0,12-698) and a positive correlation with leukocyte count ($p < 0.01$) was found. All CD26+ PB-BM matched pairs (57/62) showed superimposable results in terms of absolute number of CD26+LSCs/µL (18,28 and 18,38 respectively) while the percentage of CD26+ cells within the CD34+/CD38- fraction appeared lower in BM than in PB samples (median 28,18 and 36,86; range 0,55-77,14 and 5,59-98,57 respectively). In 30/134 (22.3%) PB samples and in 5/62 BM samples CD26+ LSCs were not detected and no one was found Ph or BCR-ABL1 positive. Pts with CD26

neg PB/BM samples were subsequently diagnosed as Idiopathic Myelofibrosis, Myelodysplastic/Myeloproliferative disorders benign neutrophilia and Ph+ acute lymphoblastic leukemia. **Conclusions**: Flow-cytometry evaluation of PB CD34+/CD38-/CD26+ LSCs is a feasible, very rapid and highly specific alternative/complementary diagnostic tool for CML. To validate these data in a larger cohort of patients we are developing a pre-titrated lyophilized antibody mixture (lyotube, BD Biosciences) to maximize sensitivity and to optimize standardization and working time, with the further aim to monitor stem cells minimal residual disease in CML patients.

056 (also Best Poster Abstract Presentation)

CD73, an ecto-5'-nucleotidase, is a commonly overexpressed aberrant marker in Acute Leukaemias

Mahima Sanyal, Pg Subramanian, Gaurav Chatterjee, Dilshad Dhaliwal, Ganesh Kumar Viswanathan, Sitaram Ghogale, Nilesh Deshpande, Yajamanam Badrinath, Ashok Kumar, Sumeet Gujral, Prashant Tembhare
Tata Memorial Hospital, MUMBAI, India

Introduction: CD73 is a membrane bound glycosyl-phosphatidylinositol linked protein. It is an ecto-5'-nucleotidase that catalyses dephosphorylation of nucleoside monophosphates. Over-expression of CD73 has been described in many solid cancers and is a potential target molecule for immunotherapy. Data on CD73-expression in hematolymphoid neoplasms is limited to few B-cell acute lymphoblastic leukaemia (B-ALL) studies with small cohort. However, expression-pattern of CD73 in acute myeloid leukaemia (AML) and T-cell acute lymphoblastic leukaemia (T-ALL) is unknown. Hence, we evaluated the expression-pattern of CD73 in T-ALL and AML as well as a large cohort of B-ALL as an additional aberrant-marker.
Materials and methods: Expression-pattern of CD73 (PECF594; Clone-AD2) was evaluated in 458 cases of acute leukaemia using 10-color flow cytometric immunophenotyping (FCI) on Navios flow-cytometer. Data was analysed using Kaluza-V1.3-software. Mean fluorescent intensity (MFI) of CD73 was determined as geometric mean (GM) on normal myeloblasts, leukaemic-blasts, monocytes, and B/T lymphocytes. CD73-positivity was defined as >10% positive blasts.
Results: A total of 458 cases were studied which included 250 B-ALL, 65 T-ALL and 143 AML. M:F ratio was 1.7 and median age was 17.1 years (range, 1-61 years). Median (range) MFI of CD73 on monocytes, negative lymphocytes (internal negative control), positive subset of B & T lymphocytes (positive control) was 0.395 (0.04-2.18), 0.235 (0.05-1.98), 7.535 (1.4-19.65) and 6.295 (1.97-16.16) respectively. CD73 was positive in 81.6% of BALL, 30.3% T-ALL, and 33.3% AML samples. Median percentage and range of CD73-positive blasts in B-ALL, T-ALL and AML were 39.9% (0.06%-99.9%), 2.3% (0.07-63.6%) and 3.7% (0.01-99.7%). Median (Range) of MFI of CD73-expression in positive (>10%-blasts) blasts of B-ALL, T-ALL and AML were respectively 6.36 (1.4-54.6), 2.5 (0.09-41) and 3.1 (0.15-23.7). Median (range) MFI of CD73-expression in normal myeloblasts [from B-ALL minimal residual disease (MRD) samples] and hematogones were 0.59 (0.52-0.65) and 0.3 (0.12-0.45) respectively. CD73-MFI was significantly higher in AML-blasts (P<0.001) and B-blasts (<0.001). Among T-ALLs, CD73 expression had strong association with early-precursor-T-ALL, ETPALL (CD73 positivity in 10/14 cases of ETPALL compared to 10/51 cases of non-ETPALL, P=0.005)
Conclusion: CD73 is most commonly expressed in B-ALL followed by AML and T-ALL. Among T–ALLs, CD73 expression is significantly associated with ETPALL. CD73 is not expressed in normal myeloblasts and B-cell-precursors. Hence, it is a valuable marker in the diagnosis and MRD monitoring in acute leukaemia. This data also shows that anti-CD73 immunotherapy has potential scope in the treatment of acute leukaemia, especially B-ALL and ETPALL.

057 (also Best Poster Abstract Presentation)

Identification of Minimal Disseminated Disease in T-Cell Acute Lymphoblastic Lymphoma by Flowcytometric Immunophenotyping

Ganesh Kumar Viswanathan, Prashant Tembhare, Nikhil Patkar, Sumeet Gujral, Gaurav Chatterjee, Dilshad Dhaliwal, Y Badrinath, Sitaram Ghogale, Nilesh Deshpane, Mahima Sanyal, Manisha Suthar, Shripad Banavalli, Gaurav Narula, Manju Sengar, Bhausaheb Bagal, Navin Khattry, Subramanian Papagudi Ganesan
Tata Memorial Centre, MUMBAI, India

Introduction: T-lymphoblastic lymphoma (T-LBL) with minimal disseminated disease (MDD) is defined as extra-medullary T-LBL with <25% morphologically identifiable blasts in the peripheral blood (PB) and/or bone marrow (BM) but with the presence of abnormal T-lymphoblasts in BM detected by flowcytometric immunophenotyping (FCM-IPT). Published literature of this rare subgroup is sparse.

Aim: This study aims at identifying MDD in cases of T-LBL with <25% blasts in PB and BM using 8-10 colour FCM-IPT and study the clinical and immunophenotypic features.
Methods: A retrospective analysis of 3 year data in 40 children of T-LBL (diagnosed on mediastinal and/or lymph node biopsy) with predominantly lymphomatous presentation and <25% blasts in PB and BM was done. Clinical and laboratory parameters were analysed with FCM-IPT data. FCM-IPT of BM aspirate was performed on a 3-laser-10-color flowcytometer and analysed using Kaluza® software. A minimum of 1,00,000 events were acquired and MDD was quantified.
Results: Mean age was 10.2 years (range:2-18 years). M:F ratio was 2.1:1. Mean (range) of hemoglobin, WBC count and platelet count were 12.7g/dl (9.4-16), 11.7 x 10^9/L (5.2-30.3) and 411 x 10^9/L (142-875) respectively. None showed morphologically unequivocally identifiable blasts in PB. LDH was raised in the majority [mean 612U/L, N< 190U/L; range (166-1450)]. CSF examination was negative in all cases indicating that it is unlikely to have CNS involvement in patients of T-LBL with <25% blasts in PB and BM.
MDD was seen in 14 cases (35%) and ranged from 0.007% to 18.5% (mean:1.4%; median:1.2%). Mean (range) morphologically identifiable bone marrow blast/hematogone count in the group without MDD was 2.7% (1-4%) and in the group with MDD was 3.7% (0-15%). Seven cases of T-LBL with MDD showed <5% blasts in BM indicating sensitivity and necessity of FCM-IPT. PET-CT did not show increased FDG uptake in BM in any case with MDD.
Conclusion: MDD is present in one-third of cases (35%) of T-LBL with <25% blasts in PB and BM. This underlines the importance of FCM-IPT in cases with <25% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as (1) limited published data are available, (2) these show inferior event free survival in T-LBL with MDD as compared to patients without MDD and (3) there is a need for post-induction BM examination for residual disease evaluation in MDD positive cases and intensification of therapy if positive.

058 (also Best Poster Abstract Presentation)

Multicenter study of the antibody VS38c, at diagnosis, MRD and patients undergoing Daratumumab treatment for Multiple Myeloma (MM)

Ricardo Morilla[1], Timothy Farren[2], Daniel Payne[3], Robert Podovei[1], David Bloxham[4], Ulrika Johansson[5], Alan Dunlop[6], Ruth de Tute[7], Alison Morilla[1]
[1]Royal Marsden Hospital, SUTTON SURREY, United Kingdom
[2]The Royal London Hospital, LONDON, United Kingdom
[3]Leicester Royal Infirmary UHL NHS Trust, LEICESTER, United Kingdom
[4]Addenbrooke's Hospital, CAMBRIDGE, United Kingdom
[5]University Hospitals Bristol, BRISTOL, United Kingdom
[6]King's College Hospital, LONDON, United Kingdom
[7]St James Hospital, LEEDS, United Kingdom

Introduction: The VS38c antibody recognizes the 63-kDa reversibly palmitoylated transmembrane protein p63 present in all plasma cells (PC). The purpose of this study was:
To assess whether the VS38c expression in PC was comparable with that seen with the membrane CD38 antibody.
To determine if VS38c could identify PC in patients on the novel Daratumumab (anti-CD38) therapy.
Methods: Seven participating UK laboratories tested VS38c-FITC in patients with multiple myeloma (MM) at diagnosis, and following therapy for MRD, including those treated with Daratumumab. All laboratories applied their own multicolour (MFC) panels, staining and analysis protocols. All panels contained the membrane antibodies: CD45/CD19/CD56/CD38/CD138/CD117 and intracytoplasmic Kappa/Lambda in one tube and another with identical membrane antibodies and intracytoplasmic VS38c FITC. The general method was to stain for membrane antibodies followed by fixation, permeabilization and finally incubation with VS38c. Acquisition was performed on both Navios (Beckman Coulter) and BD FACSCanto II (Becton Dickinson) platforms. Routine analysis of PC was performed according to local protocols with their respective data analysis software.
Results: A total of 74 BM samples from MM patients were tested, comprising of 35 diagnostic, 32 MRD and 7 MRD treated with Daratumumab. Two normal BM were included as controls. When comparing CD38 and VS38c expression on the diagnostic samples, there was a strong correlation between the two antibodies (R^2=0.9752, p<0.01), where the total PCs ranged between 0.5% and 33% of all TNCs. For MRD cases (excluding Daratumumab), again a strong correlation was observed between the two antibodies (R^2=0.9819, p<0.01), where PCs ranged between 0.01 to 2% of all TNCs. When assessing patients on Daratumumab, there was no correlation between CD38 expression or VS38c, as CD38 appeared downregulated or lost (R^2=0.1789, p=NS). 71% of Daratumumab cases were membrane CD38 negative, however the VS38c antibody detected residual PC in all cases. Despite the small number of cases, there was a significant difference observed between CD38 and VS38c (p=0.03). The expression of CD38 versus VS38c, were similar to the total percentage of PC in the other two treated patients.

Conclusions: The percentages of PC expressing CD38 and VS38c were comparable in MM patients at diagnosis and MRD following standard treatment. However, Daratumumab treated patients demonstrated a loss of membrane CD38, but retained the VS38c transmembrane expression. In summary, VS38c could be preferentially used in routine MFC MM panels both for diagnostic samples, and those receiving standard myeloma therapy or novel anti-CD38 therapeutic agents.

059 (also Best Poster Abstract Presentation)

CD44 is highly expressed in adult B-cell precursor Acute Lymphoblastic Leukemia and is a useful minimal residual disease monitoring marker

Pearl Rodrigues, Rohitkumar Kori, Pg Subramanian, Gaurav Chatterjee, Dilshad Dhaliwal, Sitaram Ghogale, Nilesh Deshpande, Yajamanam Badrinath, Ashok Kumar, Dhanlaxmi Shetty, Ganesh Kumar Viswanathan, Sumeet Gujral, Prashant Tembhare
Tata Memorial Hospital, MUMBAI, India

Introduction: CD44, a surface glycoprotein, is a cancer stem cell (CSC) marker that regulates their self-renewal, tumor initiation, metastasis, and chemoradioresistance. It plays vital role in pathogenesis of acute myeloid leukemia and chronic lymphocytic leukemia. Anti-CD44-monoclonal antibody (RG7356) holds great promise in the treatment of these neoplasms. Recently, we published its expression-pattern and role in MRD detection in childhood B-cell precursor acute lymphoblastic leukemia (BCPALL) but till date there is no data in adult-BCPALL. Hence, we studied the expression pattern of CD44 and its role in the MRD detection in the adult-BCPALL.
Methods: We studied the CD44 (FITC, clone-G44.26, BD) expression in leukemic-blasts of 170 adult BCPALL samples using 10-color flow-cytometric immunophenotyping on Navios flow-cytometer. Data-analysis was performed using Kaluza-V1.3-software. CD44-expression was considered as positive with ≥20% CD44-positivity. Mean fluorescent intensity (MFI) and coefficient-of-variation of immunofluorescence (CV-IF) of CD44 was measured on logical scale of bivariate dot-plot. Cytogenetic studies were performed using FISH and conventional methods.
Results: Median age of 170 BCPALL patients studied was 26 years (range 15 – 66 years; M:F – 2.3). CD44 was positive in 86% adult BCPALL. Mean & median (range) of MFI and percentages of CD44 on blasts at diagnosis were 18.89, 17.1, (1.42-69.4) and 81.9, 97.8. (0.02-99.99) respectively. Mean & median (range) of the MFI of CD44 in early-B-cell precursors (CD34+) and late-B-cell precursors (CD34-) were 0.32, 0.29 (0.25-0.39) and 0.31, 0.33 (0.22-0.52). Abnormal over-expression of CD44 was statistically significant with p-value <0.001 using Mann Whitney-U-test. Mean, median & SD of CV of immunofluorescence of CD44 in leukemic-blasts were 79, 74 & 29 demonstrating its relatively homogenous-expression. Cytogenetics was available in 160/170 (94.1%) samples and cytogenetic abnormalities were seen in 85/160 (53%) of cases (BCR-ABL1, 29.37; TCF3/PBX1, 6.25%; MLL-gene rearrangement, 3.75%, hyperdiploidy 7.5% and hypodiploidy 0.6%). CD44 was not associated with any underlying cytogenetics. Of 170 cases, post-induction (day-29) MRD was available in 74 cases and positive in 42/74 (56.8%) cases. In MRD-positive samples, CD44 was over-expressed in in 38/42 (90.4%) samples. Mean, median & SD of MFI and percentages of CD44 in MRD+ blasts were 23, 20.24, 15.7 and 72.4, 86.72, 36 respectively.
Conclusion: CD44 is highly expressed in adult-BCPALL and is a very useful MRD monitoring marker in adults. Hence, anti-CD44-monoclonal antibody(RG7356) therapy may have a potential scope in the treatment of adult-BCPALL.

060

Colorectal cancer CTCs detection using two FCM approaches with EpCAM/EGFR and CKs

Aris Spathis, Antonia Mourtzikou, Vassiliki Mpakou, Anna Koumarianou, Georgios Athanasas, Petros Karakitsos
National and Kapodistrian University of Athens, ATHENS, Greece

Introduction: Identification of circulating tumor cells (CTC) has been performed using molecular techniques and more advanced automated techniques as CellSearch and Amnis. Our aim was to test the possibility of identifying CTCs using a lower cost and less complex flow cytometry based method.
Methods: Peripheral blood from 31 patients undergoing surgical resection of colorectal carcinomas were used to enumerate cells with a phenotype of CTCs. From 20 patients, a second sample 24h after surgery was obtained, while blood from 9 volunteers was used as a control group. The HT29 cell line, originating from a colorectal adenocarcinoma, was used for spiking experiments, and to identify staining pattern of EpCAM and EGFR for membrane staining, and cytokeratins for intracellular staining. PBMCs were isolated via a ficoll density gradient, counted, and after an appropriate volume of FcBlocker was added to reduce non-specific binding, antibodies targeting EpCAM-Alexa488, EGFR-PE, and CD45-PECy5 were used to stain cells of interest. After incubation 1/10 of the sample was incubated with 7-AAD and a lysis-fixation buffer. The rest of the sample was used for intracellular

staining after fixation-permeabilization. Samples were analyzed on a Partec Cyflow ML with volumetric count. Statistical analysis was performed using SPSS 24.
Results: Combination of CK8/18/19 was able to stain more than 95% of HT29 cells and was selected for intracellular staining. HT29 cells were identifiable when spiking >100 cells/7.5mL for the direct protocol, while the intracellular could identify >20 cells/7.5mL. Using the direct protocol, we identified 1.55 positive cells with a Standard Error of Mean (SEM) of ±0.64 in volunteers and 2.51 ±0.90 in patients (3.32 ±1.45 prior and 1.25 ±0.41 after surgery). For the intracellular protocol the respective values obtained were 1.11 ±0.45 vs. 5.78 ±1.70 (4.35 ±1.26 prior and 8.00 ±3.88 after). We used Mann-Whitney test to evaluate statistical significance for normal vs. patients, presence of nodal metastasis and Kruskal-Wallis for staging with pooled data of prior and after surgery samples. The intracellular protocol only, presented a trend for increased mean in normal vs. patients (p=0.08), presence on nodal metastasis (p=0.048) and higher stage (p=0.042).
Conclusions: Even though, we used a simple flow cytometric method, addition of more specific for colorectal carcinoma cells, as CKs expressed in glandular epithelia and EGFR, allowed the enumeration of rare cells as are CTCs. Long term follow-up of patients will allow the evaluation of clinical importance of these preliminary results.

061

A novel 4-colour panel for the detection of leukocytic populations in bronchoalveolar lavages and correlation with morphological counting

Aris Spathis, Evaggelia Aga, Antonia Mourtzikou, Andriana Papaioannou, Dionusios Aninos, Spuridon Papiris, Petros Karakitsos
National and Kapodistrian University of Athens, ATHENS, Greece

Introduction: The quantification of major leukocytic populations in bronchoalveolar lavage (BAL) has some material specific challenges, including increased auto-fluorescence of alveolar macrophages, presence of bronchial epithelia and degradation of cells due to handling. We used a simple 4-color protocol with or without debris/dead cells exclusion to identify main leukocytic populations in BAL.
Methods: 144 BALs received from the 2nd Respiratory Medicine Department of "ATTIKON" hospital and hospitals across Athens, were collected and analyzed on a Partec Cyflow ML using a combination of CD14-FITC/CD193-PE/CD16PECy5/CD45-APC to identify macrophages (MΦ), neutrophils, eosinophils, basophils/mast cells and lymphocytes. 12 cases were also analyzed using DAPI for exclusion of dead and degraded cells and an alternate, previously published combination (CD15/HLADR/CD16/CD45). A Haemacolor stained Cytospin preparation was used for morphology counting of cells. At least 150 cells from different fields were counted per case. Statistical analysis was performed using SPSS 24 with non-parametric testing.
Results: Cells were categorized as MΦ (SSC med-high, CD14+/CD193+/CD16+/CD45+), neutrophils (SSC high, -/-/+/+), eosinophils (SSC high, -/+/-/+), basophils/mast cells (SSC low, -/+/-/+) and lymphocytes (SSC low, -/-/-/+). NK and NK-like T lymphocytes were identified as CD16+ with low SSC, while peripheral blood monocytes as (SSC med, +/-/+ -/+) with activated ones expressing CD16. The results from the two methods correlated highly (Correlation Coefficient >0.8, $p<0.001$); cytometry slightly underestimed percentage of MΦ and slightly overestimated the percentage of lymphocytes and eosinophils compared to morphology. Linear regression displayed high R square (>0.8). Exclusion of dead cells reduced counted neutrophils and increased lymphocytes ($p<0.01$, Wilcoxon). Finally, comparison of the two different tubes for leukocytic counting revealed that the CD14/CD193 tube identified more MΦs in a statistical significant manner (p=0.019, Wilcoxon).
Conclusions: Using a simple 4-color one tube strategy we were able to identify all major leukocytic populations of interest in BAL with comparable results with morphology counting. The proposed combination had similar results with a previously used combination, with enumeration of MΦ correlating better to morphology. Exclusion of dead cells with a DNA dye allowed for easier discrimination of populations, but altered obtained results for neutrophils and lymphocytes resulting in greater discordance to morphological counting.

