The Science CRO

Harnessing Next Generation Cytometry in Immuno-Oncology

Christos Nikolaou, Zuzanna Makowska, Claudia Noack, Alexandra Rausch, Oliver von Ahsen, Ralf Lesche, Juliette Pabst, Martin Lange, Barbara Nicke, Charlotte Kopitz, Krzysztof Brzezinka

NUVISAN ICB GmbH | Muellerstr. 178 | 13353 Berlin | Germany

Introduction

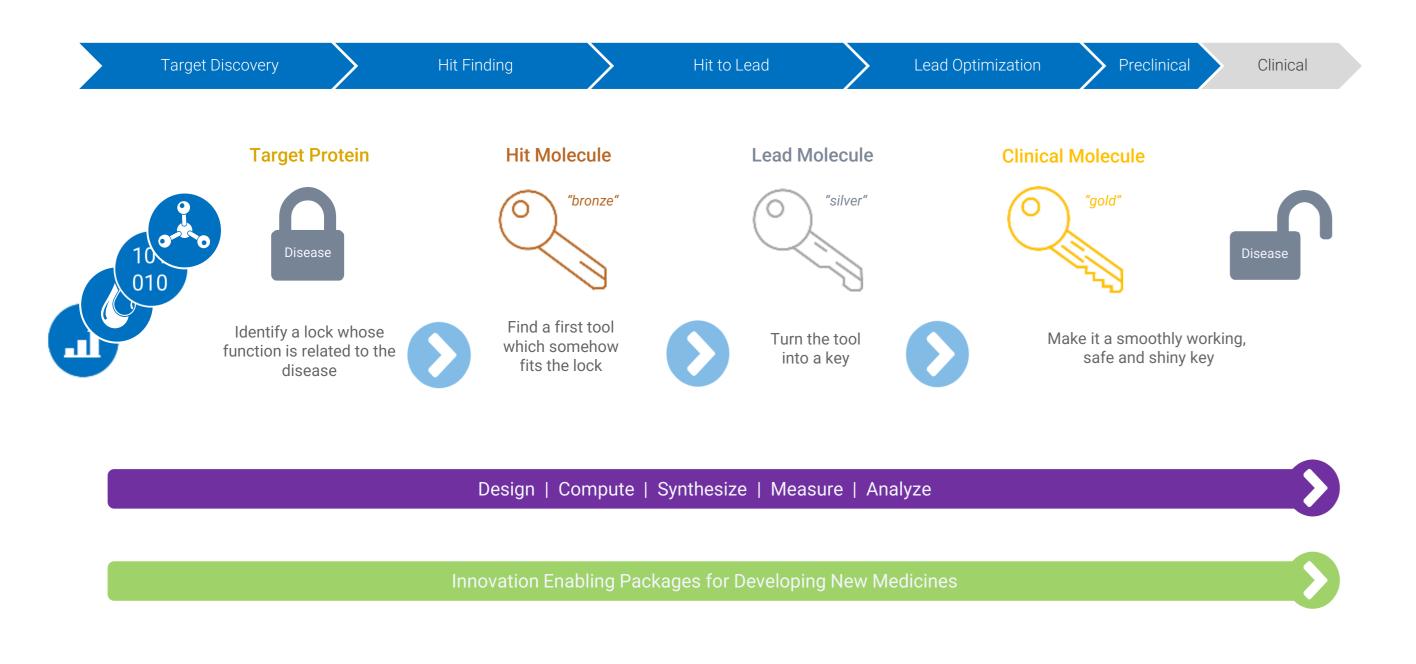
The high variability in the distribution of immune and non-immune compartments within the tumor microenvironments (TME) among patients drives their response or potential resistance to current treatment strategies. Understanding this diversity is a key to patient tailored treatment which can meet individual's needs.

