

Immune monitoring tools and their application

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Benefits of immune monitoring in advanced cell therapies

Immune monitoring plays an important role in the detection of cellular immune responses at preclinical and clinical stages and is essential for the development and clinical application of living immunotherapies.

Immune monitoring

- facilitates a deeper understanding of the immune response *in vitro* and *in vivo*
- provides crucial data on the effectiveness of treatment in preclinical models
- provides insight into basis of clinical efficacy in patients
- has the potential to identify new biomarkers or therapeutic targets

Multiple assays are used for immune monitoring in TCR-T cell therapy

Identification of TCR-T cells

- Multimer staining of T cells expressing recombinant TCR
- Digital droplet PCR for detection of recombinant TCR

Characterization of TCR-T cells

- Determination of T-memory subset composition
- Determination of T-activation / T-checkpoint status

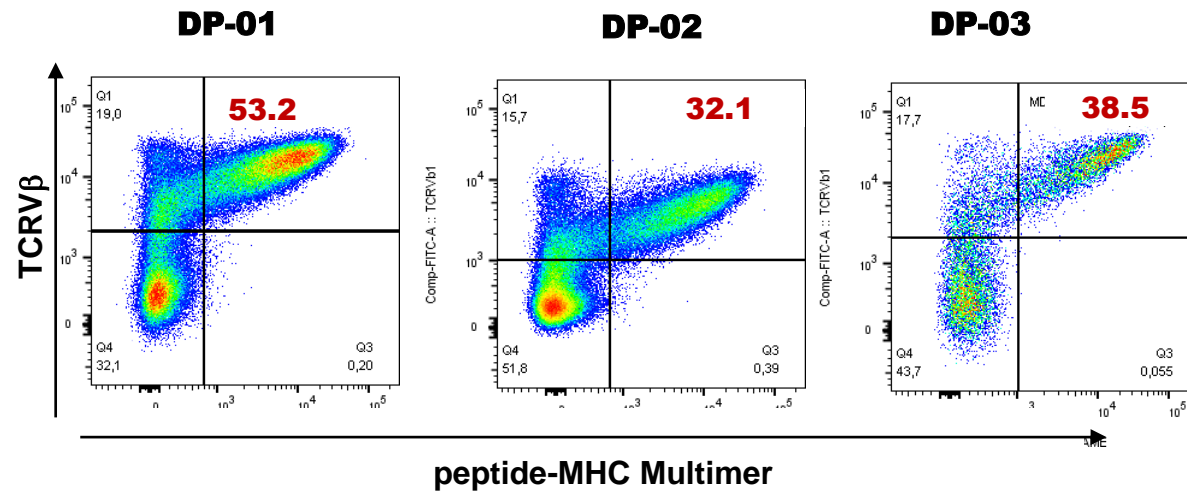
Functional analysis of TCR-T cells

- Multiplex assay of TCR-T cytokine secretion
- Intracellular cytokine staining of TCR-expressing T cells
- Single cell secretome of T cells expressing recombinant TCR
- 3D-serial killing mediated by TCR-Ts
- Proliferation of TCR-Ts after stimulation

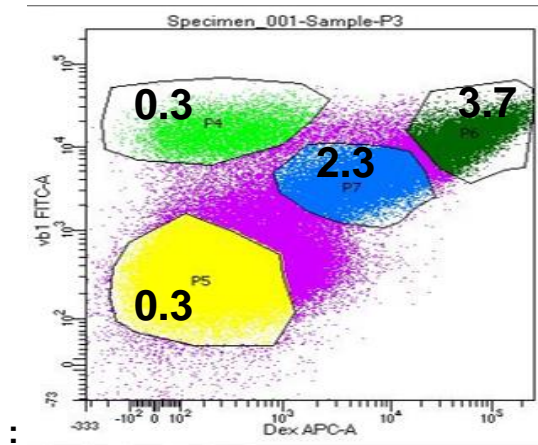
Identification of TCR-T cells

Dual staining of TCRvβ and peptide-MHC precisely identifies TCR-expressing T cells in drug products or patient samples

