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## TCRs as precision tools against B-cell and plasma cell malignancies

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# CHAPTER

# 4

## T-cell receptor-based targeting of immunoglobulin constant domains for treatment of multiple myeloma

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## ABSTRACT

In multiple myeloma (MM), transformed plasma cells produce high levels of monoclonal immunoglobulins, most of which are of IgG or IgA subtype. While surface expression of immunoglobulins on MM is usually absent, we hypothesized that peptides derived from immunoglobulins presented in HLA would pose suitable targets for T-cell receptor (TCR-) based therapy of MM. Based on a previously established HLA-peptidome database of B-cell malignancies, we identified three IgG- and four IgA-constant-domain-derived peptides presented in the commonly expressed HLA-A2 and HLA-B7. Using peptide-HLA tetramers, we isolated high-avidity CD8 T-cell clones from the allo-HLA repertoire. In total, we characterized one T-cell clone recognizing an endogenously processed and presented IgG peptide in HLA-A2, and seven T-cell clones recognizing endogenously processed and presented IgA peptides in HLA-B7. Based on recognition of isotype positive MM cell lines, TCRs of the most potent T-cell clones were sequenced, cloned and retrovirally expressed in healthy donor T cells. Upon TCR gene transfer, immunoglobulin-TCR T cells demonstrated potent and isotype-specific recognition and lysis of MM cell lines as well as MM cells in patient bone marrow samples. TCR-transduced CD8 T cells specifically depleted healthy IgA or IgG B cells in an HLA- and isotype-dependent manner. Importantly, healthy cells from non-B-cell lineages were not recognized. Furthermore, IgA-TCR T cells induced anti-tumor responses in an *in vivo* xenograft model of established IgA<sup>positive</sup> MM, demonstrating potential for clinical development of immunoglobulin-targeting TCR therapy.

## INTRODUCTION

Multiple myeloma (MM) is a malignancy caused by uncontrolled expansion of plasma cells and is largely incurable(1, 2). Chemotherapy, immunomodulatory drugs like proteasome inhibitor, anti-CD38 monoclonal antibodies and autologous stem cell transplantation extended life expectancy of patients, but patients relapsing after treatment have extremely poor overall survival(3). The only truly curative therapy for MM to date has been allogeneic stem cell transplantation (allo-SCT), but allo-SCT is generally not generally performed anymore due to high treatment related mortality(4). In recent years, the development of chimeric antigen receptor (CAR) T cells that target antigens expressed on the surface of malignant cells generated promising treatment options for relapsed/refractory MM (RRMM). Notably, B-cell maturation antigen (BCMA-)targeting CAR T cells induce responses in a majority of RRMM patients(5, 6). Despite high initial response rates and median progression free survival in RRMM exceeding one year, most patients eventually relapse(7, 8). Relapses after CAR T-cell therapy can often be attributed to immune escape of antigen low or negative tumor cells, as a consequence of single-antigen-targeting(8-11). In contrast, the effectiveness of allo-SCT has been attributed to polyclonal T-cell responses, illustrating that T-cell responses targeting multiple antigens are likely needed to induce durable complete remissions(12). Consequently, identification of additional antigens suitable for targeted T-cell therapy will be crucial for a tolerable and curative approach(11).

As MM originates from a malignant plasma cell clone, most MM cases are characterized by production of monoclonal immunoglobins(13). We hypothesized that the constant domains of immunoglobulin heavy chains could serve as targets for immunotherapy. IgG immunoglobulins are produced in 54% of MM cases, while IgA is produced in 22% of MM and is associated with a worse prognosis(14). Other subtypes such as IgM isotypes are rare, and in 20% of cases MM cells secrete immunoglobulin light chains only(15). During B-cell development, formation of plasma cells is marked by loss of BCR surface expression. Consequently, malignant plasma cells do not express a BCR on the surface, hindering recognition by CAR T cells. However, peptides derived from IgG and IgA heavy chains presented in HLA could be targeted using T-cell receptors (TCRs). The constant domains of immunoglobulin heavy chains are regions conserved within immunoglobulin isotypes and could thus serve as a universal target for malignancies expressing the respective immunoglobulin. IgG heavy chain constant domains are encoded by the *IGHG* genes and IgA heavy chain constant domains are encoded by the *IGHA* genes.

In this study, we aimed to create new options for T-cell therapy of MM by identification of TCRs recognizing peptides from the constant domains of IgG and IgA presented in the common HLA alleles HLA-A\*02:01 (HLA-A2) and HLA-B\*07:02 (HLA-B7). We

demonstrate that IgG- and IgA-specific T-cell clones with high functional avidity can be identified from PBMCs of HLA-mismatched healthy donors, and that gene transfer of immunoglobulin-targeting TCRs (immunoglobulin-TCRs) to healthy donor T cells installs potent anti-myeloma activity *in vitro* and *in vivo* without off-target reactivity towards immunoglobulin negative cells.

## RESULTS

### **IgG- and IgA-constant-domain-derived peptide identification for HLA-A2 and HLA-B7**

To identify epitopes suitable for TCR-mediated-targeting of *IGHG* or *IGHA* expressing malignancies, we made use of a previously established HLA class-I peptidome database. This database consists of the peptidomes of various patient-derived B-cell malignancy samples, EBV-LCL cell lines and the MM cell line U266(16-18). We analyzed the peptidomes of HLA-A2 and/or HLA-B7 expressing materials for peptides derived from IgG or IgA constant domain proteins according to the UniProt *homo sapiens* database, and selected those peptides for which binding to HLA-A2 or HLA-B7 was predicted by netMHC4.0. Furthermore, to allow targeting of IgG or IgA producing cells independent of additional subtyping (*IGHG1-IGHG4* for IgG and *IGHA1* and *IGHA2* for IgA), peptides were only selected when present in most variants. In total, this resulted in identification of seven peptides: two IgG-derived peptides presented in HLA-A2 and, one IgG-derived peptide presented in HLA-B7, one IgA-derived peptide presented in HLA-A2, and three IgA peptides presented in HLA-B7 (**Table 1**). IgG- and IgA-derived peptides as identified by mass spectrometry were synthetically generated and refolded with their cognate HLA molecules to generate peptide-HLA (pHLA-) monomers. Biotinylated pHLA-monomers were combined with streptavidin to form phycoerythrin (PE)-labelled pHLA-tetramers that were used for T-cell identification.

### **Identification and selection of IgG- and IgA-specific T-cell clones recognizing MM cells**

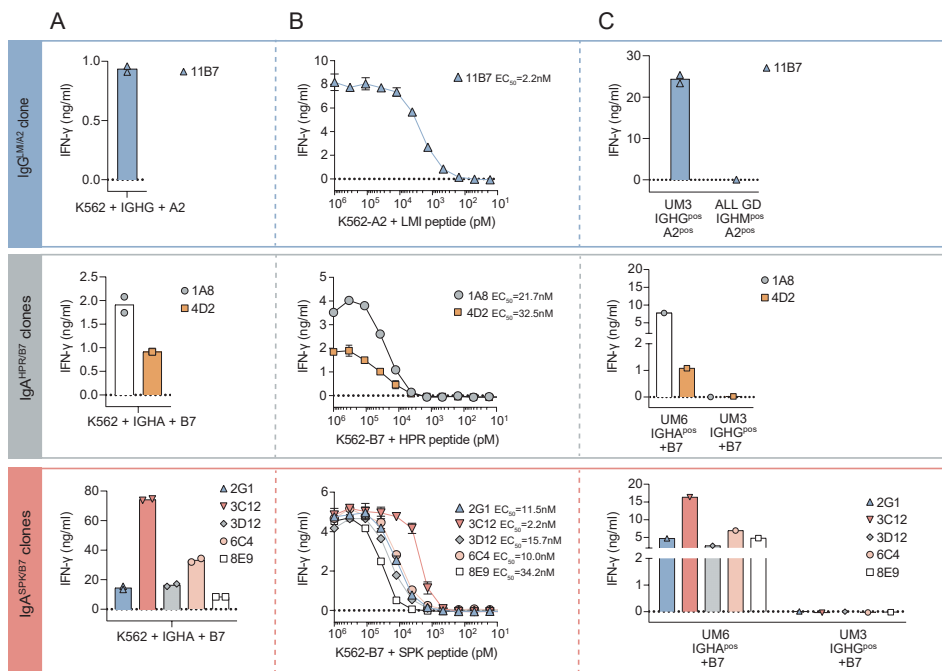
To identify high avidity T-cell clones recognizing IgG- or IgA-constant-domain-derived peptides in HLA-A2 or HLA-B7, we exploited the immunogenicity of self-peptides in the HLA-mismatched repertoire (19). To this end, peripheral blood mononuclear cells (PBMC's) from 26 HLA-A2<sup>negative</sup> and HLA-B7<sup>negative</sup> healthy donors were isolated from buffy coats. pHLA-tetramer<sup>positive</sup>, CD8<sup>positive</sup> T cells were single-cell sorted, and T-cell clones were expanded. Peptide reactivity and specificity of T-cell clones were screened in a high-throughput manner as previously described (17) using antigen negative HLA-A2 or HLA-B7 transduced K562 target cells loaded with IgG and IgA peptides. Peptide specific T-cell clones were expanded further, and reactivity to endogenously processed and presented peptide was tested using HLA-A2 or HLA-B7 and *IGHG* or *IGHA* transduced

