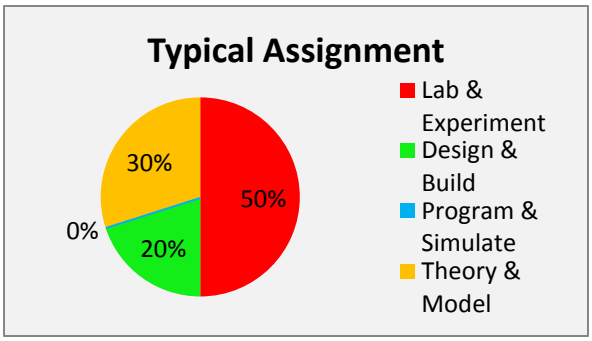
Catching metastasizing cells in the act

Novel characterization methods for circulating tumor cells

Introduction

Circulating tumor cells (CTC) are cancer cells that are disseminated from primary or metastatic tumors in cancer patients. These CTC are extremely rare (there is 1 CTC compared to $5 \cdot 10^6$ white cells and $5 \cdot 10^9$ red cells in 1 mL of blood), but clinical trials have shown that the number of CTC in blood is predictive of survival in several types of cancer, including breast, prostate and colon cancer.



Ongoing research

As CTC offer us the possibility to study cancer while metastasizing, we are interested in isolating, identifying and characterizing these cells in any way possible. In order to do this a broad range of techniques is used such as: immunofluorescence, FISH, PCR, WGA, next-gen sequencing, punching, microfluidic devices.

Projects:

Novel markers for detection of Circulating Tumor Cells

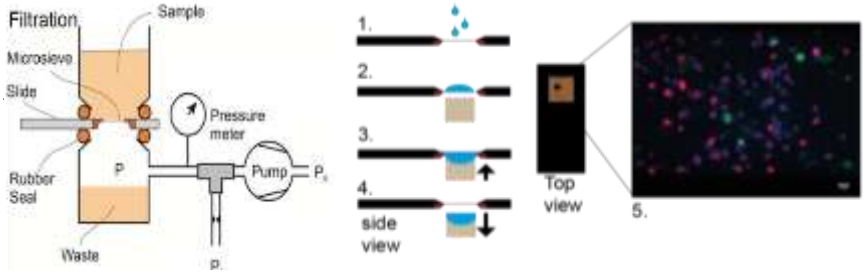
In this project we aim at finding novel markers expressed by CTC that can potentially be included in a routine diagnostic scenario. We will test new monoclonal antibodies (mABs) developed from tissue microarrays of tumor samples from breast, lung, colon and prostate cancer. Though the nature of the targeted antigens is often unknown, some of them showed consistent expression in several tumor cell lines. The mABs will need to be evaluated on cell lines, white blood cells and CTC.

RNA-seq from single Circulating Tumor Cells

Recently, methods have been developed at MCBP to isolate single CTC in a tube and to amplify the DNA of these cells. This DNA can be used to identify relevant mutations and transformations that could be indicative for the efficiency of treatment of patients. Next to DNA, also RNA expression could be informative. No proper methods are yet set up at the MCBP labs to prepare RNA from single CTC. Recently a method has been developed by Islam et al. to prepare full length barcoded cDNA from single cells in a single step. This assignment will be to set up this method and evaluate its performance.

Depletion of white blood cells

Approaches to increase the blood volume that can be analyzed are necessary to be able to detect more CTC. Instead of 7.5 mL blood, a diagnostic leukapheresis (DLA) will be used to obtain a product volume of 40 mL representing 750 mL of blood, this will be collected and analyzed for the presence of CTC using different technologies. The huge number of hematopoietic cells in the background contributes to the difficulty in isolating CTC. Depletion of white blood cells is needed to be able to isolate CTC. For this purpose, an assignment will be set-up to test multiple antibody-based CD45 depletion assays and compare performance. Subsequently methods need to be developed to identify and characterize the CTC in this depleted DLA product.



Filtration and immunofluorescence staining of CTC

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