TCR-T drug products



Sorting and determination of vector copy number



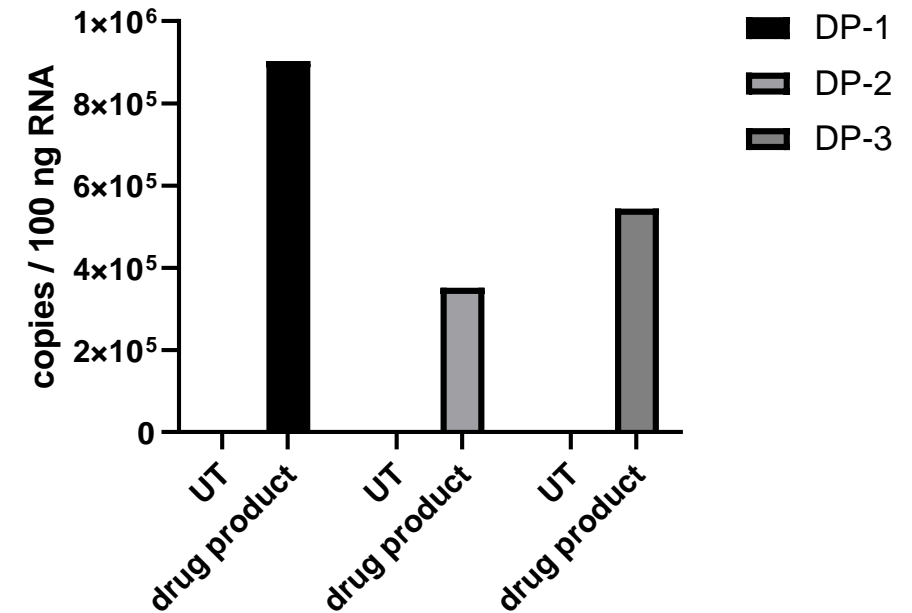
Intensity of multimer binding is dependent on the vector copy number

Possible applications:

- Determination of the transduction efficiency
- Dose calculation based on binding the relevant peptide-MHC complex
- Analysis of *in vivo* persistence by detection of TCR-T cells in patient blood and bone marrow

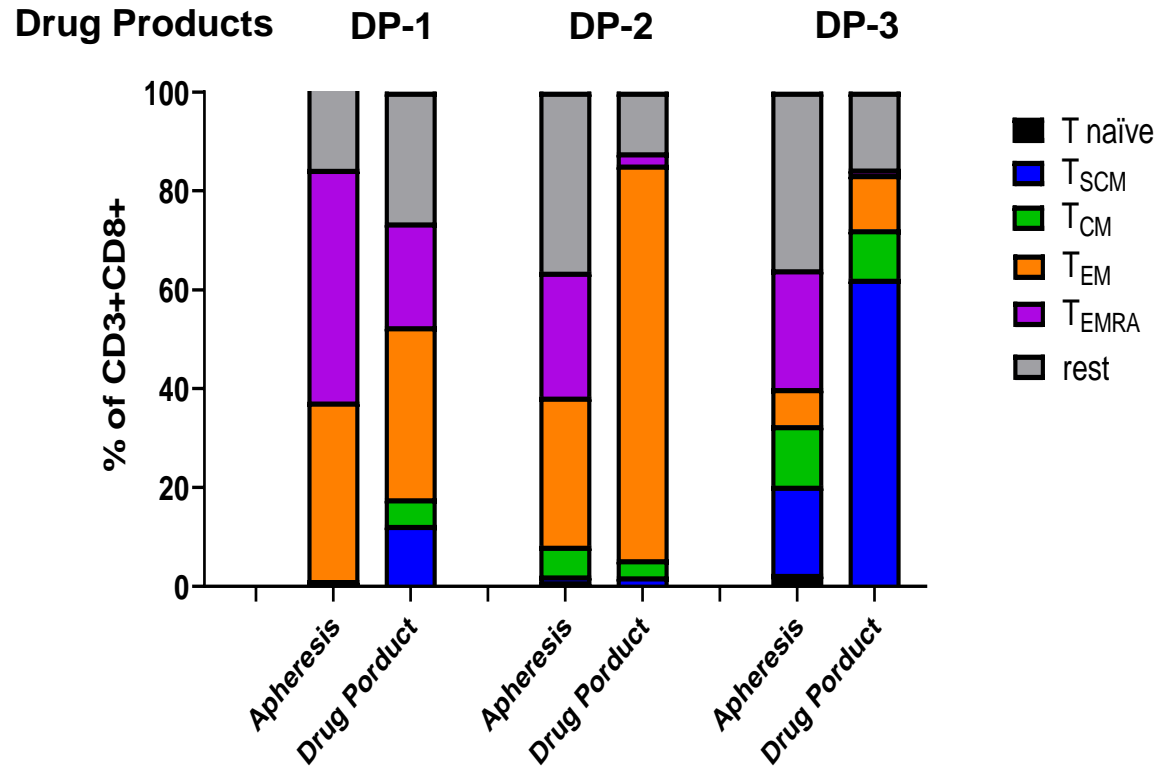
Digital droplet PCR (dPCR) identifies TCR-T cells with molecular precision