062

Phenotypic detection of endothelial progenitor and circulating endothelial cells and their proliferation capacity with exercise in chronic heart failure patients

Georgios Mitsiou[1], Aikaterinh Psarra[2], Savvas Tokmakidis[3], Eleftherios Karatzanos[4], Ilias Smilios[3], Stavros Dimopoulos[4], Eirini Grigoriou[2], A Tsirogianni[2], Serafeim Nanas[4]
[1]Evangelismos General Hospital,School of Medicine/Democritus University of Thrace, ATHENS, Greece
[2]Immunology and Histocompatibility Dept, Evangelismos Hospital, ATHENS, Greece
[3]Democritus University of Thrace, Dept. of Physical Education and Sport Scienc, KOMOTINI, Greece
[4]1st Critical Care Department, Evangelismos General Hospital, School of Medici, ATHENS, Greece

Introduction: The lack of specific markers for detection of two angiogenic cell types: endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs), as well as their small percentage in peripheral blood, make their characterization difficult and ambiguous. Exercise has been shown to be an effective stimulation in provoking EPCs and CECs proliferation.
Aim: The effectiveness of interval and continuous training protocols in promoting EPCs and CECs proliferation.
Methods: Blood samples were drawn from 17 chronic heart failure patients who participated in interval and continuous exercise training protocols before, immediately and 40min after training. The determination was performed with the BDFACS CantoII flow cytometer (Becton-Dickinson) and the FACS Diva analysis software. Boolean analysis was used to determine cell populations by applying a combination of specific surface markers. (with and without KDR) $CD34^+$ / $CD133^+$ / $CD45^-$ and $CD34^+$ / $CD133^+$ / $CD45^-$ / $VEGFR_2^+$ for EPCs, $CD3^+$ / $CD133^-$ / $CD45^-$ and $CD34^+$ / $CD133^-$ / $CD45^-$ / $VEGFR_2^+$ for CECs. Each cell population was detected in a total of 1,000,000 white mononuclear cells. The range of EPCs and CECs was estimated at 0.01 to 0.2% and 0.1 to 6.0% respectively.
Results: Overall, results indicated that $CD34^+$ / $CD133^+$ / $CD45^-$, $CD34^+$ / $CD133^-$ / $CD45^-$ increased after training (from 31.1±47.5 to 51.5±61.7 cells/10^6 enucleated cells, $p<0.01$ and from 69.7±53.8 to 95.1±69.9 cells/10^6 enucleated cells, $p<0.01$, respectively) and remained enhanced 40min after training (from 31.1±47.5 to 56.1±68.6 cells/10^6 enucleated cells, $p<0.05$ and from 69.7±53.8 to 101.1±76.4 cells/10^6 enucleated cells, $p<0.01$, respectively). $CD34^+$ / $CD133^+$ / $CD45^-$ / $VEGFR_2$ increased after training (from 10.5±11.8 to 16.5±16.3 cells/10^6 enucleated cells, $p<0.05$) but didn't show statistical significance 40min after training (from 10.5±11.8 to 18.7±21 cells/10^6 enucleated cells, $p=0.16$). In regards to CD34 + / $CD133^-$ / $CD45^-$ / $VEGFR_2^+$ they didn't show statistical difference either after training or 40min after training (from 9.7±16.1 to 10.6±8.9 cells/10^6 enucleated cells, $p=0.98$ and from 9.7±16.1 to 13.6±14.2 cells/10^6 enucleated cells, $p=0.98$, respectively).
Conclusions: Flow cytometry is a reliable and effective method not only for the phenotypic analysis of EPCs and CECs but also for the distinction of these two cell populations, though their clinical significance remains under evaluation and the impact of the appropriate exercise training protocol requires further research.

063

Immune Monitoring of Patients Receiving Rituximab Therapy

Michelle Delelys, Rachel Nicholson, Alexandra Shay, John Niles, Frederic Preffer
Massachusetts General Hospital, BOSTON, U.S.A.

Introduction: The therapeutic antibody rituximab (Rituxan^R), is used to treat patients with a broad range of conditions including autoimmune diseases (e.g. rheumatoid arthritis and Antineutrophil Cytoplasmic Autoantibodies (ANCA) vasculitis) and B-cell malignancies (e.g. chronic lymphocytic leukemia and non-Hodgkin's lymphoma). Rituximab works by targeting and rapidly depleting circulating CD20 expressing B-cells. The immune monitoring of patients that receive rituximab is useful in assessing the therapeutic efficacy and dosing of the drug. This study evaluated the performance of a flow cytometric based Lab Developed Test (LDT) compared to a commercially available reagent (Becton Dickinson (BD) Multitest^{TM} 6-color TBNK plus CD20) for measuring T and B cells in peripheral blood.
Methods: One hundred and four (104) serial, blood samples submitted for rituximab monitoring at Massachusetts General Hospital (Boston MA) between March and May 2017 were tested using the LDT (CD3, CD4, CD5, CD8, CD19, CD20 and CD45) and a BD FACSCanto^{TM} 10-color flow cytometer. Remnant patient samples were subsequently tested using the TBNK (CD3, CD4, CD8, CD19, CD16, CD56, CD45+) plus CD20 as the test method (TBNK+CD20) on a BD Canto^{TM} II flow cytometer. Linear regression was performed for lymphocyte subset absolute counts and percentages. Range (minimum and maximum), the coefficient of determination (R^2) and intercepts, slopes and their corresponding 95% confidence intervals were calculated.
Results: The results demonstrated that the LDT method was equivalent to the commercial test method (TBNK+CD20). The regression slopes ranged from 0.9 to 1.05 with 95% confidence intervals from 0.8 to 1.2 when the LDT and test method TBNK+CD20 reagent were compared. The R^2 values were above 0.88 for the test method

(TBNK+CD20) compared to the LDT. In the TBNK+CD20 results, the slopes were 1.02 (0.99 to 1.05) and 1.03 (0.99 to 1.06) for CD19 absolute counts and CD19+CD20 absolute counts, respectively. The slopes with 95% confidence were within a 10% bias interval of 0.9 to 1.1 and the R^2 values were all greater than 0.88.
Conclusion: The results of this study demonstrate good performance for the BD Multitest 6-color TBNK+CD20 reagent compared to the LDT. In our opinion, either assay may be confidently used to monitor patients receiving rituximab therapy.

064

Selected Lactobacillus strains change H. pylori induced T cell profiles via dendritic cells modulation
Anna Helmin-Basa, Malgorzata Wiese-Szadkowska, Lidia Gackowska, Izabela Kubiszewska, Anna Labejszo, Jacek Michalkiewicz
Nicolaus Copernicus University Ludwik Rydygier Collegium Medicum in Bydgoszcz, BYDGOSZCZ, Poland

Introduction: According to new studies the composition of intestinal microflora may determine the type of *H. pylori* infection (symptomatic *vs* asymptomatic), we examined an array of intestinal bacteria to determine if they (and if so, which of them) can modulate the profile of *H. pylori*-induced response. The aims of study were: 1) to determine the maturity level of DCs (phenotype, profile of cytokines) after being exposed to chosen LAB strains, *H. pylori*, LAB with *H. pylori*; 2) to analyze the response profile of naive T cells after exposure to bacteria-stimulated DCs with or without the addition of supernatant from the DC culture.
Methods: Thirty potentially probiotic strains were identified based on the sequences of genomic markers (16S rRNA). In the antagonistic studies of the *Lactobacillus* strains (relative to *H. pylori* Cag A +strain 95), a method based on simultaneous growth of the indicator strain (*H. pylori*) and LAB strain. For further studies the most antagonistic strain and non-antagonistic strain were selected (accordingly *L. rhamnosus* 900 and *L. paracasei* 915). The immunological research was conducted on the basis of the following technique and methods: 1) culture of monocyte derived dendritic cells (moDCs) with LAB, LAB+*H. pylori* or *H. pylori,* co-culture of stimulated moDCs with naive T lymphocytes); 2) flow cytometry techniques; 3) immunoenzymatic tests.
Results: *H. pylori*-stimulated DCs showed some immature phenotype ($CD80^{low}/CD83^{low}$) and tolerogenic potential. Interestingly, after stimulation with the mixtures of selected LAB strains (antagonistic and non-antagonistic one) and *H. pylori*, DCs with mature-like phenotype (mDCs, $CD80^{high}/CD83^{high}$) exerted diversified effects on the type of T cell response. Noticeably, the antagonistic strain *L. rhamnosus* 900, both alone and in mixture with *H. pylori*, inhibited induction of *H. pylori*-specific Tregs. Such reactivity profile was indirectly determined by cytokine microenvironment (IFN-γ, IL-23, and IL-12p70) which are very important in the type of *H. pylori* infection.
Conclusions: On the basis of the obtained results the antagonistic LAB strain has some proinflammatory and antibactericidal properties that limit chronic *H. pylori* infection. As we postulate the individual composition of the intestinal microflora determines the type of the inflammation induced by *H. pylori*, probably through the control of the cells reactivity in the GALT (especially by DCs that have influence on type of T cells responses). This project demonstrates the potential application of specially selected probiotic strain in combination therapy of *H. pylori* infection.

065

Lymphoid sub-populations in the bone marrow of adult and pediatric ITP patients'
Nagwa Hassanein, Bargavi Balakrishnan, Abdullah al Khorasi, Faisal al Anzi
Prince faisal cancer center, BURYDAHA, Saudi Arabia

Introduction: ITP is one of the commonest benign immune-hematological disorders which affect pediatric and adult groups. Its immune-pathogenesis is yet to be understood, detailed study of lymphoid subpopulation in the bone marrow might help uncover the mystery and boast the management.
Methods: Immunophenotyping done for Heparinized bone marrow aspirate samples extracted from 30 pediatric and 27 adult patients at prince Faisal cancer center. Using the following screening panel: CD45,CD33,CD71,CD19,CD10,CD20,CD3,CD4,CD8,CD16+56,CD34,CD117,CD15,CD38, and Kappa/Lambda. Statistical analysis performed using Excel, Ethical committee approved the study.
Results: The lymphoid cells were sub-typed as mature B –cells, hematogones, T cells (CD4 (T- helper cells), CD8 (T-cytotoxic cells), NK cells and plasma cells. Mature B- cells range was (0.9-19 %) in pediatric group while was (1-6.2%) in adult group. Hematogones was detected with higher frequency and quantity in pediatric than adult group, the range was (0.7-17%) in children while was (0.4-4.3%) in adults, It was detected in 100 % of the pediatric group in comparison to 77.8% in adults. However there was no significant correlation between age and hematogones percentage for both groups with r value measured -0.06 and -0.2 respectively. Also there was no correlation

between the value of mature B cells and hematogones in both pediatric and adult groups with r equal 0.2 and -0.1 respectively. CD3 found to be higher significantly in adults than pediatric group with p value 0.01, the range was (4.5-32 %) in adults, while (3.5-14.4%) in pediatric group. CD4/CD8 ratio was invariably disturbed in pediatric group with no single case has CD4 more than CD8, only 2/30(6.6%) cases has the same value for both of them. On the other hand, adults CD4/CD8 disturbed ratio as detected in 79% of cases while normal ratio observed in 21% of them. NK cells range in pediatric group (0.1-2.5%), while in adults, range was (0.2-5.6%). Plasma cells were detected in both groups with range in pediatric (0.1-0.3) while in adults the range was (0.1-0.8).
Conclusion: we conclude that immune response in ITP patient is activated with features as reversed CD4/CD8 ratio and increased hematogones, however more correlation with the platelet count and response for treatment is needed to judge the significance and implication of these finding.

066

Flow cytometry is a useful tool for identification of Neuroblastoma infiltrating the bone marrow
Nagwa Hassanein, Ali mr al Saif, Abdullah al Khorasi, Faisal al Anzi
Prince faisal cancer center, BURYDAHA, Saudi Arabia

Introduction: flow cytometery is an emerging tool for diagnosis of non-hematological malignancy which infiltrates bone marrow. Disseminated Neuroblastoma presenting with pancytopenia are morphologically indistinguishable from lymphoblast, this necessitate differentiating markers by flow cytometry.
Method: Staging bone marrow aspirate from Neuroblastoma patients extracted at prince Faisal cancer center, has undergone screening panel for phenotypic identification of abnormal cells, over a period of 2 years from May 2015 through May 2017, this panel include : CD45,CD3,CD4,CD8,CD19,CD10,CD20,K/L, CD34,CD117,CD15,CD33,CD38,CD71 and CD56. Acquasition done on FACS CANTOII, FACS Diva were used for analysis. This study was ethically approved.
Results: out of five staging cases, 3 cases uncover disseminated Neuroblastoma in the bone marrow which were successfully Diagnosed by flow-cytometry using the previous combination of antibody.The most important markers were CD45 and CD56, The abnormal cells were positive for CD56 while negative for CD45 and other markers in the panel, one case was also positive for CD117. These finding was correlated with immunohistochemistry markers as CD56, Synptophysin and Chromogranin applied on B.M and the original tumor biopsy respectively.
Conclusion: Flow cytometery is a helpful tool in identifying Neuroblastoma cells using a small panel. The promising markers are CD56 in association with CD45 and CD117. In addition, this panel could differentiate the cases presented with only cytopenia without knowing the original diagnosis from precursor – ALL and others.

067

Increased expression of neutrophil and monocyte CD64 in patients with active tuberculosis
Arianna Gatti[1], Bruno prof. Brando[1], Carlo dr Magnani[2]
[1]Hematology Laboratory and Transfusion Center, LEGNANO (MILANO), Italy
[2]INFECTIOUS DISEASE UNIT, Legnano General Hospital, LEGNANO (MILANO), Italy

Background: Polymorphonuclear neutrophils (PMN) and Monocytes (MO) display bactericidal responses and produce inflammatory proteins in infection caused by Mycobacterium Tuberculosis (MB). This phenomenon is particularly enhanced in respiratory MB infection. We hypothesized that the high-grade inflammatory reactions occurring in overt MB could promote an increased expression of peripheral blood PMN and MO surface CD64 (high affinity Fc receptor I), like it occurs during sepsis.
Methods: In the current study flow cytometry was used to study prospectively the quantitative expression of surface CD64 by whole blood PMN and MO from 35 inpatients with documented culture, PCR and Quantiferon positive tests, untreated Active Tuberculosis (ATB). The control groups were made by 20 intensive care unit patients with documented non tuberculosis pneumonia (NTP) and by 20 non-infected intensive care unit patients.
Results: PMN CD64 expression was significantly higher in patients with ATB (median 4577 ABC - Antibody Binding Capacity) than in non infected subjects (median 404.5 ABC; $p < 0.0001$) and was also increased in patients with NTP (2720.5 ABC; $p<0.0001$). The highest intensity of PMN and MO CD64 expression was associated with ATB rather than with NTP. Looking at CD64 intensity distributions on PMN and MO, 55% of ATB patients showed values >4500 ABC on PMN and 42% >40000 ABC on MO, respectively. Non-infected subjects and NTP patients never displayed a PMN CD64 expression higher than 4000 ABC.

Conclusions: Peripheral blood PMN and MO membrane CD64 can be up-regulated also in primary, untreated ATB patients. A very high CD64 expression by PMN and MO can be useful in discriminating patients with ATB from those with other pneumonias.

068

Evaluation of Activation and Homing Markers on Regulatory T cells using 12-Color BD FACSLyric™ Flow Cytometer

Nihan Kara, Mirko Corselli, Tri Le, Margaret Inokuma, Noel Warner, Alan Stall, Suraj Saksena
BD Biosciences, SAN JOSE, U.S.A.

Introduction: Deeper understanding of regulatory T cell (Treg) biology is critical for the successful development of therapeutic and diagnostic applications. Treg heterogeneity in terms of phenotype, function, and distribution is widely documented, thereby making a more detailed characterization of these cells critical for downstream applications. Several reports have identified markers correlating with different biological functions of Treg subsets, but detailed information about the interplay between these markers is currently lacking. We developed an 8-color plus 4-color drop-in modular flow cytometry assay to identify and characterize Treg subsets in greater depth. Specifically, we developed two different Treg characterization panels to explore critical facets of Treg biology: activation and homing. Our results highlight an interesting interplay between different Treg markers, their putative biological function, and the underlying heterogeneity within the Treg population.
Methods: An 8-color backbone panel was developed using antibodies against CD3, CD4, CD25, CD127, FoxP3, CD45RA, CD15s and CD161 for the detection of Treg subsets in fresh PBMCs from healthy donors. Two supplementary 4-color drop-in panels for activation (PI16, CD147, CD39 and HLA-DR) or homing (CXCR3, CCR4, CCR6 and CD31) enabled deep phenotypic characterization of Treg subsets.
Results: The 8-color backbone panel enabled clear identification of $CD3^{+}CD4^{+}CD127^{low/neg}CD25^{+}FoxP3^{+}$ Treg cells, further categorized as $CD45RA^{+}$ (naïve) and $CD45RA^{-}$ (activated) subsets. The inclusion of CD15s and CD161 in the backbone panel additionally enabled identification of functionally suppressive effector and/or pro-inflammatory cytokine-secreting Tregs within the heterogeneous $CD45RA^{-}$population. Addition of the 4-color drop-in activation panel (PI16, CD147, CD39 and HLA-DR) allowed us for the first time to investigate the interplay between multiple activation markers in a single tube, and identify, for example, a subset of Tregs co-expressing high levels of CD15s, HLA-DR, CD147, PI16 and FoxP3. Using a similar approach, we developed a 4-color drop-in panel to study Treg homing by analyzing the expression of CCR4, CCR6, CXCR3, and CD31 in naïve and effector Tregs. This approach helped in identifying Treg subsets phenotypically similar to T helper cell subsets. In addition, recent thymic emigrants were identified based on CD31 expression within the naïve Treg population.
Conclusions: The utilization of a modular 12-color flow cytometry assay presents a new approach enabling analysis of different aspects of Treg biology, while optimizing the workflow. Simultaneous assessment of multiple markers on Tregs enables deep immunophenotyping and reveals biologically relevant subtypes within the heterogeneous population. Further studies are needed to characterize the functional attributes of these Treg subtypes.

069

Cynomolgus macaca fascicularis as a model for pre-clinical studies with a specific biomarker important in inflammatory diseases

Rita Vicente, Julie Rossignol, Isabelle Bentz, Muriel Pelegrin, Florence Ruffel-Seguier, Brigitte Molinier, Marc Henriquet, Olivier Fedeli
Sanofi, MONTPELLIER, France

Introduction: One of the most challenging aspects of drug development is to find a candidate with significant pharmacologic but minimal adverse effects. In order to assess adverse side effects of the candidate molecules, toxicology studies are performed. In the case of therapeutic antibodies, one of the criteria to select the species for toxicology studies is the cross-reactivity of the therapeutic antibody between the selected species and humans. One of the species commonly used for safety assessment, in the case of therapeutic antibodies, are non-human primates (NHP). In this study we aim to compare a target marker on the cell surface of classical immunological populations in peripheral blood of NHP and healthy humans. The final goal was to determine if NHP were a relevant model for this particular project.
Methods: Peripheral blood analyses were performed from 48 NHP and compared to 10 Human Healthy Controls (HHC). NHP were Cynomolgus macaca fascicularis aged from 23 to 29 months that received healthcare in accordance with the Directive 2010/63/EU and the internal policy on animal protection. HHC were EFS donors (Etablissement Français du Sang) aged from 29 to 59 years old. In order to identify classical immunological

populations, whole blood was stained with fluorescent antibodies, acquired with a BD FACSVerse™ flow cytometer and analyzed with BD FACSuite software.
Results: Classical immunological populations, such as CD4, CD8 and regulatory T cells, B cells, natural killer (NK), dendritic cells and monocytes, were analyzed. The percentage of these populations expressing the specific biomarker important in inflammatory diseases was compared between NHP and HHC. Results were analyzed in absolute counts (cells per blood liter) and in percentages. Despite the differences in number of cells/L, between both species, the distribution of these classical immunological populations was similar, except for NK cells and monocytes that were significantly higher in NHP than in HHC. Concerning the target marker, most of the classical immunological populations presented similar percentage within the parent population between NHP and HHC, except for CD8 naïve T and NK cells that were higher in HHC, as well as memory and naïve B cells that were higher in NHP.
Conclusion: Globally the distribution of the classical immunological populations and their percentage of positive cells for the specific biomarker important in inflammatory diseases are equivalent between NHP and HHC. The use of NHP is an extremely relevant model for pre-clinical studies concerning this particular target marker.

070

Cytofluorimetric characterization of immune responses induced by a vaccine targeting the cancer stem cell antigen xCT in breast cancer models
Laura Conti[1], Stefania Lanzardo[1], Roberto Ruiu[1], Elisabetta Bolli[1], Gaetano Donofrio[2], John P O'Rourke[3], Federica Pericle[3], Federica Cavallo[1]
[1]UNIVERSITY OF TORINO, TORINO, Italy
[2]UNIVERSITY OF PARMA, PARMA, Italy
[3]Agilvax, Inc., ALBUQUERQUE, U.S.A.

Introduction: The several unsuccessful treatments in metastatic cancers might miss cancer stem cells (CSC), which play a critical role in cancer. The identification of oncoantigens (OA) expressed by CSC may provide new targets for cancer therapies (1).
Methods: A transcription profiling analysis of the ErbB2$^+$ TUBO cell line cultured as epithelial cells or tumorspheres was performed. Integrating data obtained with meta-analyses of 7 human breast tumor data sets we identified xCT, a channel that supports glutathione synthesis, as a new CSC OA that was validated *in vitro* and *in vivo* (2). To set up immunotherapies targeting xCT, we used xCT plasmids or an approach based on bacteriophage MS2 virus-like particle (VLP) or Bovine herpesvirus 4 based (BoHV4) technologies. Using genetic approaches, we produced VLPs displaying different xCT extracellular domains (ECD) or BoHV4 coding for xCT full protein. The induced immune response was analysed by cytofluorimetric analysis.
Results: xCT expression increases over tumorspheres passages and its silencing significantly reduces tumorsphere generation. *In vivo* immunotargeting of xCT slows established subcutaneous tumor growth and impairs pulmonary metastasis formation in mice challenged with syngeneic tumorsphere-derived cells. This effect depends on the generation of specific antibodies that alter CSC self-renewal and redox balance and is improved when combined with cytotoxic drugs.
Conclusions: We developed new vaccines targeting a freshly identified breast CSC OA, xCT, whose inhibition strongly impairs mammary tumor development and metastases. This study provides a new tool for the design of combined therapeutic approaches that efficaciously target both breast CSC and differentiated cancer cells, leading to both cancer treatment and prevention of metastases.

