Setting up of a GMP-compliant method for the production of human TCR-transduced T cells for the clinical testing

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The clinical testing of immunotherapy strategies based on TCR genetically-modified T cells requires the identification and setting up of optimal culture conditions and protocols to expand high quality T cells in compliance with good manufacturing practice (GMP) norms.

To this aim, we selected a "model" TCR, e.g codon-optimized murinized TCR150, targeting the hyaluronan-mediated motility receptor (HMMR/Rhamm) that was previously cloned into the MP71 retroviral vector and extensively characterized (Spranger et al. Blood 2012). As the first part of this study, we developed a small scale T cell production method. Peripheral blood mononuclear cells (PBMCs) were initially isolated from fresh leukapheresis of HLA-A*02 negative healthy-donors, whose isolation conditions were optimized for an enrichment of $CD3^+$ cells. Frozen PBMCs were then activated ex-vivo and transduced with TCR-150 by a double spin infection on Retronectin®-coated non-tissue culture 24 well plates. Cells were then split every other day and expanded up to 13 days. Phenotypic analysis to assess transduction rates, $CD3^+$, $CD8^+$ and $CD8^-$ expansion and expression of memory markers were performed at day 6, day 10 and day 13 on fresh cells. Samples of cells were frozen at day 10 and day 13 of culture for further phenotypic and functional analysis, aimed firstly at determining that the freezing process was not affecting any phenotypic property and secondly, at better characterizing the final T cell product, with quantification of non-$CD3^+$cells (e.g. $CD14^+$, $CD19^+$, $CD34^+$, $CD3^-CD56^+$), analysis of chemokine receptor expression (CCR7, CXCR4, CCR5) and evaluation of IFN-γ release after stimulation with both HLA-A*02-positive/HMMR-positive and HLA-A*02 negative/HMMR-positive targets.

In the present analysis we compared two different GMP-grade media (X-VIVO vs CellGro) supplemented with 5%-human serum with a different combination of T cell growth factors (IL-2 versus IL-7+IL-15). Moreover, we analyzed whether different T cell activation methods (OKT-3 vs Dynabeads® CD3/CD28) and diverse cell concentrations ($0{,}25x10^6$ cells/ml vs $0{,}5x10^6$ cells/ml) affects the quality of the expanded TCR-transduced T cells.

Finally, since in some cases (as with TCR-150) the transduced TCR is reported to be CD8-dependent it might be relevant to enrich and more selectively expand the $CD8^+$ compartment of the T cell population. To this aim, we developed and tested a protocol to enrich $CD8^+$ T cells via a CD4-depletion step performed at the beginning of the culture.

In the near future and according to results obtained from ongoing analyses, a large scale expansion protocol will be developed in collaboration with a biotech company (EUFETS).