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MAGE-C2–Specific TCRs Combined with Epigenetic Drug-Enhanced Antigenicity Yield Robust and Tumor-Selective T Cell Responses

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Adoptive T cell therapy has shown significant clinical success for patients with advanced melanoma and other tumors. Further development of T cell therapy requires improved strategies to select effective, yet nonself-reactive, TCRs. In this study, we isolated 10 TCR sequences against four MAGE-C2 (MC2) epitopes from melanoma patients who showed clinical responses following vaccination that were accompanied by significant frequencies of anti-MC2 CD8 T cells in blood and tumor without apparent side effects. We introduced these TCRs into T cells, pretreated tumor cells of different histological origins with the epigenetic drugs azacytidine and valproate, and tested tumor and self-reactivities of these TCRs. Pretreatment of tumor cells upregulated MC2 gene expression and enhanced recognition by T cells. In contrast, a panel of normal cell types did not express MC2 mRNA, and similar pretreatment did not result in recognition by MC2-directed T cells. Interestingly, the expression levels of MC2, but not those of CD80, CD86, or programmed death-ligand 1 or 2, correlated with T cell responsiveness. One of the tested TCRs consistently recognized pretreated MC2$^+$ cell lines from melanoma, head and neck, bladder, and triple-negative breast cancers but showed no response to MHC-eluted peptides or peptides highly similar to MC2. We conclude that targeting MC2 Ag, combined with epigenetic drug-enhanced antigenicity, allows for significant and tumor-selective T cell responses. ***The Journal of Immunology*****, 2016, 197: 2541–2552.**

In recent years, treatment with T cells gene engineered with TCRs demonstrated significant clinical responses in patients with metastatic melanoma, colorectal carcinoma, synovial sarcoma, and multiple myeloma (1–6). Clinical TCRs tested were HLA-A1 or HLA-A2 restricted and directed against the melanocyte-differentiation Ags melanoma-associated Ag recognized by T cells (MART)-1, gp100, melanoma-associated Ag (MAGE)-A3, New York esophageal squamous cell carcinoma (NY-ESO)-1, carcino-embryonic Ag, or p53. Collectively, these trials were performed in a total of ∼80 patients and demonstrated objective responses ranging from 12 to 67% (1–4, 7).

In some cases, the use of TCRs directed against Ags that are expressed on tumor cells but also, albeit at a lower level, on normal cells led to severe melanocyte destruction in skin and eyes or severe inflammation of the colon (2, 3). A lethal event was observed in a patient infused with TCR-transduced T cells targeting MART-1/HLA-A2 (EAAGIGILTV epitope) (8). The accompanying cytokine-release syndrome, in combination with semiacute heart failure and an epileptic seizure, resulted in the patient's death. In another study, targeting the cancer germline gene-encoded MAGE-A3/HLA-A2 (KVAELVHFL epitope) led to neurotoxicity and the death of two patients as a result of the anti–MAGE-A3 TCR recognizing shared and highly similar MAGE-A9 and MAGE-A12 epitopes (7). Also, targeting MAGE-A3/HLA-A1 (EVDPIGHLY epitope) led to cardiotoxicity with fatal outcome in two patients (9) because this TCR recognized a highly similar peptide from the muscle protein Titin. The TCRs were affinity enhanced in the latter two trials.

In this study, we assessed the preclinical value of targeting MAGE-C2 (MC2) Ags with TCRs that were patient derived and were not affinity enhanced. We chose MC2 as a target Ag for TCR gene therapy for several reasons. First, the MC2 Ag belongs to a subfamily of cancer-germline genes encoded by the X chromosome (described in more detail at http://www.cta.lncc.br). It is selectively expressed in tumors but not in normal tissue, with the exception of male germline cells (10, 11). Second, MC2 expression is found in advanced tumors of different histological origins where it is associated with poor patient survival (10) and may serve as a predictor for sentinel lymph node metastasis (12). Tumor types with significant MC2 expression include metastatic melanomas (∼40% at mRNA and protein levels) (10, 12), head and neck squamous cell cancers (∼20 and 10% at mRNA and protein levels, respectively) (13, 14), ER$^-$ invasive ductal breast carcinomas (∼30% at protein level) (10, 15), and bladder carcinomas (15% at mRNA level) (10). Third, MC2 was shown to contribute to carcinogenesis by suppressing p53-dependent apoptosis (16) and inducing epithelial-to-mesenchymal transition (15). Finally, one of its most promising features is that MC2-derived antigenic peptides are targeted by T cell responses in a fraction of cancer patients, without detectable toxicity (17).

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Abbreviations used in this article: ALK, ALKDVEERV; ALK/A2, ALK/HLA-A2; ASS, ASSTLYLVF; AZA, azacytidine; BORIS, brother of the regulator of imprinted sites; HNSCC, head and neck squamous cell carcinoma; IMGT, ImMunoGeneTics; LLF, LLFGLALIEV; LLF/A2, LLF/HLA-A2; MAGE, melanoma-associated Ag; MB4, MAGE-B4; MB10, MAGE-B10; MC2, MAGE-C2; MART, melanoma-associated Ag recognized by T cell; NY-ESO, New York esophageal squamous cell carcinoma; PD-L1, programmed death ligand-1; PD-L2, programmed death ligand-2; pMHC, peptide:MHC; qPCR, quantitative PCR; SES, SESIKKKVL; TNBC, triple-negative breast cancer; VPA, valproate.

MC2-derived antigenic peptides include $MC2_{336–344}$ (ALKDVEERV [ALK]/HLA-A2 [ALK/A2]) (18), $MC2_{191–200}$ (LLFGLALIEV[LLF]/HLA-A2 [LLF/A2]) (18), $MC2_{307–315}$ (SESIKKKVL [SES]/B44) (19), and $MC2_{42–50}$ (ASSTLYLVF [ASS]/B57) (20). Even though all four peptides have been identified with patient-derived T cells (21, 22), the ALK and LLF epitopes are of particular interest because of their restriction by HLA-A2*0101, the most common MHC class I allele among the white population. The processing of ALK depends on intermediate or immunoproteasomes, whereas that of LLF depends exclusively on intermediate proteasomes (18, 23). Exposure to IFN-γ leads to the replacement of standard proteasomes by intermediate or immunoproteasomes, and activated intratumoral T cells secreting IFN-γ are expected to increase the surface expression of these two MC2 epitopes (18, 23, 24).

In this study, we established a panel of 10 TCRαβ genes from patient-derived $CD8^+$ anti-MC2 T cell clones. T cell clones were obtained from melanoma patients who experienced significant clinical responses following vaccinations with MAGE-A1 and/or MAGE-A3 Ags that were accompanied by enhanced frequencies of CD8 MC2-specific T cells in blood, invaded lymph nodes, and regressing cutaneous metastases (17, 21, 22). We sequence identified these TCR genes, introduced them into peripheral T cells, and evaluated T cell responses toward tumor cell lines of multiple histologies. In addition, we established a pretreatment regimen consisting of azacytidine (AZA), valproate (VPA), and IFN-γ, successfully increasing the responses of TCR-transduced T cells toward $MC2^+$ tumor lines while maintaining nonresponsiveness to $MC2^-$ cell lines. A large panel of normal cell types did not express MC2 mRNA. Notably, one of the tested TCRs consistently recognized pretreated $MC2^+$ tumor cell lines but showed no response to other peptides, including those that are highly similar to MC2. We conclude that targeting MC2, together with epigenetic drug treatment of tumor cells, results in safe and effective T cell responses.