target cells. From 26 buffy coats, we identified 8 T-cell clones specific for target epitopes IgG<sup>LMi/A2</sup>, IgA<sup>HPR/B7</sup> or IgA<sup>SPK/B7</sup> that recognized HLA-A2 or HLA-B7 positive and IgG or IgA positive K562 cells to varying degrees (**Figure 1A**). Peptide titration experiments revealed a high functional avidity of these clones with EC<sub>50</sub> values ranging between 34.2nM and 2.2nM (**Figure 1B**). The identified T-cell clones were then tested for their capability to recognize *IGHG* or *IGHA* expressing MM cell lines. The IgG<sup>LMi/A2</sup> specific T-cell clone 11B7 potently recognized *IGHG*<sup>positive</sup> MM cell line UM3 and *IGHG*<sup>negative</sup> cell line ALL GD was not recognized (**Figure 1C**). All IgA HLA-B7 specific T-cell clones recognized *IGHA*<sup>positive</sup> UM6 cells while *IGHA*<sup>negative</sup> UM3 MM cells were not recognized (**Figure 1C**). IgA<sup>SPK/B7</sup> specific T-cell clones 6C4 and 8E9 recognized HLA-B7<sup>negative</sup> wild-type UM6 cells in addition to HLA-B7 transduced UM6 cells (**Figure S1**), demonstrating off-target reactivity outside the HLA-B7 molecule for these T-cell clones. IgA<sup>HPR/B7</sup> specific T-cell clone 1A8 and IgA<sup>SPK/B7</sup> specific T-cell clone 3C12 demonstrated the most potent recognition of UM6 cells corresponding with the peptide affinities of these clones. Based on these data, clones 11B7 (IgG<sup>LMi/A2</sup>), 1A8 (IgA<sup>HPR/B7</sup>) and (3C12 IgA<sup>SPK/B7</sup>) were selected for further analysis.

**Table 1. IgA (*IGHA*) and IgG (*IGHG*) derived peptides identified in HLA-I peptidome data of MM cell line U266, EBV-LCLs or patient B-cell malignancy samples presented in HLA-A2 or HLA-B7**

| Immunoglobulin gene | Sequence    | HLA-I <sup>a</sup> | Peptide code          | HLA binding confirmed <sup>b</sup> | Eluted from cell type <sup>c</sup> |
|---------------------|-------------|--------------------|-----------------------|------------------------------------|------------------------------------|
| <i>IGHG</i>         | GLYSLSSVTV  | A2                 | IgG <sup>GLY/A2</sup> | yes                                | EBV-LCL                            |
| <i>IGHG</i>         | LMISRTPEV   | A2                 | IgG <sup>LMi/A2</sup> | yes                                | EBV-LCL, CLL                       |
| <i>IGHG</i>         | QPREQVYTL   | B7                 | IgG <sup>QPR/B7</sup> | yes                                | EBV-LCL, CLL                       |
| <i>IGHA</i>         | FAVTSILRV   | A2                 | IgA <sup>FAV/A2</sup> | yes                                | ALL, MM                            |
| <i>IGHA</i>         | HPRLSLHRPAL | B7                 | IgA <sup>HPR/B7</sup> | yes                                | MM                                 |
| <i>IGHA</i>         | SPKVFPLSL   | B7                 | IgA <sup>SPK/B7</sup> | yes                                | EBV-LCL                            |
| <i>IGHA</i>         | KPTHVNVSV   | B7                 | IgA <sup>KPT/B7</sup> | yes                                | EBV-LCL                            |

<sup>a</sup> HLA allele of origin of identified peptides. HLA alleles of origin were determined based on HLA-A\*02:01 (HLA-A2) or HLA-B\*07:02 (HLA-B7) expression of materials for which peptides were identified in the peptidome combined with predicted binding to HLA-A2 or HLA-B7 according to netMHC 4.0. <sup>b</sup> Peptide binding to the respective HLA allele was investigated by peptide-HLA monomer refolding. Yes: peptide-HLA monomers were successfully refolded and remained stable. No: peptide-HLA monomers could not stably be refolded. <sup>c</sup> cell type of origin from which peptides were eluted. Abbreviations: ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; EBV-LCL, Epstein-Barr virus-transformed lymphoblastoid cell lines.



**Figure 1. Selection of immunoglobulin-specific T-cell clones that recognize multiple myeloma cell lines.** T-cell clones were co-cultured overnight with target cells and IFN- $\gamma$  production was measured by ELISA. Data is representative of two independent experiments. **A)** T-cell clones that recognize LMI peptide from IgG in HLA-A2 (upper panel), HPR peptide from IgA in HLA-B7 (middle panel) or SPK peptide from IgA in HLA B7 (bottom panel) were stimulated with K562 cells transduced with *IGHG* or *IGHA* genes encoding for IgG and IgA, respectively. K562 cells were additionally transduced with HLA-A2 (+A2) or HLA-B7 (+B7). An effector:target ratio of 1:6 was used. Technical duplicates are shown. **B)** T-cell clones were co-cultured with target HLA transduced K562 cells loaded with decreasing concentrations of immunoglobulin peptides, starting at 1  $\mu$ M. Data from A) and B) were obtained in the same experiment. Values represent means of technical duplicates. **C)** The IgG<sup>LMI/A2</sup> specific clone was stimulated with IgG expressing, HLA-A2 positive UM3 cells and IgM expressing HLA-A2 positive acute lymphoblastic leukemia cell line (ALL GD) as a negative control (right panel). IgA<sup>HPR/B7</sup> and IgA<sup>SPK/B7</sup> specific T cell clones were co-cultured with IgA positive UM6 multiple myeloma cells or IgG positive UM3 multiple myeloma cells as a negative control. Stimulator cells were transduced with HLA-B7 (+B7).

### Safety profiling of candidate immunoglobulin-targeting T-cell clones

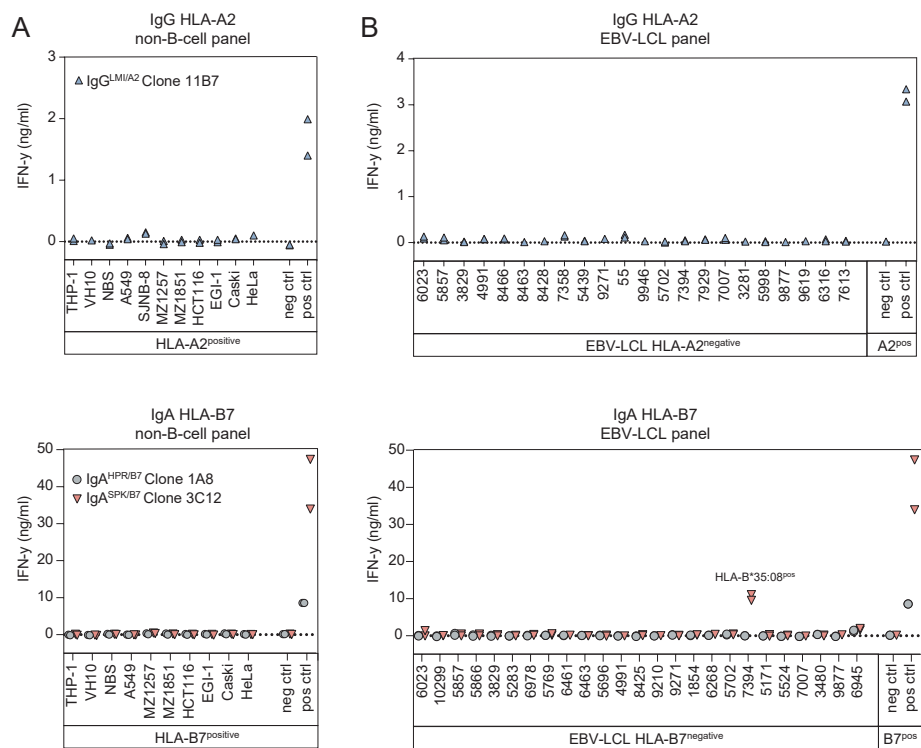
To gain insight into the target specificity of the selected T-cell clones, we tested cross-reactivity with other peptides within the same restriction element, as well as reactivity towards peptides in other HLA alleles. Cross-reactivity with peptides presented by the HLA restriction alleles was assessed by stimulating with HLA-restriction-allele positive cells from various tissue origins that were negative for immunoglobulin expression. None of the tested clones reacted towards the cell line panel, while gene transduced K562 cells (pos ctrl) induced recognition (**Figure 2A**). General T-cell stimulatory capacity

of the target cells used was verified using allo-HLA-A2 and allo-HLA-B7 T-cell clones (**Figure S2**). To test cross-reactivity with other HLA restriction alleles, immunoglobulin-targeting T-cell clones were co-cultured with a panel of Epstein-Barr virus-transformed lymphoblastoid cell lines (EBV-LCLs) expressing common HLA class-I alleles but not the target HLA restriction allele (**Table S1**). T-cell clones 11B7 (IgG<sup>LMI/A2</sup>) and 1A8 (IgA<sup>HPR/B7</sup>) did not recognize any EBV-LCL (**Figure 2B**), indicating no cross-reactivity towards any of the expressed HLA class-I alleles. T-cell clone 3C12 (IgA<sup>SPK/B7</sup>) recognized one EBV-LCL in the panel, namely EBV-LCL MMG expressing HLA-A\*01:01, -A\*32:01, -B\*35:08 and -C\*04:01 (**Table S1**). All alleles except HLA-B\*35:08 were also expressed by at least one other EBV-LCLs in the panel. Since the other EBV-LCLs were not recognized, reactivity of clone 3C12 is most likely directed to HLA-B\*35:08. While recognition could be directed to an unrelated peptide in HLA-B\*35:08, EBV-LCLs can express *IGHA*, and recognition might be directed to the SPK peptide presented in HLA-B\*35:08, which is highly similar to the target allele HLA-B\*07:02. Future research needs to be performed to identify the peptide-HLA complex inducing recognition. However, the HLA-allele frequency in the world population of HLA-B\*35:08 is 0.7%, therefore we do not consider this potential cross-reactivity detrimental to further investigation of this clone for TCR gene therapy.

