Poster #144

Development of a CD8 co-receptor independent T cell receptor specific for tumor-associated antigen MAGE-A4 for next generation T cell-based immunotherapy

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Abstract

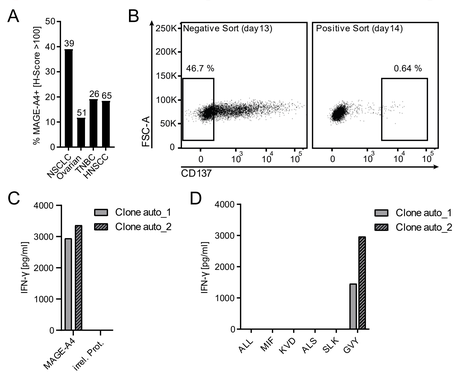
Background. The cancer-testis antigen MAGE-A4 is an attractive target for T cell-based immunotherapy, especially for indications with unmet clinical need like non-small-cell lung carcinoma or triple-negative breast cancer. Overcoming high tumor burden using adoptive transfer of T cells modified to express a transgenic T cell receptor (TCR) demands optimal recognition of the corresponding target on tumor cells by the TCR-modified T cells (TCR-Ts). Here we describe the isolation and pre-clinical characterization of high avidity TCR-Ts expressing a human leucocyte antigen (HLA)-A*02:01-restricted MAGE-A4-specific TCR that is fully functional in T cells irrespective of CD4 or CD8 co-receptor expression.

Methods. An unbiased CD137-based sorting approach was first used to identify an immunogenic MAGE-A4-derived candidate epitope that was properly processed and presented on HLA-A2 molecules encoded by the HLA-A*02:01 allele. To isolate high avidity T cells via subsequent multimer sorting, an *in vitro* priming approach using HLA-A2-negative donors (allogeneic-HLA-restricted priming approach) was conducted to bypass central tolerance to this self-antigen. Pre-clinical parameters of safety and activity were assessed in a comprehensive set of *in vitro* and *in vivo* studies of the lead TCR candidate derived from a selected T cell clone.

Results. A TCR recognizing the MAGE-A4-derived decapeptide GVYDGREHTV was isolated from primed T cells of a non-tolerant HLA-A2-negative donor. The respective TCR-T cell product bbT485, expressing the lead TCR in T cells from healthy donors, was demonstrated pre-clinically to have a favorable safety profile and superior *in vivo* potency compared to TCR-Ts made using a TCR derived from an HLA-A2-positive donor bearing a tolerized T cell repertoire to self-antigens. The natural high avidity allogeneic (allo)-derived TCR was found to be CD8 co-receptor-independent, allowing effector functions to be elicited in transgenic CD4+ T helper cells. These CD4+ TCR-T cells not only supported an anti-tumor response by direct killing of MAGE-A4-positive tumor cells, but also upregulated hallmarks associated with helper function, such as CD154 expression and release of key cytokines upon tumor-specific stimulation.

Conclusion. The extensive pre-clinical assessment of safety and *in vivo* potency of this non-mutated high avidity, CD8 co-receptor-independent, MAGE-A4-specific HLA-A2 restricted TCR provide the basis for its use in clinical TCR-T immunotherapy studies. The ability of this co-receptor-independent TCR to activate all transduced T cells (irrespective of CD4 or CD8 expression) could provide enhanced cellular responses in the clinical setting through the induction of functionally diverse T cell subsets that goes beyond what is currently tested in the clinic.

Figure 1. Tumor associated antigen MAGE-A4 harbors an immunogenic candidate T cell epitope