- TCR-T cells are identified by the RNA of a viral element present in the vector used for TCR transfer
- dPCR allows quantification of the target sequences without need for comparison against a standard curve
- dPCR is easier to validate as it has better precision, reproducibility and sensitivity
- The dPCR would be expected to be more sensitive for TCR detection. CAVEAT: a high fraction of tumor cells could contribute to failure to have adequate numbers of T cells in the processed materials due to strong tumor-derived mRNA as can be the case in blood cancers



Phenotypic characterization of TCR-T cells

Multi-color flow cytometry reveals variations in T-memory composition of TCR-T cells

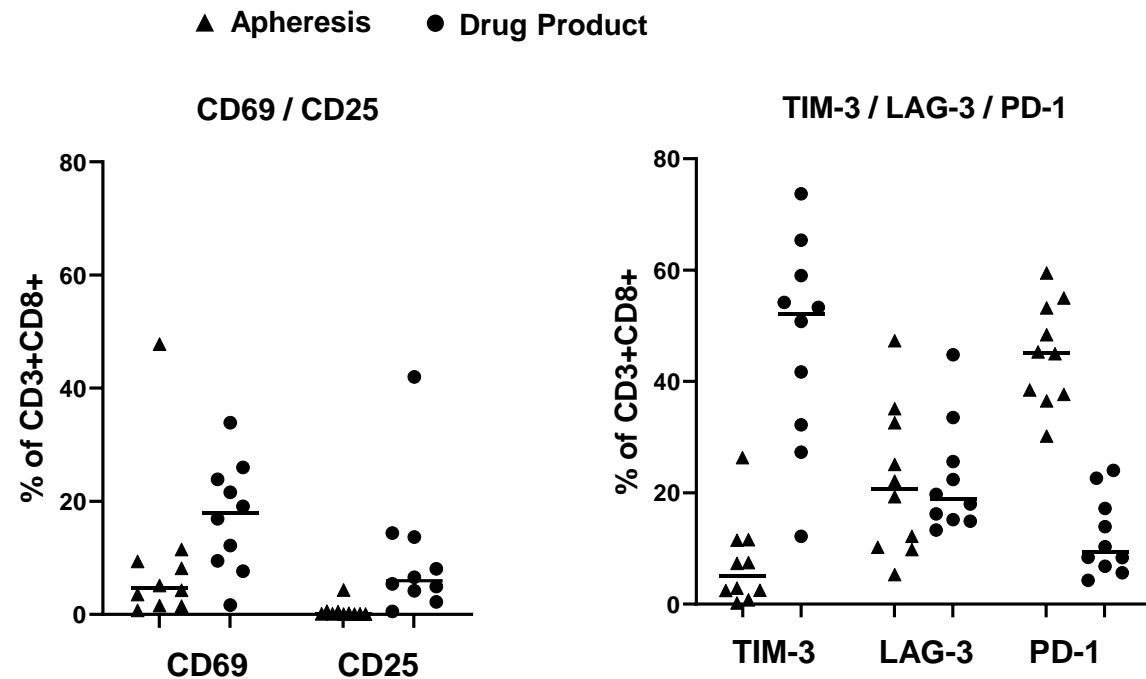


T-memory staining panel				
live/dead	CD45	CD3	CD8	CD4
pMHC Multimer	TCRvβ			
CD45RA	CCR7	CD27	CD95	

Possible applications:

- Characterization of the starting material and possible correlation to the composition of drug products
- Influence of the production process on the T-memory composition
- Characterization of TCR-T cells “*in vivo*”