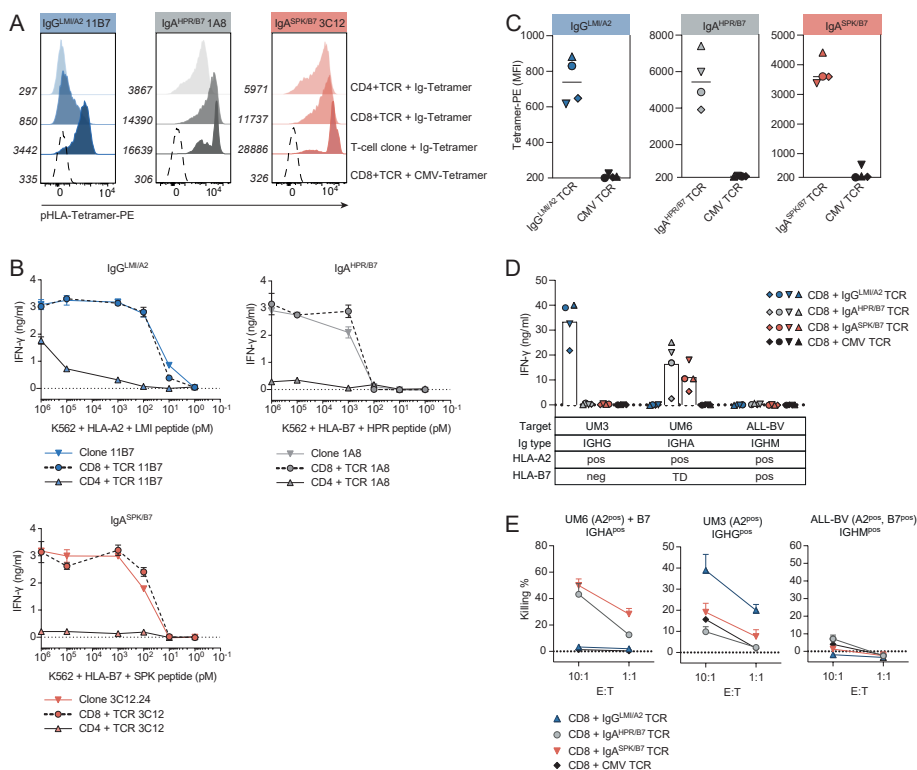
### Immunoglobulin-TCR transfer redirects CD8 T cells to antigen positive targets

Having established general target specificity and safety profiles of immunoglobulin-targeting T-cell clones, we continued to study their potential for TCR gene transfer. The TCRs of the identified T-cell clones were sequenced and cloned into retroviral expression vectors. Third-party CD8 and CD4 T cells were retrovirally transduced and enriched for transgenic-TCR expression. CD8 T cells transduced with immunoglobulin-TCRs 11B7, 1A8 or 3C12 showed specific binding to respective pHLA-tetramers, demonstrating surface expression and retainment of specificity of virally expressed immunoglobulin-TCRs (**Figure 3A**). Although mean fluorescence intensities (MFI) of bound pHLA-tetramer to TCR-CD8 T cells was lower than for parental T-cell clones, the peptide sensitivity was comparable to that of parental T-cell clones (**Figure 3B**). In TCR-CD4 T cells, high pHLA-tetramer binding was observed for TCRs 1A8 and 3C12, but in peptide titration experiments antigen specific activation of these CD4 T cells was not observed (**Figure 3B**), suggesting that CD8 is required for functional sensitivity. Successful introduction of immunoglobulin-TCRs into CD8 T cells was reproducible for multiple donors (**Figure 3C**), and immunoglobulin-TCR T cells were screened for recognition of *IGHG* or *IGHA* expressing MM cell lines and an *IGHG/IGHA* double negative acute lymphoblastic leukemia (ALL) control cell line. IgG-TCR T cells specifically recognized *IGHG*<sup>positive</sup> UM3 cells, and IgA-TCR T cells specifically recognized *IGHA*<sup>positive</sup> UM6 cells (**Figure 3D**). To further analyze the functionality of immunoglobulin-TCR T cells, lysis of MM cell lines was analyzed after short term co-culture experiments. Immunoglobulin-TCR CD8 T cells specifically lysed MM cells in an antigen dependent manner (**Figure 3E**). Together,

these data demonstrate that immunoglobulin-TCRs T cells were highly functional while specificity was maintained.



**Figure 2. Safety screenings of IgG<sup>LMII/A2</sup>, IgA<sup>HPR/B7</sup>, and IgA<sup>SPK/B7</sup>-targeting T-cell clones.** T-cell clones were co-cultured overnight with target cells in an E:T ratio 1:6 and IFN-γ production was measured by ELISA. Technical duplicates are shown. **A)** T-cell clones were stimulated with cell lines of various non-B-cell origins, namely AML (THP-1), fibroblast (VH10 and NBS), lung carcinoma (A549), neuroblastoma (SJNB-8), renal cell carcinoma (MZ1257 and MZ1851), colon carcinoma (HCT116), bile duct carcinoma (EGI-1) and cervix carcinoma (Caski and HeLa). IgG<sup>LMII/A2</sup> reactive clone was co-cultured with HLA-A2 expressing stimulator cells (upper panel), IgA<sup>HPR/B7</sup> and IgA<sup>SPK/B7</sup> reactive clones were co-cultured with HLA-B7 expressing stimulator cells (lower panel). Cells either naturally expressed HLA-A2 or -B7 or were transduced with HLA restriction alleles. Target HLA transduced K562 cells were included as a negative control (neg ctrl) and HLA transduced K562 cells additionally transduced with *IGHA* or *IGHG* were included as positive control (pos ctrl). **B)** T-cell clones from A) were co-culture with an EBV-LCL panel. EBV-LCL panels were designed to not express the target HLA restriction alleles. Controls as in A).

