Detection of PD-L1 and PD-L2 on Circulating Tumor Cells (CTCs) Using Microfluidic Based Chipcytometry

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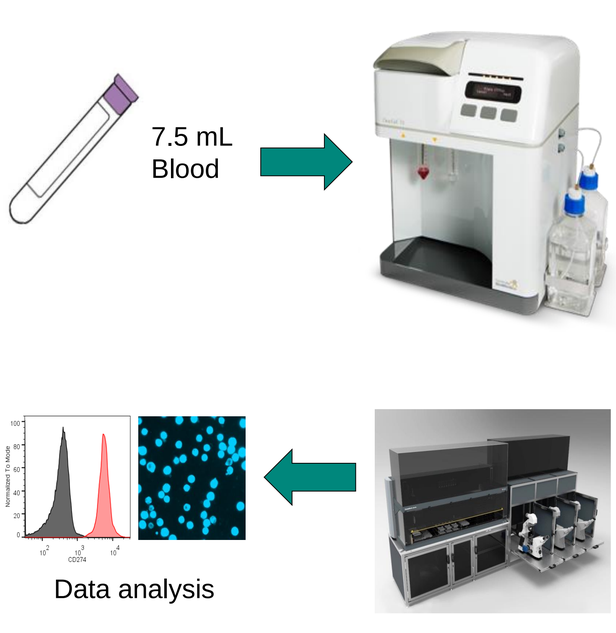
Abstract

- Recent advances in cancer therapy have demonstrated the potential of the immune system in cancer control and rejection.
- Prominent amongst these approaches has been the success of anti-PD-1 immunotherapy, to break the strong inhibitory signal, transmitted by tumor specific ligands such as PDL-1, to the PD-1 immune modulatory receptor expressed on T-cells.
- PD-L1 expression on the tumor is a clinically validated biomarker of therapeutic response to anti-PD-1 immunotherapy. However, obtaining tumor biopsies for PD-L1 interrogation is an invasive procedure not suited for frequent longitudinal monitoring during cancer therapy. Furthermore, tumor heterogeneity for PD-L1 expression may not accurately capture the PD-L1 status of the whole tumor burden in a single biopsy.
- An alternative, minimally invasive, approach is the analysis of blood samples for circulating tumor cells (CTCs) which have broken away from the tumor and entered the periphery.
- We describe the development of an assay workflow to detect and characterize circulating tumor cells in peripheral blood samples. Our approach uses a sized-based microfluidic enrichment technique, and subsequent characterization with microfluidic based cytometry (Chipcytometry).

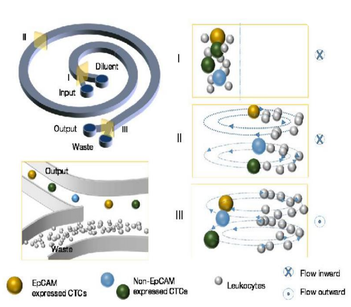
Workflow

- Human whole blood is hemolyzed and centrifuged to harvest nucleated cells. ClearCell® FX enriches CTCs based on size.
- Enrichment product is loaded onto microfluidic chips and stored at 4°C until ready for analysis.
- CTCs imobilized on microfluidic chips are phenotyped with monoclonal antibodies against tumor markers using the Zellkraftwerk CYTOBOT.



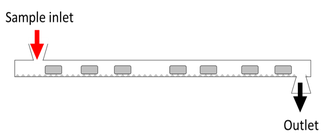
CTC enrichment by ClearCell® FX System

- Microfluidic chip enriches CTCs based on size
- Label free isolation
- Process blood volume of 7.5 mL
- Processing in approx. 1 hour

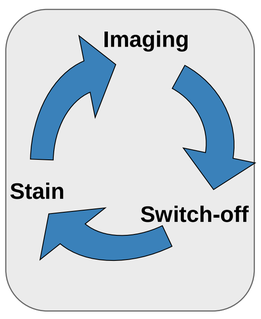


- Smaller cells (RBCs ~8 µM and leucocytes ~8–15 µM) are affected by the Dean drag and migrate to the outer wall
- Larger CTCs (~15-20 µM) experience strong inertial lift forces and are focused along the inner wall

CTC characterization by Chipcytometry



- Cells immobilized on microfluidic chip
- Fixation of immobilized cells and long term storage at 4°C



- Phenotyping by staining with monoclonal antibodies conjugated to a fluorophore and detection using CYTOBOT system
- Sequential staining of each antibody permits unlimited set of markers

- Individual cells are identified and based on their position
- Multidimensional information is layered to create a profile for each cell

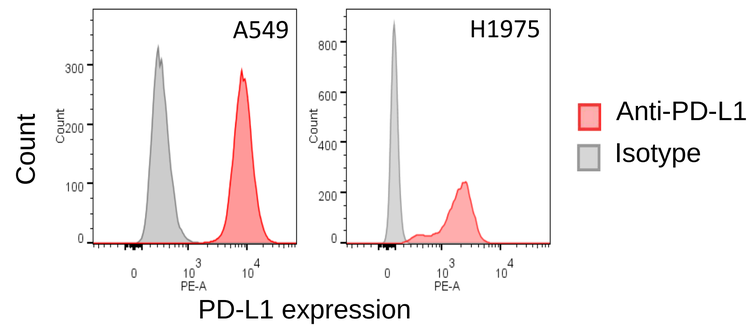
Marker	Relevance
DAPI	Validated marker of CTC identification*
CD45	Validated marker of CTC identification*
Cytokeratin	Validated marker of CTC identification*
EpCAM	Validated marker of CTC identification*
CD3	Negative on CTCs
CD15	Negative on CTCs
PD-L1	Immune checkpoint regulator
PD-L2	Immune checkpoint regulator
Vimentin	Marker of Epithelial – mesenchymal transition

