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CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE

BOOK OF ABSTRACTS
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Annual Meeting 2016

Belgian Society for Advancement of Cytometry

October 21st, 2016

Bedford Hotel Brussels

SCK•CEN
Boeretang 200
BE-2400 MOL
Belgium
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Contact: Baatout Sarah

Tel: +32 14 33 27 29

E-mail: sarah.baatout@sckcen.be

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Studiecentrum voor Kernenergie
Centre d'Etude de l'Energie Nucléaire
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Status: Unclassified

Programme

9:30 Welcome

Morning session: Highlight in clinical flow cytometry

10:00 Flow cytometric scores in MDS | Gautier Detry, Hôpital de Jolimont, La Louvière, Belgium
10:30 Assessment of maturation pathways in Kaluza: Applications in AML and MDS | Olivier Pradier, Hôpital Erasme, ULB, Brussels, Belgium
11:00 Platelet counting: Current issues and solutions | François Mullier, UCL Mont-Godinne, Namur, Belgium
11:30 Systemic mastocytosis, Flow cytometry and Molecular biology | Katrien Vermeulen, Antwerp University Hospital (UZA), Belgium
12:00 Angioimmunoblastic lymphomas and updates on other T-cell lymphomas | Adrien Randazzo, Les Cliniques du Sud Luxembourg, Arlon, Belgium
12:30 Intestinal intraepithelial lymphocytes in celiac disease | Nancy Boeckx, KULeuven, Belgium

13:00 Lunch & Vendor exhibition

Afternoon session: How to get your flow cytometry data published

14:00 Sharing and annotating data in compliance with MiFlowCyt: The Minimum Information about a Flow Cytometry Experiment | Josef Spidlen, TerryFoxLaboratory, British Colombia Cancer Agency, Canada
14:40 The role of the reporting framework MIATA within current efforts to advance immune monitoring | Marij Schoenmaekers-Welters, Leiden University Medical Center, The Netherlands

15:10 Coffee break & Vendor exhibition

15:40 Monitoring regulatory T cells in clinical samples: consensus on an essential marker set and gating strategy for regulatory T cell analysis by flow cytometry | Saskia Santegoets, Leiden University Medical Center, The Netherlands
16:05 Toward harmonized phenotyping of human myeloid-derived suppressor cells by flow cytometry | Susanna Mandruzzato, University of Padova, Padua, Italy

Storm session 5' short communications

16:30 Electroporation of Dicer-substrate siRNA duplexes targeting endogenous TCR enhance tumor killing activity of Wilms' Tumor 1 (WT1)-specific TCR-redirected cytotoxic T cells | Diana Campillo-Davo, Tumor Immunology Group (TIGr), Laboratory of Experimental Hematology, University of Antwerp, Belgium

16:36 Dental CBCT in children: In vitro and ex vivo DNA damage and oxidative stress analysis | Niels Belmans, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium

16:42 Effects of ionizing radiation on mitochondrial function in human endothelial cells | Bjorn Baselet, Radiobiology Unit, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium

16:54 Exploring radiosensitivity during neurulation: impact of X-irradiation on the incidence of various malformations, prenatal mortality and adult health | Kai Craenen, Radiobiology Unit, Institute for Environment, Health and Safety, Belgian Nuclear Research Centre SCK•CEN, Mol, Belgium

17:00 A multiparametric flow cytometric scoring system as a diagnostic tool in myelodysplastic syndromes | Morgane De Laveleye, Department of Clinical Biology, Hospital of Jolimont, Haine-Saint-Paul, Hainaut, Belgium

17:06 Gating without a gate | Christian Gosset, University of Liege, Liege, Belgium

17:15 Farewell


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Fax : +352-26503134
Email: seraglu@pt.lu


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ALEXION

Myelodysplastic score in flow cytometry

Detry G.[1] Staquet L., De Lavaleye M., Husson B[1].
1: Centre Hospitalier de Jolimont, Haine Saint Paul. Belgium

BACKGROUND

Myelodysplastic syndroms (MDS) are clonal hematopoietic diseases characterised by a persistent cytopenia and the presence of more than 10% of dysplastic cells in at least one hematopoietic lineage. The classical cytomorphological examination of these dysplastic cells is subjective, lacks sensitivity and may be difficult in some conditions (drug exposure, renal or hepatic failures). Cytogenetic abnormalities are present in about 50% of the patients. Flow cytometry, FISH and SNP analysis have been proposed as a complementary diagnosis tool.
In 2008, a first workshop of the European Leukemia Net (ELN) took place in Amsterdam and defined a consensus on how to process bone marrow cells in flow cytometry[1]. Four years later, the ELN published the minimal core markers that should allow a correct diagnostic of MDS patients in flow cytometry[2]. Guidance about the analysis of these proposed core markers and prospective studies are still needed.

OBJECTIVE

The aim of this study was to establish the diagnostic weight of each of the ELN core markers in a retrospective study and to define the best strategy for a proper diagnosis of MDS in flow cytometry.

CONCLUSION

Flow cytometry is increasingly recognized as an important tool in the diagnosis of MDS. Several markers can lead to a strong suspicion of MDS and these information can initiate a discussion between the flow cytometry and the cytomorphologist.
However, the dissection of MDS from normal bone marrow remains a struggle and requires highly trained staff as well as deep validation studies. As validated prospective studies are still lacking, each lab should rank their markers according to the antibodies' combinations in use in their lab and validate their knowledge in day to day discussions with cytomorphologists.