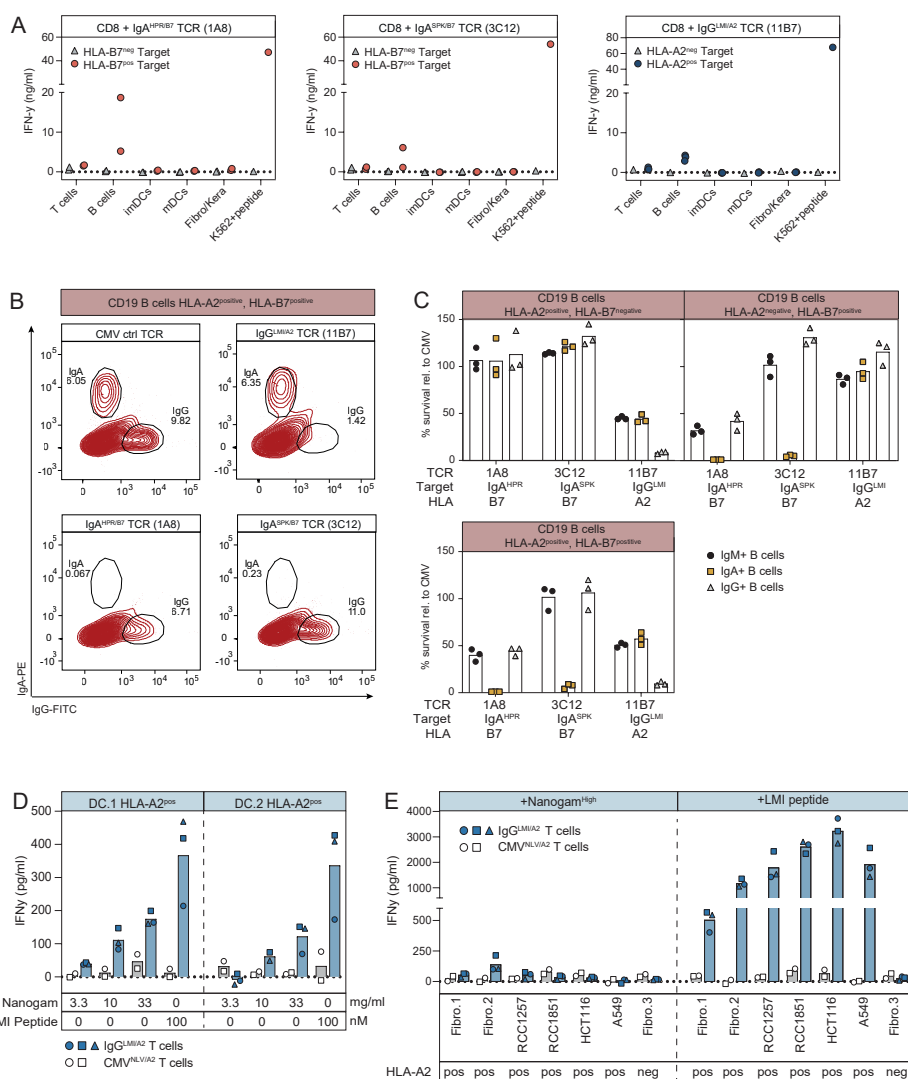


**Figure 3. Functionality of IgG- and IgA-specific TCR transduced T cells.** **A)** MACS isolated CD4 and CD8 T-cell populations were separately transduced with TCR 11B7 specific for IgG<sup>LMI/A2</sup>, TCR 1A8 specific for IgA<sup>HPR/B7</sup> or TCR 3C12 specific for IgA<sup>SPK/B7</sup>. TCR T cells were stained with the respective immunoglobulin pHLA-tetramers. Parental T-cell clones were included as positive controls. Immunoglobulin-TCR CD8 T cells were stained with CMV pp65-HLA-A2 tetramer as a negative control. Cells were analyzed by flowcytometry and TCR T cells were gated on mTCR+. **B)** Parental T-cell clones and TCR-transduced CD8 and CD4 T cells from one donor were co-cultured overnight with target-HLA transduced K562 cells loaded with decreasing concentrations of IgG or IgA peptides, starting at 1uM. IFN-γ production was measured by ELISA. Values represent means of technical duplicates. **C)** CD8 T cells from 4 donors transduced with and enriched for immunoglobulin TCRs or CMV pp65-HLA-A2 TCR as a negative control. Cells were stained with immunoglobulin pHLA-tetramers as indicated in graph titles and analyzed by flowcytometry. Mean fluorescent intensities are plotted. Each symbol represents CD8 T cells isolated from a different donor. **D)** TCR transduced CD8 T cells from C) were co-cultured overnight with IgG positive HLA-A2 positive UM3 multiple myeloma cells, IgA positive, HLA-A2 positive UM6 multiple myeloma cells additionally transduced with HLA-B7 (TD) or and IgM expressing HLA-A2 and HLA-B7 positive acute lymphoblastic leukemia cell line (ALL BV) as a negative control. IFN-γ production was measured by ELISA. **E)** Target cell killing by immunoglobulin-TCR CD8 T cells was analyzed in a 6-hour chromium release assay using the same targets as in D). CMV TCR CD8 T cells were included as a negative control. Percentage target cell lysis is displayed, values represent means and standard deviations of technical triplicates.

## Immunoglobulin-TCR T cells recognize isotype specific B cells and immunoglobulin cross-presenting APCs

As immunoglobulin-TCR T cells were highly reactive towards MM cells, we set out to gain further insight into potential on-target off-tumor reactivity. TCR T cells were co-cultured with healthy tissue subsets of both non-hematopoietic and hematopoietic origin, including peripheral blood B cells. Immunoglobulin-TCR T cells did not recognize T cells, immature dendritic cells, mature dendritic cells, fibroblasts or keratinocytes, regardless of target HLA restriction alleles being expressed (**Figure 4A**). Positive control T cells recognized all included subsets when target HLA was expressed (**Figure S3**). Peripheral blood-derived B cells were recognized by immunoglobulin-TCR T cells, but only when target HLA alleles were expressed. Recognition of mixed B cells was relatively limited compared to positive control cells, especially by IgA-TCR T cells. Limited recognition could be caused by lower frequency of IgA<sup>positive</sup> B cells in peripheral blood. To investigate if immunoglobulin-TCR T-cell reactivity was specifically directed to IgG or IgA positive B cells, we co-cultured total B cells with immunoglobulin-TCR T cells and analyzed the subtype-specific survival of IgM, IgA or IgG positive B cells by flowcytometry. IgG-TCR T cells specifically depleted IgG B cells, while IgA B cells survived (**Figure 4B**). Vice versa, when cultured with IgA-TCR 1A8 and 3C12 T cells, IgA B cells were completely eradicated whereas IgG B cells survived (**Figure 4B**). We quantified the survival of B cells isolated from 3 additional donors by flowcytometry, confirming preferential lysis of isotype positive B cells, although we observed some degree of bystander killing (**Figure 4C**). IgA or IgG B cells that did not express target HLA restriction alleles were not lysed. Together, these data demonstrate that immunoglobulin-TCR T cells can be expected to lyse healthy B cells expressing the respective immunoglobulins, while antigen negative subsets are unlikely to be targeted.

Since MM is associated with high serum immunoglobulin concentrations, we considered the possibility that this could lead to immunoglobulin uptake and cross-presentation of immunoglobulin peptides by antigen presenting cells (APCs). APCs from HLA-A2<sup>positive</sup> donors were cultured with Nanogam (normal serum immunoglobulins) at concentrations between 3.3 mg/ml and 33 mg/ml. A concentration of 33mg/ml corresponds with serum M-protein levels observed in MM patients(20). Nanogam loading of DCs induced recognition by IgG-targeting T cells in a concentration dependent manner (**Figure 4D**). While recognition of cross-presenting APCs might be beneficial for *in vivo* T-cell activation, cross-presentation by other antigen negative cells could potentially be harmful. To explore if non-APC cells would cross-present immunoglobulin peptides under inflammatory conditions, cell lines of various origins were pre-treated with IFN- $\gamma$  and loaded with 33 mg/ml Nanogam. Six out of seven target cells did not induce any recognition, whereas one stimulator cell was weakly recognized by IgG-targeting T cells (**Figure 4E**).



**Figure 4. Recognition of healthy cell subsets by immunoglobulin-TCR T cells.** **A)** IFN- $\gamma$  production after overnight co-culture of immunoglobulin-TCR Td CD8 T cells with CD40L activated B cells, immature dendritic cells, mature dendritic cells, PHA-activated T cells and keratinocytes and fibroblasts pre-treated for 48h with 100 IU/ml IFN- $\gamma$ . Each symbol represents the average value (from technical duplicate) of target cells isolated from a different donor. Target cells not expressing the relevant HLA restriction allele are depicted in grey, cells expressing the HLA restriction alleles are depicted in color. Per panel T cells with one of the immunoglobulin-TCRs are shown as indicated in the graph titles. K562 + HLA and peptide loaded K562 + HLA are included as negative and positive controls. **B)** Example of a FACS based killing experiment of CD40L activated peripheral blood B cells from and HLA-A2 and HLA-B7 positive healthy donor co-cultured overnight with CMV pp65-HLA-A2 TCR, IgG<sup>LMI/A2</sup> TCR (11B7), IgA<sup>HPR/B7</sup> TCR (1A8) or TCR IgA<sup>SPK/B7</sup> (3C12) CD8 T cells. Experiment was performed using an E:T ratio of 3:1. **C)** Killing assay as in B) of B cells isolated from 3 healthy donors with different HLA-A2 and HLA-B7 typing as indicated in graph titles. Survival of IgA and IgG B cells was analyzed after gating on live cells, single cells, CD3 negative and CD19 positive cells. Survival of IgM, IgA, IgG positive B cells was calculated [Legend continues on the next page]

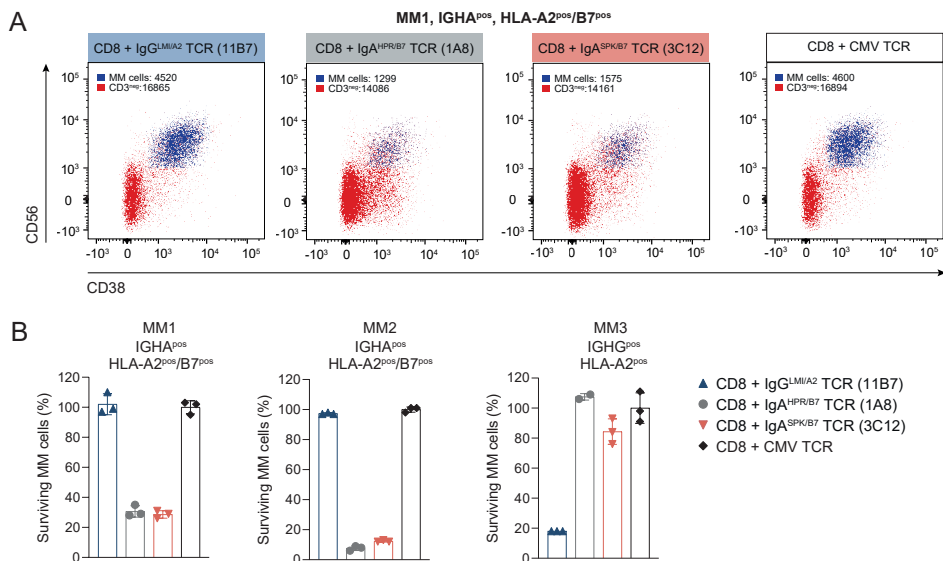
relative to negative control CMV TCR T cells. Technical triplicates are shown. **D)** IFN- $\gamma$  production by Immunoglobulin-TCR (in blue) T cells from 2 donors (round and squared symbols) and the parental T-cell clone (triangular symbol) or CMV-TCR (in white) T cells from the same donors. T cells were co-cultured with monocyte-derived matured dendritic cells (DCs) from 2 HLA-A2<sup>positive</sup> donors exposed to increasing concentrations of normal soluble immunoglobulins (Nanogam) or loaded with 100nM IgG LMI peptide. **E)** T cells from D) Co-cultured with fibroblasts and various adherent tumor cell lines with IFN- $\gamma$  exposed to the highest Nanogam concentration (33ng/ml) or IgG LMI peptide (100nM).