Materials and Methods

Patient-derived CD8 T cell clones and TCR genes

We acquired a total of eight CD8 T cell clones (Table I) from two melanoma patients. The first patient (EB81) received vaccinations with HLA-A1–restricted MAGE-$A3_{168–176}$(EVD) and MAGE-$A1_{161–169}$(EAD) epitopes. One year after vaccination, cutaneous metastases had disappeared, and the patient remained tumor-free for >10 y. MC2-specific T cell clones were derived by limiting dilution from cocultures of blood CD8 T cells and autologous tumor cells. Several anti-MC2 T cell clones were present in blood and tumors at higher frequencies after vaccination (21). In particular, frequencies of the ALK-specific T cell clone 16 increased >3000-fold in a regressing cutaneous metastasis and accounted for 9% of all $CD8^+$ T cells within an invaded lymph node (17). A second melanoma patient (LB2586) received vaccinations with autologous, monocyte-derived dendritic cells pulsed with MAGE-$A3_{168–176}$ (EVD)/HLA-A1 and MAGE-$A3_{243–258}$ (KKL)/HLA-DP4 peptides. Vaccination led to a mixed clinical response, progressing lesions were surgically removed, and the patient showed an overall survival of >5 y. The anti-MC2 T cell clone CTL A, derived as described above, was found at tumor sites with a >1000-fold higher frequency than in blood. mRNA was obtained from all T cell clones and converted into cDNA, and TCR-V–encoding regions were amplified via PCR using a set of sense primers covering all TCR-Vα or TCR-Vβ segments, according to the ImMunoGeneTics (IMGT) database, or via 5′RACE PCR primers (SMARTer Kit; Clontech Laboratories, Mountain View, CA); in both cases, it was combined with TCR-Cα or TCR-Cβ antisense consensus primers. 5′RACE PCR was optimized to recover PCR products from low T cell numbers (~10,000 T cells). Initial products from either method were reamplified by nested PCRs, cloned into the TOPO 2.1 vector (Invitrogen), and subjected to DNA sequencing. TCRα and TCRβ sequences were verified in at least five colonies. Using the IMGT database and the HighV-QUEST tool (http://www.imgt.org), the TCR V, D, and J sequences were classified according to Lefranc nomenclature. Next, TCRα and TCRβ genes were codon optimized (GeneArt, Regensburg, Germany) and cloned into the pMP71 vector (a kind gift of Prof. Wolfgang Uckert, Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany) using a TCRβ-2A-TCRα cassette via NotI/MluI (TCRβ genes) and MfeI/EcoRI (TCRα genes).

PBMC, packaging cells, and reagents

PBMCs from healthy human donors were isolated by centrifugation via Ficoll-Isopaque (density = 1.077 g/cm^3; Amersham Pharmacia Biotech, Uppsala, Sweden). T cells were cultured in RPMI 1640 medium supplemented with 25 mM HEPES, 200 mM L-glutamine, 6% human serum (Sanquin, Amsterdam, the Netherlands), and antibiotics (RPMI complete) and 360 U/ml human rIL-2 (Proleukin; Chiron, Amsterdam, the Netherlands) and were stimulated every 2 wk with a mixture of irradiated allogeneic feeder cells, as described elsewhere (25). Packaging cells 293T and Phoenix-Ampho were cultured in DMEM supplemented with L-glutamine, 10% FBS, nonessential amino acids, and antibiotics (DMEM complete).

Additional reagents included the HLA-A2–binding peptides $MC2_{336–344}$ (ALK), $MC2_{191–200}$ (LLF), MAGE-B4 $(MB4)_{160–169}$ (LVFGLALKEV), and MAGE-B10 $(MB10)_{162–171}$ (LIFGLDLKEV) (all from Eurogentec, Maastricht, the Netherlands); a previously described library of 114 known HLA-A2–restricted self-peptides (7, 26) (a kind gift of Dr. Matthias Obenaus, Max Delbrück Center for Molecular Medicine in the Helmholtz Association; originally established by Stefan Stevanovic and Hans-Georg Rammensee, University of Tübingen, Tübingen, Germany); human IFN-γ (PeproTech, Rocky Hill, NJ); and 5′-AZA and valproic acid (VPA; both from Sigma-Aldrich, St Louis, MO). The following mAbs and peptide: MHC (pMHC) reagents were used for flow cytometry: TCR–Vβ28–FITC (Beckman Coulter, Marseille, France); CD3-PerCP, CD8-allophycocyanin, CD107a-PE, and BB7.2-PE (all from BD Biosciences, San Jose, CA); and PE-labeled pMHC multimers (dextramers; Immudex, København, Denmark).

T cell transduction

PBMCs of healthy donors were activated with anti-CD3 mAb OKT3 and transduced with TCR-encoding retroviruses (TCR T cells) or empty retroviruses (mock T cells) that were produced by a coculture of 293T and Phoenix-Ampho packaging cells, as described earlier (27, 28).

Tumor cell lines and pretreatment regimens

Melanoma, head and neck squamous cell carcinoma (HNSCC), and bladder carcinoma cell lines were cultured in DMEM complete, whereas triple-negative breast-cancer (TNBC) and esophageal cancer cell lines were cultured in RPMI 1640, L-glutamine, 10% FBS, and antibiotics. Melanoma cell lines are: EB81-MEL derived from patient EB81 ($MC2^+$ (determined by PCR), HLA-$A2^+$ ($A2^+$, determined by PCR and flow cytometry), MEL78 ($MC2^+$, $A2^+$), MEL624 ($MC2^+$, $A2^+$), 518-A2 ($MC2^+$, $A2^+$), 607-B ($MC2^+$, $A2^+$), and MZ2-MEL ($MC2^-$, HLA-$A2^-$). HNSCC cell lines are: SCC9 ($MC2^+$, $A2^+$), SCC38 ($MC2^+$, $A2^+$), 93VU120 ($MC2^+$, $A2^+$), and SCC14C ($MC2^-$, $A2^-$) (kind gifts of Prof. Ruud Brakenhof, VU University Medical Center, Amsterdam, the Netherlands and Prof. Tom Ottenhof, Leiden University Medical Center, Leiden, the Netherlands). TNBC cell lines are: MDA-MB157 ($MC2^+$, $A2^+$), Sum159PT ($MC2^+$, $A2^+$), Sum225CWN ($MC2^+$, $A2^+$), and HCC1806 ($MC2^+$, $A2^-$). Bladder carcinoma cell lines are: 1207 ($MC2^+$, $A2^+$), J82 ($MC2^+$, $A2^+$), and T24 ($MC2^+$, $A2^-$). Esophageal carcinoma cell line is: OEC-19 ($MC2^-$, $MB4^+$, $A2^+$). Prior to their use in T cell assays, tumor cells were treated or not with human rIFN-γ (50 pg/ml for 48 h) or treated with a combination of AZA (1 μM for 72 h), VPA (1 mM for 48 h following AZA treatment), and IFN-γ (50 pg/ml for 48 h simultaneously with VPA).

Gene expression of MC2 Ag and T cell cosignaling ligands

mRNA was obtained from tumor cell lines and reverse transcribed, and RT-PCR was performed to assess gene expression levels of MC2 and, in some cases, MB4, HLA-A2, CD80, CD86, programmed death-ligand 1 (PD-L1), programmed death-ligand 2 (PD-L2), and GAPDH (primer sequences available upon request). PCR products were subjected to gel electrophoresis, after which the intensities of the products were quantified by densitometry (Quantity One v4.6.7 software), corrected for background noise, and standardized for GAPDH intensities. For quantification of MC2 mRNA in healthy human tissues, quantitative PCR (qPCR) was conducted with a normal human tissue cDNA panel (OriGene Technologies, Rockville, MD) and primers and TaqMan probes for MC2 (Gene ID: 51438) and GAPDH (Gene ID: 2597) (Life Technologies, Carlsbad, CA).

