Adaptive cellular therapy (ACT) has dramatically changed the landscape of immunotherapy; however, only a small proportion of solid tumor patients have benefited from these advances due to i) heterogeneity of tumor antigen expression; ii) tumor escape (e.g. only one target is addressed); or iii) off-target toxicities (e.g. expression of targets on normal tissues). ACTolog® is a personalized ACT approach, based on the work of Cassian Yee, using autologous T-cell products designed to overcome these issues. IMMA101-101 is a first-in-human clinical trial in HLA-A*02 positive patients with relapsed or refractory solid tumors using the multi-targeted ACTolog® approach in which up to four products with different tumor target-specificities are manufactured and infused for each individual patient.

We have developed two flow cytometric phenotyping assays that allow us to determine the frequency of target-specific cells in the final product and persisting cells in the blood as well as to deeply characterize the memory marker expression (Tetramer panel: CD45RA, CD69, CD27, CD40, CD40L, CD28, PD1) and immune checkpoint expression (Table panel: CD137, LAG-3, PD-1, TIGIT, TIM-3) of target-specific cells. Product characterization and initial persistence data of the first three treated patients revealed a high prevalence of persisting target-specific cells in the blood until 2 months after infusion as well as a favorable phenotype of target-specific cells, encouraging for further development of the ACTolog process.

**Figure 1:** ACTolog treatment concept. Expression of ACTolog warehouse targets is analyzed by qPCR and mass cytometry. Lymphodepletion is drawn from HLA-A*02 positive patients, whose tumors have been identified to be positive for one or more ACTolog targets. Autologous T-cells against ACTolog targets are in vitro primed in the presence of IL-2 followed by HLA tetramer-guided cell sorting and expansion prior to infusion. Infusion of target cell enriched T-cell products is conducted after patient progression. Expression of infused T-cells is supported by lymphodepletion and IL-2 regime.

**Figure 2:** Occurrence of surface markers over the course of T-cell differentiation. The top row shows the different CD8 T-cell memory phenotypes analyzed in Tmem & IC staining assays; their proliferative potential and effect function. The table below displays a list of all markers used for phenotypic characterization. In Tmem & IC staining assays and their relative expression in different memory phenotypes according to programmed cell death (PD-1) expression. The table classifies the main function of the different surface markers. For Tmem & IC assays the expression of memory markers was used to deeply characterize the phenotype of target-specific CD8 T-cells before and after adoptive transfer.

**Figure 3:** Persistence of adoptively transferred target-specific CD8 T-cells. Persistence of adoptively transferred target-specific CD8 T-cells for the first 3 IMMA101-101 patients was determined by ex vivo Tmem & IC staining assay. Data are shown for the different final products, baseline, as well as 1, 2, and 8 weeks post transfer. Flow cytometry plots on the left show multimer staining for indicated targets, pre-gated on viable CD8+ T-cells. Numbers in plots indicate frequency of respective multimer+ or multimer- cells. Line charts on right side summarize frequency of target-specific cells of CD8 T-cells [%]. For ex vivo multimer staining assay from FBNK samples were stained at indicated time points were thawed and rested overnight using 1 x antigen (0.15 and 20 kU/L) A2-3. Subsequently samples were stored for LD/CD8, followed by multimer and surface staining and acquired at 80 LSRIII flow cytometry the same day.

**Summary and Conclusion**

Product characteristic:
- Contains high frequencies (36.93 – 99.44%); median: 81.39% of target-specific CD8 T cells
- Displays elevated frequencies (8.12 – 32.68%; median: 16.07%) of favorable central memory (EM) T cells compared to other ACT programs (Fig. 4)
- Analysis of T-cell memory (Tmem) markers reveal phenotype as expected for in vitro stimulated cells (low expression of CD27, CD28, CD127, CD62L, and CD152; elevated expression of CD45RO) (Fig. 4)
- Immune checkpoint (IC) markers are rarely expressed on target-specific cells of final products (only TIM-3 and low expression of LAG-3) and hence no signs of T-cell exhaustion (Fig. 4)

**References**


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