Multi-color flow cytometry identifies activation status of TCR-T cells



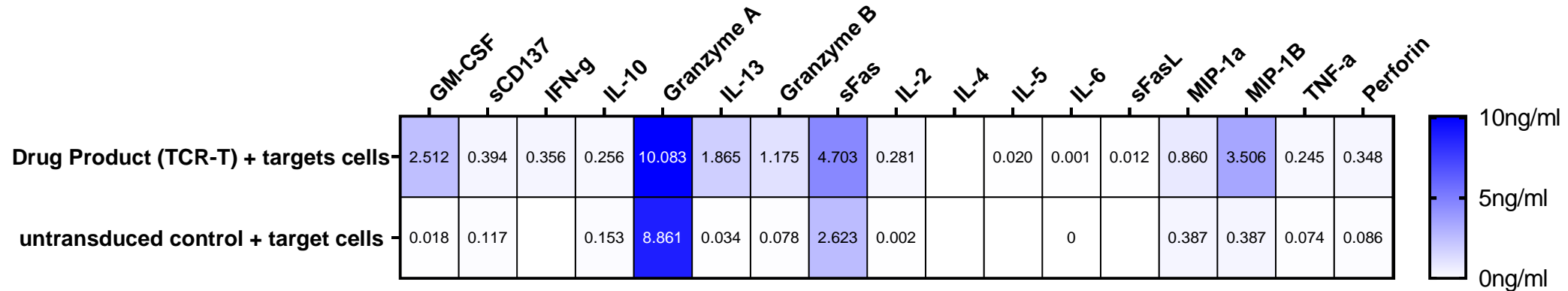
T-activation / checkpoint staining panel				
live/dead	CD45	CD3	CD8	CD4
pMHC Multimer	TCRvβ			
CD69	CD25	TIM-3	LAG-3	PD-1

Possible applications:

- Characterization of the starting material and possible correlation to the expression in drug products
- Influence of the production process on the expression of activation and checkpoint markers
- Characterization of TCR-T cells “*in vivo*”

Functional analysis of TCR-T cells

Multiplex technologies show cytokine potential of TCR-T cells

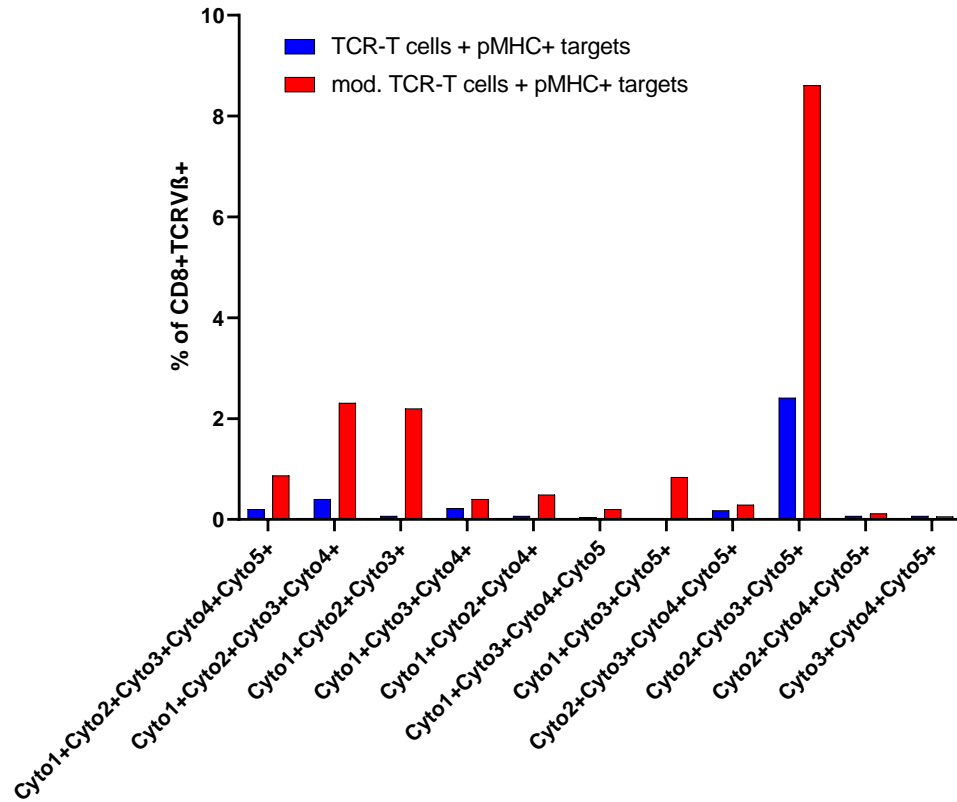


Possible applications:

