# MAGE-A1 targeting TK-8001 TCR-T cells currently being investigated in the IMAG1NE Phase 1/2 clinical trial demonstrate broad *in vitro* and *in vivo* anti-tumor activity and are superior to human-derived MAGE-A1 TCRs

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## Background

As cancer-testis antigens are self-antigens, T cells expressing high-affinity T cell receptors (TCRs) against such antigens are eliminated via negative thymic selection. Therefore, human donor-derived TCRs isolated from healthy individuals or cancer patients are typically of low affinity. As low affinity has been related to reduced antitumor activity, affinity maturation can be used to increase affinity, but this may result in reduced specificity and potential off-target toxicity. Using our proprietary MyT™ platform (1), we isolated a naturally optimized high-affinity TCR specific for the cancer-testis antigen MAGE-A1 (TCR 8001), currently being investigated in the IMAG1NE Phase 1/2 clinical trial (NCT05430555). Here, we describe preclinical evaluation of TCR 8001-transduced T cells, including anti-tumor activity against cancer cell lines derived from a wide range of tumors, and side-by-side *in vitro* and *in vivo* comparison to human donor-derived MAGE-A1 TCRs.

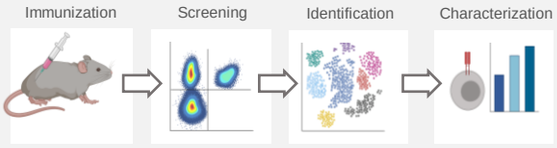
## Methods

MAGE-A1-specific TCRs were isolated from MyT platform mice immunized with the MAGE-A1 epitope KVLEYVIKV. Human donor-derived TCRs reactive to the same epitope were synthesized based on publicly available sequences. All TCRs were re-expressed in primary human T cells and compared for peptide sensitivity and potential to recognize and kill cancer cell lines with different expression levels of MAGE-A1. To determine *in vivo* functionality, a mouse model (TNA2 mice) in which MAGE-A1 and HLA-A2 are expressed in transplanted syngeneic tumors was employed (2).

### MyT™ platform


MyT™ platform mice carry the entire human TCRα/β gene loci and single or multiple HLA class-I alleles, covering approximately 80% of the HLA types found in the United States and Europe. They are engineered to lack expression of murine TCRα, TCRβ and MHC.

### TCR identification workflow




1) MyT™ platform mice are immunized with DNA encoding for the full-length antigen or with antigenic peptides. Booster immunizations are performed as required.

2) After each immunization, mice are screened for immune responses by *in vitro* restimulation of peripheral blood lymphocytes using cytokine expression as readout.

3) Antigen-specific T cells identified by the expression of activation markers are isolated from spleens and lymph nodes of responding mice and the TCR sequences are identified using single cell RNA sequencing.

4) Most frequent TCR clonotypes are cloned in expression vectors and are re-expressed in human T cells for characterization of specificity and functionality.

### 1) MyT™ platform-derived TCRs exhibit higher peptide sensitivity than human donor-derived (HDD) TCRs

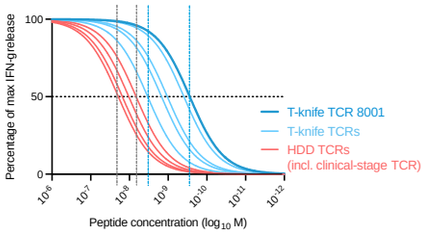



**Fig.1:** IFN-γ production of TCR-transduced human T cells in response to stimulation with MAGE-A1 peptide-loaded T2 cells. Data are normalized to maximum IFN-γ release and fitted to a four-parameter logistic model. Graph is representative of at least three experiments with different T cell donors.

### 2) T cells expressing MyT™ platform-derived TCRs recognize tumor cells with lower MAGE-A1 expression better than human donor-derived (HDD) TCRs

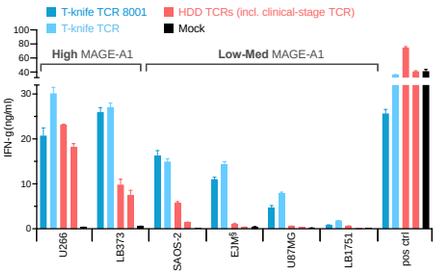



**Fig.2:** IFN-γ production of TCR-transduced human T cells upon stimulation with a range of tumor cell lines exhibiting high or low-medium MAGE-A1 expression (E:T=1:5). Mock T cells served as negative control. PMA/Ionomycin stimulation served as positive control. Data are shown as mean of duplicates ± SD. Graph is representative of n=2 donors. §EJM cells are transduced with HLA-A2.