T cell IFN-γ production

Transduced T cells (6×10^4/well in a 96-well plate) were cocultured with T2 cells (LCLxT lymphoblastoid hybrid cell line 0.1743CEM.T2) or tumor cells (2×10^4/well) in a total volume of 200 μl of T cell assay medium (RPMI 1640, L-glutamine, 10% FBS, and antibiotics) for 24 h at 37°C and 5% CO_2. T2 cells were pulsed with saturating (1 or 10 μM) or titrated

Table I. MC2-specific T cell clones obtained from metastatic melanoma patients

Epitope	Amino Acids according to MC2 Gene	Restriction Element	Clone Name	Clone ID	Reference
ALK	336–344	HLA-A2	EB81-CTL 606 C/22.2	CTL 16	(17)
			EB81-CTL 721 C/3.13	CTL 40	
LLF	191–200	HLA-A2	EB81-CTL 606 C/19.3	CTL 4	(17)
			EB81-CTL 606 C/21.7	CTL 6	
			EB81-CTL 606 A/16.2	CTL 11	
			EB81-CTL 721 C/3.2	CTL 41	
SES	307–315	HLA-B44	LB2586- CTLA	CTL A	(22)
ASS	42–50	HLA-B57	EB81-CTL C/17.3	CTL 1	(17)

T cell clones were obtained from melanoma patients who experienced a complete and durable response for >10 y following vaccination with MAGE-A1 and A3 (EB81 T cell clones) or a mixed response and an overall survival > 5 y following several injections of dendritic cells pulsed with two MAGE-A3 peptides (LB2586 T cell clone). MC2 T cell clones were derived from cocultures between peripheral CD8 T cells and autologous tumor cells, followed by limiting dilution. See *Materials and Methods* for details.

amounts (typically ranging from 1 pM to 1 μM) of MC2, MB4, or MB10 peptides (the latter two found by National Center for Biotechnology Information Blast homology searches) or a panel of different peptides commonly restricted by HLA-A2 (list of contained peptides available upon request). Subsequently, supernatants were harvested, and IFN-γ levels were determined by standard ELISA (eBioscience, San Diego, CA). For TCR6, half-maximal effective concentrations of MC2 or MB4 peptides (EC_{50}) required for T cell IFN-γ production were calculated using trend line approximations ($R^2 \geq 0.96$).

Flow cytometry

Transduced T cells or tumor cells (5×10^5) were washed with PBS and incubated with Abs at 4°C or with pMHC multimers at 37°C for 30 min. Following staining, cells were washed with PBS and fixed with 1% paraformaldehyde. Events were acquired on a FACSCanto flow cytometer and analyzed using FCS Express 4 software (BD Biosciences).

For CD107a staining, T cells (2×10^5/well of a 96-well plate) were cocultured with either T2 cells (2×10^4/well) or tumor cells (4×10^4/well) in a total volume of 100 μl of T cell assay medium for 2–4 h at 37°C and 5% CO_2 in the presence of CD107a-PE Ab. After incubation, cells were harvested, stained with CD3-PerCP and CD8-allophycocyanin Abs, measured, and analyzed for CD107a expression within CD3, $CD8^+$ T cells.

For pMHC titrations, cultured T cells were stained with CD8-allophycocyanin Ab and titrated amounts of PE-labeled pMHC multimers. To determine pMHC concentrations required to achieve half-maximal binding, results were analyzed using GraphPad Prism to determine EC_{50} values.

Formation of surface-expressed pMHC class I

Assays to determine the stability of MC2 peptides:HLA-A2 complexes were performed, as described elsewhere (29). In short, T2 cells (1×10^6/well in a 96-well plate) were incubated and loaded with titrated amounts of peptide in serum-free AIM V medium at 26°C for 14–16 h and then at 37°C for another 2 h before staining for HLA-A*0201 surface expression with BB7.2-PE mAb. Events were acquired on a FACSCanto flow cytometer and analyzed using FlowJo software (TreeStar, Ashland, OR). Cells were gated for viability, and data are presented as mean fluorescence intensities for different peptide concentrations.

Statistical analysis

Responses of TCR T cells toward $MC2^+$ versus $MC2^-$ tumor cell lines were corrected for responses of mock T cells and tested using one-tailed, unpaired Student *t* tests. Correlations between T cell IFN-γ responses and gene expressions of MC2, HLA-A2, and costimulatory and coinhibitory molecules were calculated using Pearson correlation coefficients. Differences were considered statistically significant when $p < 0.05$.

Results

Patient-derived MC2-specific TCRs mediate T cell recognition of cognate epitopes with varying avidities

We obtained TCRα and TCRβ genes from eight patient-derived, MC2-specific T cell clones from two melanoma patients who experienced durable clinical responses after vaccination therapy (17, 21, 22). T cell clones were directed against the ALK/A2, LLF/A2, ASSTLYLVF/B57, and SES/B44 epitopes (Table I), and using TCR-V–specific PCR techniques we obtained 10 pairs of TCRα and TCRβ sequences (Table II). Two clones (CTL41 and CTLA) expressed two rearranged TCRα sequences, most likely as a result of incomplete

Table II. Sequence identification and gene classification of MC2-specific and patient-derived TCRs

Clone	Epitope	HLA-	TCR Chain	V-Gene	J (D)-Gene	TCR
EB81-CTL 16	ALK	A2	α	Vα3*01	Jα3*01	TCR16[a]
			β	Vβ28*01	Jβ2-5*01	
EB81-CTL 40	ALK	A2	α	Vα13-1*02	Jα3*01	TCR40
			β	Vβ28*01	Jβ2-5*01	
EB81-CTL 4	LLF	A2	α	Vα12-2*01	Jα31*01	TCR4
			β	Vβ7-9*01	Jβ2-1*01	
EB81-CTL 6	LLF	A2	α	Vα12-2*01	Jα23*01	TCR6
			β	Vβ15*02	Jβ2-3*01	
EB81-CTL 11	LLF	A2	α	Vα14/DV4*01	Jα41*01	TCR11
			β	Vβ9*01	Jβ2-7*01	
EB81-CTL 41	LLF	A2	α_1	Vα8-1*01	Jα28*01	TCR41-I
			α_2	Vα22*01		
			β	Vβ9*01	Jβ1-2*01	TCR41-II
EB81-CTL 1	ASSTLYLVF	B52	α	Vα41*01	Jα54*01	TCR1
			β	Vβ7-2*04	Jβ2-7*01	
LB2586-CTL A	SESIKKKVL	B44	α_1	Vα12-3*01	Jα13*01	TCRA-I
			α_2	Vα9-2*02	Jα37*01	
			β	Vβ19*01	Jβ1-4*01 (Dβ1*01)	TCRA-II

cDNAs derived from T cell clones were PCR amplified using a set of TCR-Vα or TCR-Vβ sense primers and a corresponding TCR-Cα or TCR-Cβ antisense primer or 5′RACE. Following nested PCRs and cloning, TCRα and TCRβ sequences were identified using http://www.imgt.org and classified according to Lefranc nomenclature. See *Materials and Methods* for details.

[a]Underlined TCRs were surface expressed and demonstrated binding to pMHC complexes upon gene transfer into primary human T cells.

D, diversity gene segment; J, joining gene segment; V, variable gene segment.

allelic exclusion. T cells from healthy donors were transduced with pairs of TCRα and TCRβ genes and assessed for TCR surface expression and binding of pMHC multimers by flow cytometry. Focusing on HLA-A2–restricted TCRs, we observed that transductions with TCR16 (ALK), as well as TCR4, TCR6, and TCR11 (LLF), yielded populations of T cells that significantly bound cognate pMHC multimers (Supplemental Fig. 1). Transductions with TCRs 40, 41-I, and 41-II yielded populations of T cells that showed no or very limited staining with pMHC multimers (~1%). This was not the result of compromised TCR gene transductions because TCR-Vβ28 Ab staining of T cells transduced with TCR40 (same Vβ-chain as TCR16) revealed clear surface expression of the introduced TCRβ-chain (data not shown). For subsequent experiments, we FACS-sorted TCR-transduced T cells and obtained T cell populations with high (>70%) and stable binding of pMHC (Fig. 1A), with the exception of TCR11 T cells, which gradually lost pMHC binding capacity over time (data not shown).