- Characterization of the functional activity of drug products by analyzing coculture supernatants
- IMP activity by analyzing patient serum after IMP administration in line with TCR-T immunotherapy

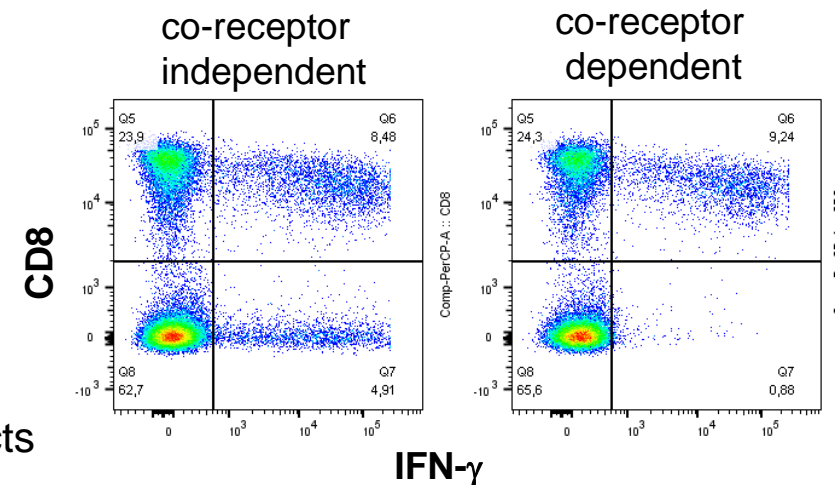
CAVEAT: Only secretion of single cytokines is analyzed – no real determination of poly-functionality

Intracellular cytokine multi-color flow cytometry identifies polyfunctional TCR-T cells



Intracellular cytokine staining				
live/dead	CD45	CD3	CD8	CD4
pMHC Multimer	TCRVβ			
Cytokine 1	Cytokine 2	Cytokine 3	Cytokine 4	Cytokine 5

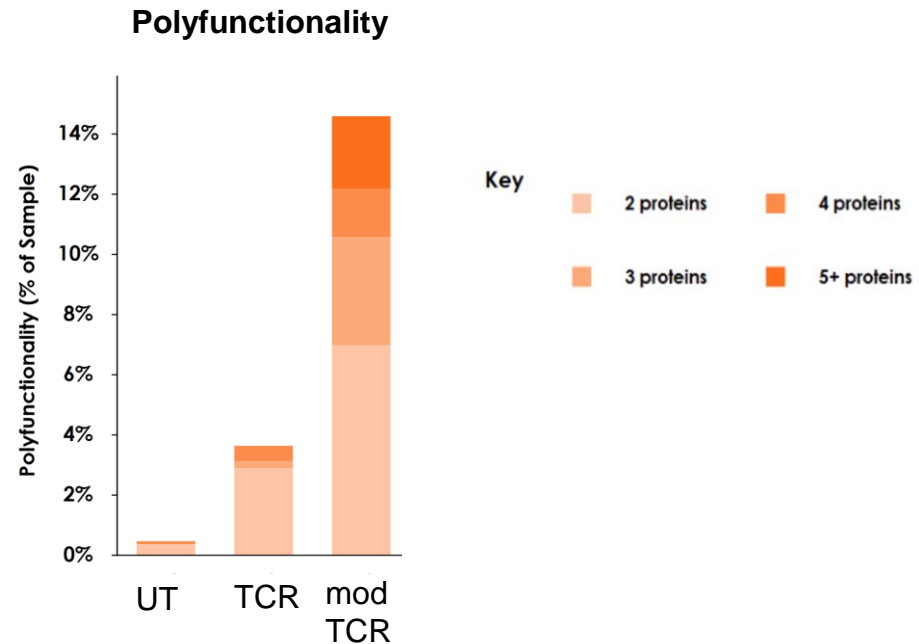
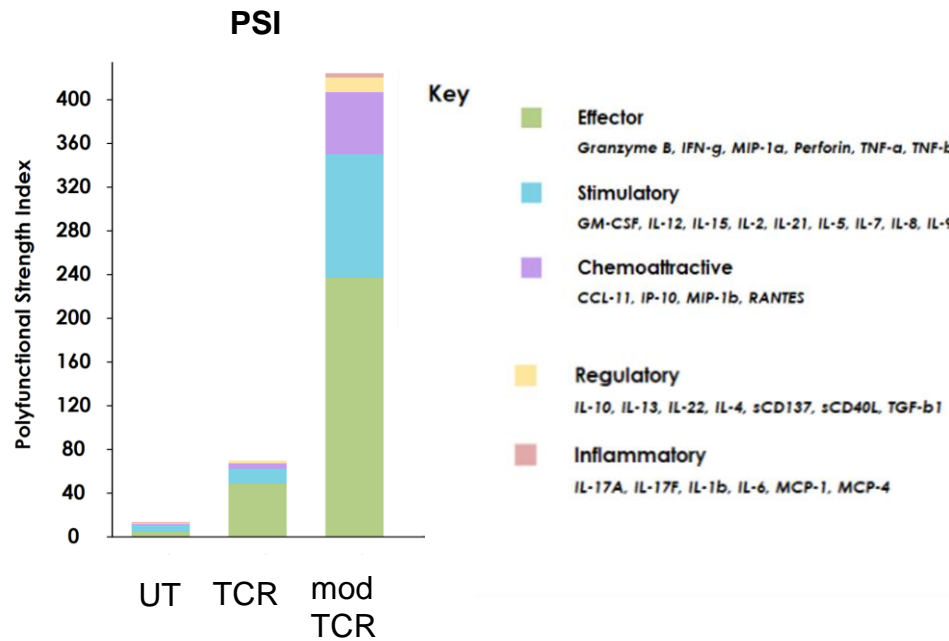
Analysis of TCR co-receptor dependency