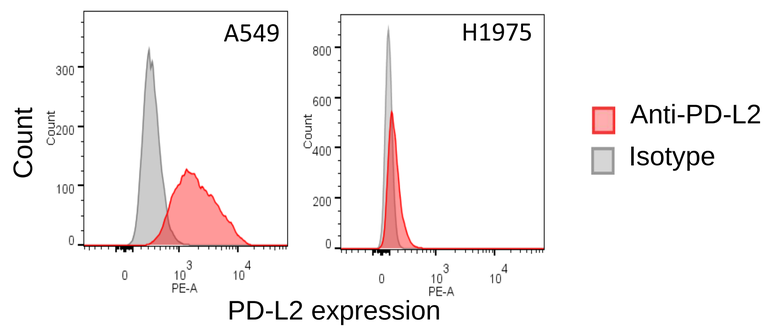
* FDA validated CellSearch system identify CTCs as DAPI+ / CD45- /Cytokeratin+ / EpCAM+

Specificity of anti-human PD-L1 and PD-L2 monoclonal antibodies determined by Flow Cytometry

- Specificity of monoclonal antibodies against PD-L1 and PD-L2 was determined on non-small cell lung cancer cell lines, A549 and H1975, using flow cytometry.
- A549 cells were incubated with IFN-γ for 24 hours prior to staining to upregulate PD-L1 and PD-L2. Cells were fixed prior to staining.
- The data demonstrates the presence of PD-L1 on A549 and H1975 cells, and PD-L2 on A549 cells.
- The specificity of the staining was demonstrated by the fact that no signal was detected for the isotype control on both A549 and H1975 cells.

Flow cytometry histograms:

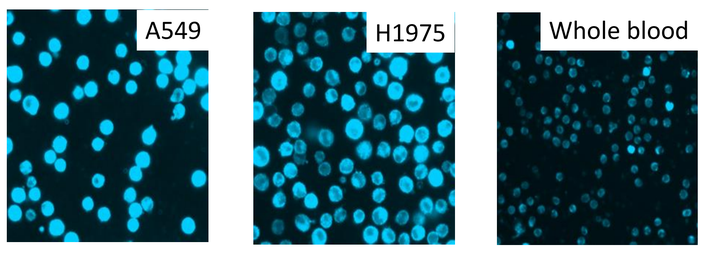




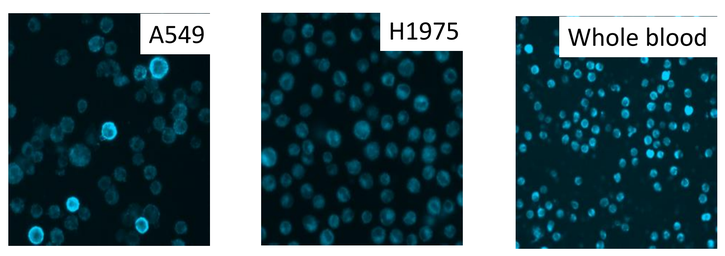
Detection of PD-L1 and PD-L2 expression by Chipcytometry

- A549, H1975 and whole blood was analyzed for PD-L1 and PD-L2 expression using the CYTOBOT chipcytometry system. A549 cells were incubated with IFN-γ for 48 hours prior to staining to upregulate PD-L1 and PD-L2. Histograms translated from images.
- Individual A549 and H1975 cells positive for PD-L1, and individual A549 cells positive for PD-L2,could be observed.

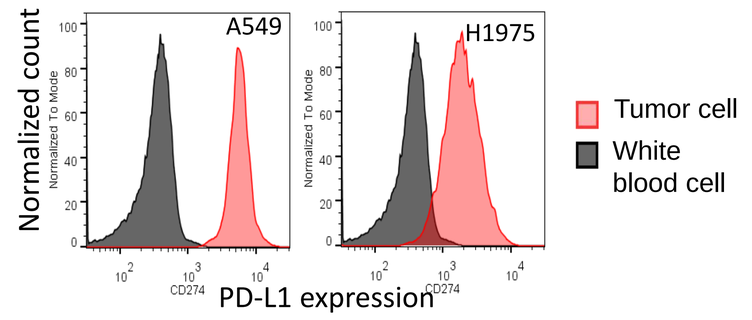
Detection of PD-L1

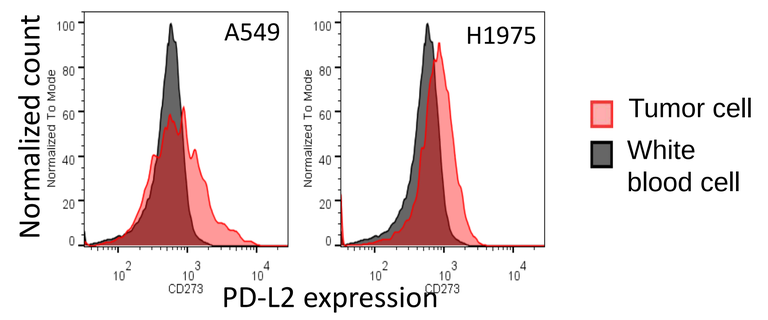


Detection of PD-L2



Histograms derived from chipcytometry image fluorescence:





Conclusions

- **Assay workflow to detect and characterise CTCs**
- **Specificity of PD-L1 and PD-L2 antibodies demonstrated by flow cytometry**
- **Established feasibility of PD-L1 and PD-L2 detection by chipcytometry**
- **Evaluation of workflow in currently on-going experiments with spiked samples and samples from breast cancer patients**