### 3) T cells expressing TCR 8001 maintain cytotoxic capacities upon repeated antigen stimulation longer than a clinical-stage human donor-derived TCRs

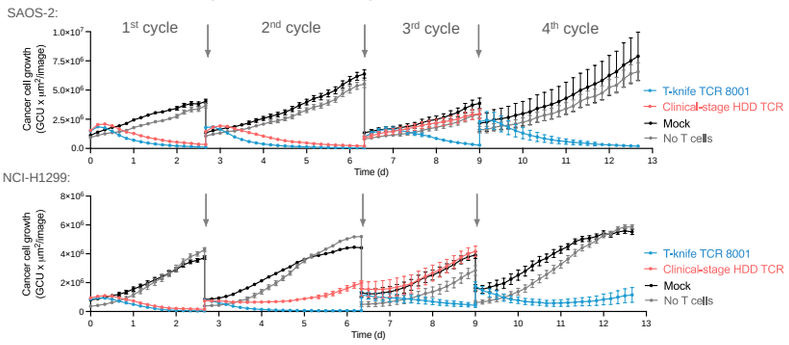



**Fig.3:** TCR-transduced human T cells were co-cultured with MAGE-A1 expressing SAOS-2 (transduced with GFP) or NCI-H1299 (transduced with HLA-A2-GFP) cells at the E:T ratio of 5:1. Target cells cultured without T cells or with Mock T cells served as negative controls. After 60-80h T cells were harvested and re-seeded on a new plate with fresh tumor cells to start a new restimulation cycle (indicated by grey arrows). While TCR 8001-transduced T cells were able to efficiently kill target cells for 4 consecutive cycles, T cells expressing a human donor-derived TCR lost cytotoxic capacities during the 3rd cycle and could not be included in the 4th cycle due to insufficient T cell numbers. All co-cultures were monitored by live cell imaging using the IncuCyte Zoom S3. Graphs display the growth of GFP expressing cancer cells depicted as the total green object area. Data are shown as mean ± SD (9 image areas per replicate well). Graphs are representatives of n=2 donors.

### 4) T cells expressing TCR 8001 exhibit superior T cell engraftment than a clinical-stage human donor-derived TCR leading to significantly enhanced relapse-free survival in a challenging tumor model

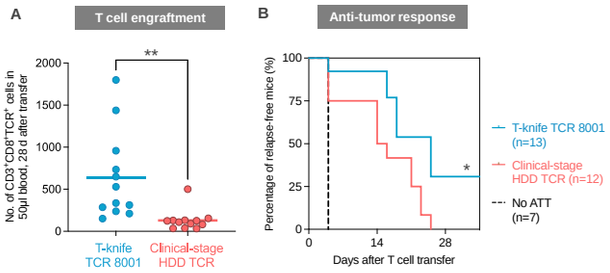



**Fig.4:** In a syngeneic model, TNA2 mice were injected with $5x10^6$ MAGE-A1/HLA-A2-transduced mouse fibrosarcoma cells (MC703) and monitored for tumor growth. 21 days post injection, mice with measurable tumors ≤ 65 $mm^3$ were randomized and treated with $10^6$ syngeneic mouse CD8+ T cells transduced with TCR 8001 or a clinical-stage human donor-derived (HDD) TCR. (**A**) Number of $CD3^+$ $CD8^+$ $TCR^+$ T cells in mouse peripheral blood, as determined by pMHC multimer flow cytometry staining, 28 days after the treatment (line indicates mean of n=12 for each group). ** p ≤ 0.005 (unpaired t-test). (**B**) Tumor relapse rates after tumor regression. While all mice treated with HDD TCR T cells relapsed within 25 days, 4 out of 13 animals receiving TCR 8001 T cells stayed relapse-free until day 35. Tumor regression was defined as 2 consecutive days of decreasing tumor size with at least 25% overall tumor reduction. The day of relapse was defined as the second consecutive day showing an increase of tumor volume after regression. Refractory Mice that never had tumor regression were defined to have an event on day 4 after adoptive T cell transfer (ATT). No ATT control mice were left untreated after tumor cell injection. * p ≤ 0.05 (Mantel-Cox Test).