### Immunoglobulin-TCR T cells lysed IgG or IgA positive MM cells from patients *ex vivo*

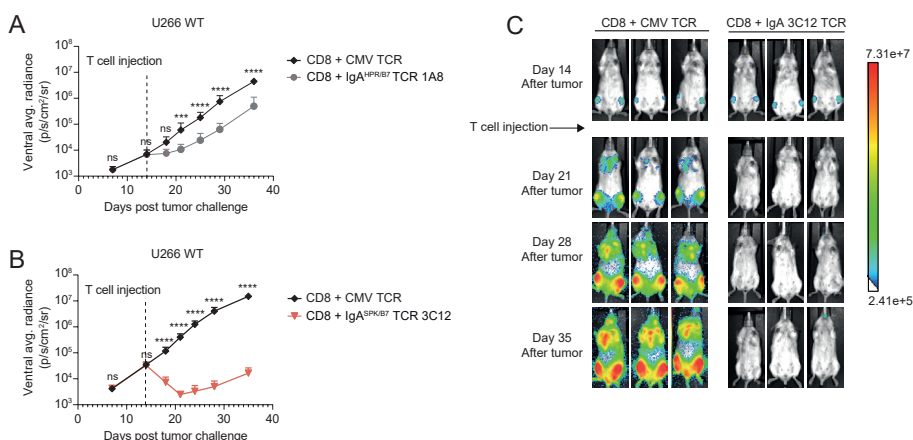
To study the implications of immunoglobulin-TCR T-cell therapy for MM, patient-derived bone marrow (BM) samples were assessed for immunoglobulin-TCR-mediated-targeting. We selected 3 BM samples from HLA-A2 and/or HLA-B7 positive patients containing MM cells expressing either *IGHG* or *IGHA* (**Figure S4**). All samples were co-cultured with immunoglobulin-TCR or control-TCR T cells and survival of MM cells was quantified using flow cytometry (**Figure 5A, 5B**). *IGHA*<sup>positive</sup> MM cells from both samples MM1 and MM2 were specifically lysed by IgA-TCR T cells but not by control or IgG-TCR T cells. Conversely, *IGHG*<sup>positive</sup> MM cells from MM3 were specifically lysed by IgG-TCR T cells but spared by control or IgA-TCR T cells. Together these data demonstrated Ig-subtype-specific and HLA-restricted lysis of MM cells, indicating potent *in vitro* recognition of patient MM cells.

### *In vivo* targeting of IgA positive MM tumors by IgA-TCR T cells

Finally, we analyzed the *in vivo* tumor-targeting capacity of immunoglobulin-TCR T cells in a murine xenograft model for established MM using the MM cell line U266. We chose for the MM cell line U266, as U266 cells endogenously express *IGHA* and, readily engraft into NSG mice(21). Despite lower expression of *IGHA* compared to UM6, U266 are potently recognized by IgA TCR T cells *in vitro* (**Figure S5**). Mice were engrafted with U266 cells and treated with IgA-TCR T cells or control T cells 14 days after tumor inoculation. After T-cell injection, anti-tumor efficacy of IgA-TCR T cells was observed within a few days (**Figure 6**). 1A8-TCR T cells inhibited tumor outgrowth compared to control treated mice, which resulted in a 12-fold lower tumor burdens on day 15 after T-cell injection (**Figure 6A, Figure S6A**). 3C12-TCR T cells drastically reduced tumor burdens within 7 days after T-cell injection, resulting in near complete tumor eradication and 160-fold lower tumor burdens for 3C12-TCR treated mice compared to control mice (**Figure 6B,C, Figure S6B**). Overall, these data demonstrated the *in vivo* anti-tumor efficacy of IgA-TCR T cells, while superior results were obtained when using 3C12 TCR T cells compared to 1A8 TCR T cells.



**Figure 5. Killing of MM cells in patient bone marrow samples.** Killing of MM cells in patient bone marrow samples was assessed in a FACS-based cytotoxicity experiment where immunoglobulin-TCR Td T cells were co-cultured with MM patient BM samples in an E:T ratio 3:1. **A)** Example of survival of an HLA-A2<sup>pos</sup>/B7<sup>pos</sup> patient sample after co-culture with CD8 T cells transduced with IgG<sup>LMI/A2</sup> TCR (11B7), TCR IgA<sup>HPR/B7</sup> (1A8), TCR IgA<sup>SPK/B7</sup> (3C12) or CMV TCR (negative control). In blue MM cells are displayed, MM cells were gated on: live cells → single cells → CD3 negative cells to exclude co-cultured T cells → CD45 negative, CD19 negative → CD56 positive, CD38 positive. MM cells (in blue) were backgated on total CD3 negative cells (in red). **B)** Percentage survival of MM cells from 3 donors after co-culture with immunoglobulin TCR T cells calculated relative to CMV TCR T cells. HLA-A2 or B7 expression of patients is indicated in graph titles. Technical triplicates are shown. Data from the same experiment as shown in A).



**Figure 6. In vivo efficacy of IgA- TCR CD8 T cells.** NSG mice engrafted with  $2 \times 10^6$  HLA-B7 positive U266 multiple myeloma cells transduced with *Luc2* luciferase were i.v. injected with  $5 \times 10^6$  TCR-transduced CD8 T cells after 14 days. CD8 T cells were transduced with IgA<sup>HPR/B7</sup> TCR 1A8, IgA<sup>SPK/B7</sup> TCR 3C12 or [Legend continues on the next page]

control CMV (pp65-NLV-HLA-A2) TCR and enriched for mTCR expression by MACS. T cells were infused 7 days after re-stimulation. Tumor outgrowth was frequently tracked by bioluminescence imaging. **A-B)** Mean and standard deviations of tumor outgrowth (average radiance) over time on the ventral side. **A)** Tumor outgrowth in CMV TCR (n=4) versus IgA<sup>HPR/B7</sup> TCR 1A8 (n=6) treated mice. **B)** Tumor outgrowth in CMV TCR (n=7) versus IgA<sup>SPK/B7</sup> TCR 3C12 (n=8) treated mice. **C)** Raw bioluminescence images of three representative CMV TCR and IgA<sup>SPK/B7</sup> 3C12 TCR treated mice from figure B) at different time points. Raw bioluminescence images of all mice are shown in suppl. Figure 6. Statistics depict two-way ANOVA comparing groups per timepoint with Sidak's multiple comparisons post hoc test.

## DISCUSSION

Cellular therapy using redirected T cells is a promising approach for the treatment of MM. Here, we set out to broaden the landscape of therapeutically targetable antigens for MM. We hypothesized that epitopes derived from immunoglobulin constant domains pose attractive targets for TCR-mediated-targeting. Peptides for TCR-mediated-targeting of IgG and IgA immunoglobulins were identified from the HLA class-I ligandome of B-/plasma cell malignancies. pHLA-multimers were used to isolate T-cell clones from HLA-mismatched healthy donors. T-cell clones specific for the LMI peptide from the IgG constant domain presented in HLA-A2 or the SPK or HPR peptides from the IgA constant domain presented in HLA-B7 were identified. TCRs of T-cell clones that demonstrated the most potent recognition of isotype expressing MM cell lines were sequenced and transferred to third-party T cells. Immunoglobulin-TCR T cells maintained specificity and recognized as well as lysed MM cell lines. Immunoglobulin-TCR T cells did not recognize healthy cell subsets except for B cells. Recognition of B cells was mainly directed to isotype positive B cells. In the absence of immunoglobulins DCs were not recognized, but DCs co-cultured with high concentrations of immunoglobulins were recognized by IgG-TCR T cells. IgA-TCR-engineered T cells demonstrated potent anti-myeloma activity against patient MM cells *ex vivo* as well as in an *in vivo* xenograft model of IgA<sup>positive</sup> MM. CAR T cells and TCR T cells have different recognition mechanisms. As CAR T cells recognize complete proteins, target proteins secreted by or cleaved from malignant cells can bind to CAR T cells and interfere with CAR T-cell functionality. This is especially of concern in BCMA-targeting CAR T-cell therapy, as MM patients often exhibit high levels of circulating soluble BCMA that render BCMA CAR T cells less sensitive(22). For TCR T cells, soluble antigen does not impair T-cell functionality, as activation of TCR T cells requires binding to protein-derived peptides presented in HLA rather than to full proteins. Instead, circulating antigen could have an indirect effect through uptake and cross-presentation by bystander cells. We demonstrated that professional APCs were indeed able to take-up soluble immunoglobulins and present immunoglobulin-derived peptides in HLA, leading to TCR T-cell recognition. The highest tested concentration corresponded to immunoglobulin concentrations found in serum of MM patients(20), and recognition was proportional to immunoglobulin concentrations. On

non-APCs, cross-presentation of immunoglobulins was absent or highly inefficient. While it is difficult to predict the precise consequences this phenomenon might elicit clinically, one could speculate that cross-presentation by APCs will lead to activation of immunoglobulin-TCR T cells *in vivo*. This may be beneficial for initial expansion and anti-myeloma activity of immunoglobulin-TCR T cells, although a potential aggravation of side effects such as cytokine release syndrome would then need to be considered. At the same time, recognition of cross-presenting APCs could lead to their depletion, which would be an undesirable effect on a long-term. However, a potential depletion of APCs should be of temporary nature, since cross-presentation of immunoglobulins will decrease when immunoglobulin-producing malignant and non-malignant cells are depleted as a result of therapy. Given the potential safety concerns, it might be desirable to limit the extent of immunoglobulin cross-presentation prior to clinical administration of immunoglobulin-TCR T cells. In this case, reducing free serum immunoglobulins using serum apheresis could be explored(23-25).