071

Immunophenotypic and functional characterization of monocytes and macrophages derived from human induced pluripotent stem cells (iPSCs)
Silvia Della Bella[1], Francesca Calcaterra[1], Elena Pontarini[2], Chen Guibin[3], Yang Dan[3], Ma Yuchi[3], Paolo Tentorio[2], Claudia Carenza[1], Chiara Pandolfo[1], Joanna Mikulak[2], Manfred Boehm[3], Domenico Mavilio[1]
[1]University of Milan - Humanitas Clinical and Research Center, ROZZANO (MI), Italy
[2]Humanitas Clinical and Research Center, ROZZANO (MI), Italy
[3]National Institutes of Health - Lab of Cardiovascular Regenerative Medicine, BETHESDA (MD), U.S.A.

Background: Human induced pluripotent stem cells (iPSCs) are generated from adult somatic cells genetically reprogrammed by enforcing expression of transcription factors important to maintain the properties of embryonic stem cells. Because iPSCs are self-renewing and pluripotent cells, they can serve as an unlimited source of personalized human cells and tissues for disease modelling, drug screening and cellular therapies. In this study, we

generated iPSC-derived macrophages (iPSC-Mϕ) and compared the immunophenotype and function of these cells with macrophages obtained from peripheral blood monocytes (PB-Mϕ). iPSC-Mϕ individuals with CCR5-Δ32 homozygous deletion (CCR5-Δ32 individuals), a rare mutation conferring natural resistance to HIV-1 infection, were also included in the study.

Methods: Dermal fibroblasts from healthy human skin were reprogrammed to iPSCs, differentiated into iPSC-derived monocytes that were in turn differentiated into resting (M0), pro-inflammatory (M1) or anti-inflammatory (M2) iPSC-Mϕ. Circulating monocytes were isolated from peripheral blood obtained from healthy donors, and similarly differentiated into M0, M1 and M2 PB-Mϕ. The expression of monocyte-macrophage lineage markers and polarization markers was analyzed by multicolor flow-cytometry. The allostimulatory and phagocytic properties of these cells were assessed by flow cytometry and confocal microscopy. Cytokine production was assessed by Luminex multiplex assay. The susceptibility of iPSC-Mϕ obtained from CCR5-Δ32 and wild-type individuals to be infected by HIV-1 was analyzed by PCR.

Results: Similar to PB-Mϕs, iPSC-Mϕs were able to correctly differentiate into M0, M1 or M2 following incubation with adequate stimuli, as assessed by expression of polarization markers. Even at functional level, iPSC-Mϕs were similar to PB-Mϕs, as they were able to serve as professional phagocytes, and promote T-cell proliferation in mixed lymphocyte reactions. Finally, all iPSC-Mϕs were susceptible to HIV infection, unless derived from CCR5-Δ32 individuals.

Conclusions: In this study we demonstrated that iPCS-Mϕs are phenotypically and functionally similar to PB-Mϕs and can therefore represent an alternative and reliable tool to study the monocytic-macrophagic compartment in physiological and pathological conditions, even in the context of rare human conditions.

072

BAFF and sCD23: modulators of the circulating B-cell compartment during pregnancy in atopic women?

Catarina Martins[1], Jorge Lima[1], Maria José Leandro[2], Glória Nunes[1], Luís Miguel Borrego[1]
[1]NOVA Medical School|Faculdade de Ciências Médicas - Universidade Nova de Lisboa, LISBOA, Portugal
[2]Center for Rheumatology Research, Dep. of Medicine, University College London, LONDON, United Kingdom

Background: Maternal atopy represents a risk factor for the development of atopy. In an attempt to better understand the underlying immune background, we have recently described the modulation of immune cells from pregnancy to postpartum, in atopic and non-atopic women. To complete these observations, we aimed to assess how pregnancy affects circulating B-cells and B-cell related factors in women with atopic asthma.

Method: Four groups of women were recruited: atopic pregnant women (AP), healthy pregnant women (HP), atopic non-pregnant women (ANP) and healthy non-pregnant women (HP). All pregnant women were assessed in the third trimester of gestation (weeks 31^{st}-36^{th}). Circulating B-cells were evaluated by flow cytometry, in a 4-color BD FACSCalibur, with a panel of monoclonal antibodies including anti-CD19, CD24, CD27, CD38, IgD, and IgM. CellQuestPro™ was used for data analysis. B-cell subsets were considered according to the Bm1-5 classification. Soluble CD23 (sCD23) and B-cell Activating Factor (BAFF) were quantified by ELISA in serum samples (Quantikine,R&D). Statistical analysis was performed with GraphPadPrism, with significance defined by P-value <0.05.

Results: 134 women were recruited (24 AP, 43 HP, 32 ANP, 35 HNP). AP and HP presented lower B-cell counts than their non-pregnant counterparts. Compared to HNP, HP presented lower transitional (Bm2'; $p<0.001$) and memory subsets (Bm1 and Bm5; $p\leq0.038$) and plasmablasts (Bm3+4; $p=0.002$), with higher percentages of naïve cells (Bm2; $p<0.001$).

Similarly, compared to ANP, AP presented decreased percentages of memory subsets (Bm1 and Bm5; $p\leq0.030$) and plasmablasts (Bm3+4; $p=0.038$), and higher percentages of naïve cells (Bm2; $p=0.018$), without alterations in transitional B-cells. No differences were observed comparing AP and HP women. As for soluble mediators, BAFF presented similar values in AP and HP women, with both groups presenting higher values than ANP and HNP ($p<0.001$), respectively. sCD23 was decreased in HP compared to HNP ($p<0.001$), but AP women presented higher values compared to both ANP and HP ($p\leq0.004$).

Conclusions: In atopic and non-atopic women, pregnancy is associated with a decreased circulating B-cell compartment, richer in naïve B-cells and poorer in more differentiated subsets (memory cells and plasmablasts). Nevertheless, BAFF levels, usually positively correlated with memory subsets, were increased in AP and HP. The cause-effect relationship between BAFF levels and B-cell subsets during pregnancy remains to be elucidated. Finally, the increase in sCD23 observed in AP only may suggest a distinctive feature of this IgE receptor in atopic women, possibly with future impact in the development of atopy in their progeny.

073

Evaluation of WC1 γδ T cells in young and old water buffaloes (Bubalus bubalis)

Francesco Grandoni[1], Mahmoud M Elnaggar[2], Gaber S. Abdellrazeq[2], Lindsay M. Fry[3], Francesco Napolitano[1], Victoria Hulubei[4], Giovanna de Matteis[1], Samy A. Khaliel[5], Helmy A. Torky[5], Stefano Papa[6], Claudio Ortolani[6], William C. Davis[4]
[1]CREA-ZA, MONTEROTONDO, Italy
[2]Washington State University/Alexandria University, Washington, U.S.A.
[3]Washington State University/ARS, PULLMAN, U.S.A.
[4]Washington State University, PULLMAN, U.S.A.
[5]Alexandria University, ALEXANDRIA, Egypt
[6]Università di Urbino, URBINO, Italy

Introduction: A large set of monoclonal antibodies (mAbs) specific for leukocyte differentiation molecules in cattle were screened and shown to identify conserved epitopes on orthologous molecules in water buffalo. This has provided an opportunity to characterize the immune system of buffalo, an important species in the livestock industry. The objective of the present study was to characterize subsets of WC1 positive γδ T cells present in ruminants believed to participate in innate immunity.
Methods: Four heifers (8-9 months of age) and four adult lactating buffaloes (12-15 years of age) were selected for this study. Three different combinations of mAbs were used with flow cytometry to compare expression of CD2 and CD8 on WC1-N2, WC1-N3, and WC1-N4 positive subsets of CD45R0 positive γδ T cells.
Results: The percentage of γδ T lymphocytes was significantly higher in young animals than adult animals (44.92%±9.86 *vs* 4.21%±0.77; P≤0.0001). There was a proportionate decrease in frequency of subsets in the different age groups that expressed WC1-N3 (25.06%±1.07 *vs* 24.96%±3.00) or WC1-N4 (58.15±7.69 *vs* 54.46±10.49), despite WC1-N2 showed a moderate increase (82.63%±9.86 *vs* 86.54%±6.75). These patterns of expression were similar to those observed in cattle. All γδ T lymphocytes expressed CD45R0, similar to cattle. The unique findings were demonstration of expression of CD8 on all γδ T lymphocytes and CD2 on a subset of WC1 positive γδ T lymphocytes with a characteristic $CD2^{dim}CD45R0^{high}$ immunophenotype. CD2 and CD8 are only expressed on subsets of WC1 negative γδ T lymphocytes in cattle.
Conclusions: This is the first report demonstrating differences in the immune systems between cattle and buffalo. Further studies are needed to determine whether the differences in expression of CD2 and CD8 on subsets WC1 positive γδ T lymphocytes in buffalo and cattle are associated with differences noted in the immune response to pathogens affecting both species. The availability of the large panel of cross reactive mAbs provides opportunity to compare the role of αβ and γδ T lymphocytes in host defense in cattle and buffalo.

074

Evaluation of the immunophenotypic profile in systemic sclerosis patients at different disease stages

Elena Trombetta, Chiara Bellocchi, Gaia Montanelli, Maurizio Marchini, Barbara Vigone, Alessandro Santaniello, Alessandra Cattaneo, Laura Porretti, Lorenzo Beretta
IRCCS Policlinico di Milano, MILANO, Italy

Introduction: Systemic sclerosis (SSc) is an autoimmune disease characterized by dysregulation of the immune system, vasculopathy and fibrosis of the skin and internal organs. In this study we aimed to characterize the distribution of peripheral blood cell populations and plasma concentrations of the molecules involved in B lymphocytes maturation and activation in SSc patients, in order to better understand their involvement in the different stages of the disease.
Methods: 50 SSc patients with fibrosis (limited and diffuse cutaneous subjects) or without fibrosis (definite, non fibrotic SSc and early SSc subjects) and 35 healthy controls (HCs) were considered. 26 cell subsets within T and B lymphocytes and dendritic cells (DCs) populations were characterized by an 8-color flow cytometer; IL-6, IL-10, BAFF, sBCMA, soluble TACI and soluble CD40L plasma concentrations were determined by a Luminex assay. Comparisons were carried with paired samples t test or with one-way analysis of variance (ANOVA); correlations were performed via Pearson's r.
Results: Naïve B cells ($CD19^{+}IgD^{+}CD27^{-}$) were increased while IgM-memory B cells ($CD19^{+}$ $CD27^{+}IgD^{+}IgM^{+}$) were reduced in SSc, especially in non-fibrotic subjects. The TACI and the CD40L to IgM-memory B cells ratios were increased in SSc, suggesting an activation of memory B cells. The BAFF/B cells ratio was increased in fibrotic subjects, suggesting a pronounced activity of B cells. Accordingly, plasmablasts ($CD19^{+}CD38^{br}IgM^{+/-}$) were increased in fibrotic SSc patients where their count correlated with the activation marker BCMA. DCs and activated plasmocytoid DCs were reduced in SSc and mostly in fibrotic SSc patients; similarly IL-6 was increased in SSc and in fibrotic subjects. IL-6 also correlated with the occurrence of interstitial lung disease (ILD), as well as the CD21low

($CD19^+CD38^-CD21^-$) B cells count. No differences were found in the T cell distribution, including Treg ($CD3^+CD4^+CD25^{br}CD127^{low}$) and Th17 ($CD3^+CD8^-IL\text{-}17^+$) cell populations.
Conclusions: Fibrotic and non-fibrotic SSc patients have distinct profiles of circulating cell populations. These differences point toward a different activation state of the B cell compartment that may also influence the presence of ILD.

075

Successful implementation of a simplified CD8+ degranulation assay for the diagnosis of Familial Hemophagocytic Lymphohistiocytosis syndrome

Marianna Tzanoudaki[1], Adina Sandu[2], Sofia Tantou[1], Eliana Gkika[1], Eleni Ploumi[1], Virginia Polaki[1], Manolis Liatsis[1], Athanasios Michos[2], Maria Kanariou[1]
[1]„Aghia Sophia„ Children's Hospital, ATHENS, Greece
[2]1rst Pediatric Clinic, National Kapodistrian University of Athens, ATHENS, Greece

Introduction: Laboratory diagnosis of Familial syndromes of Hemophagocytic Lymphohistiocytosis (F-HLH) is challenging, as the required equipment is not readily available in most clinical laboratories. In this setting, simpler and more affordable degranulation assays could serve as valuable screening tests. This is a report of the successful implementation of a simplified CD8+ degranulation assay for the diagnosis of F-HLH in a child with atypical symptoms.
Methods: The test was applied on a 4 year old girl with albinism and a family history of lethal HLH, presenting with progressive CNS deterioration and no other clinical or laboratory signs of HLH. Degranulation efficiency was assessed by Flow Cytometry, using CD107a/b-FITC either (1) on PMA/ionomycin stimulated resting CD8+ or CD56+ lymphocytes or (2) on PHA/IL-2 blasts, on the 3rd day of culture, after stimulation with PMA/ionomycin or anti-CD3 (OKT3). No CD28 beads or longer stimulation procedures were used.
Results: There was a significant difference between the patient and the normal control regarding the percentage of degranulated CD8+ cells, using either protocol. PMA/ionomycin stimulation of PHA/IL-2 blasts seemed to better discriminate patient from normal (5.3% vs 80.3%, respectively). Definite diagnosis of type II Griscelli syndrome was made based on hair microscopy and genetics in Hôpital Necker Enfants Malades, Paris. CNS involvement was postulated to be a result of intracranial HLH
Conclusions: The above simplified degranulation test is not meant to substitute the existing protocols used in international F-HLH reference centers, which should always be consulted in suspicious cases. Due to the rarity of the syndrome, we cannot comment on the sensitivity or specificity of the test. However, it is an affordable and easily applicable screening method, which was shown to have the ability to detect an atypical F-HLH patient.

076

Antigen specific memory B cell fluctuations induced by the pneumococcal conjugate and plain polysaccharide vaccine in HIV-infected patients

Paraskevi Farmaki[1], Maria Chini[2], Nikos Mangafas[2], Marianna Tzanoudaki[3], Marios Lazanas[2], Vasiliki Spoulou[1]
[1]„Aghia Sophia„ Children's Hospital, ATHENS, Greece
[2]Infectious Dis. Unit, 3rd Internal Medicine Dept, Korgialeneio-Benakeio Hospital, ATHENS, Greece
[3]Dept. of Immunology & Histocompatibility, „Aghia Sophia„ Children's Hospital, ATHENS, Greece

Introduction: Memory B cell (MBC) subsets have distinct roles in the establishment of vaccine induced immunological memory, which is related to vaccine effectiveness: IgM+ MBCs replenish the MBC pool, whereas switched IgG+ (sIgG) MBCs can differentiate into plasma-cells upon antigen reencounter. As, guidelines regarding the optimal pneumococcal vaccination schedule in HIV-infected individuals are conflicting, we aimed to assess the immunogenicity of pneumococcal conjugate vaccine (PCV13) and the plain polysaccharide vaccine (PPV23) in HIV-infected individuals, by investigating the pneumococcal serotype (PS) specific MBC fluctuations post vaccination.
Methods: 40 HIV (+) adults (27-57 years old), on ART, with undetectable viral loads and CD4+ T-cell count between 200 and 894cells/μl, received 1 dose of PCV13, followed by 1 dose of PPV23, one year apart. Blood samples were obtained prior and 1 month post each vaccination. PS3 and PS14 specific B cell levels were studied by 8 color Flow Cytometry (using PS coated beads) and were defined as "IgM+" ($PS^+CD19^+CD10^-CD27^+CD21^+IgM^+$) and "IgG+" ($PS^+CD19^+CD10^-CD27^+CD21^+IgM^-$). Results were correlated also with PS specific antibody titers.
Results: One month post PCV13 vaccination, PS14 and PS3 specific IgM+ MBCs remained stable (P=0.06; P =0.25 respectively), whereas PS14 and PS3 specific IgG+ MBCs increased significantly (P<0.05; P<0.001). In contrast, vaccination with PPV23 resulted in a statistically significant decrease in PS14 and PS3 specific IgM+ MBCs (P=0.04; P=0.05), while PS14 and PS3 specific IgG+ MBC counts were constant (P =0.23; P=0.5). Preexisting PS14 and PS3

specific IgG+ MBC counts were positively related to antibody levels at 1 month post both PCV13 (r=0.559, *P*=0.001; r=0.706, *P*<0.05) and PPV23 (r=0.796, *P*<0.05; r=0.660, *P*<0.05). Positive association was also found between preexisting antigen specific IgM+ and IgG+ MBCs post PCV13 (r=0.559, *P*=0.001; r=0.706, *P*<0.05).
Conclusions: MBC subsets were shown to have different kinetics following conjugate and polysaccharide pneumococcal vaccine. PCV13 administration enriched PS specific MBC pool, whereas PPV23 drained it, suggesting a negative effect on PCV13 induced immunological memory. The PPV23 induced MBC depletion could be a possible explanation of the "hyporesponsiveness", which compromises the protection of HIV infected patients, especially those with low CD4+ T-cell counts.

077

Antigen specific T cell subset alterations during treatment for M. tuberculosis infection in children
Eleni Ploumi[1], Marianna Tzanoudaki[1], Eliana Gkika[1], Natalia Lazopoulou[2], Virginia Polaki[1], Anastasia Linioti[1], Violetta Kapsimali[3], Despoina Lazopoulou[2], Athanasios Michos[2], Maria Kanariou[1]
[1]„Aghia Sophia„ Children's Hospital, ATHENS, Greece
[2]1rst Pediatric Clinic, National Kapodistrian University, ATHENS, Greece
[3]Microbiology Department, National Kapodistrian University, ATHENS, Greece

Introduction: *M. tuberculosis* (*Mtb*) infection is known to induce a Th1 immune response, but little is known as to the alterations of this response during treatment. In order to identify markers for disease progression and treatment success, we aimed to describe selected Purified Protein Derivative (PPD) specific T cell subset fluctuations, during treatment of latent *Mtb* infection in children.
Methods: 10 children, 3 to 12 years of age, who were diagnosed with latent *Mtb* infection, were selected for the study. Blood was drawn before initiation of treatment and at 3 and 6 months later. Childrens' PBMCs were cultured for 18h (37^{o}C, 5% CO_2) either in Complete Media, or in the presence of either PMA or PPD. Brefeldin A was used to prevent cytokine secretion. Cultured cells were stained with cIL2-FITC, cTNFα-PE, CD8-ECD, CD3-PC5.5, cIFNγ-PC7, CD4-APC and CD45-APC Alexa750, and analyzed by Flow Cytometry (Navios, Beckman Coulter). The T cell subset absolute counts were calculated by extrapolation to the basic T cell subset counts (assessed by single platform in another tube).
Results: A significant reduction of IFNγ producing T cells was observed after initiation of treatment. The reduction was obvious at 3 months, but reached statistical significance at 6 months. It was more prominent in the CD4+CD8- cell subset and, in PMA stimulated cells. More specifically, there was a reduction of the CD4+TNFα-IFNγ+ subset (*P*=0.008) and an elevation of the CD4+TNFα+IFNγ- subset percentage (*P*=0.01), as well as of the CD8+TNFα+IFNγ- subset count (*P*-=0.002) after PMA stimulation. A similar pattern was observed for the PPD specific T cells, but failed to reach significance.
Conclusions:A shift from Th1 polarization seems to occur after treatment of latent *Mtb* infection, whereas the level of TNFα production seems to be maintained. The fact that these changes were prominent after PMA, rather than PPD, stimulation might be due to the immunodominance of different antigens or may reflect an alteration of the overall cytokine profile of the T cells induced by *Mtb* infection. Further study of additional patients is scheduled in order to validate and/or enhance the significance of the above results.

