How to make a fishing rod for adoptive cellular therapy: the ACTolog® approach, a multi-targeted endogenous T-cell therapy

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Introduction

Adoptive cellular therapy (ACT) is one of the major drivers currently revolutionizing cancer immunotherapy. Immatics in collaboration with The University of Texas MD Anderson Cancer Center, USA (MD Anderson) developed a novel personalized ACT approach which is currently being tested in a phase I clinical study.

With Immatics' proprietary target discovery platform XPRESIDENT® new tumor-associated peptides (TUMAPs) have been identified and validated as promising targets for cancer immunotherapy. In the ACTolog® approach target expression is tested in patient tumor samples prior to the generation of a personalized drug product. T-cell products for up to four expressed targets (from a warehouse of eight targets) are generated in parallel by *in vitro* priming of patient autologous peripheral blood nuclear cells (PBMCs) followed by isolation and rapid expansion of target specific CD8⁺ T cells which are finally reinfused to fight patients' tumor cells. This novel personalized, multi-target approach with defined T-cell specificities is a promising strategy to improve patients' prognoses.

Here, we want to focus on the generation and quality control mechanisms of the tools used for the identi

fication and isolation of target-specific CD8⁺ T cells which can be used as effective and safe drug for cancer immunotherapy.

The ACTolog® concept

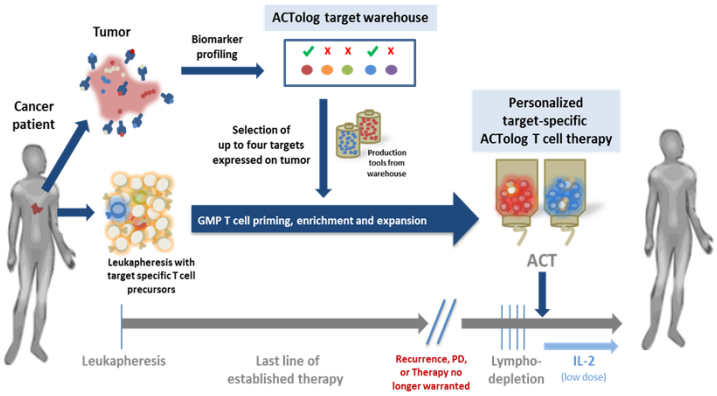
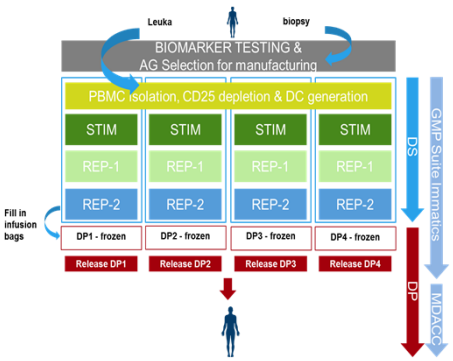


Figure 1: Overview of the ACTolog® concept.

Manufacturing process for ACTolog® drug products

Figure 2: Process flow of patients' materials.
Tumor biopsy is screened for target gene expression. Leukapheresis is taken from the patient to isolate PBMCs if at least one target is expressed. CD25 depleted PBMCs and antigen loaded autologous dendritic cells (DC) are then used for T-cell priming *in vitro* (STIM) for up to four targets in parallel. Antigen-specific T cells are sorted under aseptic conditions using in-house produced quality controlled clinical grade pHLA tetramers. After two rounds of rapid expansion (REP) *ex vivo* the drug substance (DS) is filled in infusion bags and stored until patient infusion at MD Anderson Cancer Center (MDACC). In addition to numerous in-process controls, each drug product (DP) is subjected to a final release testing.



In-house production of pHLA tetramers for sterile T-cell sorting

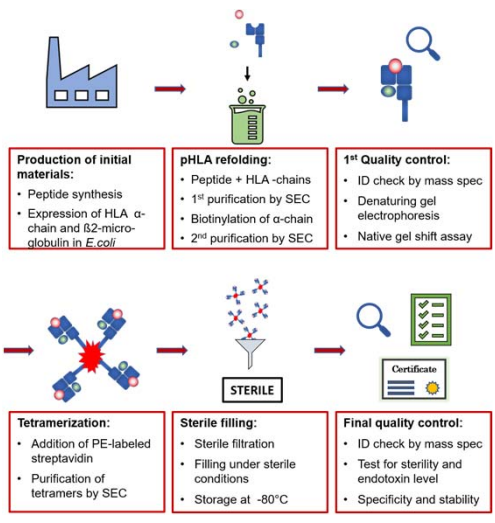


Figure 3: pHLA tetramer production.
pHLA tetramers for ACTolog® are manufactured at Immatics from initial materials to final product. Peptides are synthesized by solid-phase Fmoc chemistry and HLA chains are produced as inclusion bodies in *E.coli*. pHLA monomers are refolded under redox conditions followed by biotinylation and purification steps. In a 1st quality control peptide specificity, biotinylation and integrity of the complexes are verified by tandem mass spectrometry (MS/MS) and gel chromatography. After tetramerization to fluorochrome-labeled streptavidin, pHLA monomers, dimers and trimers are removed by a third size exclusion chromatography (SEC). The tetramer fraction is reconstituted in freezing buffer, sterile filtered and filled under sterile conditions. After passing all final quality controls a certificate is compiled and pHLA tetramers are stored until use at -80°C.