1: Arja A *et al.,* Standardization of flow cytometrey in myélodysplastic syndromes: report from the first European LeukemiaNet Working conference on flow cytometry in myelodysplastic syndromes.
2: TM Westers *et al.,* Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group. Leukemia (2012); 26:1730-1741

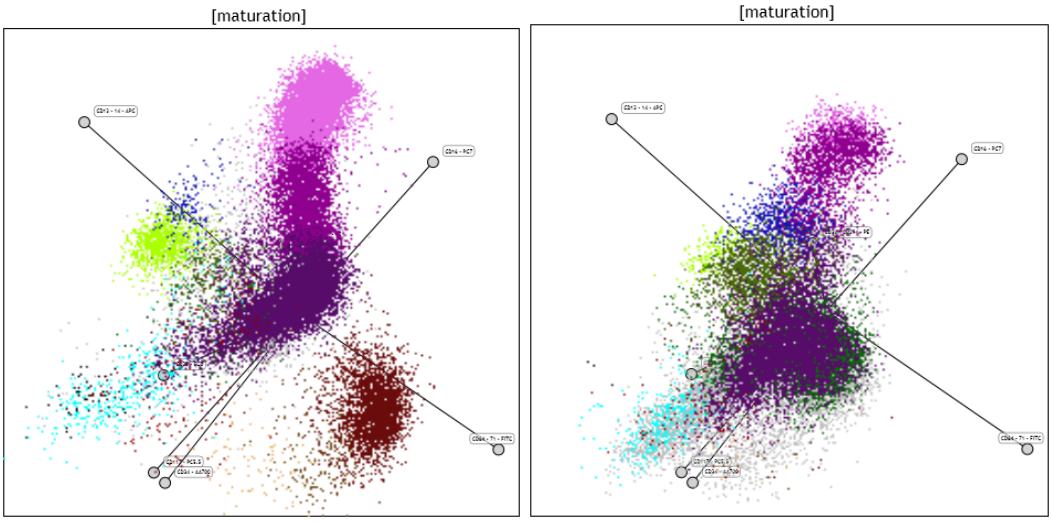
Assessment of maturation pathways in Kaluza: Applications in AML and MDS

Dr Olivier Pradier, Hôpital Erasme , ULB, Brussels, Belgium.

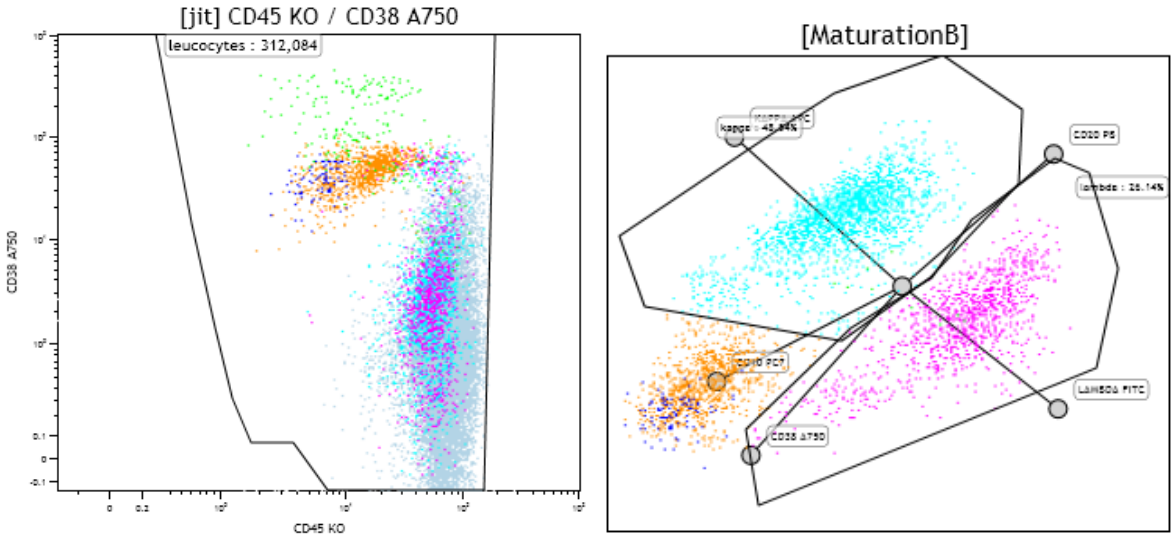
Multi-parametric flow cytometry (fcm) associated with multimab procedure that adds multiple antibodies with the same fluorochrome within the same combination, enables the design of combinations of antibody combining more than 10 markers combining immaturity and maturity of several different cell lines and thus to analyze the main cell maturation pathways of haematopoietic marrow. The vector representation of Kaluza fcm software can integrate multiple axes in the same plot and thus to combine in an image, the relative representation of multiple markers on the same cell. So, we leave the negative positive binary representation of each marker to enter an evolutionary continuum where each cell moves in a maturation pathway inscribed in a multidimensional representation. We can thus, define maturation pathways for erythroid, myeloid lineage, or monocytic lineage, the B lymphocyte or plasma cells maturation pathways. The application of this procedure in the bone marrow, can create a cytometric myelogram. Knowledge of normal channels of maturation sets the abnormality. Thus, myelodysplasia leads to cytometric distortion of its maturation pathways. Also it is easy to define the lineage of myeloid leukemia observing the maturation pathway used by leukemic cells.