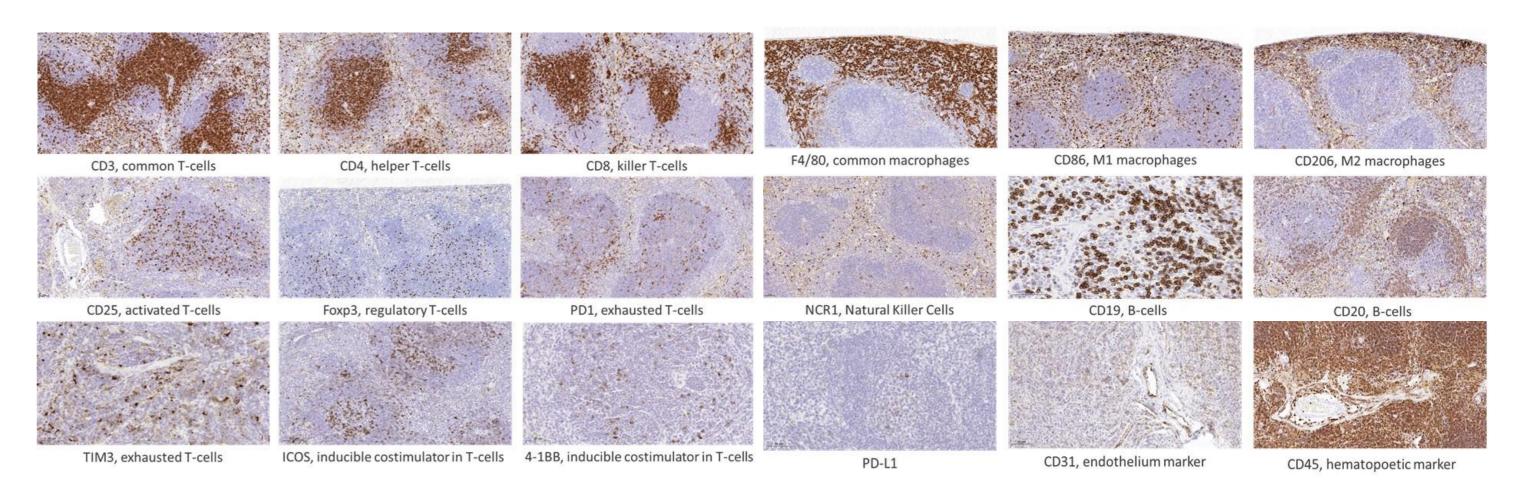
Comprehensive Spatial Profiling of Lymphoid Organs and TILs

- Immune cell profiling
- Single-cell RNA sequencing
- Tissue-based IHC
- Full spectrum flow cytometry or functional assays

are detrimental in proper disease assessment and contribute to novel drug discovery.

Fully Integrated Solutions From Target to Patient





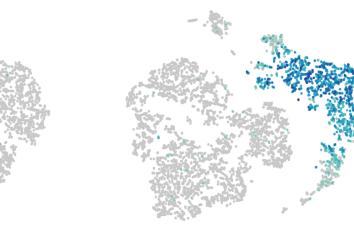
Spatial profiling of lymphocytes in tissues as well as tumor-infiltrating immune cells can shed light on mechanisms of cancer-immune evasion, thus providing opportunities for the development of novel therapeutic strategies

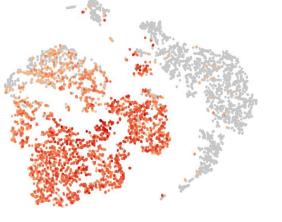
Use Case: Single Cell RNASeq of PBMCs

Barcode Rank Plot of a representative PBMC single cell sequencing result (10x Genomics v3.1)

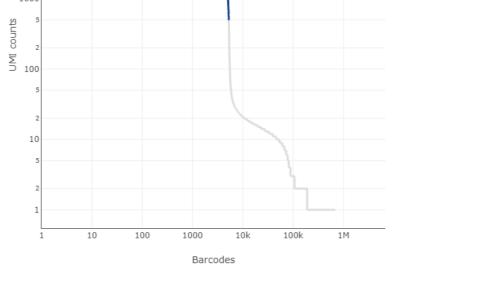


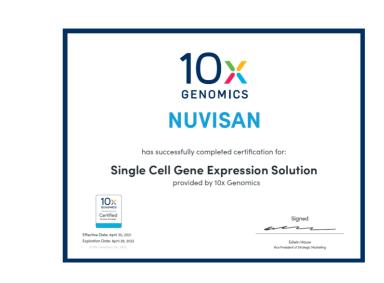
tSNE cluster analysis shows clear separation of PBMC cell populations