Key aspects of the ACTolog® concept

- **Endogenous T-cell therapy** based on pioneering work of Prof. Cassian Yee, MD Anderson.
- **Defined T-cell specificity** by isolation and expansion of specific T cells from patient PBMCs.
- **Multiple indications**: Target antigens have been shown to be naturally presented in various solid tumors.
- **Personalized:** ACTolog® cell therapy products are based on antigen expression of each patient.
- **Multi-target approach:** Targeting different antigens limits tumor escape and is assumed to result in stronger immune response.

Specificity and stability of ACTolog® pHLA tetramers

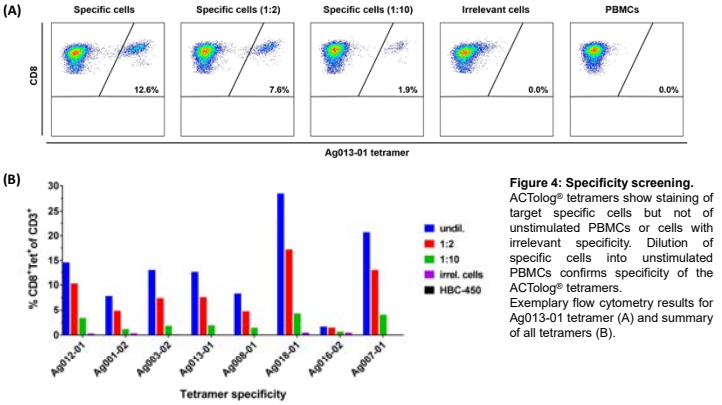


Figure 4: Specificity screening.
ACTolog® tetramers show staining of target specific cells but not of unstimulated PBMCs or cells with irrelevant specificity. Dilution of specific cells into unstimulated PBMCs confirms specificity of the ACTolog® tetramers.
Exemplary flow cytometry results for Ag013-01 tetramer (A) and summary of all tetramers (B).

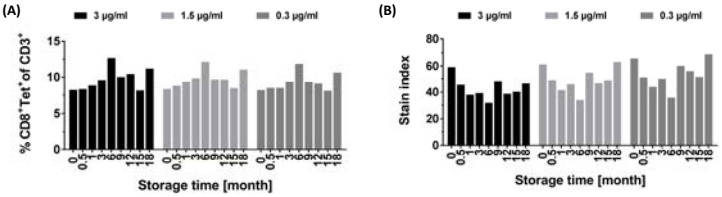
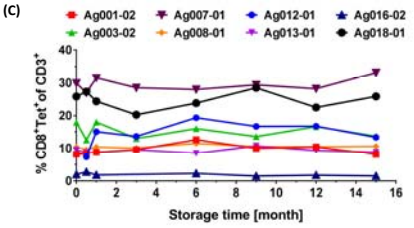


Figure 5: Long-term stability.
Stability of tetramer staining with frozen ACTolog® tetramers is tested over time. Frequency of CD8⁺Tet⁺ cells (A+C) and stain index (B) is stable at least up to 15 month after manufacturing.
Exemplary results for Ag001-02 tetramer at three different staining concentrations (A+B) and summary for all ACTolog® tetramers at 3 µg/ml (C).



Isolation of target-specific patient cells

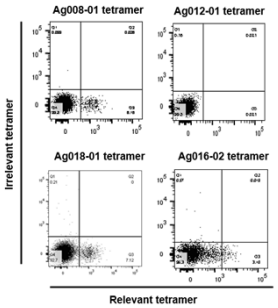


Figure 6: Tetramer staining after *in vitro* stimulation.
Exemplary results of wells screened for target specific cells by tetramer staining after *in vitro* priming. Precursor cells have been found for Ag008-01, Ag016-02 and Ag018-01 but not for Ag012-01 in PBMCs of this patient.

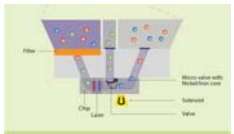


Figure 7: MACSQuant® Tyto™ system.
Microchip-based cell sorting in a fully closed cartridge system protects the cell sample from contamination.
(adapted from www.miltenyibiotec.com)

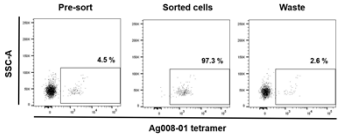


Figure 8: Evaluation of cell sorting.
Tetramer staining of pre- and post-sort samples measured on the MACSQuant Tyto for analysis of sort quality (Enrichment = 21x, Yield = 58%).

Table 1: Overview of T-cell product development.
Tetramer staining and sort results at different stages of T-cell product generation for one exemplary patient.

Patient ID	Antigen	%Tet⁺/Lymph (Pre-Sort)	%Tet⁺/Lymph (Sorted cells)	%Tet⁺/Lymph (Waste)	Enrich-ment	Sort Yield	%Tet⁺/CD3⁺CD8⁺ (after REP1)	%Tet⁺/CD3⁺CD8⁺ (after REP2)
IMA101_60_028	Ag018-01	3.74	90.2	1.3	24x	62%	52.5	52.1
IMA101_60_028	Ag008-01	4.54	97.3	2.61	21x	58%	90.1	80.8
IMA101_60_028	Ag016-01	4.11	91.7	2.8	22x	46%	86.1	45.6