To test TCR functions, transduced T cells were cocultured with T2 cells pulsed with a saturating dose (1 μM) of ALK (TCR16) or LLF (TCR4, 6 and 11) peptides. TCR-transduced, but not mock, T cells clearly produced IFN-γ upon stimulation with peptide-loaded cells, with TCR11 T cells producing less IFN-γ than T cells transduced with the other TCRs (Fig. 1B). With respect to T cell degranulation, findings were similar, and TCR (but not mock) T cells upregulated surface expression of CD107a upon stimulation with peptide-loaded cells. Again, T cells expressing TCR11 were the least responsive population (Supplemental Fig. 2A).

In addition to an initial test of T cell function, we conducted pMHC titrations as a measure of T cell avidity. These studies demonstrated that the TCRs show EC_{50} values for binding of pMHC to CD8 T cells ranging from low to high: TCR4 < TCR6 < TCR11/16 (Fig. 2A). When analyzing CD4 T cell populations, findings were similar but demonstrated that TCR11 and, in particular, TCR16 showed a significant decrease in their abilities to bind pMHC (Fig. 2B). The observed hierarchy in T cell avidity and, in particular, the CD8 dependence of TCR16 corresponded to the abilities of these T cells to upregulate CD107a expression (Supplemental Fig. 2A).

Pretreating melanoma cells with epigenetic drugs and IFN-γ increases MC2 gene expression and T cell stimulation

Next, we tested T cell reactivities toward $MC2^+$ melanoma cell lines. Because ALK and LLF peptides require processing by immune (ALK) or intermediate (ALK and LLF) proteasomes, rather than standard proteasomes (23), we pretreated melanoma cells with IFN-γ to induce the expression of immune proteasome catalytic subunits. TCR T cells did not produce levels of IFN-γ that exceeded those of mock T cells when stimulated with untreated melanoma cells and exhibited weak to moderate IFN-γ production after stimulation by IFN-γ–pretreated melanoma cells, in particular the EB81-MEL and 607B cell lines (Fig. 3). To enhance the level of MC2 T cell responses, we resorted to an additional treatment with AZA and VPA for their reported effect on the expression of MAGE Ags in cancer cells and their recognition by T cells in preclinical and clinical studies (30–36). AZA is a DNA methyl transferase inhibitor that allows expression of genes that are silenced through the demethylation of CpG islands in promoter regions. VPA is a histone deacetylase inhibitor that blocks histone

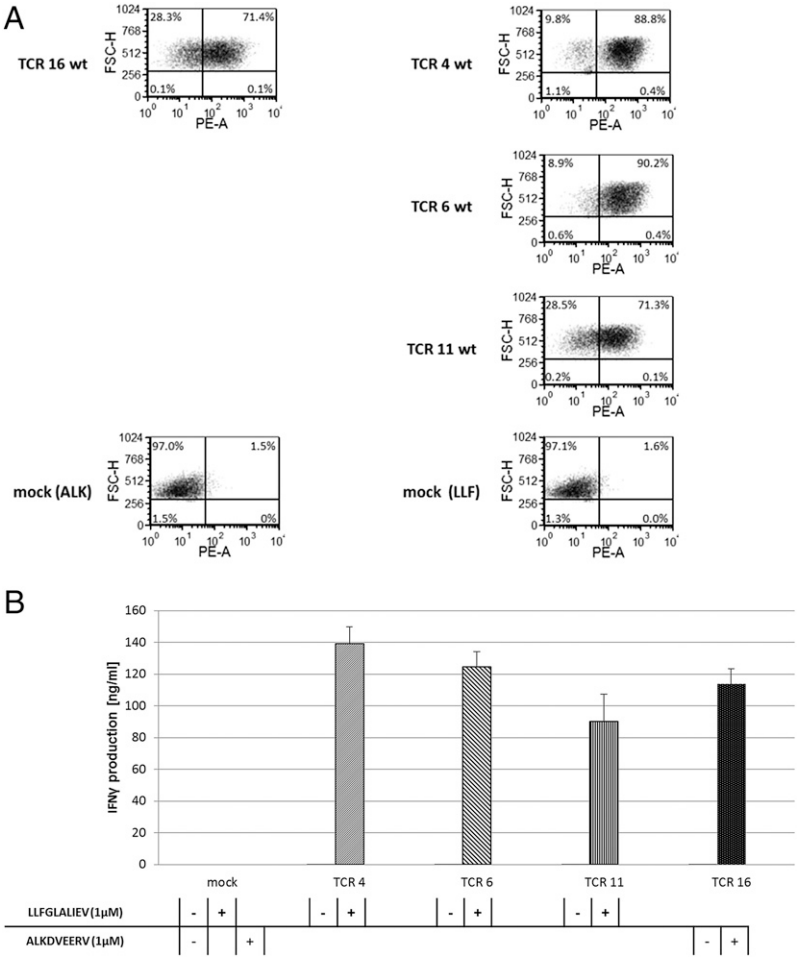


FIGURE 1. MC2 TCR T cells bind pMHC and produce IFN-γ in response to cognate epitope. T cells were transduced with anti-MC2 TCR4, TCR6, TCR11, or TCR16 or not (mock) and were FACS sorted with the corresponding pMHC multimers. (**A**) TCR-transduced and sorted T cell populations were labeled with ALK/A2-PE (TCR16) or LLF/A2-PE (TCR4, TCR6, TCR11). T cells were gated for live cells, and dot plots are representative of five experiments in two donors. Percentages in upper right quadrants represent fractions of T cells stained by pMHC corrected for background staining of corresponding mock T cells. (**B**) T cells from (A) were cocultured with T2 cells pulsed with 1 μM of ALK (for TCR16) or LLF (for TCR4, TCR6, and TCR11) peptide for 24 h. IFN-γ levels in culture supernatants were measured by ELISA and are displayed as mean ± SEM ($n = 4$ experiments). IFN-γ levels from mock T cells and T cells incubated with unpulsed T2 cells ranged from 3 to 80 pg/ml. Data are from one healthy donor of two tested with similar results.