078

Effects of hyperglicemia on cultured myeloid cells from diabetic patients
Enrica Trevisiol[1], Luisa Sambado[2], Maria Teresa Conconi[1], Maria Sambataro[2]
[1]Università degli Studi di Padova, TREVISO, Italy
[2]U.O.C. di Nutrizione Clinica e Malattie del Metabolismo, Osp. Ca' Foncello, Trev, TREVISO, Italy

Introduction: One long-term complication of type 2 diabetes (T2DM) is diabetic peripheral neuropathy that leads to nerve damage and augmented risk of ulcers or wounds in legs and foot. Myeloid cells can show a fundamental role in the inflammatory environment. A recurrent marker on almost all myeloid cells is CD33, a 67kDa protein that belongs to the SIGLEC family. The CD33-related Siglecs regulate leukocytes-mediated inflammatory and immune response. This study aims to evaluate the expression level of the myeloid marker CD33 as a possible linkage with T2DM and the related complications. Mimicking the *in vivo* hyperglycemic environment on cultured peripheral blood mononuclear cells (PBMCs), we wanted to assess if a myeloid population expressing CD33 could be strictly related to the diabetic condition.
Methods: 60 diabetic samples were divided in 4 groups: non-complicated T2DM patients (D, n=20), diabetic patients with neuropathy without (N, n=20) or with (N1, n=20) foot lesions (ulcers/osteomyelitis), T2DM patients with neuromacroangiopathy and foot lesions (NV, n=20). 20 healthy subjects were taken as controls. Human

PBMCs from 5 C and 5 NV were isolated and cultured with basal medium with or without 10mM or 30mM glucose for 24hours and 5days. $1x10^6$ CD14+ monocytes were cultured for 7days with IL-4 and GM-CSF to obtain dendritic cell differentiation (Mo-DC). CD33, CD14, CD16 and DC markers were evaluated on whole blood and cultured cells by flow cytometry.
Results: CD33 expression correlated with total monocyte blood count (p<0 001 R^2=0.9) and was elevated in diabetic complicated patients, mainly NV group (C=15,4±2,7%, D=16,7±4,7%, N=18,4±5,9%; N1=18,1±7,1%; NV=24,3±8,1%. p<0,001 vs C, D, N, N1). *In vitro*, cell number decreased from t0 to t=5days,regardless of hyperglycemia, expecially in T2DM (p<0,05 vs C, both in basal and glucose medium). CD33 expression seems to maintain in replicated cells. Almost 95% of CD33+cells coexpressed CD14 at t=5days in controls, showing a correlation between these populations. CD33+CD16+ cells were up-regulated in T2DM from 24h from seeding and also *in vivo*. Mo-DCs increased at t=7days in controls more than in diabetic samples. Glucose stimulation showed a decrease of CD33 expression in Mo-DC in particular in T2DM.
Conclusions: Evaluating different myeloid populations, we hypothesize that CD33+ cells in diabetic patients could reflect monocyte activation without Mo-DCs differentiation in T2DM. CD33+CD16+ cells, up-regulated in T2DM, could be actively implicated in the impairment of wound healing process. Next studies are necessary to investigate inflammatory pathways involving CD33 expression and CD14 and CD16 maturation response.

079

Single Platform CD34+ Cell Enumeration on the New BD FACSVia™ System
Yang Zeng
BD Life Sciences, SAN JOSE, U.S.A.

Background: Transplantation of hematopoietic stem cells and progenitor cells is critical in the treatment of malignancies and blood system disorders. Enumerating viable $CD34^+$ cells accurately and consistently has significant impact on the efficacy and safety of stem cell engraftment. Standardized flow cytometric analysis of viable $CD34^+$ cells has been widely used in laboratories that provide stem cell sources to achieve reliable and reproducible $CD34^+$ cell counts for stem cell therapies.
The BD FACSVia™ system is a newly developed flow cytometer featuring compact optical design, fixed alignment, and pre-optimized detector settings for ease of use. To perform viable $CD34^+$ cell analysis on the BD FACSVia system, we completed a study to verify this single platform method in comparison with the BD FACSCalibur™ flow cytometer, based on guidelines from the International Society of Hematotherapy and Graft Engineering (ISHAGE).
Methods: A total of 32 stem cell source samples, including fresh and frozen mobilized apheresis samples, cord blood, and bone marrow, were purchased from qualified vendors and stained using CD34 PE and CD45 FITC in BD Trucount™ tubes. Cell viability was determined using 7-AAD dye. Stained samples were lysed with 1X NH_4Cl before acquisition. Instrument QC was performed daily on the BD FACSVia system using the BD™ cytometer setup and tracking (CS&T) system. BD Calibrite™ beads were used to set up the BD FACSCalibur system. BD™ Stem Cell Controls were prepared and run on the two instruments to ensure that $CD34^+$ control cells were within the specified QC range. On the BD FACSCalibur system, cell analysis was performed using the BD™ Stem Cell Enumeration template. On the BD FACSVia system, a sequential Boolean gating template based on the ISHAGE protocol was used with BD FACSVia™ research software* to analyze $CD34^+$ cells.
Results: Linear regression results of cell absolute counts between the BD FACSVia system (test) and the BD FACSCalibur (predicate) were:
Viable $CD34^+$ cells (slope = 0.991, intercept = 1.12, R^2 = 0.998)
Viable $CD45^+$ cells (slope =0.978, intercept = -106.69, R^2 = 0.989)
Total $CD34^+$ cells (slope = 1.00, intercept = -5.82, R^2 = 0.987)
Conclusion: Results of the BD FACSVia System correlated well with the BD FACSCalibur system in absolute counts of viable $CD34^+$, viable $CD45^+$, and total $CD34^+$ cells.
*The BD FACSVia™ research software is For Research Use Only. Not for use in Diagnostic or Therapeutic procedures

080

Evaluation of Instrument QC Tracking Results for the BD FACSVia™ System

Yang Zeng, Angela Chen, Harshada Rohamare, Sasha Meyerovich, Farzad Oreizy, Maryam Saleminik, Fred Mosqueda, Kevin Judge
BD Life Sciences, SAN JOSE, U.S.A.

Introduction: The BD FACSVia™ System is a newly developed flow cytometer featuring a compact optical design, fixed alignment, and pre-optimized detector settings to facilitate ease of use. Daily one-step instrument QC using the BD™ Cytometer Setup and Tracking (CS&T) procedure provides quality control for electronics, fluidics, and optical performance as well as automatic optimization of instrument compensation. Less than five minutes is needed to complete instrument QC by running BD™ CS&T beads on the BD FACSVia System. Users do not have to adjust photomultiplier tube voltages and compensation during daily operation. Levey-Jennings (LJ) plots track daily fluctuations of instrument parameters. Software features of the BD FACSVia System improve workflow efficiency for sample acquisition and analysis.
Methods: We analyzed LJ tracking results for the CS&T instrument QC for up to 19 months on three BD FACSVia Systems that were placed at three study sites to evaluate instrument performance. Stability of fluorescence spillover values, and the median fluorescence intensity (MFI) of four fluorescence channels were also assessed using T-cell subset biomarkers. During the study time, the BD CS&T beads were acquired multiple days per week on each of the three cytometers to qualify the BD FACSVia Systems for running biological assays.
Results: Our long-term LJ tracking data showed that coefficient of variation (CV%) of the instrument sensitivity remained within 28%. Sensitivity values of all fluorescence channels were above the manufacturer's specification for the minimum sensitivity. CV% of the bright bead MFI was from 1.6% to 9.6%. For the robust coefficient of variation (rCV%) of the bright bead, variation (measured as CV%) was from 3.5% to 17.7%. The maximum CV% of Fluorescence spillover values was <7%. The MFI of each T-cell subset marker demonstrated stability that was acceptable. Over a four-month period, the CV% of marker MFI in all four fluorescence channels remained within 8%.
Conclusion: The BD FACSVia system demonstrated good long-term stability of CS&T instrument parameters, fluorescence spillover values, and MFI of biological markers.
CE marked in compliance with the European In Vitro Diagnostic Medical Device Directive 98/79/EC.

081

Evaluation of the Long-Term Stability of the BD™ CS&T Setup Workflow on the BD FACSLyric™ System

Yang Zeng, Sasha Meyerovich, Angela Chen, Harshada Rohamare, Farzad Oreizy, Kevin Judge
BD Life Sciences, SAN JOSE, U.S.A.

Introduction: For immunofluorescence applications, flow cytometer setup and QC are critical to ensure consistent and reproducible results over time and across cytometer systems. The BD™ Cytometer Setup and Tracking (CS&T) workflow on the newly developed multicolor BD FACSLyric™ system incorporates BD™ CS&T beads, BD™ FC beads, and BD FACSuite™ software. It is a user-friendly method that characterizes and maintains the performance of the instrument. The optimal biological application settings can be created in BD FACSuite software and saved using the CS&T bright bead target values for standardization of multiple cytometers with differing optical components. The CS&T setup workflow allows users to define baseline performance and perform daily QC automatically for critical instrument parameters. The workflow also performs daily checks and adjustments for optimal signal resolution, fluorescence target value positions, and laser delays. Levey-Jennings (LJ) plots track daily instrument fluctuations from baseline settings.
Methods: We verified the long-term stability of a 10-color BD FACSLyric system using the CS&T workflow with three lots of CS&T beads. The CS&T setup was run multiple days per week on the cytometer for 11 months to qualify the BD FACSLyric system for biological studies. To analyze LJ-tracking data for 330 days, we compared key parameters every 30 days relative to Day-1. A total of 11 percent difference (%diff) values ($\%diff_{Day\text{-}30}$ = (Day-30 – Day-1)/Day-1*100) were generated for each key parameter. The maximum positive or negative values of the 11 were analyzed and evaluated. The stability of spillover values (SOVs) and biomarker median fluorescence intensities (MFIs) was observed and assessed over a two-month period.
Results: For the bright bead MFI, maximum %diff within 330 days relative to Day-1 was <±2%. For all fluorescence channels, the robust coefficient of variation (rCV%) of the bright bead remained stable over 11 months. Instrument sensitivity showed stable values that were within the manufacturer's specification. Decrease of the Qr value was less than 25% over 330 days. Over a two-month period, fluorescence spillover values remained stable (CV% < 2) and the CV% values of biomarker MFIs in six fluorescence channels were within 10%.

Conclusion: Our data demonstrated stable instrument parameters on the BD FACSLyric instruments. The BD CS&T setup workflow is a comprehensive method for cytometer tracking and standardization in long-term studies.
CE marked in compliance with the European In Vitro Diagnostic Medical Device Directive 98/79/EC.

082

Utility of PNH testing involving CD24 versus CD157 as the main GPI linked protein

Timothy Milne[1], Alan Dunlop[2], Katy Sanchez[2], Austin Kulasekararaj[3], Tom Butler[1], Timothy Farren[1]
[1]Barts Health NHS Trust, LONDON, United Kingdom
[2]HMDC ViaPath, Kings College Hospital, LONDON, United Kingdom
[3]Kings College Hospital NHS Foundation Trust, LONDON, United Kingdom

Introduction: High sensitivity testing for PNH clones has long been shown to be clinically significant. Two different approaches to identify PNH populations include CD157/FLAER, OR CD24/CD14/FLAER, both with a CD45, CD15 (granulocyte) and CD64 (monocyte) backbone to focus on mature leucocytes. Whilst CCS guidelines are broad, this study demonstrates the interchangeability of two techniques, giving laboratories a choice of which reagent to choose.
Method: To assess the utility of these two approaches, to accurately determine PNH clones from 0.01 up to clone size 99.90%. 53 patient cases were analysed (38 fresh cases, 15 NEQAS samples) by two techniques: CD157/CD15/CD64/CD45/FLAER versus CD24/CD14/CD15/CD64/CD45/FLAER. For validation this was compared to a screening method including CD16/CD14/CD45/FLAER. Stress and precision analysis was performed in 5 patients. Within the 53 patients, 8 cases were used as a validation cohort between two separate centres.
Results: Strong correlation coefficient was observed between the two techniques for both granulocytic and monocytic series (R^2=0.9999 and 0.9984 respectively). Correlation with UKNEQAS consensus data for both techniques was highly significant for the granulocyte ($p<0.01$, Bias=0.2850) and monocyte series ($p<0.01$, Bias=1.014). The CD24 technique displayed better correlation within the monocytic series against the NEQAS consensus. Robustness: Stress testing showed no preference towards either technique when comparing both granulocytic and monocytic series tested at 48 hours and repeated within 120 hours, with the exception of the CD24 technique in the monocytic series (R^2=0.8208, p=ns). For validation and reproducibility, interlaboratory comparison between two independent sites for the CD157/CD15/CD64/CD45/FLAER technique was deemed statistically significant with minimal variance for both the granulocytic and monocytic series ($p<0.01$).
Conclusion: The two techniques showed an equal ability to quantitate PNH populations from 0.01% to 99.90%**.** Neither technique was superior in robustness, accuracy or precision. The monocytoid estimation may have preferential advantage by the CD24/CD14/CD15/CD64/CD45/FLAER technique, however the clinical utility in real terms maybe of little consequence, as the monocyte clone is used as confirmatory only. Of interest, one known MDS case showed partial negativity for CD24 on the granulocytes series. Whilst this phenomenon appears rare, CD157 negativity in PNH negative patients has also been observed. Previous reports highlight the CD157 technique delineating between type II and type III granulocytic clones, we did not observe this preference. Further work may be warranted to establish differing utility between methods in cases of MDS with associated PNH clones, but this study suggests the two methods have similar validity in quantitation of PNH clones.

083

In vitro evaluation of a new selective iNOS inhibitor on normal rat astrocytes and C6 rat glioma cells

Marialucia Gallorini, Cristina Maccallini, Viviana di Giacomo, Silvia Sancilio, Alessandra Ammazzalorso, Barbara de Filippis, Marialuigia Fantacuzzi, Letizia Giampietro, Rosa Amoroso, Amelia Cataldi
University of G. d'Annunzio, CHIETI, Italy

Introduction: Malignant gliomas are highly lethal brain tumors that portend an inauspicious prognosis for patients. Expression of inducible nitric oxide synthase (iNOS) in malignant gliomas has been extensively documented and nitric oxide (NO) has been known to promote *ex novo* tumor vascularization, thereby facilitating glioma cells growth and malignancy. Recently, a N-[(3-Aminomethyl)benzyl] acetamidine derivative, structurally related to the leading scaffold 1400W, was synthesized and short named CM554. In this work, the biological response of rat normal astrocytes and C6 rat glioma cells towards CM554 was evaluated.
Materials and Methods. Cells were exposed to 1.5 mM of both 1400W and CM554. Cell metabolic activity and cytotoxic cell response were evaluated by means of MTT test and LDH released, respectively. Oxidative and nitrosative stress occurrence (ROS and RNS) was analyzed by flow cytometry as well as cell cycle progression. Finally, the expression of NOSs, Nrf2, ERK 1/2, p38 and PARP-1 was analyzed by Western blotting.

Results: Initially, a significant decrease of cell metabolic activity of C6 rat glioma cells exposed to a range of concentrations of CM554 (0-2 mM) after 24, 48 and 72 h is detected, while normal astrocytes appear active and metabolizing. Lactate Dehydrogenase (LDH) is clearly released by glioma cells starting from 48 h of exposure to CM554, disclosing cytotoxicity occurrence. The analysis of cell cycle confirms MTT data, showing the highest percentage of rat glioma cells blocked in G1 phase after 72 h of exposure to 1.5 mM CM554 (71.79% 1.5 mM *vs* 63.97 % 0 mM). CM554 significantly increases the generation of ROS and $ONOO^-$ after 3 h of exposure while it appears ineffective on NO free production. Oxidative and nitrosative stress triggers Nrf2 and ERK 1/2 up-regulation as a mechanism of chemoresistance and cell survival. Quite the opposite, the expression of phospho-p38 is found down-regulated after 6 h of exposure to CM554 thus activating PARP-1, DNA fragmentation and chromatin condensation leading to cell cycle arrest in G1 phase and necrotic cell death.
Conclusions. Data here reported suggest that CM554 is highly selective *in vitro* for C6 rat glioma cells mainly at 1.5 mM concentration. Notably, CM554 exerts a substantial cytotoxic effect, decreasing cell proliferation rate of glioma cells and leading to oxidative stress-related cell death.

084

Demonstration of extended open vial stability of CE-IVD conjugated antibodies

Ankitha Channabasappa[1], Samee Saif[2], David Faye[3], Emmanuel Gautherot[3], Valerie Mallet[3], Krishnamurthy Thyagarajan[2], Julie Vernier[3]
[1]Beckman Coulter, BANGALORE, India
[2]Beckman Coulter India Pvt Limited, Bangalore Development Centre, BANGALORE, India
[3]Beckman Coulter Inc., MARSEILLE, France

Introduction: Conjugated antibodies have a wide array of applications in the field of Flow cytometry and imaging. However, their usage depends on the closed vial and open vial stability which, may differ from each other as the opened vials may be subjected to light exposure, oxidation and room temperature handling; thereby limiting the usage. Beckman Coulter has investigated the performance of its CE-IVD conjugated antibodies, already available in the market, to increase their open vial stability from 3 months to 6 months.
Methods: 110 CE-IVD conjugated antibodies were studied using a retrospective approach. Their 6 month usage was simulated by opening the vials and rejecting calculated volumes at different time points. On Day 0, the opened vials were tested against a reference (closed vial). The % recruitment of target cells, mean fluorescence intensity of positive and negative cells were analyzed.
Results: The difference in % recruitment of target cells between test and reference was <5% when % target was ≥15% and <3% when % target was <15%, the mean fluorescence intensity ratio was ≥75% (positive) and ≤125% (negative).
Conclusions: This study demonstrates the robustness of Beckman Coulter CE-IVD conjugated antibodies in terms of open vial stability thus enhancing their utilization.

085

Assessment and validation of internal acceptance criteria for sample stability for specimens undergoing clinical flow cytometric analysis

Katy Sanchez, Conor Stanley, Sophie Steward, Angela Giorgini, Deborah d'Costa, Melissa Bullard, Charles Stanley-Manogaran, Alan Dunlop
Viapath Analytics LLP, LONDON, United Kingdom

Introduction: Sample quality is of the upmost importance to the data quality produced by clinical flow cytometry and its interpretation leading to clinical diagnosis and treatment. Sample aspiration is an invasive procedure and time-dependant to the clinical course often making them unrepeatable. Immunophenotyping reference laboratories are reliant on the delivery of acceptable samples for analysis and when delayed, samples may require processing close to, or even after, published guidance. The problem then lies with the laboratory to decide if the results obtained after the recommended time-frame are accurate. To avoid misinterpretation or refusal of precious patient material, this study aimed to internally investigate the accuracy of data acquired and the ability to provide reproducible interpretation from samples tested beyond 48 hours from aspiration.
Methods: Multiparameter flow cytometry was performed for the following investigations using peripheral blood or bone marrow as appropriate; chronic lymphoid diseases, acute leukaemia, plasma cell neoplasms and Paroxysmal Nocturnal Haemoglobinuria (PNH). Baseline analysis were performed on samples less than 24 hours from aspiration then repeated on days 3, 4 and 5 when data was collated and analysed using Infinicyt™ software. Where possible, a minimum of 3 samples were used per antibody panel type resulting in 34 datasets over 11

panels. The ability to identify target populations and maintain their consistent percentage of total nucleated cells was assessed. Key acceptance criteria also included the maintenance of the baseline immunophenotypes and detection of low level populations to ensure no differences in interpretation for high sensitivity MRD assays.
Results: This study validated the sample stability up to 5 days for diagnostic chronic lymphoid diseases and acute leukaemia's (sensitivity 0.1%), 3 days for plasma cell analysis (sensitivity 0.01%) and up to 4 days for both red and white cell analysis for PNH (sensitivity 0.01%). The study also suggested the sample stability for Chronic Lymphocytic Leukaemia Minimal Residual Disease (CLL-MRD) analysis could be extended to 5 days (sensitivity 0.01%), however, this was a smaller dataset. Critically, B cell Acute Lymphocytic Leukaemia (B-ALL) MRD analysis (sensitivity 0.01%) is only stable for 48 hours after which disease quantification became unreliable.
Conclusions: This study allowed the observation of sample stability beyond the recommended 48 hours which is advantageous to reference laboratories. It has internally validated the extension of this time frame for a number of flow cytometry panels allowing greater confidence in the results or the addition of substantiated comments of the assays limitations.

086

CD7 expression in a case of chronic lymphocytic leukemia
Kira Matokhina, Ekaterina Zakharko, Elena Rybkina, Darya Drokova, Bella Biderman, Andrey Sudarikov, Valentina Dvirnyk
National Research center for Hematology, MOSCOW, Russian Federation

Introduction: Chronic lymphocytic leukemia (CLL) is the most common hematological disease in adults. Malignant cells mostly express B cell antigens with rather specific aberration (CD19+CD20low+CD22-/+CD79b-/+CD23+CD5+). In some rare cases co-expression of T-cell markers (CD8+, CD4+, CD7+) has been also reported.
Methods: peripheral blood samples from 180 primary CLL patients have been investigated in our laboratory during the last 2 years. 8 color flow cytometry (BD FACS Canto II) with BD reagents (CD45, CD3, CD5, CD4, CD8, CD7, CD19, CD20, CD22, CD23, CD38, CD43, CD79b, CD81, CD200, CD10, CD305) was used in each case of measurement.
Results: Only 1/180 patient had a T-cell marker (CD7) co-expression within this study. An 86-year-old male was admitted to our hospital with B-symptoms (fatigue, night fever and sweating) and lymphadenopathy (submandibular, axillary, inguinal, retroperitoneal). Examination of peripheral blood revealed elevated leukocyte count (21000/μL) with lymphocytosis (14900/μL). Monoclonal B-cell population with immunophenotype CD19+CD20low+CD22+CD23+CD5+cyKappa+CD200+CD43+CD38+CD7+ was found by flow cytometry. PCR (polymerase chain reaction) analysis according to Biomed-2 protocol confirmed monoclonal rearrangement of the immunoglobulin heavy chain genes. Sequencing of rearranged IGVH gene had shown 100% homology with germinal IGHV1-2*02 sequence. CDR3 sequence belonges to stereotyped receptor CLL#1 subset indicating very high risk of progression. Also mutation of PEST domen of NOTCH1 gene was found. Cytogenetic analysis identified trisomy 12 but there was no evidence for 13q14, 17p13/TP53, 11q22ATM deletion or IGH/14q32, cMYC/8q24 translocation. The patient had stable clinical course during 19 months of observation without a specific treatment.
Conclusion: expression of T-cell markers could be found in rare CLL cases. However prognostic value of this phenomenon requires further investigation.

