In **in vitro** evaluation of TCR efficacy and toxicity using 3D spheroid models

**Abstract**

Clinical studies using TCR-transduced T cells for adoptive T cell therapy revealed the efficacy of the therapeutic approach but also uncovered possible toxic effects against healthy tissues, such as neuronal or cardiac cells, caused by off-target TCR or off-target toxicity. (Morgan et al., 2014; Linette et al., 2013) Due to the lack of adequate in vitro models for prediction of TCR efficacy or potential TCR-mediated toxicity against healthy tissues, physiologically relevant in vitro models need to be developed. The usage of 3D spheroids as targets for TCR-transduced T cells represents a powerful strategy to test killing capacity or possible safety issues of TCRs, which could fill the gap between 2D co-cultures and animal models.

Compared to 2D cell layers, cells within a 3D spheroid structure more closely resemble the physiology in vivo regarding cell interactions, proliferation rates, apoptosis, and gene expression.

We describe the use of 3D spheroids generated from tumor cell lines for assessment of TCR efficacy or healthy primary cells/PSC-derived cells for evaluation of potential TCR-directed toxicity. HLA-A2-positive cells served as target cells in functional co-culture assays with CD8+ T cells transduced with an HLA-A2-restricted TCR. Spheroids were generated using a soft agar attachment plates or via the hanging-drop culture method. Spheroids were co-cultured with TCR-transduced T cells and killing of spheroids was analyzed in real-time using the IncuCyte live cell imaging system. For better visualization of spheroid killing, tumor cell lines were transduced using a lentivirus encoding a nucleoside- and neomycin-resistant selectable marker or by adding Annexin V red fluorescent reagent for detection of apoptotic cells. Thymic spheroids derived from healthy cells were analyzed via phase contrast and evaluation of epithelial morphology.

Tumor cell lines showing robust killing of target cell spheroids, confirming the results generated in a 2D system. Furthermore, 3D spheroids generated from healthy cells (e.g. normal human lung fibroblasts or PSC-derived cells, e.g. cardiomyocytes or neurons) were not killed via TCR killing. Cells were harvested and analyzed using an Annexin V red fluorescent reagent for detection of apoptotic cells. TCR-transduced T cells were also uncovered possible toxic effects against healthy tissues, such as neuronal or cardiac cells, caused by on-target killing.

In summary, we showed that functional in vitro assays using 3D spheroid structures using tumor cell lines or healthy cells represent an elegant approach to assess both efficacy and potential toxicity of a given TCR in vitro. The analyzed TCR showed potent efficacy as well as a favorable safety pattern in 3D in vitro cultures.

**References**

- Morgan et al., 2014: Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy.
- Linette et al., 2013: Cardiovascular toxicity and tissue necrosis mediated by affinity-enhanced T cells in myocardium and endocardium.