Possible applications:

- Comparison of different TCR-T cells with regard to their polyfunctionality
- Identification of a cytokine signature relevant for potent TCR-T drug products
- Co-receptor dependency

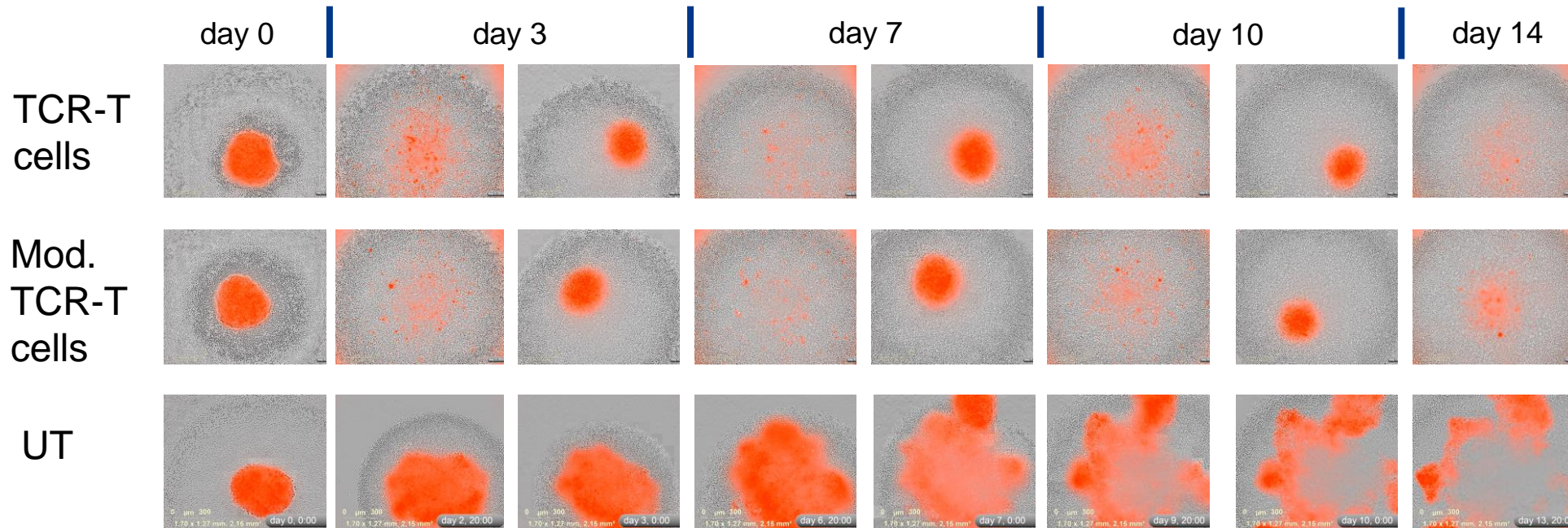
Single cell secretome analysis (Isoplexis) shows polyfunctionality of TCR-T cells