087

A Real-Time Flow Cytometric Assay of Platelet Activation in Whole Blood of Dolphins (Tursiops truncatus) Using Human-Reacting Monoclonal Antibodies
José-Enrique O'Connor[1], Mar Felipo-Benavent[2], Alicia Martínez-Romero[3], Consuelo Rubio-Guerri[4]
[1]The University of Valencia, VALENCIA, Spain
[2]Cytomics Laboratory. Joint Research Unit UVEG-CIPF., VALENCIA, Spain
[3]Cytomics Core Facility, Principe Felipe Research Center, VALENCIA, Spain
[4]Oceanogràfic Foundation-Valencian Community, VALENCIA, Spain

Introduction: Dolphins are long-lived cetaceans in the top of the aquatic food chain and can thus bioaccumulate environmental contaminants. In addition, there is a growing interest on the effects of stress in marine mammals, including physiological responses to capture and handling which lead to increased production of catecholamines and other stress hormons. Since many pollutants as well as stress signals have been clearly related to abnormalities of platelet function, we were interested in developing assays of platelet activation that might be indicators of alterations in hemostasia related to toxicity, stress or pathology, as they are well established in humans.However, the lack of species-specific antibodies against dolphin blood cells may limit the validity of those assays. For this reason, we have tested several antibodies recognizing markers of human platelets and leukocytes on whole-blood

samples of dolphins. After identifying a suitable marker for platelets and leukocytes, respectively, we have evaluated the functionality of the platelets in healthy dolphins living in controlled aquarium environment.
Methods: Citrated whole blood samples from healthy common bottlenose dolphins (*Tursiops truncatus)* were obtained from the Oceanogràfic aquarium (Valencia, Spain). Samples were stained systematically with several anti-human CD41-PE and anti-human leukocytes specific markers. For the platelet functional assay, whole blood samples were stained with anti-human CD41-PE (Clone P2, Beckman Coulter) to gate-in platelets, and with anti-human CD11a-PE-Cy5 (Clone HI111, Biolegend) to identify leukocytes. The Ca^{2+} probe Fluo4-AM (TermoFisher) was used to determine platelet activation responses to ADP by a kinetic assay, as previously described by us (Curr Protoc Cytom. 2003 May;Chapter 9:Unit 9.20. doi: 10.1002/0471142956.cy0920s24.)
Results: After testing several antibodies recognizing markers of human platelets and leukocytes on whole-blood samples of dolphins, anti-human CD41-PE (Clone P2, Beckman Coulter) and anti-human CD11a-PECy5 (Clone HI111, Biolegend) were found to label specifically leukocytes and platelets respectively. In addition, real-time cytometric analysis of Ca^{2+} mobilization demonstrated that ADP triggered platelet activation, in a similar way to human platelets in the same experimental conditions. Coexpression of CD41 and CD11a allowed to assess the interaction of platelets with other blood cells.
Conclusions: Anti-human CD41-PE (Clone P2, Beckman Coulter) and anti-human CD11a-PECy5 (Clone HI111, Biolegend) can be used to identify respectively platelets and leukocytes in dolphins. This allows to perform easily whole blood kinetic assays of platelet activation based on ADP signalling, that may be used as functional biomarkers in toxicological and physiopathological studies.

088

Flow Cytometric Analysis of Phagocytosis and Oxidative Burst in Animals Using Whole-Blood Assays Designed for Human Diagnostics

José-Enrique O'Connor[1], Beatriz Jávega[1], Guadalupe Herrera[2], Alberto Miralles[1], Inmaculada Cerrada[3], Ignacio Mesa[3]
[1]The University of Valencia, VALENCIA, Spain
[2]Incliva Foundation, VALENCIA, Spain
[3]Hospital Veterinario Valencia Sur, MASSANASA, Spain

Introduction: Phagocytosis is a first-line protective mechanism against microorganisms and infections, linked to intracellular destruction of ingested pathogens by oxidative reactions (oxidative burst) as well as oxygen-independent processes. Phagocytosis and oxidative burst are considered as indicators of the status of innate immune function. In fact, analysis of phagocytosis/oxidative burst by flow cytometry (FCM) is now a frequent application in human clinics, with several commercial products and assay systems available for routine studies. In spite of this availability of human-targetted phagocytosis assays, there are no reports of their use to systematic testing of phagocytosis and oxidative burst in animals, where analysis rely mostly in non-standardized assays. Accordingly, we have tested in several animal species the performance of two commercially available assays designed for the evaluation of phagocytosis or oxidative burst in humans.
Methods: Heparinized blood samples from healthy dogs, pigs, rabbits and laboratory mice were obtained from Valencia Sur Veterinary Hospital, Massanasa, Spain (Dogs), Department of Physiology, Valencia University (Rabbits) and Animal Housing Facility, UCIM, Valencia (Pigs and Mice) taking advantage of scheduled sampling for clinical or experimental purposes. Phagocytosis and oxidative burst were studied using, respectively, IngoFlowEx® and FagoFlowEx® kits (both from EXBIO, Prague, Czech Republic), following manufacturer's instructions and using appropriate controls.
Results: Application of the human-oriented IngoFlowEx® and FagoFlowEx® kits to whole-blood samples of the several animal species tested by us showed that granulocytic and monocytic populations could be distinguished and that phagocytosis of FITC-Labelled Escherichia coli (IngoFlowEx®) and oxidation of dihydrorhodamine 123 (FagoFlowEx®) could be detected and quantified. The phagocytic and oxidative responses were more evident in granulocytes than in monocytes in all the species tested.
Conclusions: The phagocytosis assay with the commercially available IngoFlowEx® and FagoFlowEx® kits can be easily applied in several animal species, namely dogs, pigs, rabbits, and mice, in a similar way as they are used for human samples. These assays may be useful to detect alterations in the immune functions in animals as a consequence of disease, stress or toxicity.

089

Flow cytometry analysis of CD30 expression in acute T-lymphoblastic leukemia/lymphoma patient

Ekaterina Zakharko[1], Elena Rybkina[2], Darya Drokova[1], Valentina Dvirnyk[1]
[1]National Research center for Hematology, MOSCOW, Russian Federation
[2]National Research Center for Hematology, MOSCOW, Russian Federation

Introduction: less than 1% of normal peripheral blood cells express CD30. This level can be increased by virus infection or autoimmune process. This antigen is very specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and tumor cells of anaplastic large cell lymphoma but variable for other lymphomas. The level of CD30 expression in acute leukemia, especially in onset of T-lymphoblastic leukemia/lymphoma, is not very clear.
Methods: 12 bone marrow samples of T-lymphoblastic leukemia/lymphoma patients were studied during last 1 year. There were 9 males and 3 females with age median 24 year. 11/12 were newly diagnosed patients, 1/12 was late relapse patient. An 8-color flow cytometry (Becton Dickinson (BD) FACS CantoII, reagents (BD): CD30, CD45, CD3) was used for surface CD30 detection in each case of study.
Results: tumor cells with co-expression of CD30 were detected in 6 (50%) patients and comprised 14,4 – 99,9% of the blast population (CD30+ blasts level was >20% in 5 cases and in 1 case it was 14,4%). There was no correlation of CD30 expression with EGIL variant or genetic changings. Complete remission was achieved in 8 (80%) patients (5/6 among CD30 negative T-ALL and 4 among CD30 positive T-ALL). And It was not possible to collect a follow-up data in 2 patients. Relapse was diagnosed in 3 patients (1 late among CD30 negative T-ALL, 2 early in CD30 positive group). Among patients with CD30 negative T-lymphoblastic leukemia/lymphoma, all 6 patients are alive and continue therapy. In the CD30 positive T-ALL group 4 patients are dead (1 due to refusal of treatment, 1 due to infection, 2 due to relapse), and 1 has remission.
Conclusions: CD30 antigen is not specific and can be co-expressed by tumor cells in acute T-lymphoblastic leukemia/lymphoma in 50% of cases according to results of this study. Prognostic significance requires future investigation.

090

Ten-color and 12-antibodies flow cytometry panel for high sensitivity detection of minimal residual disease in B-lymphoblastic leukemia

Rodolfo patussi Correia, Laiz cameirão Bento, Rodrigo de souza Barroso, Nydia strachman Bacal, Paulo vidal Campregher, Nelson Hamerschlak
Hospital Israelita Albert Einstein, SAO PAULO, Brazil

Introduction: The improvements in multiparametric flow cytometry (FCM) have allowed increasing sensitivity of minimal residual disease (MRD) assay. This next generation flow requires more than 5 million of events to achieve the high sensitivity (HS) MRD below current clinical threshold of 0.01%. In this study we report a new FCM assay to detect MRD in B-lymphoblastic leukemia (B-ALL) with sensitivity of at least 10^{-5} to increase the limit of MRD detection by FCM.
Methods: The B-cells were evaluated by a ten-color and 12-antibodies tube:
CD58FITC/CD73+CD304PE/CD38ECD/CD10PC5.5/CD34PC7/CD123APC/CD19+CD22APC-AlexaFluor700/CD81APC-H7/CD20PB/CD45KrO. This panel was applied in 30 not pathological patients and in 45 B-ALL samples, 16 at diagnosis and 29 post-treatment. For HS MRD assay, the whole sample volume required was lysed with chloride ammonium solution, concentrated in low volume, stained, lysed again if necessary, washed and resuspended for acquisition in Navios Flow Cytometer (Beckman Coulter, BC). The data were analyzed in Kaluza software (BC), using Boolean gates and Radar.
Results: The mean of events acquired was 7,489,690 (2,701,571 to 11,900,000). The maximum number of events was obtained acquiring 7 consecutive times the same tube to obtain 11,900,000 events in Kaluza merge file, being 1,700,000 by listmode file. The parameter Time and fluorescence intensities were used for acquisition and data merge control. In MRD analysis, Radar was configured to generate two distinct regions and discriminate normal and abnormal B-cell populations according to underexpression of CD10, CD34, CD38, CD45 and CD81, or overexpression of CD10, CD20, CD34, CD58, CD73+CD304 and CD123. We applied this strategy in 29 post-treatment samples comparing their phenotype with 30 not pathological patients, in a single merged file, and easily detected MRD in 15 post-treatment samples. The low level of MRD in this study was 0.002% (149 events in total of 6.800.000). Furthermore, this technique detected more than 5 million of events in 77.8% samples, and detected MRD in patient with anti-CD19 therapy.
Conclusions: We tested a new strategy (antibodies, technical procedure and data analysis) for FCM MRD assay. It was applicable in all samples investigated, increased the sensitivity to levels below the clinical threshold of 0.01%, and facilitated the MRD analysis by the use of Radar and comparison with normal phenotypes. The clinical

significance of these findings require further studies, but the HS MRD assay described here, can give important information for follow-up of B-ALL patients, especially when the MRD levels are below the limit of detection by conventional flow cytometry.

091

Channel-free & compensation-free dead cell exclusion: N+1 panel design

Roy Edward[1], Alfonso dr Blanco-Fernández[2]
[1]BioStatus Limited, SHEPSHED, United Kingdom
[2]UCD-Conway Institute, DUBLIN, Republic of Ireland

Introduction: Exclusion of dead cells is a common requirement for flow cytometry using a viability dye, historically with DAPI, PI and 7-AAD. A new far-red fluorescing alternative DRAQ7 has been robustly tested (e.g. Akagi, et al., 2013; Smith, et al., 2013) and shown to have improved separation between negative and positive events (Moshaver et al., EBMT Conference 2013; Beckman Coulter, FLOW-1710APP05.16-A, 2016).
Conventional flow cytometry is limited by spectral "space" and preference for bright chromophores to label antibodies to aid sensitivity increases need for compensation. At its simplest, far-red DRAQ7 benefits panel design e.g. no overlap with FITC/R-PE pairs makes it highly compatible with single platform CD34+ cell counting.
Of specific interest, DRAQ7 is optimally orange-red excited (599nm, 644nm) yet is usefully excited with blue, green, yellow laser lines, permitting deployment on basic cytometers. In principle, DRAQ7 occupies multiple fluorescence channels but this enables a unique display of dead cell events in a "virtual channel"; plotting pairs of red/far-red channels from different excitations against each other one can choose a suitable pairing that gives the preferred separation of a double-positive population for a chromophore panel. These double-positive events define dead-cells to be excluded by a live cell gate and from other channels without compensation.
Methods: We employed a selection of common flow cytometry platforms to fully exemplify the concept. Multi-colour phenotyping panels containing various red/far-red chromophores were compensated for optimal performance and when applied to cell preparations had DRAQ7 added. In all cases, data was displayed to show bivariate plots for available combinations of relevant fluorescence channels. Initially, each was examined for suitability to describe a live cell gate excluding double-positive DRAQ7 events for that panel.
Results: In all cases it was possible to determine a bivariate plot that reliably and simply described the dual-excited DRAQ7 dead cell events to the exclusion of the single-excited chromophores on either axis of the bivariate plots.
Evidently, care should be taken, where possible, to avoid the labeling of co-expressed antigens with red/far-red chromophore tagged antibodies. However, it should still be possible to display sufficient bivariate plots that this combination can be overcome and a suitable alternative chosen.
Conclusions: This method permits "N+1" cytometry to design a phenotyping panel without consideration of inclusion of a viability probe, added subsequently into samples alongside antibody cocktails without requirement for compensation, and where no viability dye had been accommodated originally.

092

Fast and accurate prediction of positive and negative urine cultures by flow cytometry

Bijan Moshaver[1], Foppie de Boer[2], Heidi van Egmond-Kreileman[2], Ellen Kramer[2], Coen Stegeman[3], Paul Groeneveld[2]
[1]University Hospital Geneva, GENEVA, Switzerland
[2]Isala, ZWOLLE, Netherlands
[3]University Medical Center Groningen, GRONINGEN, Netherlands

Background: Urinary tract infection (UTI) is a widespread infectious disease in humans. Urine culture, a huge workload in the microbiology laboratory, is still the standard diagnostic test for UTI, but most of the cultures are negative. A reliable screening method could reduce unnecessary cultures and quicken reporting of negative results.
Methods: We evaluated the usefulness of a flow cytometry (FC) screening method in the prediction of positive urine culture to reduce the number of urine cultures. The urine specimens sent to the laboratory for culture were tested with the flow cytometer Accuri C6. FC bacterial counts were compared to standard urine culture results to assess the best cut-off values.
Results: Two hundred nine urine samples were included, of which 79 (37.8 %) were culture positive. On comparing the culture and the FC data in the ROC curve, the FC bacterial counts of $\geq 10^6$ bacteria/mL provided a reliable screening for bacteriuria with a sensitivity and specificity of 99 and 58 %, respectively. All negative FC results ($<10^6$ bacteria/mL) showed a negative predictive value of 99 % with a negative likelihood ratio of 0.02. The FC bacterial counts of $\geq 10^8$/mL showed a positive predictive value of 99 % with a positive likelihood ratio of 60.9.

Conclusions: Counting bacteria in human urine samples by the FC is a fast, accurate and cost-effective screening method for bacteriuria. Our results showed that FC is able to rule out UTI, which can lead to a substantial reduction (36 %) of urine cultures. It also demonstrated that this method predicts positive cultures accurately.

093

Automated flowcytometric identification of disease specific cells by the ECLIPSE algorithm

Leo Koenderman[1], Rita Folcarelli[2], Roel Bouman[2], Selma van Staveren[1], Bart Hilvering[1], Gerjen Tinnevelt[2], Geert Postma[2], Lutgarde Buydens[2], Jeroen Jansen[2]
[1]University Medical Center Utrecht, UTRECHT, Netherlands
[2]Radboud University, Institute for Molecules and Materials, Analytical Chemistry,, NIJMEGEN, Netherlands

Introduction: Flow Cytometry (FC)-based gating allows the selection of cellular (pheno)types based on their expression of surface markers. Current operational methods such as multiple gating are biased, because it is generally performed by trained personnel. Also every uni- or bivariate gating step removes potentially relevant information present in the data not used for gating. Only multivariate approaches can extract all aspects of cell variability from the data, including those associated with co-expression of multiple surface markers.
Methods: We have developed an algorithm, ECLIPSE, that allows the extraction of disease specific signals. The method makes use of 3 essential steps: 1 models the specific variability of control samples = control cells; 2 *Equal to normal* as principle to eliminate healthy cells in patients;3. Visualize diseased cells in the multidimensional space. ECLIPSE was used for the analysis of blood leukocytes before and after induction of acute inflammation evoked by challenge with systemic endotoxin. In a second analysis the algorithm was applied to identify minimal residual disease in multiple myeloma.
Results: ECLIPSE could clear identify two new neutrophil subsets only found in the blood of healthy individuals challenged with LPS. The identification did not require any supervision. The algorithm enabled the detection of minimal residual disease in the bone marrow multiple myeloma patients at low levels as low as 0.01 % of all bone marrow cells. Again no supervision was required.
Conclusions:ECLIPSE allows the identification and kinetics of disease specific profiles of multiple cell populations in chronic disease as well as rare cells such as found in minimal residual disease in leukemia and lymphoma's. As no human interference is required the analysis by ECLIPSE through an intuitive graphical user interphase can be implemented in point-of-care equipment for health and disease monitoring.

094

Investigating the role of CD25, CD69, CD74, CD133 and CD135 in detecting minimal residual disease in childhood acute myeloid leukemia

Dilshad Dhaliwal, Manisha Suthar, Rohit Kori, Pg Subramanian, Sitaram Ghogale, Nilesh Deshpande, Gaurav Chatterjee, Yajamanam Badrinath, Gaurav Narula, Shripad Banawali, Sumeet Gujral, Prashant Tembhare
Tata Memorial Centre, NAVI MUMBAI, India

Introduction: Minimal residual disease (MRD) status is the most relevant indicator for predicting impending relapses in acute myeloid leukemia (AML). Flow-cytometry(FC) based MRD (FC-MRD) is a widely applicable and sensitive method for MRD monitoring. Availability of new markers has improved the applicability of FC-MRD up to 95-98% in B-cell acute lymphoblastic leukemia. However, applicability of FC-based AML-MRD is still limited to 75-80% due to reasons like limited stable markers, variability in antigen-expression and post-chemotherapy immunomodulation. Hence, there is a necessity to investigate new robust markers to increase the sensitivity and applicability of FC-MRD in AML. We investigated the expression-pattern of five new markers in childhood-AML for their utility in the FC-MRD monitoring.
Methods: We studied the expression-pattern of CD25 (PE-CF495, clone-Bl-49.9), CD133(APC, clone-AC133), CD69 (FITC, clone-FN50), CD135 (BV421, clone-4G8), and CD74(PE, clone-LN2) in 45 childhood-AML patients (age, 0.9-14 years) at diagnosis and minimal residual disease (MRD) monitoring and in five control samples. FC was performed using 10-color-assay on Navios flow-cytometer. The expression-levels i.e. mean fluorescent intensity (MFI) was measured as geometric mean (GM) using Kaluza software v-1.3. Positive-expression was defined with >20%-positive of total blasts.
Results: Mean/Median/SD of CD25, CD69, CD74, CD133 and CD135 in leukemic blasts of childhood-AML at diagnosis and in normal myeloid-blasts from five control samples were respectively 9.5/9.8/4.7, 4.6/4.1/2.8, 13.6/13.4/11.6, 4.7/4.4/2.0, 16.3/12.5/17.9 and 1.93/1.79/0.65, 1.26/1.34/0.25, 3.01/2.5/1.31, 1.50/1.31/0.37, 1.8/1.63/0.65. Abnormal-expression of these markers on leukemic-blasts against normal myeloblasts was found statistically significant evaluated using Mann-Whitney-U test (p-value<0.01). Mean, Median, SD of positive-

percentages of CD25, CD69, CD74, CD133 and CD135 in leukemic-blasts at diagnosis were 15.4/4.5/24.8, 21.2/1.8/33.5, 24.8/5.6/30.0, 43.4/35.3/35.3, and 30.6/4.7/38.3 respectively. Of 45 AML, post-induction MRD, post-consolidation MRD, and sub-sequent MRD were available in 22/45 (48.8%), 14/45 (31.1%), 10/45 (22.2%) respectively. Of all 52 samples, MRD was positive in 19/52 (36.5%) and median (SD) MRD-values was 12.9% (7.2%). Mean/Median/SD of CD25, CD69, CD74, CD133 and CD135 in MRD-positive blasts were 7.4/6.67/5.07, 4.09/4.63/2.26, 7.14/6.43/4.14, 4.43/4.3/1.60 and 9.08/7.71/4.55. Of 19 MRD-positive samples, abnormal over-expression of CD25, CD69, CD74, CD135 in 3/19 (16%), 6/19 (31.6%), 14/19 (74%), and under-expression of CD133 was found in 3/19 (16%) respectively. Of 33 MRD-negative samples, these new markers detected MRD in four samples and corrected their false negative status.
Conclusion: Of the five markers studied,CD74 followed by CD135 and CD69 were most useful markers to improve the applicability and accuracy of MRD detection in childhood-AML.