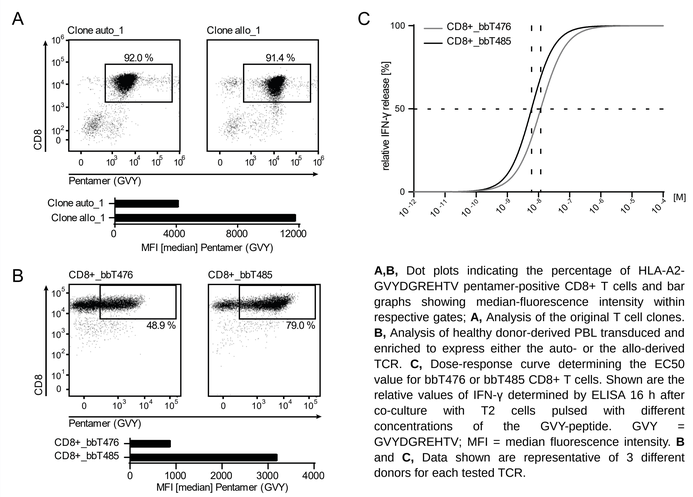
A, Formalin-fixed paraffin-embedded (FFPE) human tumors of different histologic subtype (primarily excisional biopsies) were stained with an anti-human MAGE-A4 antibody and evaluated by a study pathologist. The intensity of staining was scored based upon a 3-point scale, the percentages of cells assigned to each score were estimated and a Histoscore (H-score) was assigned. Numbers above bars indicate the number of analyzed samples. **B,** CD8+ T cells from an HLA-A*02:01-positive healthy donor were expanded for 12 days by stimulation with autologous MAGE-A4 expressing monocyte-derived dendritic cells. Shown are dot plots depicting the gating strategy of expanded CD8+ T cells 16 h after co-culture with A2+K562 cells. CD137-negative bulk sorted cells (left) were co-cultured with A2+K562 cells transduced with MAGE-A4; 16 h later CD137-positive cells (right) were single cell-sorted. **C,D,** Single cell sort-derived T cell clones were expanded for 15 days and tested for reactivity towards MAGE-A4. Shown are bar graphs displaying IFN-γ concentrations in supernatants of two positive T cell clones 16 h after co-culture with target cells for screening. **C,** Co-culture with A2+K562 cells transduced with MAGE-A4 or an irrelevant protein (irrel. Prot.). **D,** Co-culture with A2+K562 cells pulsed with different candidate peptides.

Table 1. MAGE-A4-derived HLA-A*02:01-restricted candidate epitopes

Peptide	Length (aa)	Predicted affinity*	Comment
KVLEHVVRV	9	16.7	100% sequence match to MAGE-A8
ALLEEEEGV	9	23.4	Peptide tested (Figure 1 D)
MIFGIDVKEV	10	40.6	Peptide tested (Figure 1 D)
FLWGPRALA	9	46.4	100% sequence match to MAGE-A1/8
ALAETSYVKV	10	49.2	100% sequence match to MAGE-A1/8
KVDELAHFL	9	60.7	Peptide tested (Figure 1 D)
ALSNKVDEL	9	125.1	Peptide tested (Figure 1 D)
SLKMIFGIDV	10	163.5	Peptide tested (Figure 1 D)
GVYDGREHTV	10	1751.8	Peptide tested (Figure 1 D); Multimer Sort

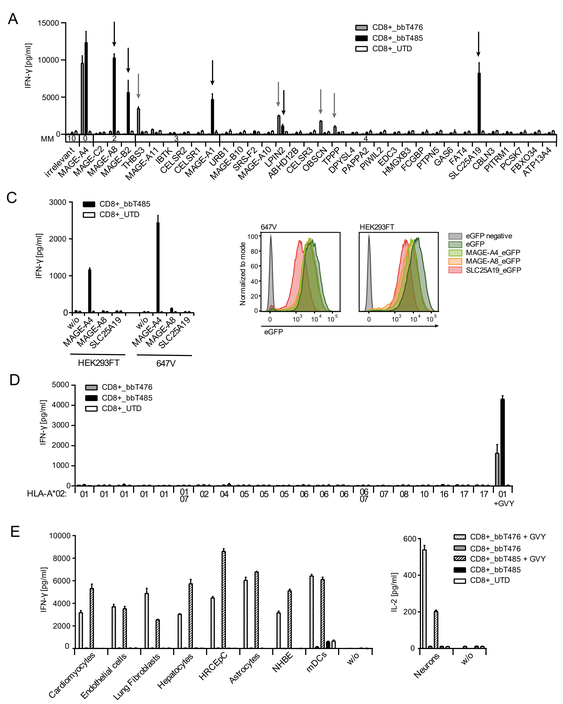
Sequences of listed epitopes selected based on predicted binding affinity towards HLA-A*02:01 based on NetMHC data (Andreatta & Nielsen, 2016; Nielsen et al., 2003) and previously reported experimental data. Pos = position within MAGE-A4 amino acid sequence, nm = nanomolar units.