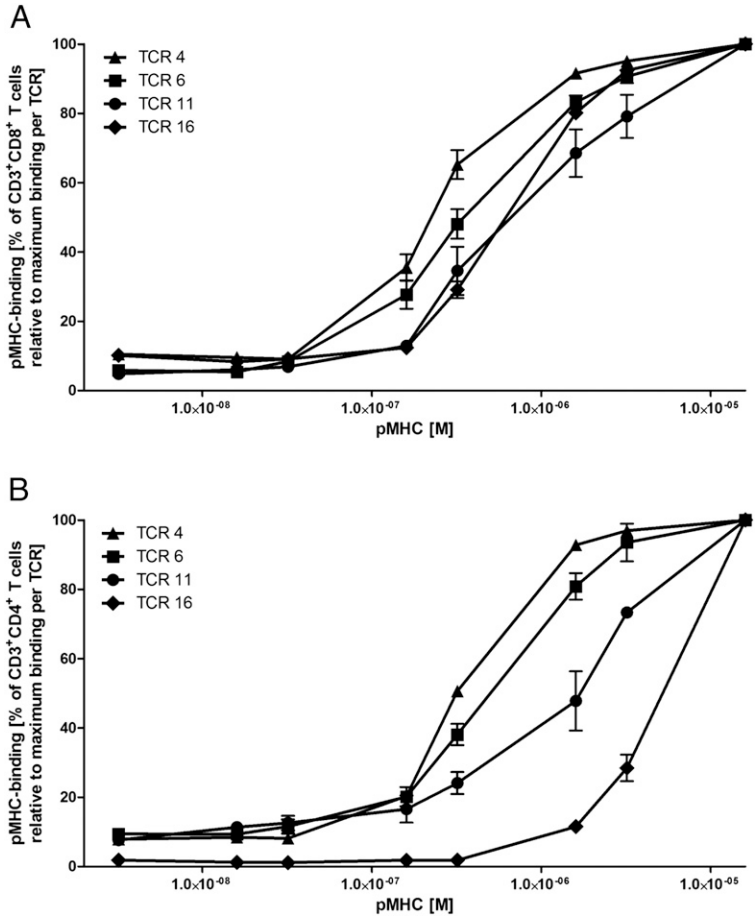


FIGURE 2. MC2 TCRs provide T cells with different avidities for MC2 peptide:HLA-A2 complexes. For pMHC multimer titrations, MC2 TCR T cells were stained with CD8-allophycocyanin Ab (**A**) or CD4-FITC Ab (**B**) and different concentrations of ALK/A2-PE (TCR16) or LLF/A2-PE (TCR4, TCR6, TCR11). Maximal percentages of T cells that bound pMHC multimer were set at 100%, and resulting curves and EC_{50} values were analyzed using GraphPad Prism (CD8: TCR4 = 2.26×10^{-7} M, TCR6 = 3.48×10^{-7} M, TCR11 = 6.67×10^{-7} M, TCR16 = 6.63×10^{-7} M; CD4: TCR4 = 3.16×10^{-7} M, TCR6 = 5.01×10^{-7} M, TCR11 = 1.7×10^{-6} M; EC_{50} for TCR16 could not be determined). Data are displayed as mean ± SEM (n = 3).

deacetylation, thereby relieving chromatin condensation and increasing gene expression. Timing and dosage of AZA and VPA are based on the work of Goodyear et al. (30). We tested a combination of IFN-γ, AZA, and VPA on six melanoma lines, the noncancerous keratinocyte cell line HaCaT, and primary fibroblasts. Fig. 4A shows that, upon treatment with AZA/VPA/IFN-γ, MC2 gene expression was upregulated in four of six tumor cell lines but notably not in the noncancerous cells. Treatment variably regulated gene expression of HLA-A2, which was only upregulated in two of six melanoma lines. In addition, we assessed MC2 protein expression in pretreated tumor cells via flow cytometry using the Ab LX CT10.9 that was validated previously for immune histochemistry (37). Staining confirmed PCR data and demonstrated upregulated MC2 protein expression following AZA/VPA/IFN-γ treatment (data not shown). Finally, melanoma and normal cells were tested for their ability to stimulate the production of IFN-γ or upregulation of surface CD107a by MC2 TCR T cells (Fig. 4B, Supplemental Fig. 2B). All tested $MC2^+$ melanoma lines stimulated two or more of the tested TCRs. These data demonstrate that melanoma cells, but not normal cells, exhibit improved recognition by MC2 TCR T cells following treatment with AZA/VPA/IFN-γ.

T cells directed against MC2 show significant responses against head and neck, breast, and bladder cancer cells following pretreatment with AZA/VPA/IFN-γ

To extend our results to nonmelanoma tumor cell lines, we explored the gene expression of MC2 and HLA-A2 in HNSCC, TNBC, and bladder carcinoma cell lines. As shown in Fig. 4C, MC2 gene expression was upregulated in the majority of tumor cell lines following treatment (9 of 11, with the exception of TNBC cell lines MDA157MB and Sum159PT), and HLA-A2 gene expression was upregulated to a more variable extent in tumor cell lines following treatment (6 of 11, with the exception of HNSCC cell line SCC 14C, TNBC cell lines MDA157MB and HCC1806, and bladder cancer cell lines J82 and T24). Importantly, treatment of HNSCC, TNBC, and bladder carcinoma cell lines clearly resulted in an enhancement of IFN-γ production (Fig. 4D) and upregulation of CD107a (data not shown) by MC2 TCR T cells. All $MC2^+$ tumor cell lines tested yielded a significant response by at least one of the TCRs. As was observed for melanomas, AZA/VPA was required, in addition to the IFN-γ pretreatment, to maximize MC2-specific T cell responses.

MC2 T cell responses are governed by expression level of Ag but not expression of costimulatory or coinhibitory molecules

Even though pretreatment of target cells elicited enhanced responses of MC2 TCR T cells, such T cell responses were heterogeneous with respect to different TCRs and different tumor target cells. In an effort to better understand this heterogeneity, we first assessed the relationship between individual TCR and T cell IFN-γ responses. When analyzing the breadth of T cell responses, TCR6 was the only TCR able to mediate a T cell response against all $MC2/A2^+$ tumor cell lines tested, irrespective of the tumor type (100% response, n = 13 tumor cell lines), whereas TCR4 and TCR16 mediated T cell responses against 50% of these target cells, and TCR11 mediated responses against only 15% of them (Fig. 5A). Notably, TCR6 mediated T cell responses against all four tumor types, yet TCR4 and TCR16 demonstrated a preference toward melanomas, and TCR11 mediated responses against HNSCC and bladder carcinomas (Fig. 5A). In addition to the breadth of the response, we analyzed the quantities of IFN-γ

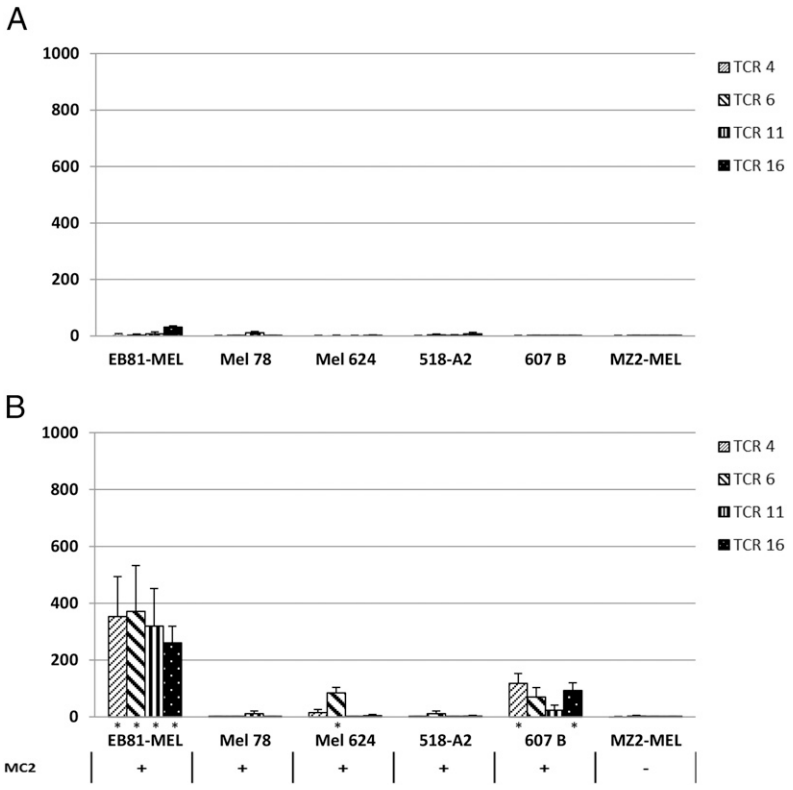


FIGURE 3. T cell recognition of MC2$^+$ melanoma cells is limited. MC2 TCR T cells were cocultured with tumor cells that were positive or negative for MC2 (based on RT-PCR data, indicated as "+" or "−") at an E:T ratio of 3:1 for 24 h. Melanoma cells were either left untreated (**A**) or pretreated with human IFN-γ (50 pg/ml for 48 h) (**B**). Levels of IFN-γ produced by T cells in culture supernatants were measured by ELISA and are displayed as means ± SEM (n = 3). IFN-γ production by MC2 TCR T cells was corrected for IFN-γ production by mock T cells (same donor and tumor cell line); IFN-γ levels produced by mock T cells ranged from 10 to 95 pg/ml. Data are from one healthy donor out of two tested with similar results. *$p < 0.05$, responses of TCR T cells toward MC2$^+$ versus MC2$^-$ target cells, Student t test.