Possible applications:

- Comparison of different TCR-T cells with regard to their polyfunctionality
- Identification of a cytokine signature relevant for potent TCR-T drug products
- Possible distinction between non-responders and responders in clinical settings based on the PSI

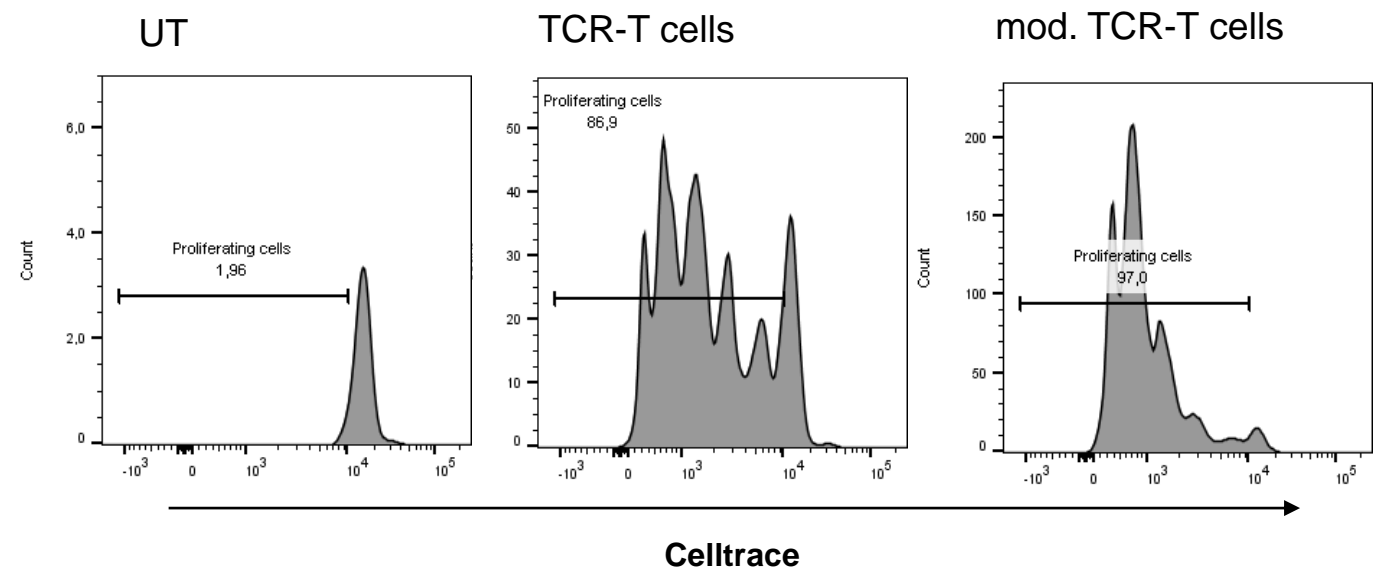
Live cell imaging using IncuCyte S3 shows 3D serial killing by TCR-T cells



Possible applications:

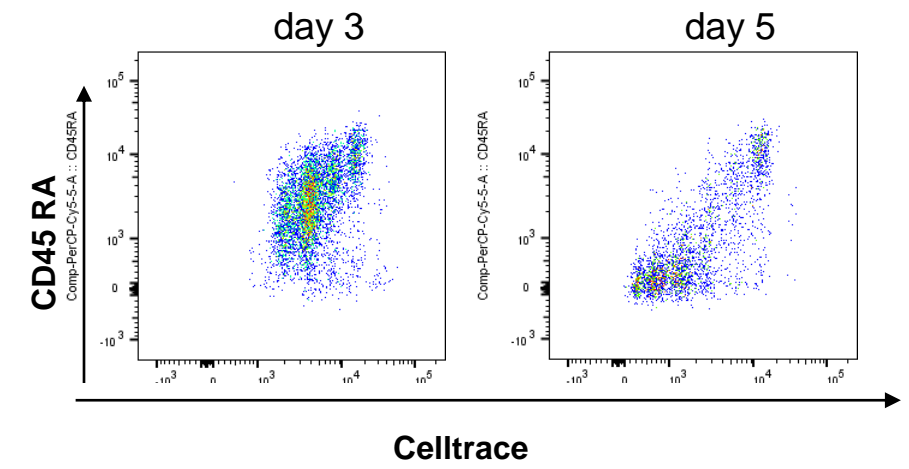
- Comparison of different TCR-T cells with regard to their killing capacity
- Analysis of the killing of tumor target cells expressing different amounts of relevant peptide-MHC
- Serial challenges of TCR-T cells with tumor spheroids resemble an intermediate step between *in vitro* and *in vivo* experiments

