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The Science CRO

Harnessing Next Generation Cytometry in Immuno-Oncology

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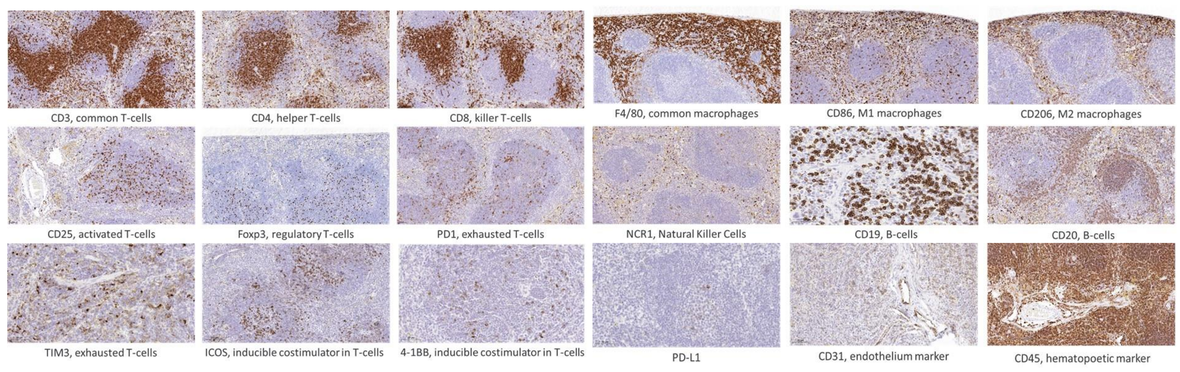
Introduction

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.

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

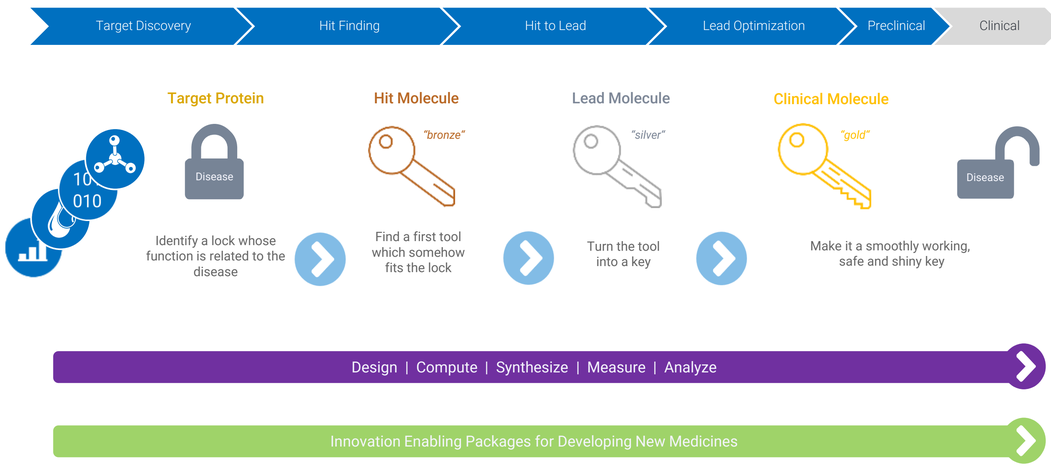
are detrimental in proper disease assessment and contribute to novel drug discovery.

Comprehensive Spatial Profiling of Lymphoid Organs and TILs



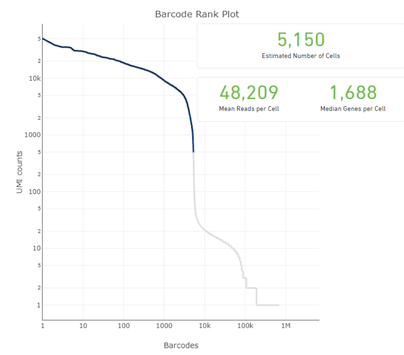
- Spatial profiling of lymphocytes in tissues as well as tumor-infiltrating immune cells can shed light on mechanisms of cancer–immune evasion, thus providing opportunities for the development of novel therapeutic strategies