produced, which again were highest for T cells expressing TCR6 (median IFN-γ production = 267 pg/ml), lower for T cells expressing TCR16 and TCR4 (156 and 136 pg/ml, respectively), and lowest for T cells expressing TCR11 (97 pg/ml) (Fig. 5B). Interestingly, the highest quantities of IFN-γ were found with melanoma target cells, followed by bladder carcinoma cell lines, and then HNSCC and TNBC lines. Collectively, these findings favor TCR6, a TCR that provides T cells with intermediate to high avidity (Fig. 2), with respect to MC2-specific anti-tumor T cell responses. Second, we assessed the impact of the expression of genes coding for surface molecules critical for T cell stimulation, such as the MC2 Ag, HLA-A2, the costimulatory molecules CD80 and CD86, and the coinhibitory molecules PD-L1 and PD-L2. CD80 and CD86, which were only negligibly or not expressed in tumor or normal cell lines, showed no change following AZA/VPA/IFN-γ pretreatment, whereas expression of PD-L1 and PD-L2 was increased in some cell lines following pretreatment (Fig. 6A). This argues that AZA/VPA/IFN-γ treatment does not generally enhance gene expression but that the effect is restricted to genes such as the cancer germline gene MC2. Testing whether T cell responses depended on the expression levels of any of these genes, a significant correlation was observed with the expression level of MC2 and not with any of the other investigated molecules (Fig. 6B, shown for TCR6 T cells, but observed for all TCRs, data not shown). When categorizing tumor types according to the levels of MC2 expression, it appears that melanoma cells expressed the highest levels, followed sequentially by bladder carcinoma, TNBC cells, and HNSCC cells (Fig. 6B). These results most likely explain the earlier observation that melanoma cells stimulated the highest T cell responses and support the pretreatment of tumor cells to enhance MC2-specific T cell responses.

TCR6 shows no on-target or off-target toxicity in vitro

We conducted a series of assays to exclude possible on- and off-target toxicities, thereby enhancing the potential clinical value of MC2 TCRs. First, qPCRs were performed for MC2 gene expression using a library of 48 human tissues; the only tissue positive for MC2 gene expression was testis (Fig. 7A), for which expression was 51-fold lower than within our positive control, the EB81-MEL cell line. Spiking experiments demonstrated a sensitivity of these MC2-specific qPCRs of 1 MC2$^+$ cell in 10^3 MC2$^-$ cells. Second, T cells transduced with TCR6, the TCR that scored best according to our in vitro experiments, were cocultured with T2 cells loaded with HLA-A2–eluted self-peptides. These T cell stimulations demonstrated no cross-recognition of TCR6 against any of these peptides, with the only noticeable recognition being the one against the MC2 peptide (Fig. 7B). Third, TCR6-transduced T cells were cocultured with the MB4 peptide LVFGLALKEV and the MB10 peptide LIFGLDLKEV, which are both highly homologous to the MC2 peptide LLFGLALIEV, according to National Center for Biotechnology Information Blast searches (amino acids different from the MC2 LLF peptide are underlined). TCR6 T cells showed some response to MB4 peptide, ∼15% of the response seen against MC2 LLF peptide, but not to the MB10 peptide. Further titrations with MB4 peptide revealed that it had a higher EC_{50} value than the MC2 LLF peptide. Importantly, TCR6-transduced T cells did not recognize target cells, such as the esophageal cancer cell line OEC-19, expressing native MB4 (Fig. 7C–E). Collectively, these results provide clear evidence against risks for on- or off-target reactivities when targeting MC2 via TCR6.

Discussion

In this study, we tested the preclinical value of targeting MC2 epitopes with patient-derived and nonaffinity-enhanced TCRs, in particular TCRs specific for the HLA-A2–restricted epitopes ALK and LLF. When studying HLA-A2–restricted TCRs, we observed that three TCRs (of seven) did not enable transduced T cells to bind pMHC. T cells expressing the four other TCRs (TCR4, TCR6, TCR11, and TCR16) were used to determine EC_{50} values for their binding of pMHC multimers (Fig. 2). These TCRs yielded T cells