We present routine application used for three years at the Erasmus Hospital and for analyzing the equivalent of a bone marrow aspirate by flow cytometry. This is a panel of three tubes; one dedicated to the analysis of myeloid and erythroblast maturation, since the hematopoietic stem cell CD34 + CD38- to the most mature elements. The second allows you to analyze in T lymphocyte populations and the plasma cell maturation is the third, analyze B lymphoblastic maturation, lambda kappa monoclonality and functional B cells populations.

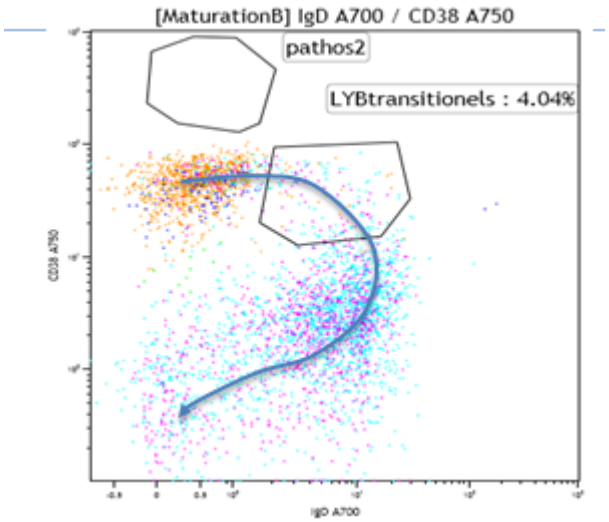
As an example, one of the Kaluza representations on bone marrow maturation pathways. Black dots; hematopoietic stem cells, cyan; myeloid blasts, purple and pink; neutrophil maturation (central pathway), brownish; erythroid maturation (in the right), green monocytic maturation (on the left, dark blue dots represent non classical monocytes). Lymphocytes, plamocytes, éosinophils and basophils are removed from this representation and counted elsewhere. Plot B is an example of myelodysplasie



Example of B lineage maturation. Black dots; B blasts, orange cluster are the hematogones, Cyan; kappa cells, pink; lambda cells and green plasmocytes. Classical dot plot rCD38/CD45 representation and a vector view showing the acquisition of kappa and lambda in the transitional stage



Classical CD38-IgD vue of B cells maturation from B blasts , hematogones, transitional, naïve and memory B cells.



For bone marrow analysis the panel we use a 10 colors Navios from Beckman Coulter and for the analysis of the hematopoietic marrow, the panel includes three combinations of antibodies; one for myeloid differentiation, one for T cells subpopulations and plasmocytes maturation and one for B cells maturation

FITC	PE	ECD	PC5.5	PC7	APC	AA700	AA750	PB	KO
CD64 CD71	CD36 cd294	CD33	Cd117	CD16	CD13 CD14	CD34	CD38	CD15	CD45
CD8 CD64	CD27 cd138	CD56 – DR	CD5	CD19 *Tcrγ–δ*	CD4 CD14	CD34 CD16	CD38	CD3 CD20	CD45
LAMB	CD19	CD27	CD5	CD10	KAPPA	IGD	CD38	CD20	CD45

Leukemic lineage infidelity are determined in the two others combination.

Prof F.Mullier, Prof B.Chatelain

Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC-NARILIS), Laboratory of clinical biology

The clinical decision to proceed with prophylactic platelet transfusions is widely based on platelet counts determined by haematological analysers. Conventional automated platelet counting methods are unable to provide consistently accurate results in this low thrombocytopenic range. This inaccuracy might have a significant impact in under- and overtransfusion of platelet concentrates to patients. In addition, these analysers are also used to count platelets in platelet concentrates. This presentation will review the methods available for platelet quantification (microscopy, impedance, optical, fluorescence and flow cytometry). The variables, interferences, advantages and drawbacks of each method will be addressed. The standardization of other platelet parameters (i.e mean platelet volume, immature platelet fraction, most frequent platelet volume and platelet large cell ratio) will also be discussed.

Systemic mastocytosis: flow cytometry and molecular biology

Vermeulen K[1], Deiteren K[1], Maes MB[1], Sabato V[2], Van den Bossche J[1], Malfait R[1]

[1]Laboratory of Hematology University Hospital Antwerp, Edegem, Belgium
[2]Immunology-Allergology-Rheumatology, University Hospital Antwerp, Edegem, Belgium

BACKGROUND
Systemic mastocytosis (SM) is a rare mast cell disease due to clonal, neoplastic proliferation of mast cells and can occur in a variety of forms. In the revised World Health Organization (WHO) classification (2016) mastocytosis is no longer considered a subgroup of the myeloproliferative neoplasms but is now a separate disease category. Histological, cytological, immuunphenotypical, molecular and biochemical information on mast cells is necessary to diagnose SM.

OJECTIVE
The low mast cell frequency in both normal bone marrow and bone marrow from patients with mastocytosis demands for reliable and sensitive immunophenotypic and molecular assays.