Multi-color flow cytometry identifies proliferating TCR-T subsets

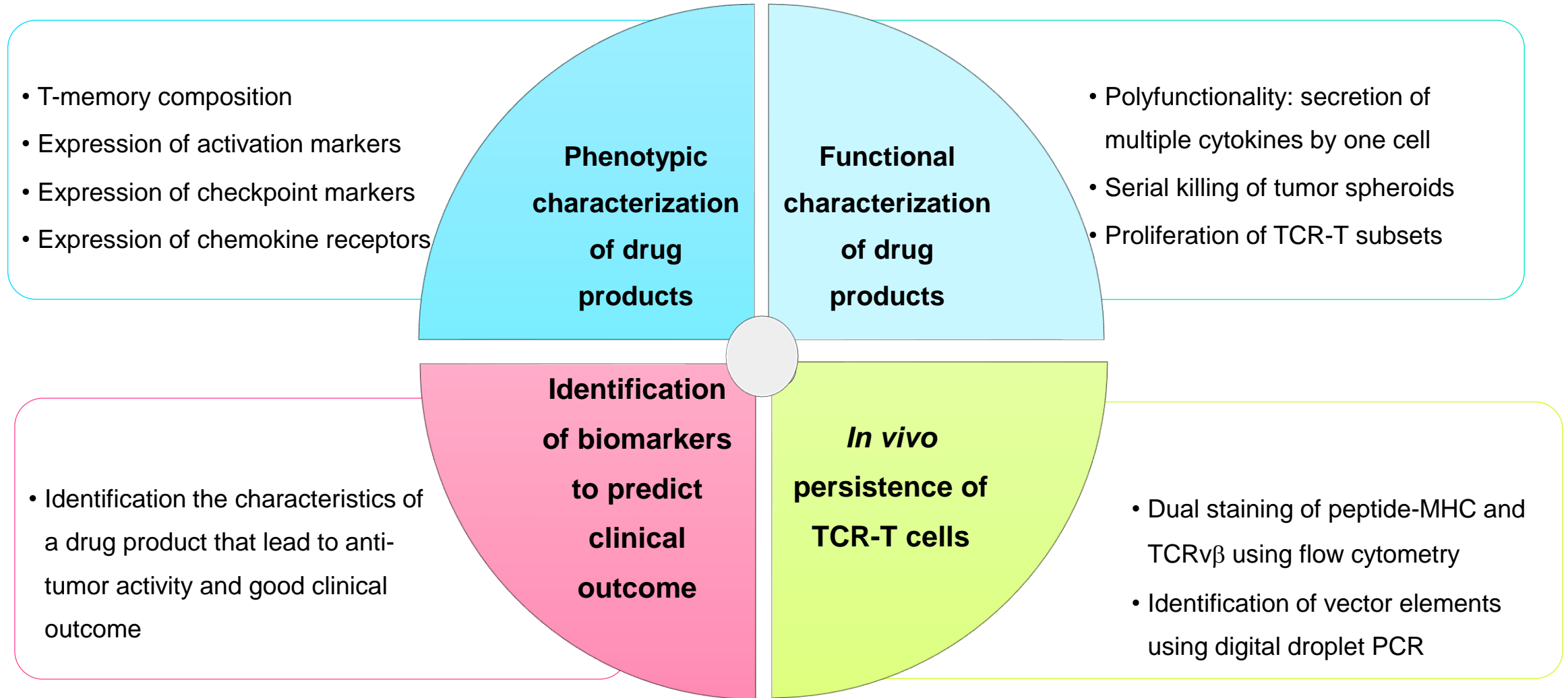


Possible applications:

- Comparison of different TCR-T cells with regard to their proliferative capacities
- Analysis of proliferation-induced changes in marker expression
- Identification of TCR-T cell subsets with the highest proliferative potential



A wealth of information is gained with multiple immune monitoring approaches



Thank you