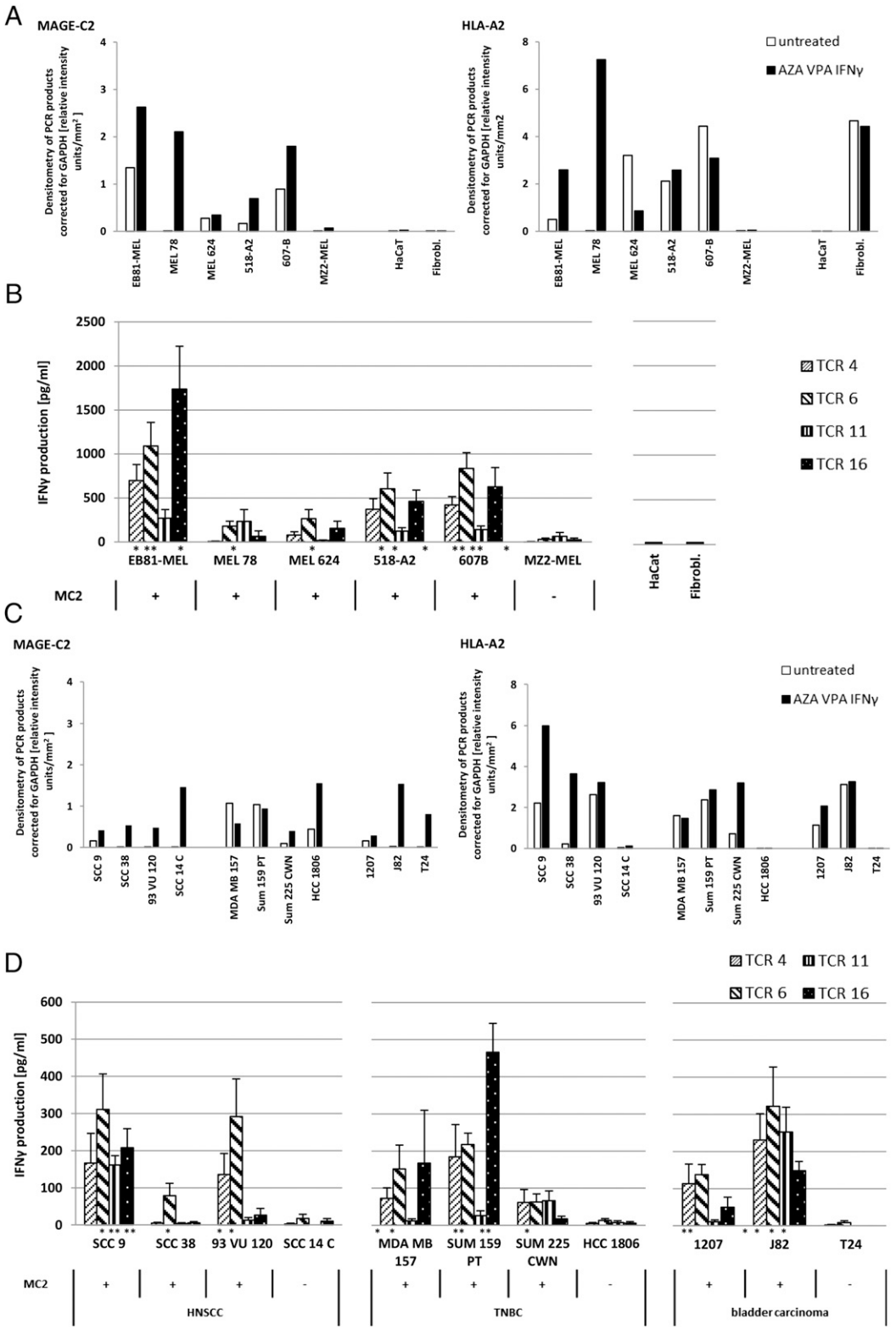


FIGURE 4. Treatment of melanoma, head and neck, bladder, and breast carcinoma cells with AZA and VPA enhances MC2 gene expression and T cell recognition. (**A**) Melanoma cell lines ($n = 6$), HaCaT keratinocytes, and fibroblasts (Fibrobl.) were pretreated or not with AZA (1 μM for 72 h), VPA (1 mM for 48 h), and IFN-γ (50 pg/ml for 48 h). RNA was isolated from 1×10^6 cells, followed by cDNA synthesis and RT-PCR with primers for detection of MC2, HLA-A2, and GAPDH transcripts. PCR products were subjected to gel electrophoresis, and intensities of MC2 and HLA-A2 bands were quantified by Quantity One (version 4.6.7), corrected for background noise and standardized for GAPDH levels, and displayed in relative intensity units/mm^2. (**B**) MC2 TCR T cells were cocultured with pretreated melanoma cells from (A) at an E:T ratio of 3:1 for 24 h. IFN-γ levels in culture supernatants were measured by ELISA and are displayed as mean ± SEM ($n = 5$). IFN-γ production by MC2 TCR T cells was corrected for IFN-γ production by mock T cells; IFN-γ levels produced by mock T cells ranged from 2 to 300 pg/ml. (**C**) Cell lines from HNSCC ($n = 4$), TNBC ($n = 4$), and bladder carcinoma ($n = 3$) were pretreated as described in (A). RNA was isolated, used for RT-PCR, and subjected to analysis of MC2 and HLA-A2 expression. (**D**) MC2 TCR T cells were cocultured with pretreated tumor cell lines from (C) and analyzed for IFN-γ production, as described in (B). IFN-γ production of mock T cells ranged from 9 to 180 pg/ml. Data are from one healthy donor out of two tested with similar results. $^*p < 0.05$, $^{**}p < 0.005$, responses of TCR T cells toward MC2$^+$ versus MC2$^-$ target cells, Student t test.

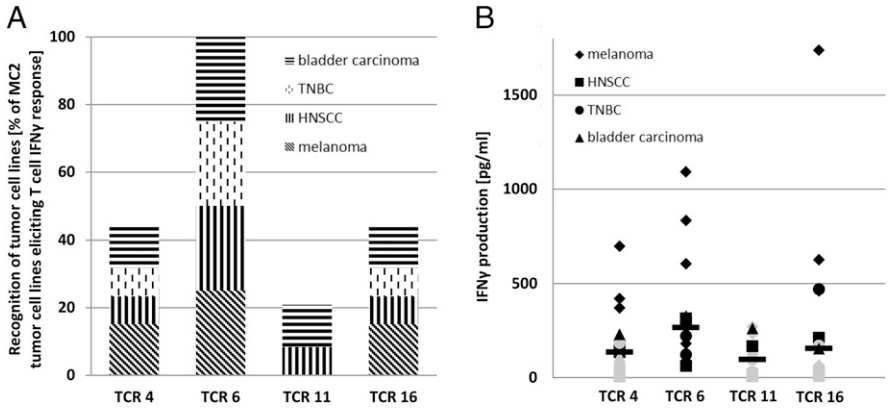


FIGURE 5. MC2 TCR6 performs best with respect to tumor cell recognition. Breadth (**A**) and intensity (**B**) of T cell responses of each TCR were quantified based on the results shown in Fig. 4A and 4D. (A) Proportions of tumor lines, out of a total of 13, that resulted in significant IFN-γ production by TCR-transduced T cells; maximal contribution by each individual tumor type was set to 25% (melanoma: five cell lines; HNSCC: three cell lines; TNBC: three cell lines; bladder carcinoma: two cell lines). (B) Levels of IFN-γ produced by TCR-transduced T cells following coculture with tumor lines ($p < 0.05$ = black; $p > 0.05$ = gray). The median production per TCR is indicated by the horizontal black line ($n = 5$ experiments; 13 tumor lines per experiment).

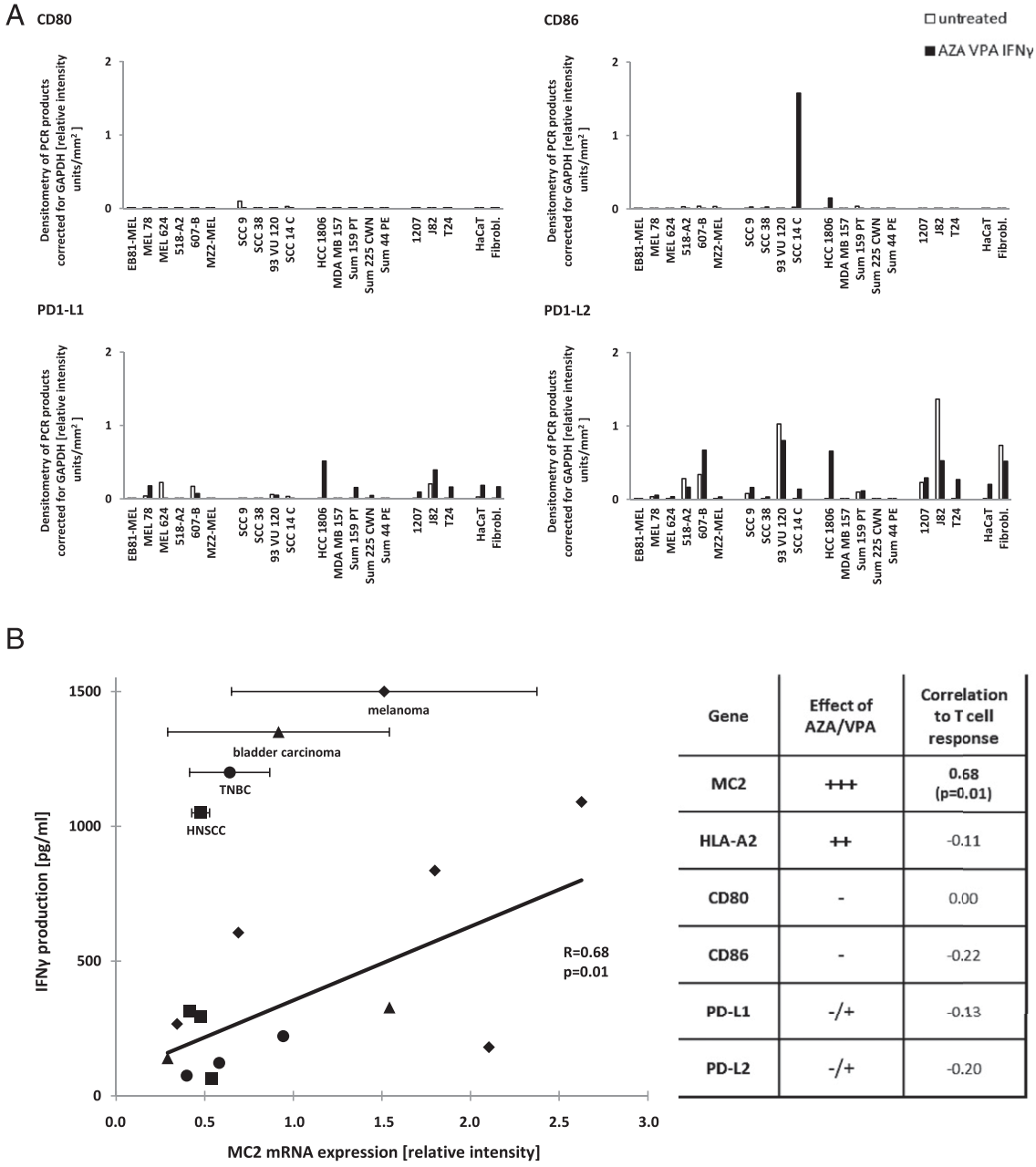
with varying T cell avidities toward pMHC; when analyzing CD8 T cells, and in particular CD4 T cells, we observed that TCR16 and TCR11 mediated the weakest binding, TCR6 mediated an intermediate binding, and TCR4 mediated the strongest binding of pMHC. However, the observed variation among TCRs is in line with reported T cell avidities of the parental CTL clones obtained from patient EB81 (18). For example, CTL 16, as well as our TCR16-transduced T cells, exhibited the lowest avidity among the tested CD8 T cell clones, whereas this CTL clone was present at the highest frequencies in patient blood and an invaded lymph node (17). This suggests that high affinity of a TCR is not a prerequisite for effective in vivo stimulation by naturally presented tumor Ags (38, 39). Reasons for differences in pMHC binding, apart from TCR affinity, may include the fact that certain TCRs show enhanced proneness to mispair with endogenous TCR chains, which results in dilution of surface TCR expression (40), and that certain TCRs are less dominant with respect to CD3 assembly and surface expression (41). To minimize the occurrence of TCR mispairing, we introduced TCRs into the pMP71 vector using a TCRβ-2A-TCRα cassette. Nevertheless, TCR mispairing cannot be completely excluded. Although not a primary topic of the current study, we explored additional strategies to limit TCR mispairing and showed that incorporation of a CD3ε-CD28 cassette (42) into TCR16 maximally enhanced the fraction of pMHC-binding T cells (data not shown).