CD14: monocytes





Nuvisan ICB Flow Cytometry Core Facility

Analysers Equipment (S1)

- Cytek Aurora (VBR lasers)
- BD Canto II (VBR lasers)
- Miltenyi MACSQuant X (VBR lasers)
- Sartorius Intellicyt iQueScreener PLUS (VBR lasers)

Cell sorting Equipment (S2)

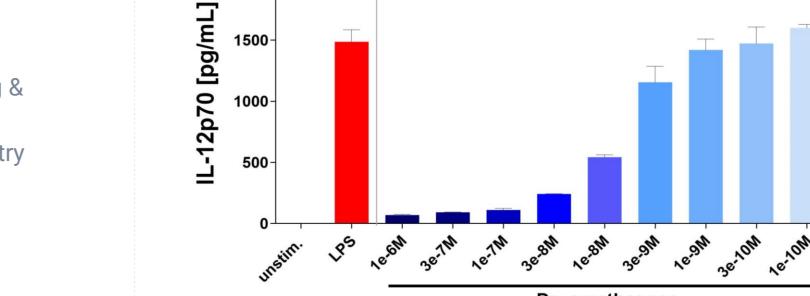
• Sony MA 900 (VBYGR lasers)

Applications

- Immunophenotyping Cell proliferation/ killing assays
- Cytokine & Functional profiling
- Antibody screening
- Cell line development
- Primary cell screening
- Target identification with siRNA / CRISPR
- Monitor treatment efficacy
- Phosphorylation assays
- Cell sorting for subsequent single cell sequencing

Service

- Scientific consultation (mouse, human, rat, NHP)
- Panel design (>25 parameters)
- High throughput screening
- Preparation of samples (solid or liquid tissues)
- QC & Acquisition
- Data analysis (manual gating & unsupervised clustering)
- Instrument/Analysis Cytometry Training



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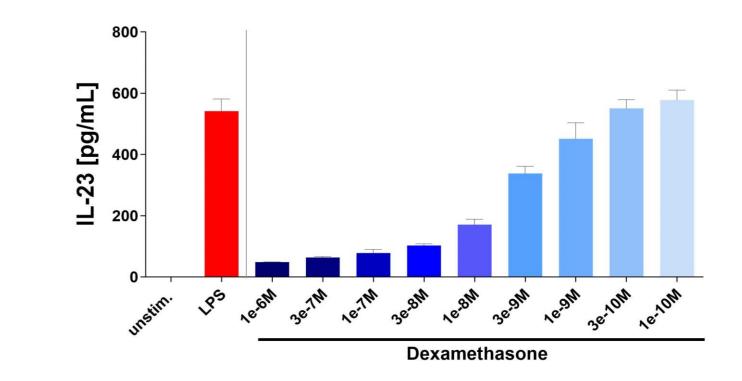
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Use Case: IL-23 and IL12p70 Secretion in human LPS-stimulated mDCs

Dose-dependent cytokine inhibition by Dexamethasone in LPS-stimulated DCs:











