High-Parameter Profiling of Psoriatic Tissue

Anja Mirenska[1], Heath Guay[2] and Jose Estevam[2]
[1]Zellkraftwerk, Germany; [2]Takeda Pharmaceuticals Inc., Cambridge, MA USA

PURPOSE

Understanding the abundance and the spatial interactions of immune cells in heterogeneous tissue samples is pivotal to improving clinical outcomes for psoriasis patients undergoing immunotherapy. Various multiplexed imaging platforms have been recently developed to visualize immune cell subtypes in tissue samples with varying distribution patterns involved in autoimmunity.

However, the lack of tools that allow for the analysis of spatial patterns is a major barrier for systematic analysis of cellular interactions in the dermal microenvironment. Here, we present data on immune cell distribution of lesional skin biopsies from psoriatic tissue using hi-plex immune fluorescence Chipcytometry technology.

MATERIAL AND METHODS

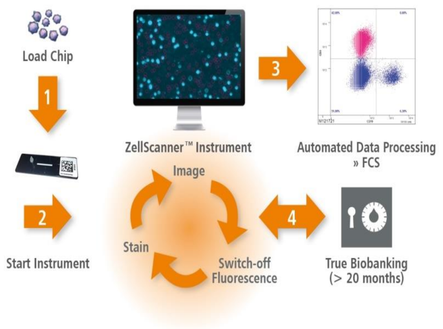
Chipcytometry is an image cytometry platform that has previously been used in biomedical research to perform immune cell phenotyping and cell function assessments, such as intracellular cytokine production, cell proliferation, apoptosis and tissue cytometry [1,2,3,4].

Using fresh-frozen tissue biopsy samples from two psoriasis patients, we developed a prototype assay for monitoring FoxP3 positive cells and checkpoint signaling using commercially available antibodies.

11-PLEX IMMUNE CELL PHENOTYPING

Marker	Antibody Clone	Vendor	Dilution	Fluorophore	Compartment
CD3	UCHT1	BD Biosciences	1:30	BUV395	Surface
CD4	RPA-T4	Biolegend	1:30	PE	Surface
CD8	RPA-T8	BD Biosciences	1:30	BUV395	Surface
CD39	A1	Thermo Fisher	1:250	Alexa Fluor 488	Surface
CD45	HI30	BD Biosciences	1:100	BUV395	Surface
CD278	ISA-3	eBioscience	1:30	PE	Surface
CD279	MIH4	eBioscience	1:30	PE	Surface
Collagen IV α1	1043	Novus Biologicals	1:30	PE	Surface
Laminin γ1	A5	Novus Biologicals	1:30	PE	Surface
Pan-Cytokeratin	C-11	Novus Biologicals	1:30	PE	Cytoplasm
FoxP3	236A/E7	eBioscience	1:30	PE	Nucleus

PRINCIPLE AND EQUIPMENT

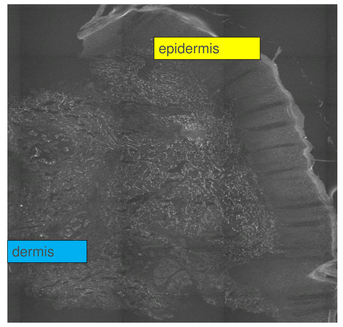


The Chipcytometry Workflow

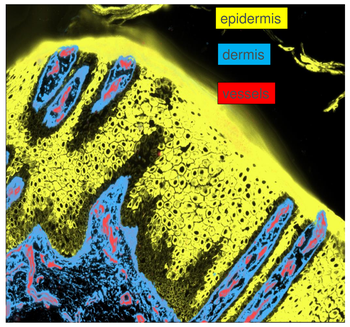
Cytobot® - Chipcytometry Instrument

WORKFLOW

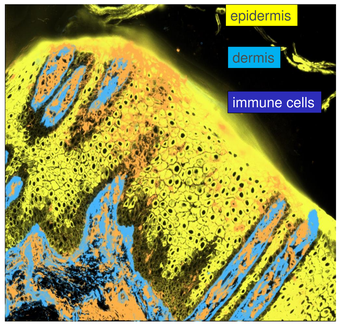
STEP 1: TISSUE ORIENTATION (TRANSMITTED LIGHT)



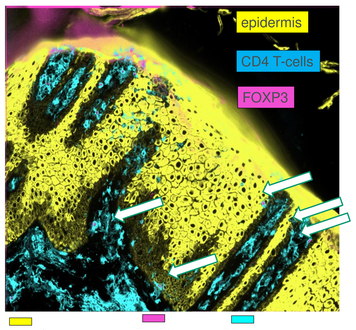
STEP 2: AREA OF INTEREST SELECTION



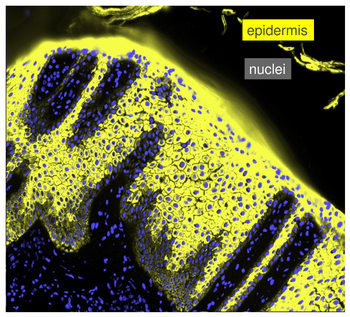
STEP 3: QUALITY CONTROL



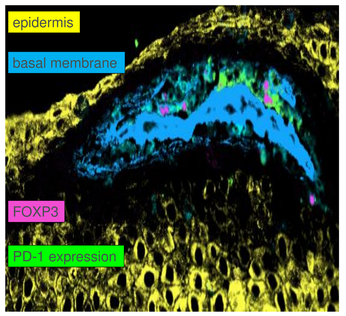
STEP 4: PHENOTYPE



STEP 5 (OPTIONAL): SEGMENT/QUANTIFY



STEP 6: LOCALISE CHECKPOINT SIGNALING



RESULTS AND SUMMARY

Population	PD-1 expression
Overall T cells	Low
FoxP3 T-cells	Medium-high

- 11 biomarkers were successfully stained on a lesional tissue section of a psoriatic patient.
- Characteristic morphology (enlarged epidermis) was detected by staining with tissue orientation biomarkers (pan-cytokeratin, laminin, collagen IV).
- CD45 was used as fitness biomarker for sample quality control (to provide confidence that biomarkers have not been damaged during sample handling).
- Immune cell infiltrates containing large T cell numbers including CD4+ T helper cells, CD8+ T effector cells and FoxP3 T-cells were detected in psoriatic skin.
- Some FoxP3 T-cells were found to express the checkpoint protein PD-1.
- Cytokine staining (IFNγ, IL-17A, IL-17F, TNFα) was negative, confirming previous findings that cytokines should only be stained on digested tissue (Welzenbach et al., 2015)

CONCLUSIONS

We conclude that high parameter tissue phenotyping of psoriasis tissue samples is technically feasible and can be used to generate high quality biomarker data. Profiling of the target expression in diseased tissues is a useful tool for drug development not only in psoriasis but also in a large range of other diseases that are driven or accompanied by altered immune responses.

REFERENCES

[1] Henning et al. *Cytometry A* (2009). A versatile platform for comprehensive chip-based explorative cytometry.
[2] Happle et al. *Science Translational Medicine* (2014). Pulmonary transplantation of macrophage progenitors as effective and long-lasting therapy for hereditary pulmonary protenosis.
[3] Roesner et al. *Journal of Investigative Dermatology* (2015). Der p1 and Der p2-specific T cells display Th2, Th17, and Th2/Th17 phenotype in atopic dermatitis.
[4] Zeng, et al. Poster at *NBC 2015*: Comparison of human whole blood immunophenotyping by ChipCytometry and Flow Cytometry: Potential applications for biomarker identification and immunomonitoring in clinical studies.

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