METHODS
In a multiparametric flow cytometric analysis the markers CD33, CD34, CD45, CD117, CD203 were used to separate and quantitate the total mast cell population while CD2, CD25, CD30 were used to discriminate between normal and aberrant mast cells.
To detect the typical CKIT D816V mutation a digital droplet PCR using one allel specific primerset and two competitive probes, one detecting the WT allele and one detecting the mutant allele was evaluated and implemented.

RESULTS
In patients with SM a mast cell frequency of 0.014-2.4% was observed, in the control population a frequency of 0.0036-0.68% was found. The specificity of all three tested aberrant markers was very good (≥96%) with CD25 having the greatest sensitivity (94%). CD30 seems, beside CD2 and CD25, to be a specific and sensitive marker for neoplastic mast cells.
All patients diagnosed with SM tested positive for CKIT mutation with a mutation burden between 0.001-47%. All samples in the control population tested negative. Reproducibility experiments revealed coefficients of variation between 1.1-7.6%. Sensitivity was as low as 0.001% (dependent on DNA input).

CONCLUSION
In our laboratory multiparametric flow cytometry and digital droplet PCR were implemented in daily practice to meet the required sensitivity in detecting rare aberrant mast cells and rare mutations in both peripheral blood and bone marrow.

Angioimmunoblastic lymphomas and updates on other T-cell lymphomas

Adrien Randazzo[1], Nicolas Hougardy[1], Jean-François Classen[1]

[1]Cliniques du Sud Luxembourg, Arlon, Belgium

ABSTRACT

Lymphoma is the most common blood cancer. T-cell lymphomas account for approximately 10 to 15 percent of all non-Hodgkin lymphomas. There are many different forms of T-cell lymphomas, some of which are extremely rare. Nowadays this kind of pathology represents a significant clinical problem and is often associated with a bad prognosis. Diagnosis is often difficult because of the variability of their morphology and immunological features. Identification of sub-entities of T-cell lymphomas is an essential challenge for the future.

The WHO describes the angioimmunoblastic T-cell lymphoma as *"a peripheral T-cell lymphoma characterized by systemic disease, a polymorphous infiltrate involving lymph nodes, with a prominent proliferation of high endothelial venules and follicular dendritic cells."* It occurs in the middle-aged and elderly, with an equal incidence in males and females and accounts for approximatively 15 – 20 % of peripheral T-cell lymphomas, which represents 1 – 2 % of all non- Hodgkin lymphomas. Clinical features are typically generalized lymphadenopathy, hepatosplenomegaly, systemic symptoms and polyclonal hypergammaglobulinemia. Association with Epstein Barr Virus (EBV) is also suggested.

We present two cases of angioimmunoblastic lymphomas illustrating the variability of their presentation. We will talk about cytological, cytometric and pathologic aspects. Then we will proceed to a literature review of the possibilities offered by flow cytometry to improve the diagnosis and the characterization of circulating T-cell lymphomas.

Detection of aberrant intra-epithelial lymphocytes in refractory celiac disease

N. Boeckx

University Hospitals Leuven, Leuven, Belgium

Celiac disease (CD) is a chronic small-intestinal, immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals. The only treatment is a strict, lifelong gluten-free diet (GFD). However, a small subset of CD patients experience persisting or recurring symptoms despite strict adherence to a GFD: these patients are referred as having refractory celiac disease (RCD).

RCD patients are subdivided into RCD type I and RCD type II patients, based on the number of aberrant intra-epithelial lymphocytes (IELs) in duodenal biopsies: <20% and ≥20% aberrant IELs respectively. These aberrant IELs lack surface expression of CD3, CD4 and CD8, but have the cytoplasmatic CD3 and T-cell receptor (TCR) molecules contained inside the cell. While RCD type I patients show no increased risk to develop enteropathy associated T-cell lymphoma (EATL) and have a normal 5-year survival, patients suffering from RCD type II are at high risk for development of EATL and have a poor 5-year survival. Therefore discrimination between both types of RCD is of utmost importance.

Different methods can be used to identify the aberrant IELs in RCD patients, including immunohistochemistry and TCR-gene analysis, however, flow cytometric immunophenotyping of small-bowel biopsies are considered to be the golden standard.

Sharing and annotating data in compliance with MIFlowCyt: the Minimum Information about a Flow Cytometry Experiment

Josef Spidlen[1], Ryan Brinkman[1,2]

[1] Terry Fox Laboratory, British Colombia Cancer Agency, Canada
[2] Department of Medical Genetics, University of British Columbia, Canada

BACKGROUND
A fundamental tenet of scientific research is that published results are open to independent validation and refutation. Minimum data standards aid data providers, users, and publishers by enumerating what is needed to interpret experimental findings. The availability of annotated data is increasingly required by scientific journals and funding agencies. Deposition to public repositories is the preferred way of data sharing.

OBJECTIVE
Our main objective was to facilitate data sharing and reproducible research in flow cytometry by developing a minimum information standard and a public repository for scientist to share their cytometry data.

METHODS
Therefore, we brought together a large cross-disciplinary international collaborative group of bioinformaticians, computational statisticians, software developers, instrument manufacturers, and clinical and basic research scientists to develop the Minimum Information about a Flow Cytometry Experiment (MIFlowCyt). We then developed FlowRepository by extending and adapting Cytobank, an on-line tool for storage and collaborative analysis of cytometric data.

