Immune monitoring tools and their application

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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

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”

Drug Products | DP-1 | DP-2 | DP-3
---|---|---|---
Apheresis | Drug Product | Apheresis | Drug Product | Apheresis | Drug Product
% of CD3+CD8+

T-memory staining panel:
- live/dead
- CD45
- CD3
- CD8
- CD4
- pMHC
- TCRvß
- Multimer
- CD45RA
- CCR7
- CD27
- CD95
Multi-color flow cytometry identifies activation status of TCR-T cells

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

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

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<th>Intracellular cytokine multi-color flow cytometry identifies polyfunctional TCR-T cells</th>
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Analysis of TCR 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

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<th>day 0</th>
<th>day 3</th>
<th>day 7</th>
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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

- T-memory composition
- Expression of activation markers
- Expression of checkpoint markers
- Expression of chemokine receptors

Phenotypic characterization of drug products

- Polyfunctionality: secretion of multiple cytokines by one cell
- Serial killing of tumor spheroids
- Proliferation of TCR-T subsets

Functional characterization of drug products

Identification of biomarkers to predict clinical outcome

- Identification the characteristics of a drug product that lead to anti-tumor activity and good clinical outcome

In vivo persistence of TCR-T cells

- Dual staining of peptide-MHC and TCRβ using flow cytometry
- Identification of vector elements using digital droplet PCR

A wealth of information is gained with multiple immune monitoring approaches.
Thank you