Core Facility for Flow Cytometry – The Gade Institute
University of Bergen / Haukeland University Hospital

One-day flow cytometry seminar Thursday 15th March 2012

Building for Basic Biological Research, Jonas Liesvei 91, 3rd floor, University of Bergen

9.00-12.00 Auditorium 4 **13.00-16.45 Auditorium 1**

TIME	LECTURE	NAME
9.00 – 10.00	**Official opening of the Core Facility for Flow Cytometry** **Historical aspects of flow cytometry and cell sorting**	**MOF- Dekan Nina Langeland** **Ole Didrik Lærum:** The Gade Institute, University of Bergen and The Finsen Laboratory /Copenhagen University
10.15-11.00	**Basic principles of multicolor flow. Presentation of Euroflow**	**Einar Kristoffersen:** The Gade Inst./Dept. of immunology and Transfusion Medicine, Haukeland University Hospital, Bergen
11.15-12.00	**Single cell intracellular staining in health and disease.** **Phospho flow basics**	**Jørn Skavland**: Inst. of Medicine, University of Bergen, Bergen
13.00-13.45	**Cytometry and cancer biology: Assessment of cell proliferation and death**	**Derek Davies:** London Research Institute, Cancer Research UK, United Kingdom
14.00-14.45	**Cytometry of epithelial and mesenchymal stem cells isolated from normal and malignant tissues**	**Vera Donnenberg:** University of Pittsburgh School of Medicine, Dept. of Cardiothoracic Surgery, USA
15.00-15.45	**Cancer-targeted T cells revealed by flow cytometry**	**Johanna Olweus:** Dept. of Immunology at the Institute for Cancer Research, Oslo University Hospital Radiumhospitalet, Oslo
16.00-16.45	**Diagnostics of primary immunodeficiency syndromes using flow cytometry-based functional assays.**	**Yenan T. Bryceson:** The Gade Inst.,UiB / Center for Infectious Medicine, Karolinska institutet, Stockholm, Sweden

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ABSTRACTS

Title: **Cytometry and Cancer Biology: Assessment of cell proliferation and death**

Derek Davies
London Research Institute, Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3LY

The study of cell proliferation was one of the earliest applications of flow cytometry and the balance between proliferation and cell death is crucial to understanding of how cells behave *in vivo* in normal and disease states.
Traditionally cell proliferation has been studied by single fluorochrome DNA analysis which is quick and easy but does have some significant drawbacks – it is not possible to distinguish all phases of the cell cycle and no kinetic information is available. It is possible to use additional markers such as phosphorylated Histone H3 to identify mitotic cells and the bromodeoxyuridine (BrdU) method is often used to provide information about how many cells are cycling. But even by using a pulse labelling it is difficult to follow cells through more than two cell divisions. Dye dilution studies using dyes such as CFSE allow cells to be followed through several rounds of division (up to 6) but they lack information about cell cycle status. Similarly there is a plethora of methods available to study cell death or apoptosis such as organelle analysis, analysis of cell membrane changes or alteration to cellular DNA.
We have recently developed a flow cytometric method to combine cell division analysis using Cell Trace Violet with propidium iodide staining to look at DNA content information and identification of mitotic cells using an antibody against phosphorylated H3. This allows identification of cells in different rounds of cell division and allows us to observe in detail the cell cycle effects of chemotherapeutic agents. By using drugs known to target cells in either S phase (etoposide and 5-fluorouracil) or mitosis (nocodazole and demicolcine) we can assess the effects on cell cycle status within each division round. In addition it is possible to detect other molecules important in death pathways such as caspase-3. This method can also be combined with imaging cytometry allowing additional information concerning the stages of mitosis to be assessed making it potentially useful in studies of asymmetric division

Title: **Diagnostics of primary immunodeficiency syndromes using flow cytometry-based functional assays**

Yenan T. Bryceson
The Gade institute/Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden

Diagnostics of primary immunodeficiency syndromes using flow cytometry-based functional assays
Defects in lymphocyte cytotoxicity can cause life-threatening hyperinflammatory disorders such as hemophagocytic lymphohistiocytosis (HLH). Rapid differentiation of primary, genetic forms from secondary forms of HLH is crucial for treatment decisions. We prospectively evaluated the performance of degranulation assays based on surface upregulation of CD107a on NK cells and CTL in a cohort of 494 patients referred for evaluation for suspected HLH. Overall, resting NK cell degranulation below 5% provided a sensitivity for a genetic degranulation disorder of 96% and a specificity of 88%. Thus, degranulation assays allowed a rapid and reliable classification of patients, benefitting treatment decisions. Methods to define other primary immunodeficiency syndromes affecting lymphocyte function as well as strategies to further refine assays for functional evaluation lymphocyte defects will be discussed.

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Title: **Cytometry of epithelial and mesenchymal stem cells isolated from normal and malignant tissues**

Vera S. Donnenberg, University of Pittsburgh

This presentation will review the current state of the art in the identification and isolation of hematopoietic progenitor cells, multipotent mesenchymal stem cells, adipose stem cells, epithelial progenitors and pericytic stem cells using multiparameter flow cytometry combine with multi-color immunofluorescent microscopy and molecular techniques. Questions that will be addressed: What markers have been used to detect stem cells in normal human bone marrow, epithelial and adipose tissues? Do markers define populations with biologically unique properties? Must a self-replicating cell be rare or multipotent to be considered a stem cell? What is the difference between a stem cell and a progenitor cell? I will provide practical suggestions for disaggregation of tissues into single cell suspensions; focus on avoiding some of the technical pitfalls encountered when performing multi-color flow cytometry on disaggregated tissues. Specifically, these include recognizing sources of bias in cell recovery, use of doublet discrimination, use of DAPI to detect and remove hypodiploid events, coping with autofluorescence, and optimal use of dump gates.

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Title: **Cancer-targeted T cells revealed by flow cytometry**

Johanna Olweus
Department of Immunology, Institute for Cancer Research, Oslo University Hospital Radiumhospitalet, and the University of Oslo

Standard cancer treatment is not specific for cancer cells, and serious side effects limit therapeutic intensity. The immune receptors of T cells (T cell receptors) comprise a library of specificities that in principle can recognize any protein fragment with a high degree of specificity. This is the reason why infections are efficiently cleared without side effects. In contrast, the immune system of patients who have developed cancer normally fails at rejecting the cancer. The main reason is that the cancer cells are not recognized as foreign and dangerous. In contrast, transfer of immunity by T cells in allogeneic (from donor to patient) hematopoietic stem cell transplantation can cure a number of hematological malignancies. However, inability to separate T cells killing leukemia cells from those causing severe side effects due to attack of normal cells, limit the applicability of this treatment. The Olweus group has recently developed an approach that utilizes flow cytometry to identify and isolate T cells that selectively mediate the positive graft-versus-leukemia effect

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