RESULTS
MIFlowCyt includes recommendations about experimental overview, descriptions of the specimens and reagents, the configuration of the instrument and data analysis details. It is an approved ISAC standard that has also been adopted by journals, including Cytometry A. FlowRepository is recommended by the main cytometry societies (including ISAC, ICCS and ESCCA) and several scientific journals, including Cytometry A, and publishers (PLOS, Nature Publishing Group). Currently, FlowRepository contains 85,000 FCS files organized into 800 data sets. So far, it has seen over 20,000 users who collectively downloaded over 20,000 data sets. FlowRepository is included in the Data Citation Index (Thomson Reuters, Web of Science) and the PLOS repository integration partner program.

CONCLUSION
Transparency and public availability of protocols, data, analyses, and results are crucial to make sense of the complex biology of human diseases. Together MIFlowCyt and FlowRepository provide a mechanism for researchers to access, review, download, deposit, annotate and share cytometry datasets. During this talk, we will review MIFlowCyt and provide guidelines and tips on how to properly and efficiently annotate and share your data.

The role of the reporting framework MIATA within current efforts to advance immune monitoring

M.J.P. Welters

Medical Oncology, Leiden University Medical Center, Leiden, The Netherlands

BACKGROUND: With immune monitoring as a well-recognized effort in the immunology field, it is important that the results obtained are interpretable and reproducible. It is challenging to establish robust assays for measuring antigen-specific T-cells. This was the aim of harmonization and standardization activities of several research groups. Furthermore, findings are shared by publication in peer-reviewed journals, a prerequisite for moving the field forward. However, there is a lack of conformity in reporting the results of T-cell assays.

OBJECTIVE: A reporting framework has been established that enables an objective and thorough interpretation of published results from T-cell assays which is called "**MIATA"** (**M**inimal **I**nformation **A**bout **T** cell **A**ssays). ELISPOT and HLA-peptide multimer staining are common T-cell assays but recently the MIATA guidelines are also set up for flow cytometric analysis.

METHOD: Proficiency panels are run by the CIMT immunoguiding working group (CIP) and Cancer Immunotherapy Consortium (CIC) in order to harmonize data generated by flow cytometry. The MIATA guideline contains 5 modules in which the critical information for transparent data reporting is requested: the sample, the assay, the acquisition, the analysis (interpretation) of raw data and the laboratory environment.

RESULTS: The results of the proficiency panels organized by CIP and CIC showed that even when the acquired data of flow cytometry is provided a large variation is obtained due to gating procedure. Applying the MIATA guideline for reporting is a prerequisite for multiple reasons: transparency in the variable experimental factors, the improved interpretation of the results, increased visibility and potential higher citation rate of the published work. Currently 8 journals endorse the authors to use the MIATA guidelines.

CONCLUSION: Adherence to MIATA will be a quality label for the published work and will improve transparency, interpretation and reproducibility of published data.

Detection and functional assessment of regulatory T cells in clinical samples

Saskia J.A.M. Santegoets, Eveline M. Dijkgraaf, Judith R. Kroep, Marij J.P. Welters and Sjoerd H. van der Burg.

Department of Clinical Oncology, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands.

BACKGROUND

Regulatory T cell (Treg)-mediated immunosuppression is considered a major obstacle for successful cancer immunotherapy. Given their profound effect on the outcome of immunotherapy trials, Tregs are being studied extensively in clinical trials. However, the multitude of Treg definitions in the reported studies makes correct interpretation of data and comparisons between studies difficult. Also because unambiguous enumeration of Tregs by flow cytometry is hampered by the absence of an exclusive, highly specific marker and the inability to directly measure their function in immunomonitoring of clinical trials. The Cancer Immunoguiding Program (CIP) recently reached consensus with leading experts in the field concerning the use of an essential marker set comprising antibodies to CD3, CD4, CD25, CD127, Foxp3, Ki67 and CD45RA and corresponding gating strategy to define human Treg cells. The use of these markers was validated in a series of PBMC and tissues samples from healthy donors and cancer patients.

OBJECTIVE

To prove that the cells identified through the consensus marker set are indeed Tregs with suppressor function, we are currently developing assays to assess Treg functionality in the setting of (limited) clinical trial samples.

METHODS

We compared two flow cytometry-based approaches with a recently described, highly efficient 3H-Thymidine-based protocol for measuring Treg suppressive activity (Tim Tree et al). With the latter protocol Treg suppressive potential could be tested with only 10,000 cells in an APC-dependent and APC-independent way.

RESULTS

We were able to demonstrate that Treg suppressive activity could be measured efficiently with the 3H-based approach, whereas no inhibition of responder T cell could be detected in parallel with the flow cytometry-based assays.

CONCLUSION

The 3H-based assay is a highly sensitive assay for measuring Treg function of freshly-isolated Tregs in the setting of (limited) clinical trial samples.

Toward harmonized phenotyping of human myeloid-derived suppressor cells by flow cytometry

Susanna Mandruzzato[1]
[1]Oncology and Immunology Section, Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy

BACKGROUND – There is an increasing interest for monitoring circulating myeloid-derived suppressor cells (MDSCs) in cancer patients, which are able to divert T cell functions. Circulating levels of MDSCs have been correlated to tumor burden and overall survival in different types of cancers and they demonstrated a prognostic role for the outcome of chemotherapy, thus they might represent a promising biomarker also for immunotherapy response. However, there are also divergences in their phenotypic definition.