Figure 2. bbT485 TCR-Ts expressing an allo-derived TCR exhibits superior epitope binding characteristics compared to bbT476 expressing the auto-derived TCR



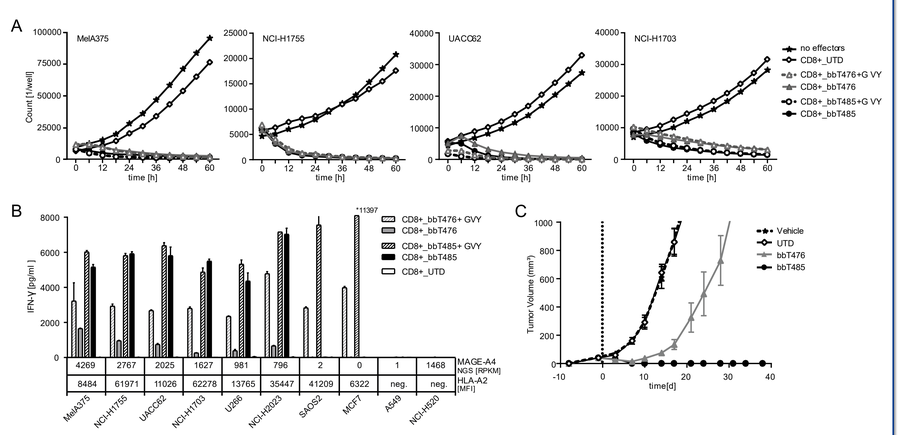
A,B, Dot plots indicating the percentage of HLA-A2-GVYDGREHTV pentamer-positive CD8+ T cells and bar graphs showing median-fluorescence intensity within respective gates; **A,** Analysis of the original T cell clones. **B,** Analysis of healthy donor-derived PBL transduced and enriched to express either the auto- or the allo-derived TCR. **C,** Dose-response curve determining the EC50 value for bbT476 or bbT485 CD8+ T cells. Shown are the relative values of IFN-γ determined by ELISA 16 h after co-culture with T2 cells pulsed with different concentrations of the GVY-peptide. GVY = GVYDGREHTV; MFI = median fluorescence intensity. **B** and **C,** Data shown are representative of 3 different donors for each tested TCR.

Figure 3. bbT476 and bbT485 TCR-Ts both display excellent safety profiles for adoptive cell therapy



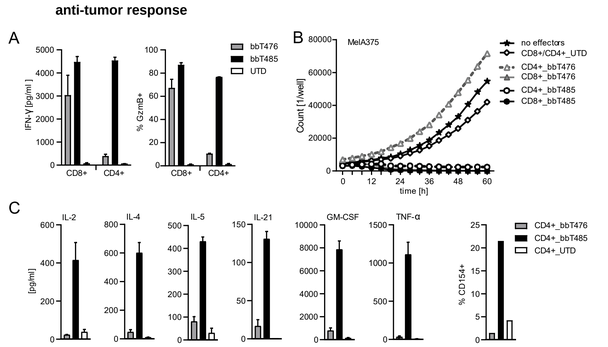
Bar graphs displaying IFN-γ concentrations in supernatants of untransduced (UTD) and TCR-expressing (bbT476 or bbT485) CD8+ T cells 16 h after co-culture with target cells relevant for safety testing: **A**, T2 cells loaded with predicted mismatched peptides using Expitope or GVY peptide. Parental protein-names indicate respective mismatched peptide. MM = number of amino acid mismatches compared to GVYDGREHTV. Arrows mark recognized mismatched peptides. **B**, A2+K562 cells transfected with ivt-RNA encoding sequence fragments containing the mismatched peptide sequences or GVYDGREHTV (MAGE-A4). An example of a midi-gene construct is shown above. Parental protein-names indicate transfected midi-genes. Arrows mark recognized transfectants. * = bbT485 not tested, # = bbT467 not tested **C**, 647V cells and HEK293T cells transduced with indicated full-length genes linked to eGFP. eGFP expression is shown for all engineered cell lines. **D**, EBV-transformed human B-cell lines (lymphoblastoid cell lines, LCLs) expressing different HLA-A*02 sub-alleles as indicated on x-axes. LCL loaded with the GVY peptide serve as positive controls. **E**, Healthy tissue cells (primary: human lung fibroblasts; normal human lung bronchial epithelial cells; NHBE, normal human renal cortical epithelial cells; HRCEpC, monocyte-derived dendritic cells; mDCs or iPSC-derived: Cardiomyocytes, Endothelial cells, Hepatocytes, Astrocytes, Neurons) loaded and not loaded with GVY peptide; concentrations for IFN-γ (left panel) or IL-2 (right panel) are depicted. w/o = without target cells. Data shown are representative of 3 different donors for each tested TCR.