Immunoglobulin-TCR T cells could have a benefit over treatment with CD19 or BCMA CAR T cells, which deplete the entire healthy B-cell compartment compromising B-cell immunity resulting in the need for antibody substitution therapy. We demonstrate that IgA- or IgG-TCR T cells deplete peripheral blood B cells expressing the respective isotype, while B cells of the other isotypes remained. This observation likely extrapolates to plasma cells meaning that plasma cells of isotypes not targeted by the TCR T cells will survive therapy. When applying IgA-TCR T cells for therapy of MM or other IgA<sup>positive</sup> malignancies, leaving IgG B and plasma cells untouched would have a major advantage because IgG is the main antibody isotype providing systemic protection against pathogens. Leaving this part of B-cell immunity intact is expected to eliminate the need for immunoglobulin substitution treatment after T-cell therapy. Overall, these patients would have better health and need less therapeutic intervention, improving their quality of life.

Finally, application immunoglobulin-TCR T-cell therapy could be extended for the treatment of other malignant and non-malignant diseases beyond MM. Immunoglobulin-TCR T-cell therapy could be applied for treatment of refractory B-cell mediated autoimmune disease. Most autoimmune diseases are associated with autoreactive B cells of an IgG immunoglobulin subtype. Recently, CAR T cells have been successfully applied for the treatment of refractory B-cell mediated autoimmune diseases including systemic lupus erythematosus and antisynthetase syndrome (26, 27). Compared to CD19 directed CAR T cells that induce general B-cell aplasia, using IgG-specific T cells would provide the unique advantage to selectively target B cells of that subtype, sparing B cells of other subtypes such as IgA and IgM.

Taken together, we have established the immunoglobulin heavy chain constant domains as targetable antigens for TCR-based treatment of MM. Immunoglobulin-TCRs induce efficient and isotype specific lysis of target cells *in vitro* and *in vivo* without inducing off-target recognition, highlighting potential for further pre-clinical and ultimately clinical development.

## MATERIALS AND METHODS

### **IgG- and IgA-constant-domain-derived peptide identification using HLA class-I ligandome data**

To identify target peptides for TCR-based-targeting of *IGHG* or *IGHA* expressing cells, previously established HLA class-I peptidome data of patient-derived B-cell malignancy samples, EBV-LCL cell lines and the MM cell line U266 was used (16-18). To generate this HLA-I ligandome data,  $>10^8$  cells were lysed and peptide HLA class-I complexes were isolated by immunoaffinity using an anti HLA class-I (Clone W6/32) antibody. Peptides were eluted from the HLA molecules and identified by mass spectrometry. Ligandomes were investigated for peptides originating from IgG (*IGHG*) or IgA (*IGHA*) protein sequences according the Uniprot *Homo sapiens* database. Peptides were assigned to most likely HLA of origin, by overlapping HLA alleles expressed by materials with predicted HLA binding according to netMHC4.0. Peptides from commonly expressed HLA alleles: HLA-A\*02:01 or HLA-B\*07:02 were selected. Synthetic peptides were generated in house using standard Fmoc chemistry. MS/MS spectra of synthetic peptides were acquired and compared to spectra of eluted peptides to validate correct sequence identification. Peptide-HLA monomers were generated for the selected peptide-HLA combinations and monomers were subsequently used to produce PE-labelled peptide-HLA (pHLA-) tetramers as described (28).

### **pHLA-tetramer T-cell isolations**

PBMCs were isolated from complete buffy coats obtained from HLA-typed healthy donors (Sanquin). Per experiment donors were selected to be negative for the HLA alleles of interest. pHLA-tetramers were used to isolate immunoglobulin-specific T-cell clones as previously described with minor modification (17). In short, PBMCs were incubated with pooled PE-labelled pHLA-tetramers (2  $\mu\text{g/ml}$ ) for 15 minutes at 37 degrees after which PE-bound cells were magnetic activated cell sorting (MACS-) enriched using anti-PE microbeads (Miltenyi Biotec). Next, enriched fractions were single-cell sorted for  $\text{CD8}^{\text{positive}}$  pHLA-tetramer $^{\text{positive}}$  cells in 96-well round bottom plates containing feeder mix. For single-cell T-cell sorts, feeder mix contained  $0.5 \times 10^6$  35Gy irradiated PBMCs and  $0.5 \times 10^5$  50Gy irradiated EBV-LCL JY cells per ml in T-cell medium (TCM) with 0.8 mg/ml phytohemagglutinin (PHA; Oxoid Microbiology Products, Thermo Fisher Scientific).

After expansion T-cell clones were screened for target peptide recognition as previously described and peptide-specific T-cell clones were restimulated(17).

### **T-cell culture**

T cells were cultured in TCM consisting of IMDM (Lonza), 1% Penicillin/Streptomycin (Pen/Strep; Lonza), 1.5% glutamine (Lonza), 100 IU/ml IL-2 (Proleukin; Novartis Pharma), 5% fetal bovine serum (FBS; Gibco, Life Technologies) and 5% human serum. T-cell clones were functionally tested between day 10-15 after (re-)stimulation. T-cell clones were restimulated every 10-15 days or cryopreserved until further use. For restimulation  $0.3 \times 10^6$  T cells per 24 well were stimulated with 1 ml feeder mix containing  $1 \times 10^6$  35Gy irradiated PBMCs,  $1 \times 10^5$  50Gy irradiated EBV-LCL JY cells and 0.8 mg/ml PHA.

### **Target cell generation and culture conditions**

Standard culture medium for cell lines consisted of IMDM (Lonza), 1% Pen/Strep (Lonza), 1.5% Glutamine (Lonza) and 10% FBS (Gibco, Life Technologies). For MM cell lines UM3 and UM6, standard culture medium was supplemented with 10ng/ml IL-6. ALL cell lines were cultured as previously described(29). Based on HLA typing, and target gene expression target cells were retrovirally transduced as previously described where indicated(30). Target genes were expressed in MP71 retroviral backbone vectors containing CD34, mouse CD19 or truncated nerve growth factor receptor (NGF-R) as a marker gene. Transduced cells were enriched for marker gene expression by MACS or fluorescence-activated cell sorting (FACS). When indicated adherent cells were pre-treated with 100 IU/ml IFN- $\gamma$  for 24-48h to mimic inflammatory conditions and increase HLA expression prior to recognition assays. For safety screening various healthy subsets were isolated from PBMCs and cultured as previously described(21). For cross-presentation experiments adherent target cells were pre-treated with IFN- $\gamma$  after which cells were cultured overnight with various concentrations of normal human immunoglobulins (Nanogam 100mg/ml, Sanquin) prior to experiments. Monocyte-derived mature DCs were used as professional APCs. To allow optimal cross-presentation Nanogam was added 16h before maturation cytokines were added. Prior to recognition assays, target cells were washed to remove IFN- $\gamma$  and Nanogam. For analysis of patient-derived MM cell lysis, patient BM samples were thawed and rested overnight in medium containing 10% human serum before use in cytotoxicity experiments.

### **T-cell recognition experiments**

T-cell recognition assays were performed in 384-well flat bottom plates. 5,000 T cells were co-cultured overnight with 30,000 stimulator cells. For adherent cells 10,000 stimulator cells were used instead. After overnight culture, IFN- $\gamma$  concentrations in the supernatant was measured by ELISA (R&D systems). For peptide titration experiments target cells were loaded with serial dilutions of peptide starting at 1uM. When peptide

loaded targets were included as positive control a concentration of 100nM was used.

### **TCR sequencing and transfer to CD4 and CD8 T cells and pHLA-tetramer staining**

TCR $\alpha$  and TCR $\beta$  sequences of selected T-cell clones were identified as previously described(17). Codon optimized TCR $\alpha$  and TCR $\beta$  variable chains were inserted in MP71-TCR-flex retroviral vectors containing codon optimized and cysteine modified murine TCR (mTCR) constant domain sequences. For TCR transduction CD8 or CD4 T cells were isolated from healthy donor PBMCs using anti-CD8 or-CD4 microbeads (Miltenyi Biotec) and MACS. T cells were activated with irradiated autologous PBMCs at  $1.0 \times 10^6$  cell per ml TCM and PHA. For  $0.3 \times 10^6$  T cells 1 ml feeder mix was used. T cells were retrovirally transduced on day 2 after activation. mTCR transgenic T cells were enriched using an mTCR-APC antibody (BD Pharmingen) and anti-APC MACS on day 7 after activation and included in functional experiments on day 10-14. To confirm specific TCR expression TCR-T cells were stained with mTCR-APC and pHLA-tetramers (2 ug/ml) and analyzed by flowcytometry (LSRII, BD Bioscience).

### **Cytotoxicity experiments**

TCR-mediated lysis of various cell lines, patient BM samples or PBMC-derived CD40L activated B cells was analyzed using 6hr  $^{51}\text{Cr}$ -release experiments or flowcytometry-based killing assays. Chromium release experiments were performed as described using E:T ratios of 10:1 and 1:1(17). Flowcytometry-based killing experiments were performed by overnight co-culture of 50,000 target cells in an E:T ratio of 3:1. B-cell co-cultures were stained using anti-CD3 (Alexa Fluor 700, BD pharmingen), anti-CD19 (PE-Cy7, BD pharmingen), anti-IgA (PE, Miltenyi Biotec), anti-IgG (FITC, DAKO) and anti-IgM (APC, BD). MM patient BM were co-cultured with TCR T cells and stained using anti-CD3, anti-CD19 (APC, BD pharmingen), anti-CD45 (FITC, BD), anti-CD38 (PE-Texas Red, Invitrogen) and anti-CD56 (PE-Cy7, BD pharmingen). MM cells were defined as CD3<sup>neg</sup>, CD19<sup>neg</sup>, CD45<sup>int/neg</sup>, CD38<sup>pos</sup>, CD56<sup>pos</sup>. Sample were acquired by flowcytomtry (Fortessa, BD Bioscience) using fixed time and flow rates. Target cell survival was calculated relative to negative control TCR T cells.