OBJECTIVE – The Cancer Immunoguiding Program (CIP) under the umbrella of the Association of Cancer Immunotherapy (CIMT) is coordinating a proficiency panel program that aims at harmonizing MDSC phenotyping.

METHODS – A two-stage approach was planned to harmonize human MDSC phenotype. In the first step, an international consortium of 23 laboratories immunophenotyped 10 MDSC subsets on pre-tested, peripheral blood mononuclear cells to assess the level of concordance and define robust marker combinations for the identification of circulating MDSCs. At this stage, no mandatory requirements to standardize reagents or protocols were introduced.

RESULTS - Data analysis revealed an expected intra-laboratory variance, but also a very high inter-laboratory variance for all MDSC subsets, especially for the granulocytic subsets. In particular, the use of a dead-cell marker altered significantly the reported percentage of granulocytic MDSCs, confirming that these cells are more sensitive to cryopreservation and/or thawing. Importantly, the gating strategy was heterogeneous and associated with high intercenter-variance.

CONCLUSION - These results document the high variability in MDSC phenotyping in the multi-center setting if no harmonization/standardization measures are applied. Although the observed variability depended on a number of identified parameters, the main parameter associated with variation was the gating strategy. Based on these findings we propose further efforts to harmonize marker combinations and gating parameters to identify strategies for a robust enumeration of MDSC subsets.

Electroporation of Dicer-substrate siRNA duplexes targeting endogenous TCR enhance tumor killing activity of Wilms' Tumor 1 (WT1)-specific TCR-redirected cytotoxic T cells

Diana Campillo-Davo[1], Fumihiro Fujiki[2], Johan Van den Bergh[1], Evelien Smits[1,3,4], Haruo Sugiyama[2], Viggo F.I. Van Tendeloo[1], Zwi Berneman[1,3,5]

[1] Tumor Immunology Group (TIGr), Laboratory of Experimental Hematology, Vaccine & Infectious Disease Institute, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium
[2] Department of Cancer Immunology, Osaka University Graduate School of Medicine, Osaka, Japan
[3] Center for Cell Therapy & Regenerative Medicine, Antwerp University Hospital, Edegem, Belgium
[4] Center for Oncological Research (CORE), Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium
[5] Division of Hematology, Antwerp University Hospital, Edegem, Belgium.

BACKGROUNG
In adoptive cellular immunotherapy, T cells can be genetically engineered to express a novel T-cell receptor (TCR) that recognizes a tumor-associated antigen. However, mispairing between transgene and endogenous TCR chains may result in a reduction of transgene TCR expression and potentially harmful off-target reactivities.

OBJECTIVE
Here, we sought to develop a novel clinically safe strategy to promote transgene expression of a Wilms' tumor 1 (WT1)-specific TCR by Dicer-substrate small interfering RNA (DsiRNA)-mediated silencing of the endogenous TCR.

METHODS
First, we isolated and cloned an HLA-A*0201-restricted WT1 peptide-specific TCR derived from a leukemia patient who demonstrated clinical benefit after receiving a WT1-targeted DC vaccine. Next, we produced a codon-optimized TCR sequence from the wild-type TCR construct. In order to suppress the translation of endogenous TCR mRNA in $CD8^+$ T cells, DsiRNA duplexes were designed to specifically target the constant regions of wild-type TCR α- and β-chains, but not the codon-optimized TCR. We further developed a double electroporation protocol in which DsiRNA electroporation was performed prior to TCR mRNA transfection.

RESULTS
Our results show more than 2-fold increase in WT1-specific TCR expression by HLA-A2/WT1 tetramer staining after DsiRNA treatment as compared to TCR mRNA electroporation only. Specific cytotoxicity against WT1 epitope-bearing target cells was significantly enhanced in TCR mRNA-electroporated T cells following DsiRNA silencing of the endogenous TCR expression. Accordingly, DsiRNA/TCR mRNA transfected $CD8^+$ T cells presented increased epitope recognition, higher levels of $CD8^+$ T-cell activation markers and cytokine secretion upon TCR triggering as compared to the non-DsiRNA treated T cells.

CONCLUSION
In conclusion, we show a marked enhancement of transgene WT1-specific TCR expression and functionality of redirected T cells upon silencing of the endogenous TCR by combining DsiRNA and TCR mRNA electroporations. These results pave the way for developing a clinically safer strategy for T cell-based adoptive immunotherapy of patients with WT1-expressing malignancies.

Dental CBCT in children: *In vitro* and *ex vivo* DNA damage and oxidative stress analysis

Niels Belmans[1,2], Marjan Moreels[1], Ivo Lambrichts[2]

[1]Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium
[2]Faculty of Medicine and Life Sciences, Biomedical Research Institute, Hasselt University, Hasselt, Belgium

BACKGROUND
Cone Beam Computed Tomography (CBCT) is a multipurpose radiographic tool for diagnosis, treatment planning, follow-up and research in dental practice, mostly in the field of pediatric orthodontics. CBCT is considered a low dose imaging modality, however, it is uncertain that using CBCT in a pediatric population is completely without risk, since children are known to be more radiosensitive than adults.