Figure 4. bbT485 TCR-Ts are more efficacious than bbT476 TCR-Ts in vitro and in vivo



A,B, Reactivity of untransduced (UTD) and TCR-expressing (bbT476 or bbT485) CD8+ T cells towards MAGE-A4-positive tumor cell lines was tested using different readouts. + GVY indicates pulsing of tumor cells with GVY peptide before co-culture assay **A**, Cytotoxicity assay showing the cell numbers of MAGE-A4-positive tumor cells expressing NucLight Red live-cell labeling reagent (mKate) in co-culture with T cells over time. No effectors = without T cells. MAGE-A4 expression of tumor cells is ordered from highest (left) to lowest (right). **B**, Bar graphs displaying IFN-γ concentrations in supernatants of T cells 16 h after co-culture with tumor cells. MAGE-A4 NGS [RPKM] = MAGE-A4 RNA level according to TRON Cell Line Portal (67), HLA-A*02:01 [MFI] = HLA-A2 expression level defined by the median fluorescence intensity as evaluated by flow cytometry. Data shown are representative of 3 different donors for each tested TCR. **C,** Female NOD-Cg-Prkdc^{scid} IL2rg^{tm1Wjl}/SzJ mice received subcutaneous (s.c.) injections of MAGE-A4-positive HLA-A*02:01-positive A375 melanoma tumor cells to establish s.c. xenografts. At 9 days post tumor implantation, randomized groups of 5 mice each received a single intravenous (i.v.) injection of either media alone, or cell suspensions containing TCR+ T cells of either bbT476 or bbT485, or an equivalent number of untransduced T cells. Mice were monitored until day 37 post T cell injection and tumor volume was measured via calipers ~2 times a week and calculated using the formula: length X width X height X π/6.

Figure 5. Co-receptor independence of the allo-derived TCR enables CD4+ T cell-mediated anti-tumor response



Graphs showing different read outs of activation of CD8+ and CD4+ T cells expressing transgenic TCRs (bbT476 or bbT485) or not (UTD = untransduced) in co-culture with MelA375. **A**, Bar graphs displaying IFN-γ concentrations in supernatants (left panel) and Granzyme B (GzmB) expression among CD8+ and CD4+ T cells determined by flow cytometry (right panel) 16 h after co-culture. **B**, Cytotoxicity assay showing the cell number of MelA375 cells expressing NucLight Red live-cell labeling reagent (mKate) in co-culture with T cells. No effectors = without T cells. **C**, Quantification of cytokine levels in supernatants (left panel) and CD154 expression among CD4+ T cells determined by flow cytometry (right panel) 16 h after co-culture. Data shown are representative of 3 different donors for each tested TCR.

Summary for bbT485 TCR-Ts

- **MAGE-A4-specific HLA-A2-restricted TCR (T cell product: bbT485 TCR-Ts)**
- **Original TCR was isolated from a non-tolerized T cell repertoire (allo-derived TCR)**
  - **→ natural high avidity TCR (no affinity maturation needed)**
- **bbT485 TCR-Ts show excellent safety profiles**
- **Highly efficacious TCR-Ts as shown in in vitro and in vivo experiments**
- **CD8 co-receptor-independent**
  - **→ CD4+ TCR-Ts support anti-tumor response by direct killing**
  - **→ Strong immunomodulatory-capacity by fully functional CD4 repertoire**

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