095

The detection of coated platelets is a sensitive flow cytometric method to identify dasatanib induced side effects

János Kappelmayer[1], Ildikó Beke Debreceni[1], Adrienne Kerényi[1], Viktor Treszkai[1], Gabriella Mezei[2], Péter Batár[2]
[1]University of Debrecen, Department of Laboratory Medicine, DEBRECEN, Hungary
[2]University of Debrecen, Department of Medicine, Division of Hematology, DEBRECEN, Hungary

Introduction: Tyrosine kinase inhibitors (TKI) are a very effective group of drugs that considerably prolong survival in patients with chronic myeloid leukemia (CML). Several lines of evidence suggest that dasatinib (Sprycel) may induce bleeding due to its effect on platelets. We hypothesized that dual agonist activated platelet formation (coated platelets) may be a useful marker to monitor TKI induced side-effects in addition to classical platelet function tests.
Methods: Citrate-anticoagulated blood samples from healthy volunteers were incubated with or without dasatinib and platelet aggregometry and coated platelet formation were detected. This latter test was executed by dual activation of gel-filtered platelets by the snake venom convulxin and thrombin to simultaneously activate the collagen and thrombin receptors. In addition peripheral blood derived from CML patients were analyzed at 0 hour and 1 and 4 hour after witnessed drug administration.
Results: A supra-therapeutic dasatinib concentration (400 nM) prevented platelet aggregation and ATP release after1 µg/ml collagen and 500 µg/ml arachidonic acid challenge and similar inhibitory effect could be observed down to 150 nM dasatinib concentration. Coated-platelet formation was significantly inhibited by preincubation with 400 nM dasatinib resulting in 4±1.9% of coated platelet formation versus controls with no TKI 38±9 % (p=0.001). The effect of dasatinib was dose-dependent and a significant inhibitory effect could be observed already at 10 nM dasatinib that is the low therapeutic concentration of the drug (coated platelet = 28±10.9, p=0.023), however this low dasatnib concentration had no inhibitory effect on platelet aggregation results. In samples derived from CML patients, platelet aggregation for both agonists became impaired at 1 hour after drug ingestion but was normalized by 4 hours, while coated platelet formation was suppressed significantly at both 1 and 4 hours after drug ingestion. Contrary to dasatinib, another second generation TKI, nilotinib (Tasigna) did not exert any effect on aggregation and on the formation of coated platelets.
Conclusion: We suggest that the measurement of coated platelets is a more sensitive marker than platelet aggregometry to detect TKI associated side effects in platelets.

096

CD81 negative expression predicts a poor cytogenetic and prognostic risk group in chronic lymphocytic leukemia

Giovanni Rossi, Potito Rosario Scalzulli, Giovanni Pio de Cillis, Valeria Paduano, Rossella Valvano, Maria Marta Minervini, Daniela Valente, Saverio Mantuano, Nicola Pio Sinisi, Nicola Cascavilla
IRCCS „Casa Sollievo della sofferenza„, SAN GIOVANNI ROTONDO (FG), Italy

Introduction: Several factors can predict the outcome in chronic lymphocytic leukemia (CLL), including immunophenotipic features. The expression of CD49d and CD38 represented the most important prognostic markers in CLL but no further markers have been investigated among those newly used. On the other hand, no markers seem to predict cytogenetic aberrations. CD81 is a tetraspasmin widely espressed on B cells and weakly expressed on CLL cells. Immunophenotipic studies of CD81 expression in patients with CLL are scanty and its value in predicting cytogenetics lesions as well as the outcome remains unknown. Thus, the aim of this study was to evaluate the potential role of CD81 in indentifying different cytogenetic and prognostic risk groups in CLL.

Methods: Samples of bone marrow from 71 patients with CLL were investigated for the surface expression of CD81 reported as percentage of CLL cells expressing the antigen and MFI. Thirty-one patients were treated and studied for minimal residual disease (MRD).
Results: The best cut-off points for CD81 were sought by constructing ROC curves, so that values greater than 20% of CLL cells and 530 by MFI were considered positive samples (CD81 pos) while the expression below these levels defined negative samples for this marker (CD81 neg). CD81 neg samples were significantly associated with unfavorable cytogenetic aberrations (67% vs 33%) such as 17p and 11q deletions, while CD81 pos samples were associated with favorable cytogenetic aberrations (96% vs 4%) such as 13q deletion and +12 trisomy (p=0.000). The majority of patients showing a negative cytogenetic profile with respect to previous aberrations had CD81 pos CLL cells (72% vs 28%)(p=0.000). There was an agreement between CD81 neg and CD38 positive expression(p=0.002). No significant correlations were found between CD81 expression and clinical features of CLL. When the only patients who underwent chemotherapy were considered, a significant association was showed between CD81 neg patients and a positive MRD (p=0.015). Interestingly, CD81 neg patients showed a significantly lower disease free survival (DFS) than those CD81 pos (p= 0.038).No significant associations were found between CD81 expression and the overall survival.
Conclusions: The absence of CD81 expression on CLL cells identifies patients who had unfavorable cytogenetic aberrations and a lower DFS compared to patients with positive CD81 expression, thus confirming the predictive role of CD81 in CLL.

097

Fully automated enumeration of CD34+ hematopoietic stem cells using the MACSQuant Analyzer 10
Philipp Gert, Martin Büscher, Katrin Lange, Susanne Höher-Peters, Christian Dose, Manuela Herber, Stefanie Pflitsch
Miltenyi Biotec, BERGISCH GLADBACH, Germany

Introduction: Flow cytometric enumeration of viable $CD34^+$ hematopoietic stem cells (HSC) from different sources provides critical information in various applications, e.g. the evaluation of stem cell grafts or the determination of optimal timing for donor apheresis. Due to the low frequency of HSCs, this rare-event analysis can prove to be a demanding and complicated procedure, creating the need for a standardized and reproducible workflow. The guidelines established by the International Society of Haematotherapy and Graft Engineering (ISHAGE) were a major step towards standardization, but operator variance can still be an issue. Here we demonstrate a fully automated process of HSC enumeration, including staining, lysis, acquisition and analysis using the MACSQuant Analyzer 10 (Miltenyi Biotec).
Methods: Automation of the complete workflow was realized by an Express Mode, including automated pipetting, sample transfer, acquisition and analysis of samples according to the ISHAGE guidelines. Finally, a report summarizing the results of each measurement, including the volumetrically determined absolute HSC count, can be generated. For the evaluation of the process, cord blood and the CD-Chex CD34 control reagent (Streck) were used. The results of the Express Mode samples were then compared to manually handled samples.
Results: With the automated workflow described here, it was possible to process six samples in parallel within less than 45 minutes for determination of viable $CD34^+$ HSC counts in cord blood or CD-Chex CD34 control reagent. These automatically obtained cell counts of HSCs were within the expected range for the different levels of the control reagent and similar to the manual controls. In addition, a spike-in experiment with magnetically isolated HSCs confirmed the linearity of the HSC count determination.
Conclusions: The results shown here demonstrate that the MACSQuant Analyzer 10 can be utilized to establish a standardized and automated workflow for HSC enumeration in order to reduce the intra- and interassay variability.

098

Quality assurance of flow cytometry assays: Results of three-year semi-annual immune monitoring inter-site comparisons of the TCRab-Haplo2010 trial

Stefanie Biedermann[1], Michael Schumm[2], Johannes Rachor[3], Sandra Karitzky[1], Christiane Siewert[1], Christian Dose[1]
[1]Miltenyi Biotec GmbH, BERGISCH GLADBACH, Germany
[2]Children's University Hospital, TÜBINGEN, Germany
[3]Children's Hospital University Clinics, WÜRZBURG, Germany

Introduction: Since cellular, immune and gene therapy are evolving and promising medical fields in research and daily routine, immune monitoring has become an essential tool for determining treatment-induced effects on immune cells. Flow cytometry offers the opportunity to monitor immunological parameters in clinical trials on low amounts of cellular material (e.g. whole blood) with a reasonable amount of time. However, reproducibility of flow cytometry-based immune monitoring is a matter of high importance, especially when multiple sites are involved. Here we present results of the semi-annual inter-site comparisons conducted over 3 years as a quality assurance measure of flow cytometry-based immune monitoring for the TCRab-Haplo2010 trial.
Methods: Blood from a healthy donors were centrally collected and distributed to the participating labs. Samples were assayed within 25 to 30 hours after blood collection by four operators using four different MACSQuant Analyzer devices at three different sites. Immune monitoring was performed according to written standard operating procedures using a two-platform approach with absolute cell count assessment by hematocounters and flow cytometric determination of the frequency cell populations of interest. Cell subset concentration data (cells/µl) of each lab, participating in the semi-annual inter-site comparison were analyzed. The reproducibility of cell subset concentration determination was analyzed by coefficient of variation (CV).
Results: Independ from technical inconsistencies (operator and device), cell concentration means were comparable for all analyzed immune cell subsets. Furthermore, immune cell subsets, which are relevant to evaluate immune reconstitution after haploidentical hematopoietic stem cell transplantation with TCRαβ-CD19 depleted stem cell grafts (T cells, T helper cells, cytotoxic T cells, B cells, NK cells, $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ T cells) showed an average CV of 6.28 % (± 3.89 %) for percentage of subsets among $CD45^+$ cells and 6.99 % (± 4.31 %) for cells/µl (two platform approach). Additionally, Monocytes (7.30 % ± 2.97 %) and Neutrophils (5.01 % ± 3.17 %) were highly reproducible in this inter-site comparison. Only subsets with low cell concentrations showed slightly increased CV values compared to all other investigated cell subsets.
Conclusions: The inter-site comparisons of immune monitoring showed low CVs of subset cell concentrations and therefore low variability/high reproducibility over six independent immune monitoring experiments with six different healthy donors over a three year period. Furthermore, these results confirm reliability and comparability of patient immune monitoring data obtained in the TCRab-Haplo2010 flow cytometry core labs.

099

Assessment of the degree of inter-centre flow cytometry standardization of Lymphoid Screening Tube

Lourdes Cordón[1], Irene Luna[2], Paula Amat[3], Lola Linares[2], María Teresa Orero[2], Victoria Fornés[4], Manuel Fernández-Delgado[2], María José Remigia[3], Fabián Tarín[2], Amparo Sempere[5]
[1]Grupo de Investigación en Hematología, Instituto Investigación Sanitaria La Fe, VALENCIA, Spain
[2]Hematology Department, Hospital General Universitario, VALENCIA, Spain
[3]Hematology Department, Hospital Clínico Universitario, VALENCIA, Spain
[4]Data Science Unit, Instituto de Investigación Sanitaria La Fe, VALENCIA, Spain
[5]Hematology Department, Hospital Universitario y Politécnico La Fe, VALENCIA, Spain

Introduction: Standardization of flow cytometry (FC) in diagnostic of haematologic neoplasms is essential. The standardized operational procedures for calibration of Euroflow Group are complex and time-consuming. The BD Oneflow™ system (Becton Dickinson -BD-, San Jose, CA) simplifies and reduces the time, but it is necessary to validate this methodology in the routine care. This study aimed to assess the FC standardization between institutions employing this system and comparing the results obtained with the BD Lymphoid Screening Tube (LST).
Methods: Peripheral blood from 40 subjects without haematologic neoplasms were collected in 4 centres in Comunidad Valenciana (March-May 2017). Sample aliquots were sent to each centre, preserved at room temperature and processed within 24-36 hours in a FACSCanto-II cytometer. A control of linearity was performed using BD Cytometer Setup & Tracking beads (CS&T). BD Oneflow™ beads were used to calibrate and compensate the fluorescence channels. Cells were stained with the BD Oneflow™ LST (CD20+CD4 HV450, CD45 HV500, CD8+Ig Lambda FITC, CD56+Ig Kappa PE, CD5 PerCpCy5.5, CD19+TCRgd PECy7, CD3 APC, CD38 APCH7) as recommended by BD. Data were analysed using Infinicyt™ software (version 1.8). To compare results and measure the degree of standardization, the percentages of leucocyte populations and the median of fluorescence intensity (MedFI) of

lymphoid populations in the appropriate fluorescence channel were reported. Concordance correlation coefficients (CCC) were calculated in each MedFI. Linear mixed regression was performed to study the differences between centres. Analyses were accomplished with R software (version 3.4.0).
Results: Percentages obtained for the different leukocyte populations: eosinophils, neutrophils, monocytes, lymphocytes T (CD4+, CD8+, TCRgd), lymphocytes B (Ig Kappa, Ig Lambda), NK cells, basophils, and dendritic cells were similar in all centres. CCC considering all samples together showed low values, being only over 40% for CD3 and CD5. Mean and confidence interval in each MedFI in all institutions showed a similar distribution with high variability in the following populations and channels: T lymphocytes in HV500 and PerCPCy5.5, NK $CD56^{bright}$ in PE, B lymphocytes Ig Kappa in PE and Ig Lambda in FITC.
Conclusions: The distribution of MedFI observed in different centres was acceptable and showed similar results. The variability within MedFI was as expected, except for $CD45^{+}$ and $CD5^{+}$ in T lymphocytes, $CD56^{bright}$ in NK cells, Ig Kappa and Lambda in B lymphocytes, possibly due to differences in samples. Common standardized procedures in clinical laboratories are key to obtain reproducible results between centres.

100

Dried Leukocytes (Lysed Whole Blood) as Controls in Flow Cytometry
Yanli Liu, Andrew Smith, Yuming Tang, Manuela Franzblau, Marybeth Sharkey, Jennifer Lazar
BD Biosciences, SAN JOSE, U.S.A.

Introduction: Controls are used to establish a baseline to compare results against introduced variables in flow cytometry. Control cells can be used as positive and procedural controls in flow cytometric applications such as diagnostic of leukaemia & Lymphoma, immune monitoring and drug discovery. Using fresh blood as controls lacks convenience, stability and consistency due to 24 hr shelf life and donor to donor variance. Meanwhile, the current control market sees major challenges of commercial control cells include short shelf life, deteriorated preservation of labile markers and decreased resolution of dim populations. We hypothesized that the dried leukocytes or lysed whole blood prepared using BD Biosciences' proprietary stabilizing and drying technology can overcome those shortcomings and offer convenience, consistency and superior staining performance of control cells to flow cytometry end users.
Method: The lysed whole blood are first stabilized and encapsulated with drying matrices. The bound water is then removed with define parameters including time, humidity and temperature. The dried cells are screened with over 300 antibody clones using high throughput screening method. The cells are also tested for long term stability using the Arrhenius Model to determine the shelf life.
Results: The data suggest that BD Biosciences' dried cells maintain the morphology comparable to fresh blood as well as resolutions of all relevant leukocyte populations including small populations like basophils and dendritic cells. BD Biosciences' dried cells can be stained and detected with over200 markers. Accelerated stability study predicts long shelf life at 2 to 8 degree.
Conclusion: Our studies show that using our dried cells as daily quality controls have the potential to eliminate the need of fresh blood controls in the lab. Compared to other control manufactures, BD Biosciences' dried cells offer better staining performance to flow users.

101

Acoustophoretic Orientation of Red Blood Cells for Diagnosis of Red Cell Health and Pathology
Jordi Petriz[1], Laura G. Rico[1], Jordi Juncà[1], Mike Ward[2], Jolene Bradford[2]
[1]Jose Carreras Leukaemia Research Institute, BADALONA (BARCELONA), Spain
[2]Thermo Fisher Inc, EUGENE, OREGON, U.S.A.

Introduction: Distortions of the normal bi-concave disc shape for RBCs appear in a number of pathologies resulting from defects in cell membrane skeletal architecture, erythrocyte ageing, and mechanical damage. We present here a reagent free method for studying red blood cell (RBC) health and pathology that takes advantage of acoustophoretic orientation effects in the Attune™ NxT Flow Cytometer. The acoustic standing wave field the instrument applies to samples, orients non-spherical cells like RBCs prior to sheath focusing and laser analysis. This has the effect of modifying the distribution of the average light scattering cross-section of individual non-spherical cells, resulting in reproducible light scatter patterns indicative of the relative shapes in a red cell population.
Methods: Blood from healthy donors, from patients with hereditary spherocytosis, from ageing and transfused blood, and blood from a patient with an artificial heart valve, were used in this study. Two microliters of EDTA anticoagulated blood were mixed with 1 mL of Hank's Balanced Salt Solution. Samples were acquired on the Attune NxT Flow Cytometer. Acquisition was performed at the lowest possible sample rate of 12.5 µL/minute.

Acquisition was stopped when 15 µL of 1:500 diluted blood were collected, approximately 100,000 total cells.
Results: Our results show distinct RBC scatter patterns for fresh blood from healthy donors, blood from hereditary spherocytosis patients, ageing and transfused blood, and blood from a patient with an artificial heart valve. The unique patterns in the acoustic focusing instrument are compared with patterns in conventional hydrodynamic focusing instruments as well as with patterns produced when the acoustic field is alternately switched on and off.
Conclusions: Here we show the potentials of combining light scatter and acoustic flow cytometry to develop new approaches for evaluating the effects of storage and ageing on changes or damage to RBCs membranes, or to immediately evaluate the quality of erythrocytes following blood donation. Moreover, such approaches could also be very helpful across a number of research fields studying RBC health in diseases and other pathologies, including heart valve hemolysis as a consequence of mechanical trauma in patients with artificial valves, thermal damage or osmotic fragility. Abnormal distributions of erythrocytes can typically be detected after just 30 to 45 seconds of acquisition time using 1-to 2µL starting blood volumes.

102

Yellow-green laser next generation-based flow cytometry for CD34+ progenitor cell counting
Jordi Petriz[1], Laura G. Rico[1], Jordi Juncà[1], Mike Ward[2], Jolene Bradford[2]
[1]Jose Carreras Leukaemia Research Institute, BADALONA (BARCELONA), Spain
[2]Thermo Fisher Inc, EUGENE, OREGON, U.S.A.

Introduction: Current flow cytometry methods for human hematopoietic progenitor cell counting consist of a series of consensus steps. Most accepted and standardized protocols include a single-platform strategy, absolute counting beads, lyse no-wash procedures and single laser excitation at 488nm for PE-CD34+/FITC-CD45dim/7-AADneg cell counting. The ISHAGE protocol is the most widely accepted method for CD34+ progenitor cell counting. This methodology follows international optimized guidelines for different monoclonal antibody clones and fluorescent conjugates or nucleic acid dyes for excluding dead cells. Phycoerythrin (PE) is the most preferred flurochrome for CD34 monoclonal antibody conjugation for the identification of human hematopoietic progenitor cells. PE is an intensely bright phycobiliprotein, its maximum absorption peak is at 565nm with a secondary absorption peak at 496nm. The availability of yellow-green (YG) laser equipped next generation flow cytometers has enabled us to revisit the ISHAGE-based method to propose a series of improvements aimed at CD34+ progenitor cell automated counting using no color compensation and blue and yellow laser excitation.
Methods: Blood samples were obtained from patients receiving G-CSF for stem cell mobilization and prepared following the ISHAGE guidelines. All cell measurements were done using the Attune™ NxT Flow Cytometer (Thermo Fisher Scientific). Side scatter (SSC) was detected using the blue laser at 488 nm and a 488/10 bandpass filter. FITC and 7AAD were detected using the blue laser at 488 and a 530/30 and 695/40 band pass filter, respectively. PE was detected using the blue laser at 488 nm and a 590/40 bandpass filter and the YG laser at 561 nm and a 584/16 bandpass filter.
Results: In this study, sample preparation, gating strategy and analysis method accurately followed the ISHAGE guidelines. By combining blue laser PE emission with yellow-green PE emission, an interesting approach for CD34+ cell counting was obtained. Plotting PE signal from the different lasers against each other results in the display of CD34+ cells in a defined narrow line, with few exceptions. This line is a result of the ratio of specific spectral signal from PE fluorescence from the 561 nm laser and the combined PE and FITC fluorescence in the PE channel from the blue laser.
Conclusions: This new approach shows the feasibility of CD34+ counting without color compensation for the YG laser and that combined excitation of FITC-CD45 and PE-CD34 with YG lasers can be applied to develop new ratiometric strategies aimed at automated CD34+ counting methods.

103

Detection of endogenous alkaline phosphatase activity in intact leukemic cells
Jordi Petriz[1], Laura G. Rico[1], Jordi Juncà[1], Mike Ward[2], Jolene Bradford[2]
[1]Jose Carreras Leukaemia Research Institute, BADALONA (BARCELONA), Spain
[2]Thermo Fisher Inc, EUGENE, OREGON, U.S.A.

Introduction: Alkaline phosphatase (ALP) is an enzyme highly expressed in pluripotent stem cells, embryonic stem cells, and embryonic germ cells. It has been classically used as a histochemical marker for the detection of these cells. Alkaline phosphatase activity is altered in some disease states, such as leukemia and lymphoma. With the aim to detect candidate malignant primitive progenitor populations in human leukemia and lymphoma cells, we modified the original ALP stem cell detection method based on the identification of alkaline phosphatase

fluorescent cells in combination with flow cytometry immunophenotyping.
Methods: Peripheral blood and bone marrow samples from leukemia and lymphoma patients were studied at diagnosis, for minimal residual disease monitoring, and relapse. ALP staining was combined with leukemia immunophenotyping and no-lyse no-wash methods using the Attune™ NxT Flow Cytometer (Thermo Fisher Scientific). Blue Side scatter (B-SSC) and violet SSC (V-SSC) were detected to discriminate leukocytes. Vybrant™ DyeCycle™ Violet stain (DCV) was also used to discriminate nucleated cells from erythrocytes debris. Alkaline Phosphatase Live Stain was obtained from Thermo Fisher Scientific.
Results: Preliminary data obtained in our laboratory have shown that ALP can be expressed at high levels in leukemia. By using these newly developed panels, leukemic cells can be classified into different ALP functional states. Prospective comparison and classification of ALP+ cells also shows different subsets of primitive leukemic cells.
Conclusions: Our results suggest that the main differences in the activity of the enzyme, accordingly with previous observations showing that primitive stem cells express the highest phosphatase activity, could help to identify and differentiate new oligoclonal/pseudoclonal populations in patients with neoplastic malignancies. We have verified that this method gives accurate and reproducible measurements and our preliminary results suggest that ALP^{high} leukemic cells appear to sustain leukemogenesis over time.