### **qPCR for immunoglobulin expression**

*IGHG* and *IGHA* expression was measured by quantitative real-time polymerase chain reaction (qPCR) as previously described(17). *IGHG* forward primer: 5'AACTCACACATGCCACCG 3'and reverse primer: 5' GATCATGAGGGTGTCTTGGG 3'. *IGHA* forward primer: 5' CCTTCACCTGGACGCCC 3' and reverse primer: 5' GGCAGGACACTGGACACG 3'. *GUSB*, *VPS29* and *PSMB4* were included as housekeeping genes (HKGs). Gene expression was calculated relative to HKGs.

### ***In vivo* MM targeting by IgA-TCR T cells**

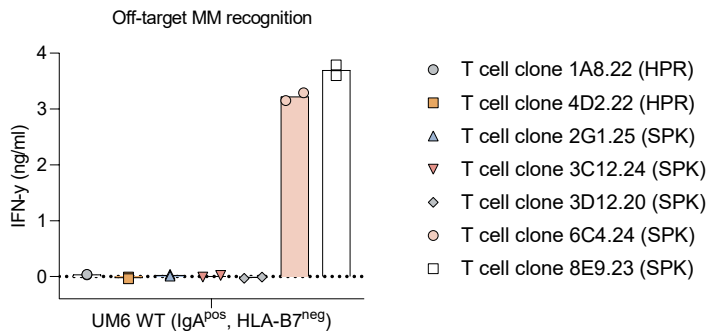
U266 cells were transduced with and enriched for Luciferase-tdTomato Red. NOD-scid-IL2Rgamma<sup>null</sup> (NSG) mice (The Jackson Laboratory) were intravenously (i.v.) injected with  $2 \times 10^6$  U266 cells. After 14 days, mice were i.v. injected with  $5 \times 10^6$  purified IgA-TCR CD8 T cells or CMV-TCR T cells as a negative control. Tumor outgrowth was measured regularly after subcutaneous (s.c.) injection of 150  $\mu$ L 7.5 mM D-luciferine (Cayman Chemical) using a CCD camera (IVIS Spectrum, PerkinElmer). Experiments were terminated when control mice reached an average luminescence of  $1 \times 10^7$  p/s/cm<sup>2</sup>/sr. The study was approved by the national Ethical Committee for Animal Research (AVD116002017891) and performed in accordance with Dutch laws for animal experiments.

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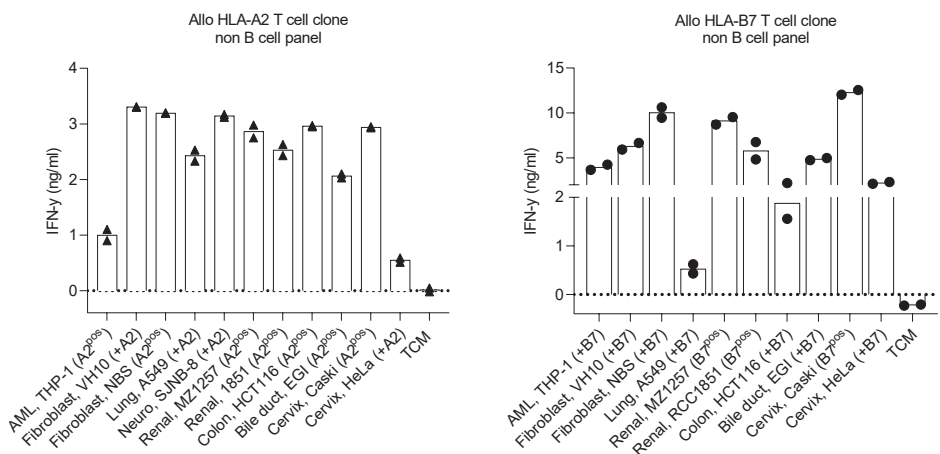
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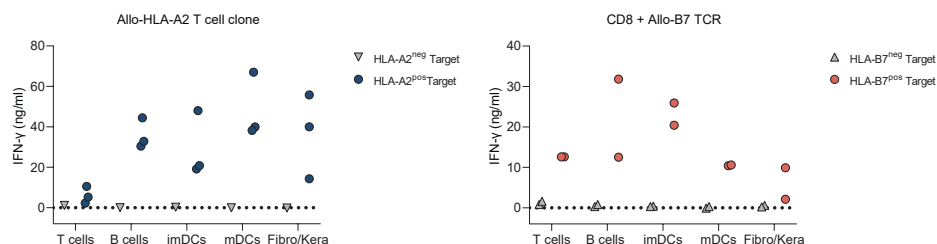
# SUPPLEMENTARY MATERIAL



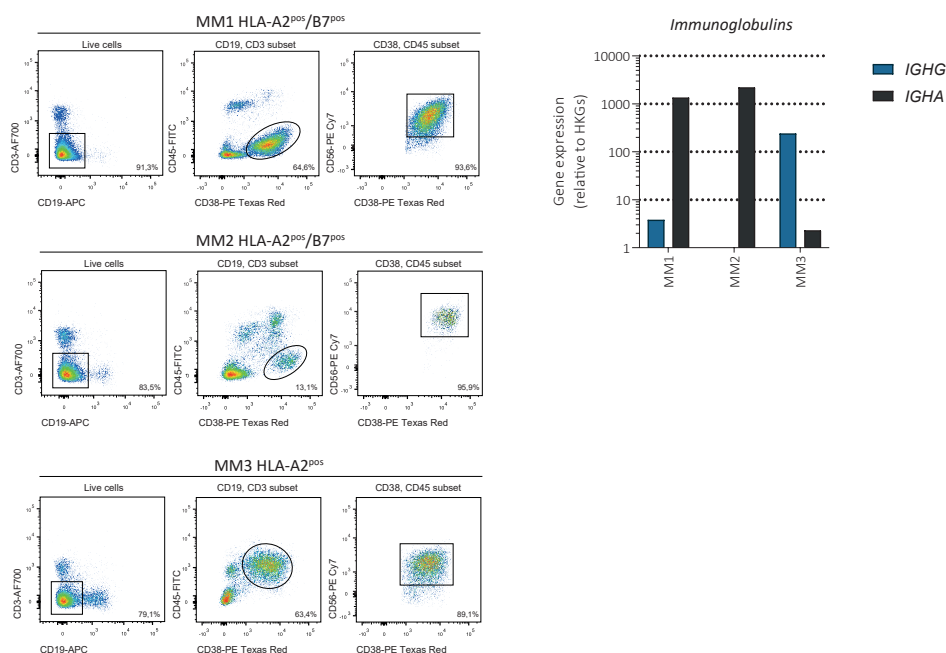
**Suppl. Figure 1.** IFN- $\gamma$  secretion of IgM HLA-B7 specific T cell clones after overnight incubation with WT IgA<sup>pos</sup> HLA-B7<sup>neg</sup> multiple myeloma cell line UM6.



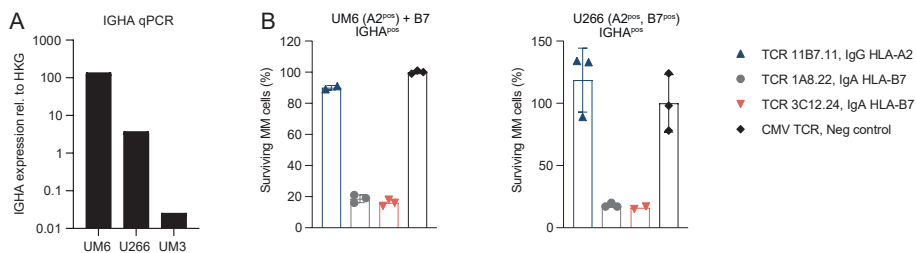
**Suppl. Figure 2.** Validation of stimulatory capacity of cell lines used for safety testing of Ig specific T cell clones. Indicated target cells were incubated with allo HLA T cell clones recognizing peptides derived from ubiquitously expressed genes presented in HLA-A2 (left) or HLA-B7 (right).