OBJECTIVE
To characterize potential biological risks involved in dental pediatric CBCT imaging. The focus is on measuring markers of DNA damage & repair response *in vitro* and *ex vivo* as well as measuring oxidative stress levels in saliva.

METHODS
Studying DNA damage and repair kinetics *in vitro* and *ex vivo* over time by microscopical visualization of γH2AX/53BP1 in dental stem cells after X-irradiation (≤ 100 mGy) and exfoliated buccal cells before and after CBCT exposure. The oxidative state was measured in saliva samples using 8-OHdG ELISA. *Ex vivo* samples were obtained from consenting children and adults.

RESULTS
We showed a dose dependent increase in the amount of DNA damage 30 minutes to 1 hour post-irradition for doses higher than 20 mGy *in vitro*. This damage is resolved 24 hours post-irradiation. DNA damage analysis in buccal cells from pediatric and adult patients reveals no significant increases in the amount of DNA damage after CBCT examination. Data from adult patients show that oxidative stress levels do significantly increase in saliva after CBCT examination.

CONCLUSION
Preliminary data indicate that low dose X-irradiation increases DNA damage *in vitro*, but CBCT examination does not lead to increased DNA damage in buccal cells from pediatric and adult patients. Adults show increased salivary oxidative stress levels after CBCT examination. These data indicate that CBCT X-ray energies might be insufficient to cause DNA double strand breaks, but cause significant increases in oxidative stress levels.

ACKNOWLEDGEMENTS
The DIMITRA project has received funding from the FP7-OPERRA project under grant agreement n°604984. Niels Belmans is a recipient of a SCK•CEN – UHasselt PhD grant.

Effects of ionizing radiation on mitochondrial function in human endothelial cells

Baselet Bjorn[1,2], Aerts An[1], Baatout Sarah[1], Sonveaux Pierre[2]

[1] Radiobiology Unit, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium
[2] Pole of Pharmacology and Therapeutics, Institute of Experimental and Clinical Research (IREC), University of Louvain Medical School, Brussels, Belgium

BACKGROUND
Mitochondria are often regarded as the powerhouses of the cell because they supply most of the cellular energy, meanwhile however generating substantial amounts of reactive oxygen species. Yet, mitochondria are also involved in other tasks such as oxidative stress signaling, calcium regulation and apoptotic control. **Dysregulation of mitochondrial functions** can result in the promotion of apoptosis and senescence, and in an increased inflammatory status. All of these adverse events are observed in the onset and progression of **atherosclerosis**, the main cause of cardiovascular disease (CVD).

OBJECTIVE
To assess the effect of ionizing radiation exposure on the mitochondrial function and related pathways in endothelial cells.

METHODS
Assays for mitochondrial DNA (mtDNA), cellular metabolism, senescence and cytokine quantification were used to compare sham- and acutely X-ray-irradiated **endothelial cells** over time.

RESULTS
We could demonstrate a differential response in the amount of **mitochondrial DNA** after exposure to medium and high dose ionizing radiation. This observation was accompanied with a **glycolytic switch**, consisting of a decreased mitochondrial respiration rate and an increased glycolytic activity. Moreover, every dose of ionizing radiation induced premature endothelial cell **senescence**, which could be partly explained by the **inflammatory reaction** occurring after exposure to ionizing radiation at the medium and high dose range.

CONCLUSION
Our results indicate that mitochondrial function is changed after exposure to ionizing radiation and could explain in part the **endothelial cell dysfunction** observed after radiation exposure. This observation indicates that mitochondria are an interesting cellular target for **cardiovascular risk-reducing strategies**, aimed to prevent radiation-induced CVD.

ACKNOWLEDGEMENTS
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Exploring radiosensitivity during neurulation: impact of X-irradiation on the incidence of various malformations, prenatal mortality and adult health

Authors

Kai Craenen[1,2], Jasmine Buset[1], Mieke Verslegers[1], Sarah baatout[1], Lieve Moons[2], Mohammed Abderrafi Benotmane[1]

Affiliations

[1] Radiobiology Unit, Institute for Environment, Health and Safety, Belgian Nuclear Research Centre SCK•CEN, Boeretang 200, 2400 Mol;

[2] Laboratory of Neural Circuit Development and Regeneration, Animal Physiology and Neurobiology section, Department of Biology, Faculty of Science, KU Leuven, Naamsestraat 61 bus 2464, 3000 Leuven, Belgium;

BACKGROUND – Epidemiology after the A-bombings in Japan and the Chernobyl disaster suggested that prenatal irradiation may lead to morphological and functional brain defects. More specifically, *in utero* exposure to ionizing radiation (IR) has been linked to an increased prevalence of neural tube defects (NTD, e.g. exencephaly) and eye defects (ED, e.g. microphthalmos). In addition, cognitive impairments during later stages of life have been shown to occur following prenatal IR exposure.

OBJECTIVE – Knowledge on radiosensitivity during neurulation is limited. Thus, we assessed the most radiosensitive stage during neural tube closure for elliciting microphthalmos and exencephaly. In addition, pre-/postnatal survival and viability was inspected.