104

Enhanced cellular discrimination using a novel optical microsystem
Antoine Leblanc-Hotte[1], Sylvie Lesage[2], Yves-Alain Peter[1], Jean-Sébastien Delisle[2]
[1]Polytechnique Montreal, MONTREAL, Canada
[2]Centre de recherche Hôpital Maisonneuve-Rosemont, Université de Montréal, MONTREAL, Canada

Introduction: Flow cytometry uses two non-fluorescent parameters, forward-scatter (FSC) and side-scatter (SSC), for distinguishing cellular physical properties. Additional non-fluorescent cellular characteristics that could be exploited to facilitate the separation of distinct cell types include the cell refractive index and mechanical deformability. As such, we developed a novel optical interrogation method which can perform refractive index distribution measurements along mechanically deformed cells. This novel micro-optical sensor is a silicon-based optical resonant cavity yielding a high-throughput measurement rate.
Methods: Myeloid cells derived from a promyelocytic leukemia cell line (HL60) were differentiated into basophils, neutrophils or maintained in a baseline differentiation state. Human osteosarcoma cell line (U2OS) were synchronized in the G0/G1 and G2/M phases. Following injection in a microchannel and sheathless focusing, cells were analyzed while flowing through the optical cavity volume. An engineered microfluidic network allowed for shear-stress induced mechanical deformation on cells to be promoted or repressed while maintaining their viability. This network also precisely focused and ordered cells with preferred interparticle spacing for an optimal measurement configuration. Resonant optical power variations were recorded for each cell passing through the sensor's optical cavity. The resulting curves were then compared against each other based on multiple extracted parameters.
Results: Our microsystem offered a substantial improvement on the discrimination of cellular populations compared to flow cytometry. In all replicated experiments, neutrophils showed segmented nuclei, were slightly smaller and less dense thus yielded reduced refractive index curves compared to basophils and the baseline condition. Importantly, the FSC-SSC based flow cytometry could not distinguish between any of the three differentiated myeloid cells populations whereas the microsystem could readily distinguish two of these cell types based exclusively on the refractive index. Moreover, osteosarcoma cells in G0/G1 and G2/M phases yielded distinct asymmetrical refractive index curves due to their respective induced deformation.
Conclusions: The differentiated myeloid cells cannot be effectively separated based on FSC and SSC, whereas the refractive index distribution measurements in our microsystem facilitated the resolution of cellular populations. Asymmetric refractive index distributions were recorded with deformed osteosarcoma cells. Employing deformability should further improve cellular resolution. Thus, cellular refractive index distribution associated with deformability is a new physical characteristic enhancing cellular discrimination and improving flow cytometry capabilities. The presented microsystem readily offers complementary measurements to FSC and SSC. We project to integrate our microsystem within a flow cytometry unit to improve cellular resolution and provide crucial information for research and clinical professionals.

105

Immunophenotypic features of bone marrow tumor cells in Burkitt lymphoma/leukemia: implications for B-lineage acute lymphoblastic leukemia diagnostics

Irina Demina[1], Tatiana Verzhbitskaya[2], Svetlana Kashpor[1], Svetlana Plyasunova[1], Maria Dubrovina[1], Larisa Fechina[2], Natalia Myakova[1], Elena Samochatova[1], Alexey Maschan[1], Alexander Popov[1]
[1]National Research Center for Pediatric Hematology, Oncology and Immunology, MOSCOW, Russian Federation
[2]Regional Children Hospital / Research Institute of Medical Cell Technologies, EKATERINBURG, Russian Federation

Introduction: Bone marrow tumor blasts immunophenotyping is an essential part of Burkitt lymphoma/leukemia (BL) and B-cell precursor acute lymphoblastic leukemia (BCP-ALL) differential diagnostics. Nevertheless immunoglobulin heavy and light chains detection on the cell surface could meet several biological and methodological pitfalls. In addition, the weak expression of IgM could be found on the blasts surface in patients with BCP-ALL. Thus the aim of the present study was development of additional BL immunophenotypic criteria.
Methods: Totally 105 pediatric patients (55 boys and 50 girls) aged from 1 to 16 years were studied. Leukemic blasts' antigen profile in 21 BL cases (study group) and 84 children with BCP-ALL (control group) was compared in a retrospective way. Immunophenotyping of bone marrow tumor cells was performed using 6-8 colors flow cytometry. Expression of CD10, CD19, CD20, CD22, CD24, CD34, CD45, CD58, CD38, iCD79a, NG2, CD13, CD33, CD117, CD15, heavy and light chains of immunoglobulins was studied.
Results: Antigen expression patterns in BL and BCP-ALL were significantly different. In addition to the well-expected differences in surface and cytoplasmic light and heavy IgM chains expression, the number of patients positive for CD20, CD45, CD34, CD58 and myeloid antigens also was not the same. In 47 (56%) BCP-ALL cases coexpression of at least one of the myeloid markers was detected on blast cells. Myeloid antigens were never found in BL patients. All patients with BL also lacked expression of CD34 and CD58, while CD10, CD38, CD20 and CD45 were expressed very homogeneously. The intensity of CD10 expression in BCP-ALL was significantly higher than in BL ($p<0.001$). Moreover due to vacuolization BL cells presented higher side-scatter ($p=0.007$). Nevertheless there was no single marker with high accuracy in distinguishing BL and BCP-ALL. Multiparametric analysis allowed much more precise differentiation between these two tumor types. In present study all cases without myeloid coexpressions and with CD34-negativity and high CD20-positive cells proportion belonged to BL (diagnostic accuracy 100%).
Conclusions: It was shown that by surface markers expression BL is significantly different from BCP-ALL. Thus even in case of ambiguous or negative results for surface IgM staining these two tumors distinguishing is still possible by complex immunophenotype analysis.

106

Immunophenotypic heterogeneity of acute leukemia meeting the early thymic precursor (ETP) criteria

Olga Illarionova[1], Tatiana Verzhbitskaya[2], Grigory Tsaur[2], Larisa Fechina[2], Svetlana Plyasunova[1], Alexander Karachunskiy[1], Alexander Popov[1]
[1]National Research Center for Pediatric Hematology, Oncology and Immunology, MOSCOW, Russian Federation
[2]Regional Children Hospital / Research Institute of Medical Cell Technologies, EKATERINBURG, Russian Federation

Introduction: Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) is a rare subgroup of ALL with distinct biological features and poor outcome. ETP-ALL immunophenotypic features by definition are: dim CD5 expression (less than 75% of positive cells), CD1a- and CD8- negativity, positivity for at least one of myeloid or stem cell markers (E. Coustain-Smith et al, Lancet Oncol., 2009). The aim of present study was to describe immunophenotypic variability of ALL cases fulfilling these criteria.
Methods: Totally 34 (13 adults and 21 children) ALL patients with ETP-immunophenotype were studied. Initial immunophenotypic diagnostics was performed by multicolor flow cytometry. T-lineage ALL subtypes as well as biphenotypic AL (BAL) and bilineage AL were classified according EGIL scoring system. Mixed-phenotype AL (MPAL) was established according to WHO criteria.
Results: Among studied patients 8 (23.5%) were diagnosed as TI-ALL, 16 (47.1%) – as TII-ALL, while 5 (14,7%) – as BAL. In 5 bilineage AL cases (14.7%) one of two blast cell populations was classified as ETP-ALL. Moreover 10 patients (29.4%) also met MPAL criteria. In 30 of 34 cases (88.2%) tumor cells expressed at least one stem cell marker (CD34 and/or CD133), while in 10 cases (29.4%) both of them were present. In 27 patients (79.4 %) blasts were positive for at least one myeloid antigen including two cases of MPO expression. Median number of expressed myeloid antigens was 2. The most frequent myeloid antigens were CD33 (19 cases) and CD117 (17 cases), while CD13, CD11b and CD15 were found less frequently (10, 10 and 9 cases respectively). 13 of 16 TII-ALL cases were CD2-positive, while only 8 patients were completely CD5-negative. CD5 was weakly expressed by at least 20% cells in remaining 8 ones. ETP-ALL was diagnosed due to cells positivity for only one myeloid/immature

marker in 5 cases. B-lineage antigens CD19 and CD10 were concomitantly expressed in one and two patients respectively.
Conclusions: Thus ETP-ALL is a rather heterogeneous AL subgroup including not only patients with very immature T-cell phenotype (TI-ALL by EGIL score) but also more mature TII-ALL cases as well as various types of AL of ambiguous lineage. Moreover myeloid immunophenotypic features were found not in all studied cases. Thus probably stricter immunophenotypic criteria (i.e. complete CD5-negativity etc) should be introduced for more precise diagnostics of early thymic precursor leukemia.

107

Pre-activated mesenchymal stromal cells prolong allograft survival in a pre-clinical model of corneal transplantation
Kevin Lynch, Oliver dr. Treacy, Xizhe dr. Chen, Grace ms O'Malley, Nick mr Murphy, Paul dr. Lohan, Serika D dr. Naicker, Aideen dr. Ryan, Thomas prof. Ritter
Regenerative Medicine Institute, GALWAY, Republic of Ireland

Introduction: The long-term (>5 years) prognosis of high-risk corneal transplantation is quite low (≤50%). Novel therapies, independent of or in coordination with, corticosteroids are needed. The ideal treatment would induce a state of immune unresponsiveness without dependency on systemic immunosuppressive drugs. Pre-activated syngeneic mesenchymal stromal cells (MSC) have demonstrated an ability to modulate immune cells *in vivo*. In this study, transforming growth factor beta 1 treated (TGF-β) MSC (TGFβ-MSC) were administered as a treatment in a murine model of corneal transplantation and rejection free survival (RFS) of corneal grafts were observed over 30 days.
Methods: MSC were isolated from BALB/c mice and extensively characterized *in vitro*. To determine if TGFβ-MSC displayed enhanced immunoregulatory ability, they were co-cultured in mixed lymphocyte reactions (MLRs). MSC and TGFβ-MSC were co-cultured at different MSC:T-cell ratios for 96hrs. T-cell proliferation, activation, death and differentiation were determined by flow cytometry.
Balb/c mice served as recipients to fully allogeneic C57BL/6 donor corneas. MSC were administered to recipient mice at day +1 ($1x10^6$ cells intravenously) and +7 ($1x10^6$ cells intravenously) post-transplantation. Mice were untreated, treated with MSC or TGFβ-MSC and RFS of grafts were observed over 30 days using corneal opacity as the main indicator of rejection. Neovascularization and corneal oedema were also observed as secondary indicators.
Results: *In vitro*, TGFβ-MSC displayed an enhanced ability to inhibit the proliferation of lymphocytes when compared to MSC alone (n=4). TGFβ-MSC inhibited the proliferation of hyper-stimulated $CD3^+TCR\text{-}\beta^+CD4^+$ lymphocytes (43.8% proliferation ±7% to 16.37% proliferation ±7.7%, n=4). Also, significantly increased $CD3^+TCR\text{-}\beta^+CD4^+FoxP3^+$ lymphocyte frequency was observed in TGFβ-MSC (8.71%±1.64) cultures when compared to MSC alone (4.45%±1.6%, n=4).
In vivo, untreated allogeneic control grafts were uniformly rejected (RFS 20.5±7.75d, n=12). Untreated MSC were not efficacious in prolonging RFS (RFS 18.28±6.71d, n=14) with no difference noted with or without treatment. in RFS. In contrast, corneal allograft rejection was significantly delayed in 70% of TGFβ-MSC treated allograft recipients (RFS 27.23±4.32d, n=13) with significantly lower mean corneal neovascularization and corneal oedema scores observed at d17-19.
Conclusions: Syngeneic TGFβ-MSC have an enhanced ability to prolong RFS when compared to untreated MSC (21% to 70%) in the absence of any other immunosuppressive therapy. They also significantly reduce both neovascularization and corneal oedema scores at the average day of rejection indicating a lower infiltration of immune cells. This study points toward the potential of using MSC as a therapeutic treatment to improve and diversify cellular-based therapies.

108

Thrombosis and Paroxysmal Nocturnal Hemoglobinuria Clones
Jos Pouwels, Martin Horst van der, Miranda Krabben
Treant Zorggroep location Scheper, EMMEN, Netherlands

Introduction: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare disorder which can be life-threatening. The disease is caused by abnormalities in the X-linked phosphatidyl-inositol-glycan class-A gene resulting in the inability to make glycosyl-phosphatidyl-inositol (GPI) anchors. Red blood cells in this patients show an abnormal sensitivity to complement-mediated lysis due to the deficient GPI-bound proteins CD55 and CD59. This hemolysis can lead to thrombosis initiated by several mechanisms. Some publications suggests that a small population of GPI-deficient

cells can be found in patients with otherwise inaccountable venous thromboembolism (VTE). These publications show a large difference in both the prevalence of GPI-deficient subjects and clone size. Our goal was to verify these percentages thereby support decision making to screen GPI-expression in *de novo* idiopathic VTE-patients. **Methods:** The study was approved by the institutional review board (NL56506.042.16). We included patients with one or more episodes of objectively demonstrated idiopathic DVT and/or PE or mesenterial or cerebral thrombosis. Blood samples of 201 patients were collected after obtaining informed consent. The analyzes were performed according to the guidelines published by Borowitz et al. for testing on GPI-deficient cells. Neutrophilic granulocytes and monocytes were tested by flow cytometry with a FACSCanto II-BectonDickinson within 48h. Stopping gate was on the neutrophilic granulocytes at 250.000 cells. Consequently, the sensitivity of the test was at least 0,01%. Red blood cells were not tested because hemolysis may underestimate the size of the clone. **Results:** Two patients (1%) had a detectable number of GPI-deficient cells in the neutrophilic granulocytes population (size 0,03%). Both patients had also GPI-deficient cells in the monocyte population although the percentage did not exceed the sensitivity limit due to the small number of tested cells. Two patients had a percentage of 0,01% GPI-deficient cells in the granulocytic population which was the treshold of the test. The patients were in the group of DVT. Due to the small number of GPI-deficient cells, all patients did not have symptoms of (pan)cytopenia and/or hemolysis. **Conclusions:** This small study did not support the conclusions of other investigations that screening of VTE-patients on GPI-expression might be of additional value for prognosis and therapy. Screening of VTE-patients might be better constrained to patients with signs of hemolysis as stated by Lazo-Lagner et al. Based on our findings and the results of Lazo-Langner we decided not to test, on a regular basis, *de novo* idiopathic VTE-patients on the presence of small GPI-deficient clones.

109

Human bone marrow mesenchymal stem cells expansion and multipotency is strongly influenced by the electroactivity of the culture substrate

María Noel Tamaño[1], Estela Carvalho[2], Lourdes Cordón[3], Leonor Senent[3], Daniela M Correia[2], Clarisse Ribeiro[2], Senentxu Lanceros-Méndez[2], Roser Sabater[4], José Luis Gómez-Ribelles[4], Amparo Sempere[3]
[1]Universidad Politécnica de Valencia, VALENCIA, Spain
[2]Departamento de Física Universidade do Minho, BRAGA, Portugal
[3]Grupo de Investigación en Hematología, Instituto Investigación Sanitaria La Fe, VALENCIA, Spain
[4]Centro de Biomateriales e Ingeniería Tisular Universidad Politécnica Valencia, VALENCIA, Spain

Introduction: Mesenchymal stem cells (MSCs) can be expanded adhered on flat supports to high numbers, while maintaining their differentiation potential towards cells belonging to the musculoskeletal lineages, such as adipocytes, osteoblasts, and chondrocytes. Nevertheless, cell-material interaction plays an important influence on cell response in terms of proliferation and maintenance of multipotency. Poly(vinylidenefluoride) (PVDF) is a biocompatible polymer that has been proposed for the culture of MSCs under electro-mechanical stimulation due to its piezoelectric properties (generation of an electrical potential variation upon mechanical stimulation and *vice versa*) when crystallized in the highly polar b phase. On the other hand, α-PVDF is non-polar and does not show piezoelectricity. In this work we analyze the response of human bone marrow MSCs, (hBMMSCs) cultured in PVDF substrates in different crystalline phases.
Methods: hBMMSCs were cultured on α-phase or β-phase PVDF films in basal medium. Cell viability, proliferation and morphology were evaluated at day 5 (before reaching confluence) and at day 28. Cell surface markers of hBMMSCs (CD105, CD90 and CD73) were analyzed by flow cytometry (FC), while expression of adipocyte, chondrocyte or osteoblast spontaneous differentiation was assessed by Q-PCR. Tissue culture polystyrene (TCPS) was used as control. All substrates were coated with fibronectine before culture and cells were seeded without serum.
Results: First FC analysis revealed that all hBMMSCs were negative for the markers CD34, CD45, CD19 and CD14, only around 8% of the cells were positive to HLA-DR, and positive for the mesenchymal marker CD105 (up to 99%), the cell adhesion molecule CD90 (around 80%) and the membrane-bound enzyme CD73 (up to 98%). Cells attached and proliferated similarly to the TCPS control in both types of PVDF substrates before becoming confluent. Nevertheless, for longer culture times β-phase inhibited proliferation, decreased the expression of CD105 and CD90 markers, significantly influenced cell morphology, while the behavior on α-phase PVDF remained similar to control.
Conclusions: The crystalline order of PVDF polymer chains at the surface of the culture support has a strong influence on cell response even if the chemical structure remains unchanged. Cell-biomaterial interaction mediated by adhesion proteins is determined by the presence of net electrical surface charge related to the oriented dipoles of the β-phase PVDF *all-trans* chains.

110

Cerebro-Spinal Fluid lymphocyte counts in various neurologic conditions

Ling Yang, Komal Abhishek Dudhatra, Te Chih Liu
National University Hospital, SINGAPORE, Singapore

Introduction: Traditional testing of Cerebro-Spinal Fluid (CSF) has focused largely on the determination of Central Nervous System (CNS) infections. The availability and enhanced accuracy of Flow Cytometry (FCM) has increasingly prompted physicians to also send a sample for lymphoma screening when investigating CSF taken from patients with unexplained neurologic symptoms. We report here a retrospective review of these requests and compare them against their eventual neurology diagnosis at discharge.
Methods: 125 paired samples, without blood contamination (RBC <1000/μL), were analysed between 2013 and 2017. The patients were grouped into 8 disease categories based on their eventual diagnosis at discharge (*Stroke/dementia, Degenerative Spine / Disc disease, Metabolic disorders, Malignancy, Demyelinating disorders, Immune-mediated disorders, infections, Others*). CSF samples were collected in Transfix medium (Cytomark, UK), pre-washed once and then stained with Euroflow lymphoma screening lyotube (BD Bioscieces, USA). Twelve markers in 8 colors are included in this panel: CD8+smLamda FITC, CD56+smKappa PE, CD5 PerCP 5.5, CD19+TCRγ/δ PE-Cy7, CD3 APC, CD38 APC-H7, CD4+CD20 V450 and CD45 V500-C. Samples were then lysed, washed, fixed and acquired with BD FACS CantoII flow cytometer. Percentages of WBC subsets were assessed by flow cytometry. Absolute WBC and RBC count of undiluted CSF samples were manually performed with a neubauer counting chamber.
Results: There were 2 cases of lymphoma in the study cohort and are grouped with the *malignancy* category. As expected, patients with *infection* (meningitis, encephalitis etc) had high lymphocyte and monocyte counts. Patients with *Degenerative Spine / Disc diseases, Stroke / Dementia* had low monocyte and lymphocyte counts (median lymphocyte count <1/μL). The lymphocyte population present in these patients comprises almost entirely T-cells. The other disease categories had mild monocytosis and lymphocytosis (median lymphocyte count 0-2.9/μL). Their CSF also showed small numbers of B (median 0.8% – 3.1%) and NK-cells (median 0.7% - 1.6%) though T-cells remain the predominant lymphocyte population present. There was no difference in the CD4/CD8 ratio between any of the 8 categories.
Conclusions: While lymphomatous involvement of the CNS is a rare finding, many diverse aetiologies can give rise to neurologic symptoms. Our results suggest that many neurologic conditions are associated with mild increases in the monocyte, total lymphocyte, B and NK-cell counts. A 'normal' CSF sample without these changes may indicate that the cause of the neurologic symptoms is of an anatomic nature as seen with *Degenerative spine / disc disease, Stroke or dementia* rather than an inflammatory cause.