Fully Integrated Solutions From Target to Patient

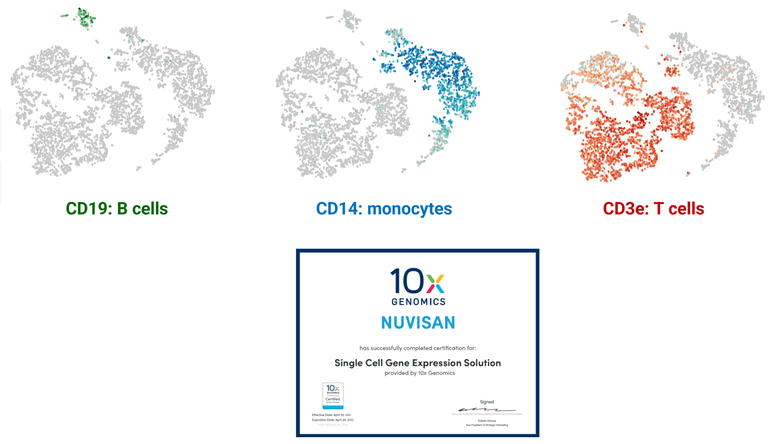


Use Case: Single Cell RNASeq of PBMCs

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



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

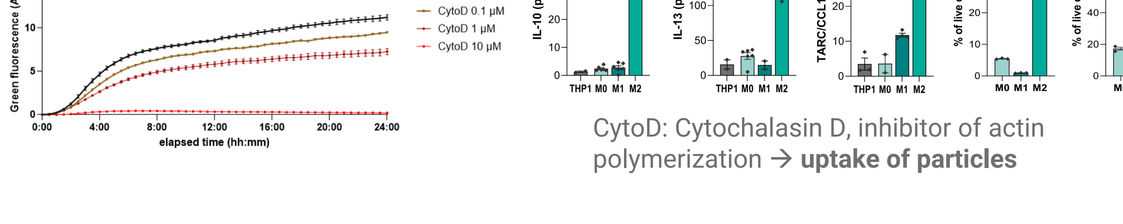
- Immunophenotyping
- Cell proliferation/ killing assays
- Cytokine & Functional profiling
- Antibody screening
- Cell line development
- Primary cell screening
- Target identification with siRNA / CRISPR
- Monitor treatment efficacy
- Phosphorylation assays
- Cell sorting for subsequent single cell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

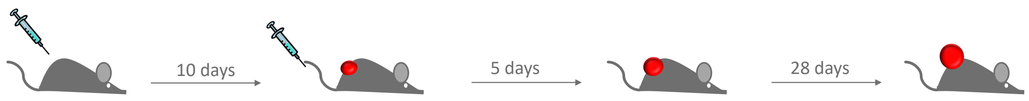
Use Case: IL-23 and IL12p70 Secretion in human LPS-stimulated mDCs

- 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)

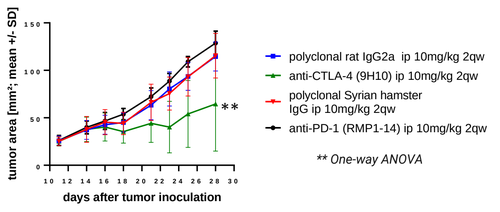


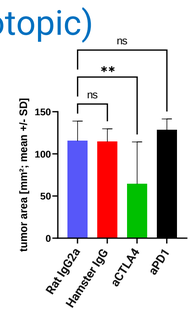
s.c. or orthotopic injection of tumor cells (e.g., 4T1, LLC, Renca, CT26, Hepa1-6 lines) into WT mice

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

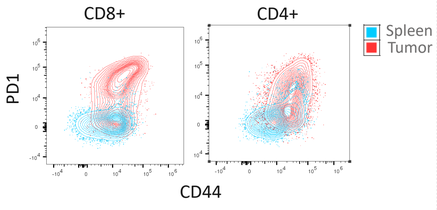
Mice are euthanized at **A)** Baseline **B)** Day 5 and **C)** Day 28 post treatments (i.p aPD1 and aCTLA4 2x weekly) . Spleenocytes and tumor-infiltrating leukocytes are isolated and analyzed on CYTEK using 25 surface markers as well as using IHC.

Murine Breast Cancer 4T1 (orthotopic)





T cells have an effector activated phenotype



Use Case: Macrophage Functionality Assay

THP1 macrophages – S. aureus phagocytosis

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

CytoD: Cytochalasin D, inhibitor of actin polymerization → **uptake of particles**

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