METHODS – Pregnant mice were irradiated at discrete stages during development (embryonic days E7, E7.5, E8, E8.5 or E9) with X-rays (0.5 Gy or 1.0 Gy) and inspected macroscopically (E18) under a stereomicroscope. Furthermore, animals irradiated at E7.5 were grown until 5 weeks of age (W5)/W10 and were scored for survival and body/brain weight respectively.

RESULTS – Among other maformations such as gastroschisis and agnathia, the prevalence of microphthalmos and exencephaly were most significantly increased after irradiation with 1.0 Gy X-rays at E7.5. Furthermore, prenatal mortality and embryonic weight were significantly impacted by irradiation during neurulation. In terms of prenatal IR-induced health effects after birth, survival chances at W5 were only significantly lower in the 1.0 Gy irradiated group, while at W10 brain and body weight were not affected by irradiation.

CONCLUSION – Our study established a more detailed insight on embryonic radiosensitivity throughout neurulation and its impact on malformations, embryonic weight, prenatal mortality and postnatal mortality. Our results suggest that mouse embryos are most prone to developing radiation-induced microphthalmos/exencephaly and to a lowered survival after irradiation at E7.5. These findings offer a good model to further study IR-induced congenital defects and underlying molecular players.

A multiparametric flow cytometric scoring system as a diagnostic tool in myelodysplastic syndromes

De Laveleye M.[1], Staquet L.[1], Husson B [1], Detry G.[1]

[1]Department of Clinical Biology, Hospital of Jolimont, Haine-Saint-Paul, Hainaut, Belgium

BACKGROUND

Myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases with heterogeneous clinical manifestations and morphological findings. The diagnosis of MDS is therefore particularly challenging, especially in early stages. In this context, flow cytometry (FC) is becoming an increasingly important tool for the diagnostic work-up of MDS. However, even if several MDS scoring systems were developed worldwide, none of these have achieved the sufficient analytical performance to date.

OBJECTIVE

The aim of this study was to develop and validate a flow cytometric scoring system (FCSS) for use in the diagnosis.

METHODS

64 bone marrow (BM) samples from MDS patients and 68 BM samples from non-MDS patients were characterized by multi-dimensional FC using an extensive panel of monoclonal antibodies. Data were acquired using a FACSCanto II cytometer (BD Biosciences, San Jose, CA). 37 parameters were analysed mainly based on the report published by the European LeukemiaNet Working Group in 2012. Mann-Whitney test was used to assess statistical differences between groups and box and whisker plots to show the distribution of data. For each of the 19 parameters selected, the receiver operating characteristic (ROC) curve analysis was performed to choose a cut-off value to differentiate between the MDS and the non-MDS patients. Finally, a score was attributed to each bone marrow samples and the analytical performance of the method was determined.

RESULTS

We assessed the diagnostic power of the scoring system for the 132 BM samples. A correct diagnosis was obtained in 49/64 cases (sensitivity 76.6%). There were 11 false-positive cases among 68 non-MDS patients (specificity 83.8%). Based on a cut-off value of 8 (1 point per criterion), the positive and negative predictive values were 81.6% and 79.2%, respectively.

Gating without a gate

Christian Gosset[1], Jacques Foguenne[2], Mickael Simul[2], André Gothot[1,2]

[1] University of Liege, Liege, Belgium
[2] Department of Laboratory Medicine, Unilab Lg, CHU Liège

BACKGROUND

Discriminating cell populations by flow cytometry has always been carried out by visual inspection of plots. Manual gating is still considered a gold standard in cell population recognition, although it is yet being considered as subjective and time-consuming, demonstrating the need for different approaches.
Recently, with the availability of powerful data processors, clustering techniques may be developed to provide an automated way of cell population identification in flow cytometric data, which could outperform human analysis.

OBJECTIVE

Here, we aim to fully automate clustering workflow as an alternative to manual gating in a T, B, NK-cell panel within a comparative cohort. The goal of this study was to automatically assign individual cell events to a defined cell population based on previous supervised learning procedure.

METHODS

We used an association of SSC, FSC, CD3 FITC, CD4 PE, CD8 APC-H7, CD16-56 PE-Cy7, CD19 APC and CD45 V500 antibodies in order to distinguish the T, B and NK cell populations. After manual gating, raw data was extracted for each cell population comprised in an FCS. file with Infinicyt and stored in txt. format comprising a cell subset identifier (0 or 1). The workflow to learn the clustering algorithm was constructed based on 500 sample computations representing 11 million events. Combined with Statistica supporting multi-core computations, a TreeNet Gradient Boosting algorithm was generated and to objectively evaluate clustering performance, a validation procedure was integrated and composed of 100 new samples.

RESULTS

Correlation between manual and algorithmic gating showed a nearly perfect linear relationship with a correlation coefficient (r) of 0.9982, 0.9992, 0.9991, 0.9988, 0.9995 for CD3+, CD4+, CD8+, NK and B cell percentages respectively. Furthermore, the differences between manual and algorithmic gating represented on Band-Altman plots displayed excellent agreement. Indeed, CD3+, CD4+, CD8+, NK and B cell percentages showed a bias of 0.34, 0.03, 0.37, 0.04 and 0.07 respectively.

CONCLUSION

The study suggests a promising tool for automated gating, allowing for more objectivity and standardization than manual gating. The reliability of automated gating should now be assessed on cell fractions with complex and/or abnormal phenotypes.