111

Endoplasmic Reticulum (ER) stress and lysosomal/mitochondrial re-organization induced by lysates of different Campylobacter jejuni strains

Barbara Canonico[1], Gianna di Sario[2], Raffaella Campana[2], Erica Cesarini[2], Ozan Gundogdu[3], Francesca Luchetti[2], Sara Gabrielli[2], Loris Zamai[2], Claudio Ortolani[2], Maria Gemma Nasoni[2], Wally Baffone[2], Stefano Papa[2]
[1]UNIVERSITY OF URBINO CARLO BO, URBINO, Italy
[2]University of Urbino Carlo Bo, URBINO, Italy
[3]London School of Hygiene and Tropical Medicine, LONDON, United Kingdom

Introduction: *Campylobacter jejuni* bacterium is a common component of mammal intestinal microbiota associated with most gastroenteritis in humans. Guillain-Barrè syndrome (GBS) is a neuropathy strictly correlated to *C. jejuni* infection. Recent studies have suggested that monocytes/macrophages may play a critical role in the onset of GBS. We studied the interaction of peripheral blood monocytes and U937 (human leukemic monocyte lymphoma cell line) cells with lysates of two CDT (cytolethal distending toxin) producer wild type strains, *C. jejuni* ATCC 33291 and *C. jejuni* ISS 1, and a *C. jejuni* 11168H *cdtA* mutant strain.
Methods: Cells were analysed in order to investigate CD14, CD54 and CD59 alterations, mitochondrial and lysosomal features, p53 and Bcl-2 status, ER stress and lysosomal exocytosis. Rapamycin administration (2 hours before lysate addition) affected subcellular events, particularly for lysosomal and ER alterations.
Results: U937 cells preincubated with lysates from *cdtA* mutant and ATCC 33291 strains showed significant differences with or without rapamycin addition, whereas cells preincubated with ISS 1 strain showed an almost constant behaviour.

Conclusions: CDT seems to induce multiple signaling pathways, modulated by mTor (Mammalian target of *rapamycin)* signaling. Following interactions with the different lysates, autophagic cell death coexist with apoptosis, partly promoted through ER stress and lysosomal re-organization in a degree depending on the specific strains.

112

Preanalytical phase in flow cytometric evaluation of bone marrow

Mikhail Drokov, Popova Natalia, Yulia Davydova, Valentina Dvirnyk, Anna Kuchmiy, Alexander Vdovin, Larisa Kuzmina, Daria Dubnyak, Irina Galtseva, Nikolay Kapranov, Vera Vasilyeva, Olga Koroleva, Ekaterina Mikhalcova, Zoya Konova, Grigory Efimov, Elena Parovichnikova, Valery Savchenko
National Research Center for Hematology, MOSCOW, Russian Federation

Background: During last 25 years multiparametric flow cytometry (MFC) become a meaningful tool in evaluation of patient's bone marrow (BM). Despite standardization of staining protocols and antibody panels, preanalytical phase for MFC test particulary for rare events (e.g. MRD) is still ignored except the common words of "first drop".
Aim: Evaluate preanalytical phase in multiparametric flow cytometry evaluation of bone marrow
Material and methods: Two portions of BM were obtained from same puncture hole on posterior superior iliac spine by Jamshidi-type aspiration needle (9G) from healthy BM donors (n=10). Cellularity (number of cells per µl) of first (1^{st}) (500 µl of BM in EDTA tube) and second (2^{nd}) portion were evaluated on Sysmex XE-2100 hematology analyzer.
Results: Cellularity in 1^{st} portion of bone marrow was $133.1x10^9$ cells/µl ($42.2\text{-}321.5x10^9$ cells/µl) and $40.5x10^9$ cells/µl ($13.8\text{-}157.9x10^9$ cells/µl) in 2^{nd} portion respectively **(p=0.007*)**.
Discussion: Here we reported that cellularity of BM samples even from healthy donors can vary up to 10 times due to sequence of BM portion. In MFC MRD tests this "differences", due incorrect preanalytical phase, lead to significant decrease in sensitivity to the same number of times as changes in cellularity of different BM portions (up to one $\log_{10}$). For tests with high sensitivity such as MFC -based MRD test it can be very important and disregarding or ignoring preanalytical phase lead to mistakes not only on analytical phase (in an area of responsibility of laboratory staff), but on treatment phase also, particularly when MRD-based strategy of treatment used .

113

Development and validation of flow cytometry panels: A laboratory experience

Chryso Pierides, Laura Koumas, Andrie Mitsidou, Rafaella Gavrielidou, Elena Socratous, Paul Costeas
Karaiskakio Foundation, NICOSIA, Cyprus

Introduction: Flow cytometry serves as a powerful analytical tool for the diagnosis and monitoring of hematological malignancies. As a matter of good laboratory practice, health care institutions are required to validate their assays, leading to their accreditation which indicates compliance with standards developed by an official agency. However, there are currently insufficient guidelines for the validation of flow cytometric assays. Herein, we report our laboratory experience for the validation and accreditation of our methods.
Methods: It is known that when designing a flow cytometry panel, one must consider matching the fluorochromes by brightness to lowest density antigens, minimize spill over and normalize the signal to the background. Accuracy, recovery, precision and linearity are also some of the parameters used for the development and validation of antibody panels and assays. Once the panel is designed and the right fluorochromes are selected, antibodies are titrated and the gating strategy is established. The panels are then tested against available reference material and quality control (QC) samples and the accuracy of the method is evaluated. Method effectiveness is determined by calculating the recovery rate, whereas precision is based on result repeatability and reproducibility. Finally, linearity is expressed in both graphical and mathematical formats, determining the dependent variable.
Results: Method validation was performed for Lymphocyte Subpopulation Immunophenotyping (CD3+, CD3+/CD4+, CD3+/CD8+, CD19+ and CD3-/CD16+CD56+), General Investigation and Lymphoproliferative Disease Investigation Immunophenotyping, PNH Detection (presence or absence of PNH clones in Red Blood Cells (RBCs) and Granulocytes/Monocytes), ALL-MRD Detection (MRD Population %), CD34 Stem Cell Enumeration (CD34+% Total WBCs, CD34+ Number/µl and CD34+/Patient Kg) and T cell enumeration (Lymphocytes, CD3+, CD3+/CD4+ and CD3+/CD8+). For the purpose of the validation/verification of our methods and for every parameter tested, we determined trueness, accuracy, precision and uncertainty. Based on the values obtained, the methods were indicated as appropriate and trustworthy for the purpose of our operation. Our laboratory is ISO 15189:2012 accredited for flow cytometry, as it satisfied all requirements for quality and competence.

Conclusion: Even though there is a published approach by Euroflow in clinical flow cytometry standardization, modular design of panels may be needed in order to adjust to the everyday routine of each laboratory. The development of standard guidelines for the validation of flow cytometric assays based on the above mentioned parameters and appropriate strategies, will increase their capacity and at the same time reduce variability.

114

Assessment of mesoporous silica nanoparticles toxicity by flow cytometry

Iris Garrido-Cano[1], Iris Garrido-Cano[1], Vicente Candela-Noguera[2], Guadalupe Herrera[1], Ana Lluch[3], Ramón Martínez-Máñez[4], Pilar Eroles[3]
[1]INCLIVA, VALENCIA, Spain
[2]IDM, VALENCIA, Spain
[3]CIBER de Oncología (CIBERONC), VALENCIA, Spain
[4]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), VALENCIA, Spain

Introduction: Mesoporous silica nanoparticles (MSNs) are biocompatible solid materials with a porous structure. Their vital advantages are large surface area, big pore size, chemical and physical stability. Additionally, it is possible to regulates their morphology and pore size[1]. Because of its peculiar features, MSNs are being studied for multiple biomedical applications such as medical imaging, diagnostic, biosensors, or controlled drug delivery [2]. Therefore, studying the cellular toxicity of MSNs is essential for the biomedical field applications.
Methods: To carry out this study, we synthesized the MCM-41, which is a widely used MSNs. The human breast cell lines MDA-MB-231 and MCF10A were exposed to different concentrations (0 - 250 µg/mL) of nanoparticles from 0 to 24 hours and stained with tetramethylrhodamine (TMRM), monochloromobimane (MCB), 2',7'-dichlorodihydrofluorescein diacetate (H2DCF-DA) or FLUO-4 along with propidium iodide or Zombie Aqua Fixable Viability Kit (Biolegend) as viability markers. Samples were acquired using BD LSRFortessa (BD Biosciences), and data were analysed by FlowJo (FlowJo, LLC). Results were normalized respect to negative control.
Results: The comparison between MSNs-exposed cells to non-exposed cells, revealed that there are no morphological changes (FSC/SSC) and no significant differences in TMRM, MCB, H2DCF-DA, or FLUO-4 staining (p_value>0.05), indicating that MSNs do not cause mithocondrial damage, ROS production or cell death, even at high concentrations of nanoparticles.
Conclusions: Our results indicate that flow cytometry is a suitable technique to evaluate toxicity at cellular level, which can be useful to study the toxicity of diverse MSNs with different cargos and coating molecules. Furthermore, we have demonstrated that MSNs do not cause cellular damage in human breast cells, which is essential for the acceptance of MSNs for clinical use. Thus, it is necessary to study the toxicity level of nanoparticles to unravel how the nanoparticles can affect different tissues.
Wang, Y. *et al.* Mesoporous silica nanoparticles in drug delivery and biomedical applications. *Nanomedicine Nanotechnology, Biol. Med.* **11,** 313–327 (2015).
Mamaeva, V., Sahlgren, C. & Lindén, M. Mesoporous silica nanoparticles in medicine-Recent advances. *Adv. Drug Deliv. Rev.* **65,** 689–702 (2013).

115

Endothelial Progenitor Cells and Diabetic Peripheral Neuropathy

Nikolitsa Kafasi[1], Natalia Dimitrakopoulou[2], Ioanna Eleftheriadou[2], Aglaia Dimitrakopoulou[3], Edward Jude[4], Nikolaos Tentolouris[2]
[1]Laiko General Hospital, GALATSI, Greece
[2]1st Dept of Propaedeutic and Internal Medicine, Medical School, National and Kap, ATHENS, Greece
[3]Dept of Immunology & Histocompatibility, Laiko General Hospital, Athens, Greece, ATHENS, Greece
[4]Tameside General Hospital, Ashton-Under-Lyne, Lancashire, UK, ASHTON-UNDER-LYNE, United Kingdom

Background and aims: Endothelial progenitor cells (EPCs) are a population of adult stem cells with the ability to differentiate into epithelial cells and to promote endothelial regeneration and neo-vascularization in response to tissue ischemia. Several studies have already reported an association between EPCs reduction/dysfunction and diabetic macrovascular complications. Although Peripheral Neuropathy (PN) has been associated with changes in the microcirculation and reduced endothelial-dependent and endothelial-independent vasodilation, regardless of the presence of macrovascular disease, data about its association with EPCs are scarce. The aim of the present study is to evaluate the relationship between PN and EPCs in patients with type 2 diabetes mellitus (DM).

Materials and methods: A total of 69 patients with DM (30 without PN (♀/♂: 14/16, aged 61.1±7.8) and 39 with PN (♀/♂: 19/20, aged 62.9±8.1) and 20 healthy controls (♀/♂: 15/5, aged 58.5±9.4) were recruited. Participants were non-smokers and had no clinical macrovascular disease. After venipuncture, Peripheral Blood Mononuclear Cells (PBMCs) were obtained and stained with monoclonal antibodies against CD45, CD34, CD309 and CD133. $1x10^6$ events per subject were acquired and analyzed with the six-color flowcytometer BD FACSCanto using the modified ISHAGE protocol. EPCs were defined as cells expressing the $CD45^{dim}/CD34^{+}/CD309^{+}/CD133^{+}$ phenotype. Statistical analysis was performed with SPSS 17.
Results: Our non-PN patients had: median disease duration 13 ys (range 5.0-20.0), retinopathy 4/30 (13.3%), hypertension 20/30 (66.7%), dyslipidemia 22/30 (73.3%), BMI 32.2±5.9 kg/m2, HbA1c 6.7% (range 6.1-7.4). The PN patients had: median disease duration 12 ys (range 10.0-20.0), retinopathy 10/39 (25.6%), hypertension 31/39 (79.5%), dyslipidemia 25/39 (64.1%), BMI 31.4±5.2 kg/m2, HbA1c 6.6% (range 6.2-7.1). Characteristics of the control group: 2/20 (10.0%) presented hypertension, 4/20 (20.0%) dyslipidemia, BMI 26.5±4.4 kg/m2, HbA1c 5.3% (range 5.2-5.4). EPCs median was found in the non-PN patients 24 (range 13-38), in PN 43 (range 29-67) and in controls 18 (range 12-38).
EPCs numbers differed significantly between the 3 groups of participants (p=0.001). Sub-analysis showed that patients with PN had significantly higher number of EPCs when compared with patients without PN (p=0.005) and controls (p=0.001). No significant difference was observed in EPCs numbers between patients without PN and controls (p=0.372).
Conclusions: EPCs numbers were significantly higher in patients with PN in comparison with patients without PN and controls. This may imply an effort for restoration of the damaged peripheral nerves. More research is warranted to clarify the role of EPCs in diabetic PN.

116

Validation of a Flow cytometry density assay to support anti-CD38 antibody clinical program in Multiple Myeloma patients
Wilfried Passe-Coutrin
Sanofi R&D, ALFORTVILLE, France

A receptor density (RD) assay using a direct staining was developed using the flow cytometry approach to measure the CD38 receptor absolute density on plasma cells, monocytes, lymphocytes and PMN in bone marrow samples collected from Multiple Myeloma (MM) patients. In addition to buffers, lysis solution and fixating reagents, the core RD kit is designed with a paired reagents consisting of standardized calibration phycoerythrin-coated beads (PE-calibrator; BioCytex) and characterized phycoerythrin-conjugated anti-CD38 antibody. The RD assay was fully validated on cell lines and whole blood samples from healthy volunteers. The real time stability and performance of the kit is routinely monitored. Using bone marrow aspirates from MM patients, the assay performance was evaluated based on the stability of sample before processing, then on the stability of immunostained samples after cell fixation. In addition, the repeatability and reproducibility of the beads coating were assessed. This assay is currently used worldwide on clinical sites; samples are processed by well-trained operators on clinical sites and the sample acquisition and data analysis are centralized.

117

Detection of Circulating Tumor Cells: Comparison between molecular and cytometric methods
Dimitrios Athanasios Ntanovasilis, Panagiotis mr. Apostolou, Ioannis dr. Papasotiriou
Research Genetic Cancer Center S.A., FLORINA, Greece

Introduction: Circulating tumor cells (CTCs) detach from the primary tumor site and enter blood circulation. Accessing and analyzing these cells constitutes a non- invasive alternative to tissue biopsies. CTCs are FDA approved for prognosis of the major cancer types, namely Breast, Colon and Prostate. However in order to take advantage of the diagnostic and clinical relevance of CTCs we need reliable methods of detection and molecular characterization of these cells. In the past years many platforms have been developed for the purpose of detecting CTCs. The aim of this study was to compare two widely used methods, Quantitative Real Time-PCR (qRT-PCR) and Flow Cytometry, and determine their specificity and accuracy.
Methods: Commercial cancer cell lines provided by European Collection of Authenticated Cell Cultures (ECACC), representing different types of cancer, were spiked in blood from healthy donors. Cells were isolated with magnetic beads and used for molecular-based analysis using specific primers (CK18, CK19). In addition, immunocytochemistry and flow cytometry methods, involving specific antibody panels, were performed to determine the presence of CTCs.

Results: Results demonstrated that molecular based techniques (qRT-PCR) have an R^2 of 0.9794 when tested on cancer cells alone and an R^2=0.6618 when spiked in peripheral blood. Flow cytometric panels used in our laboratory however proved sensitive (LOD=1.66) and linear (R^2=0.9998) in detecting CTCs as low as 10^{-6}. The immunocytochemistry data confirmed the presence of CTCs, based on their expression in cancer specific markers (EpCAM, Cytokeratins) and were distinguished from the rest blood cells which were positive for CD45.
Discussion: In this study, we compared two widely used techniques to detect CTCs in patients' blood. qRT- PCR, while being a highly sensitive method, proved to be less linear when used for the detection of spiked cancer cells in whole blood, simulating a clinical sample. On the other hand Flow Cytometry was very linear and sensitive and surpassed commercially available kits that isolate and detect CTCs based on size or certain antigen expression. Flow cytometry is also compatible with other techniques used to isolate CTCs, enabling further downstream applications.

118

The BD FACSLyric™ System* and Assay Portability for Phenotyping T-, B- and Natural Killer Lymphocytes

Imelda Omana-Zapata[1], Doreen Taufmann[2], Dominique Sauer[2], Sandra Tessmer[2], Alan M. Sanfilippo[3], John L. Schmitz[3], Sarah Gibson[4], Wendy Shallenberger[4], Claire Turko[4], Ruth Bates[4], Kevin Judge[1], Farzad Oreizy[1], Harshada Rohamare[1], Yang Zeng[1], Kimberly Dean[1], Beverly Lu[1], Caren Mutschmann[5]
[1]BD Biosciences, SAN JOSE, CALIFORNIA, U.S.A.
[2]SYNLAB Pharma Institute, BERLIN, Germany
[3]Dept Pathology, University of North Carolina, CHAPEL HILL, NORTH CAROLINA, U.S.A.
[4]University of Pittsburgh Medical Center, PITTSBURGH, PA, U.S.A.
[5]SYNLAB International GmbH, BERLIN, Germany

Introduction: The BD FACSLyric™ system consists of a flow cytometer available in different optical configurations, BD FACSuite™ Clinical software, with optional BD FACS™ Universal Loader, and BD FACSLink™ software for data transfer to a laboratory information system (LIS). The BD FACSuite Clinical software, when used with BD™ FC beads and BD™ CS&T IVD beads, supports universal setup for performance QC, instrument control, data acquisition and storage, online/offline data analysis, and instrument standardization. The objective of the study was to demonstrate the degree of precision of the BD FACSLyric system supporting portability of the assay across multiple laboratories.
Methods: Four laboratories participated in the study (one in Germany and three in the USA) using the BD FACSLyric 10-color configuration. The samples consisted of a single lot with two levels of CD-Chex™ control materials that were prepared following the BD Multitest™ 6-color TBNK protocol to identify T-, B- and natural killer (NK) lymphocyte sub-populations following manufacturer's instructions; data was analyzed using BD FACSuite™ Clinical software. Testing was carried out during five non-consecutive days, two-runs per day.
Results: The absolute count results for T-, B- and NK- cells were analyzed using percentage coefficient of variation (%CV) for T-, B- and NK- lymphocytes, for total between-laboratory and within-run variation per site. For T- and NK-cells, the total between-laboratory variation %CV values were ≤9.2%, and the within-run variation was ≤8.0%. For the B-cells, the %CV values were ≤10.2% for total between-laboratory variation, and ≤10.0% for within-run variation.
Conclusions: Our results support that the BD FACSLyric provides a high degree of precision for the measurement of lymphocyte subsets, leading to improved comparability of test results and assay portability across multiple sites.
*BD FACSLyric with BD FACSuite Clinical software is not available for sale in the USA.
CD-Chex™ is a trademark of Streck, Inc.

23-19898-00

119

Enumeration of T-, B- and NK- cells in whole blood with the BD FACSLyric™ system*: A multisite study.

Imelda Omana-Zapata[1], Alan M. Sanfilippo[2], John L. Schmitz[2], Caren Mutschmann[3], Doreen Taufmann[4], Dominique Sauer[4], Sandra Tessmer[4], Sarah Gibson[5], Wendy Shallenberger[5], Claire Turko[5], Ruth Bates[5], Ruba Hsen[6], Rachel Mclean[6], Kevin Judge[1], Farzad Oreizy[1], Harshada Rohamare[1], Yang Zeng[1], Kimberly Dean[1], Beverly Lu[1], Maurice O'Gorman[6]

[1]BD Biosciences, SAN JOSE, CALIFORNIA, U.S.A.

[2]Dept Pathology, University of North Carolina, CHAPEL HILL, NORTH CAROLINA, U.S.A.

[3]SYNLAB International GmbH, BERLIN, Germany

[4]SYNLAB Pharma Institute, BERLIN, Germany

[5]University of Pittsburgh Medical Center, PITTSBURGH, PA, U.S.A.

[6]Children's Hospital Los Angeles, LOS ANGELES, CA, U.S.A.

Introduction: The BD FACSLyric™ system consists of a flow cytometer available in different optical configurations, BD FACSuite™ Clinical software, with optional BD FACS™ Universal Loader, and BD FACSLink™ software for data transfer to a laboratory information system (LIS). BD FACSuite Clinical software, when used with BD™ FC beads and BD™ CS&T IVD beads, supports universal setup for performance QC, instrument control, data acquisition and storage, online/offline data analysis, and instrument standardization.

Methods: Five clinical sites collected the data (one in Germany and four in the USA) for this method comparison study; the BD FACSLyric 10-color configuration was used to test de-identified whole blood specimens from HIV-infected and uninfected patients. The specimens were prepared to enumerate T-, B- and NK-lymphocytes, following the BD Multitest™ IMK kit and BD Multitest™ 6-color TBNK reagent system manufacturer's instructions. Samples were analyzed on the BD FACSLyric system using BD FACSuite Clinical software and the results were compared with results obtained on the standard-of-care system, the BD FACSCanto™ II flow cytometer with BD FACSCanto™ clinical software and BD FACS™ 7-color setup beads.

Results: A total of 336 specimens were tested; the T-, B- and NK- cells absolute count and percentage of lymphocyte results were analyzed using Deming regression and Bland-Altman methods. The absolute count and percentage of lymphocytes regression results for T-, B- and NK- cells gave R^2 ≥0.98, with slope values ≥0.94, with range between 0.90–1.05. The %bias values were <10% for T- and NK-cells, and <15% for B-cells.

Conclusions: The BD FACSLyric system and the BD FACSCanto II system generated comparable T-, B- and NK-cell measurements using BD Multitest reagent systems. The study was sponsored by BD Biosciences.

*BD FACSLyric with BD FACSuite Clinical software is not available for sale in the USA.

23-19897-00