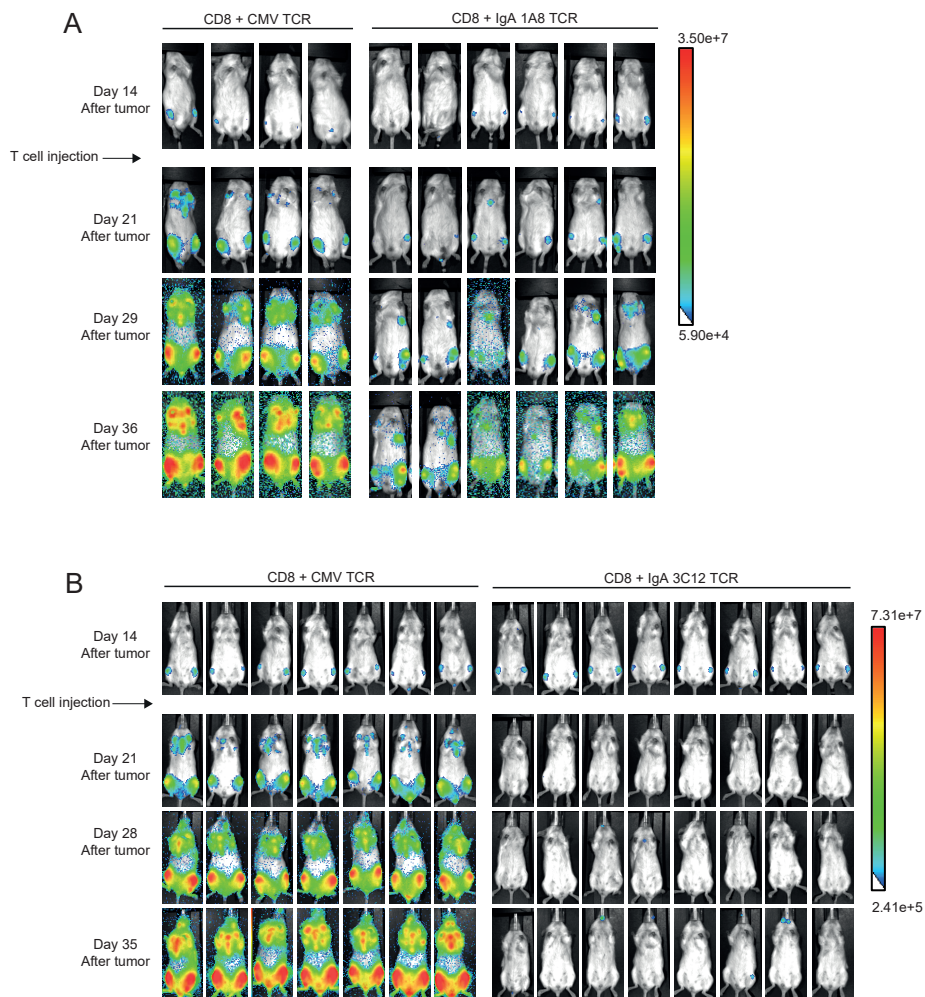
**Suppl. Figure 3. Validation of stimulatory capacity of healthy target cells from hematopoietic origin used for safety testing of Ig specific TCR T cells.** Indicated target cells were incubated with allo HLA T cell clones recognizing peptides derived from ubiquitously expressed genes presented in HLA-A2 (left) or HLA-B7 (right) and cytokine secretion after overnight coculture was measured by ELISA. Individual dots represent target cells derived from individual donors (n=3).



**Suppl. Figure 4.** Flow cytometry gating strategy used for quantification of killing of primary multiple myeloma cells from patient bone marrow samples (left). MM cells were gated on: live cells → single cells → CD3 negative cells to exclude co-cultured T cells → CD45 negative, CD19 negative → CD56 positive, CD38 positive. Quantification of IGHG (blue) or IGHA (black) expression as measured by qPCR in MM cells sorted from patient bone marrow samples (right).



**Suppl. Figure 5. A)** Quantification of *IGHA* expression in indicated cell lines as measured by qPCR. Expression values were normalized to expression of housekeeping genes (HKGs). **B)** Indicated TCR transduced T cells were incubated overnight with UM6 (left) or U266 (right) multiple myeloma cell lines and specific target cell lysis was quantified using flow cytometry.



**Suppl. Figure 6. *In vivo* efficacy of IgA-B7 TCR CD8 T cells.** NSG mice engrafted with  $2 \times 10^6$  HLA-B7 positive U266 multiple myeloma cells transduced with *Luc2* luciferase were i.v. injected with  $5 \times 10^6$  TCR transduced CD8 T cells after 14 days. CD8 T cells were transduced with IgA<sup>HPR/B7</sup> TCR 1A8, IgA<sup>SPK/B7</sup> TCR 3C12 or control CMV (pp65-NLV-HLA-A2) TCR and enriched for mTCR expression by MACS. T cells were infused 7 days after re-stimulation. Tumor outgrowth was frequently tracked by bioluminescence imaging. **A)** Raw bioluminescence images of CMV TCR (n=4) and IgA<sup>HPR/B7</sup> TCR 1A8 (n=6) treated mice. **B)** Raw bioluminescence images of CMV TCR (n=7) and IgA<sup>SPK/B7</sup> TCR 3C12 (n=8) treated mice.

**Table S1. Overview of HLA typing of EBV-LCLs used for safety assessment**

| EBV-LCL | HLA-A                                    | HLA-B                                   | HLA-C                                       |
|---------|------------------------------------------|-----------------------------------------|---------------------------------------------|
| 6023    | 03:01/03:03/03:04-<br>11:01/11:02/11:03  | 40:02/40:35/40:37 - 56:01               | 01:02/01:06/01:07-<br>02:02/02:04/02:08     |
| 10299   | 02:01- 11:01                             | 44:05- 51:01                            | 02:02:02- 14:02                             |
| 5857    | 30:04- 68:02                             | 38:01- 55:01                            | 03:03- 12:03                                |
| 5866    | 02:01- 11:01                             | 35:01- 51:01                            | 04:01- 14:02                                |
| 3829    | 01:01- 68:01                             | 44:02- 44:02                            | 05:01- 07:04                                |
| 5283    | 24:02/24:09/24:11- 66:01/66:04           | 14:02- 39:06                            | 07:02/07:10- 08:02/08:07                    |
| 6978    | 02:01- 02:05                             | 15:01- 45:01                            | 01:02- 06:02                                |
| 5769    | 02:01- 68:01                             | 35:03- 37:01                            | 04:01- 06:02                                |
| 6461    | 02:01                                    | 40:02:00                                | 02:02                                       |
| 6463    | 02:01                                    | 57:01:00                                | 06:02                                       |
| 5696    | 02:05                                    | 58:01:00                                | unknown                                     |
| 4991    | 26:01/26:08/26:02-<br>31:01/31:02/31:06  | 14:01- 49:01                            | 07:01/07:05/07:06- 08:02/08:07              |
| 8425    | 23:01- 02:01                             | 41:01- 40:01                            | 17:01- 03:04                                |
| 9210    | 02:01- 02:01                             | 15:01- 51:01                            | 03:03- 15:02                                |
| 9271    | 33:01/33:03/33:05-<br>66:01/66:04/66:08  | 58:01/58:02/58:11<br>-58:02/58:25/58:11 | 03:02/03:33/03:36-<br>06:02/06:07/06:12     |
| 1854    | 02:01- 30:02                             | 15:01- 39:01                            | 03:03- 12:03                                |
| 6268    | 02:01- 24:02                             | 35:02- 44:02                            | 04:01- 05:01                                |
| 5702    | 32:01- 68:01                             | 35:03- 52:01                            | 12:02- 12:03                                |
| 7394    | 01:01- 32:01                             | 35:08- 35:08                            | 04:01- 04:01                                |
| 5171    | 02:01- 66:01/66:04                       | 40:01/40:11/40:14- 41:02                | 03:04/03:08/03:09- 17                       |
| 5524    | 02:01/02:07/02:09-<br>31:01/31:02/31:06  | B*15:01/15:33/15:34+-<br>B*15:17        | C*03:04/03:08/03:09-<br>C*07:01/07:05/07:06 |
| 7007    | 02:05- 29:02                             | 27:05- 44:03                            | 01:02- 16:01:01                             |
| 3480    | 26:01- 01:01                             | 38:01- 18:01                            | 12:03- 07:01/07:06                          |
| 9877    | 01:01- 23:01/23:17                       | 08:01- 41:02                            | 07:01- 17:03                                |
| 6945    | 03:01- 25:01                             | 18:01- 35:01                            | 04:01- 12:03                                |
| 8466    | 23:01- 23:01                             | 51:01- 41:01                            | 17:01- 15:02                                |
| 8463    | 11:01- 01:01                             | 51:01- 50:01                            | 15:02- 06:02                                |
| 8428    | 11:01- 01:01                             | 57:01- 35:01                            | 06:02- 04:01                                |
| 7358    | 03:01- 25:01                             | 07:02- 44:02                            | 05:01/05:11- 07:02/07:37                    |
| 5439    | 03:01- 25:01                             | 15:17- 18:01/18:03/18:05                | 07:01/07:05/07:06- 12:03/12:06              |
| 55      | 68/69- 01                                | 53- 08                                  | 04-jul                                      |
| 9946    | 03:01/03:63/03:127-<br>11:01/11:12/11:20 | 35:01/35:42- 40:01                      | 03:04- 04:01                                |
| 7929    | 01:01- 01:01                             | 35:02/35:81- 52:01/52:11                | 04:01/04:15- 12:02/12:10                    |

Table S1. Continued.

| EBV-LCL | HLA-A        | HLA-B           | HLA-C                    |
|---------|--------------|-----------------|--------------------------|
| 3281    | 01:01- 32:01 | 08:01- 45:01    | 06:02- 07:01/07:06/07:07 |
| 5998    | 24:02- 68:02 | 14:02- 38:01    | 08:02- 12:03             |
| 9619    | 01:01- 33:03 | 44:03:02- 51:01 | 07:06/07:18- 14:02       |
| 6316    | 29:02- 30:01 | 13:02- 44:03    | 06:02- 16:01             |
| 7613    | 01:01- 24:02 | 15:01- 37:01    | 03:03- 06:02             |