Immune cell composition-Tumor bearing mice

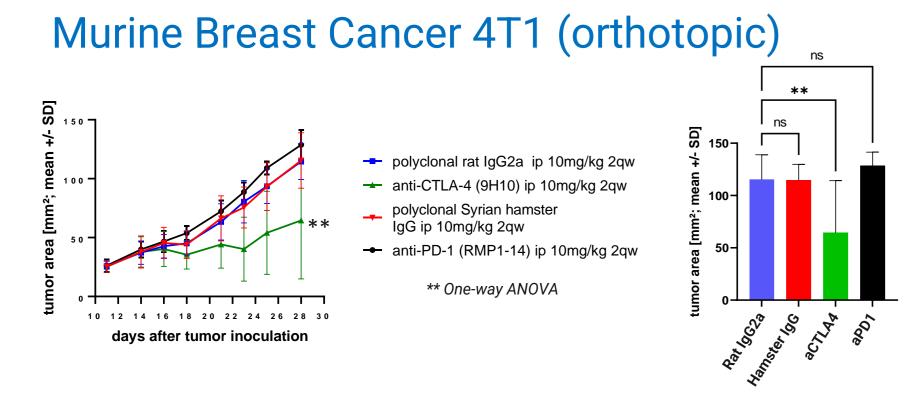
Experimental design 4 groups (aPD1 vs isotype control, aCTLA4 vs isotype control)



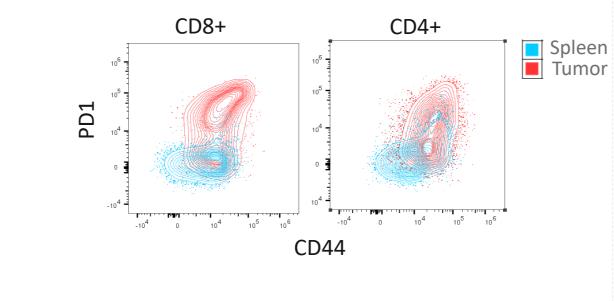
injection of tumor cells (e.g., 4T1, LLC, Renca, CT26, Hepa1-6 lines) into WT mice

Baseline & randomization day Initiation of i.p. Immune checkpoint Inhibitors (10mg/kg) vs isotype controls, 2x weekly

treatments (i.p aPD1 and aCTLA4 2x weekly) . Spleenocytes and tumor-infiltrating leukocytes are isolated and analyzed on CYTEK using 25 surface markers as well as using IHC.



T cells have an effector activated phenotype

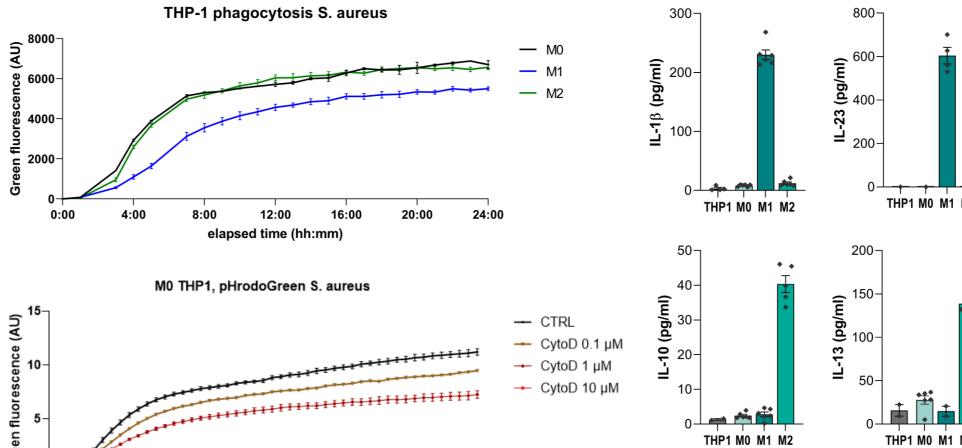


Use Case: Macrophage Functionality Assay

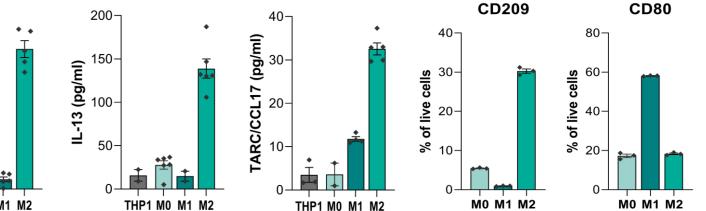
THP1 macrophages – S. aureus phagocytosis

Dexamethasone

THP1 differentiated with PMA into M0 macrophages, 24h polarisation into M1/M2







CytoD: Cytochalasin D, inhibitor of actin polymerization \rightarrow uptake of particles