### 5) T cells expressing TCR 8001 exhibit long-term cytotoxicity against a broad panel of different tumor cell lines even at very low E:T ratios

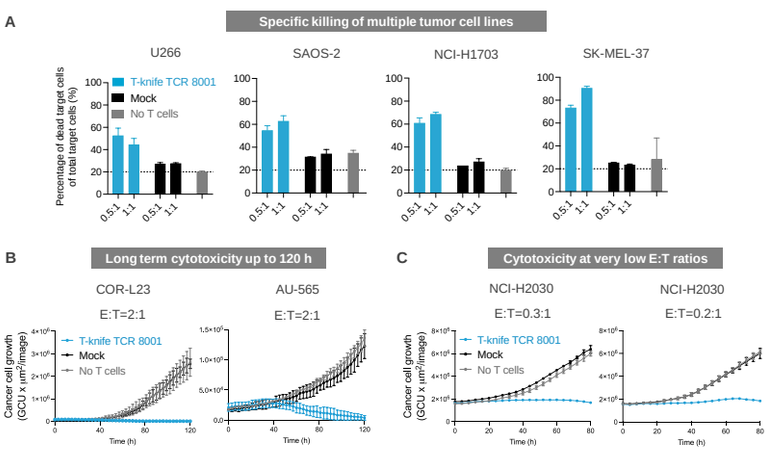



**Fig.5:** TCR 8001-transduced human T cells were co-cultured with different MAGE-A1 expressing tumor cell lines to detect T cell-induced target cell death. (**A**) Tumor cells with endogenous HLA-A2 expression were labelled with CFSE and co-cultured with T cells at the E:T ratios of 0.5:1 or 1:1. Target cells cultured without T cells or with Mock T cells served as negative controls to determine spontaneous cell death (visualized by dotted line) or unspecific target cell killing, respectively. Cell death was measured by flow cytometry after 18h (U266 and SAOS-2) or 44h (NCI-H1703 and SK-MEL-37). Data are shown as mean ± SD from n=2 donors. (**B-C**) Tumor cells with transgenic HLA-A2 and GFP reporter expression were co-cultured with T cells at (**B**) the E:T ratio of 2:1 or (**C**) very low E:T ratios of 0.3:1 and 0.2:1. Co-cultures were monitored by live cell imaging using the IncuCyte Zoom S3. Graphs display the growth of GFP expressing cancer cells depicted as the total green object area. Data are shown as mean ± SD (3 wells for each condition, ≥ 4 image areas per well) with (**B**) n=2 donors, (**C**) n=1 donor.

### 6) T cells expressing TCR 8001 secrete IFN-γ upon stimulation with tumor cell lines exhibiting even low levels of MAGE-A1 protein expression

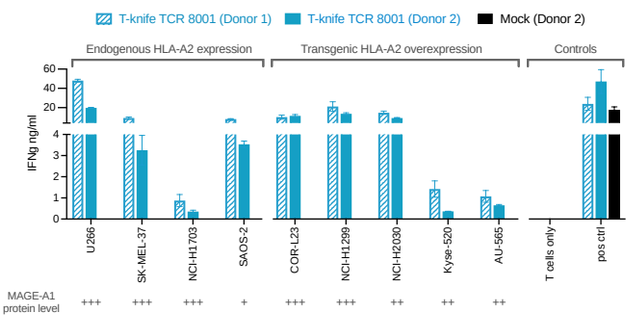



**Fig.6:** IFN-γ production of TCR-engineered and Mock T cells upon stimulation with target cells at the E:T ratio of 1:5. TCR 8001-transduced human T cells from 2 donors were co-cultured with different HLA-A2 positive (endogenous or transgenic) tumor cell lines with varying levels of MAGE-A1 protein expression (assessed by flow cytometry). Cell lines are derived from a wide range of tumors, including multiple myeloma (U266), melanoma (SK-MEL-37), non-small-cell lung cancer (NCI-H1703, NCI-H1299, NCI-H2030, COR-L23), osteosarcoma (SAOS-2), breast adenocarcinoma (AU565) and esophageal carcinoma (Kyse-520). PMA/Ionomycin stimulation served as positive control. Data are shown as mean of duplicates ± SD.

## Conclusions

Human T cells engrafted with the high-affinity MAGE-A1-specific human TCR 8001 discovered using the MyT platform, recognize and kill cancer cell lines derived from various tumor indications, including cell lines with low levels of MAGE-A1 and endogenous HLA-A2 expression. Preclinical *in vitro* and *in vivo* comparison of TCR 8001 to human donor-derived TCRs demonstrates that TCR 8001 is a superior candidate. A phase 1/2 trial evaluating the safety and efficacy of a TCR-T therapy incorporating TCR 8001-transduced T cells (TK-8001) in MAGE-A1 expressing solid tumors has been initiated (NCT05430555).

## References

1. Li LP. *et al*. Nat Med 2010;16:1029-1034
2. Leisegang, M. et al.. J Clin Invest. 2016; 126:854–858