Within the panel of LLF-specific TCRs, our results suggest that TCR11 has the lowest ligand-binding affinity, which is substantiated by two other lines of evidence. This TCR mediates the lowest level of IFN-γ production in response to MC2/A2$^+$ tumor cell lines (Fig. 6), and it mediates the lowest rate of proliferation, as well as the most rapid drop in numbers of TCR$^+$ T cells during culture of T cells (data not shown). In addition, TCR11-transduced T cells have a decreased ability to produce high levels (i.e., plateau levels) of peptide-specific IFN-γ compared with TCR4- or TCR6-transduced T cells (Fig. 1B). TCR11's lower level of performance may be related to a lesser ability to serially trigger T cells (43) or to an enhanced ability to upregulate expression of T cell PD1 (44), although we could not confirm the latter option experimentally (data not shown).

When testing MC2 TCRs toward melanoma tumor targets, whether pretreated with IFN-γ to stimulate immune and intermediate proteasome-dependent processing of the ALK and LLF epitopes (18, 23), we observed limited T cell responses (Fig. 3) (27). Pretreatment of melanoma cells was extended with the epigenetic drugs AZA and VPA, which resulted in selective gene and protein expression of MC2 in tumor cells, but not normal cells, and enhanced T cell responsiveness. Importantly, we observed similar findings in multiple tumor types, including HNSCC, TNBC, and bladder carcinoma. These results extend earlier studies that showed enhanced gene expression of NY-ESO-1, MAGE-A1, and MAGE-A3 in cancer cell lines pretreated with epigenetic drugs but not in normal respiratory epithelia or lymphocytes (32, 34, 45). Interestingly, in our experiments, different tumor cells responded differently to pretreatment. For example, melanoma cell lines revealed a base level of MC2 expression that was enhanced by AZA/VPA, whereas HNSCC cell lines only expressed MC2 following pretreatment (Fig. 4C). Because earlier studies reported an MC2 positivity of only 8% for primary HNSCC (14), the above finding highlights the potential to increase patient populations eligible for MC2-specific T cell therapy when combined with epigenetic drug pretreatment. Moreover, recent research indicates that epigenetic drugs sensitize tumors to T cells, which goes beyond enhanced gene expression of target Ags (46, 47). For example, epigenetic drugs were reported to induce IFN type I gene and related genes, which can contribute to tumor immunogenicity (48), and these drugs are able to enhance expression of chemoattractants, increase CD8 T cell infiltration, and improve the therapeutic efficacy of adoptive T cell therapy (49).

Our results with respect to drug-enhanced expression of MC2 by tumor cells are of particular interest to clinical trials with adoptive transfer of T cells because AZA and VPA were shown to be safe in patients with advanced hematological and solid malignancies (30, 50–54). The selective effects of pretreatment toward cancer cells versus normal cells may be related to an initiating event in the deregulation of cancer-germline gene expression, which is the recruitment of the transcription factor brother of the regulator of imprinted sites (BORIS) to cancer-germline promoters. Reversal of NY-ESO-1, MAGE-A1, and MAGE-A3 repression coincides with DNA demethylation, dissociation of polycomb proteins, and the presence of euchromatin marks within the respective promoters (inter alia, a shift to BORIS at the cancer-germline promoter site) (45). BORIS is a mammalian CCCTC-binding factor paralog that is absent in normal cells, prevents binding and function of CCCTC-binding factor, and results in the opening and activation of DNA chromatin (55, 56).

Pretreatment of tumor cells results in significantly enhanced responses of MC2 TCR T cells, although such responses are heterogeneous. Attempting to define parameters that are critical for



Gene	Effect of AZA/VPA	Correlation to T cell response
MC2	+++	0.68 (p=0.01)
HLA-A2	++	-0.11
CD80	-	0.00
CD86	-	-0.22
PD-L1	-/+	-0.13
PD-L2	-/+	-0.20

FIGURE 6. Expression of MC2 Ag, but not HLA-A2, costimulatory, or coinhibitory molecules, correlates with T cell response. Cell lines from melanoma, HNSCC, TNBC, bladder carcinoma and keratinocytes (HaCaT), and fibroblasts (Fibrobl.) were pretreated or not with AZA, VPA, and human IFN-γ, as described in the legend for Fig. 4. (**A**) RNA was isolated, used for RT-PCR, and subjected to analysis of CD80, CD86, PD-L1, and PD-L2 expression, as described in Fig. 4A. (**B**) IFN-γ response of TCR6-transduced T cells as a function of MC2 expression of pretreated tumor cell lines of different histologies (left panel). Ranges of MC2 expression levels per tumor type are indicated above the trend line (mean ± SEM, $n = 4$). In addition, IFN-γ response of TCR6-transduced T cells is presented as a function of HLA-A2, CD80, CD86, PD-L1, or PD-L2 expression of pretreated tumor cell lines (right panel). Calculations are based on Pearson's correlation coefficient. +++, very strong effect (>75% of tested cell lines); ++, strong effect (>50% of tested cell lines); −/+, low effect (>5% of tested cell lines); −, no effect (<5% of tested cell lines).

MC2 T cell responses, we demonstrated that MC2-specific T cell responses are most optimal in vitro with TCR6 and benefit most from higher expression levels of MC2, but not CD80, CD86, PD-L1, or PD-L2. The superiority of TCR6 may be explained, in part, by affinity, considering the possible higher affinity of TCR4 and its weaker T cell response; it may also be explained, in part, by TCR structure and level of TCR expression (41). The importance of Ag expression level for MC2 TCR T cell responses builds on earlier studies (57), but Ag being a driver of MC2 T cell recognition, irrespective of the expression of classical costimulatory and coinhibitory molecules, is a novel finding. Notably, the LLF epitope, the target of TCR11, TCR4, and, in particular, TCR6, forms highly stable peptide:HLA-A2 complexes (Supplemental Fig. 3), potentially as a result of the LLF peptide harboring the preferred peptide-binding motif for HLA-A2 (i.e., XLXXXXXXV/L) (58, 59). The EC_{50} values for HLA-A2 binding put the LLF epitope, but not the ALK epitope, below a critical threshold of 10 nM, which was reported to enable peptide cross-presentation by stromal cells and enhance antitumor T cell responses (60).

In this study, we selected MC2 as an effective and safe T cell target Ag, based on its selective expression by cancer cells. The fact that MC2 peptides are not presented by normal cells is a

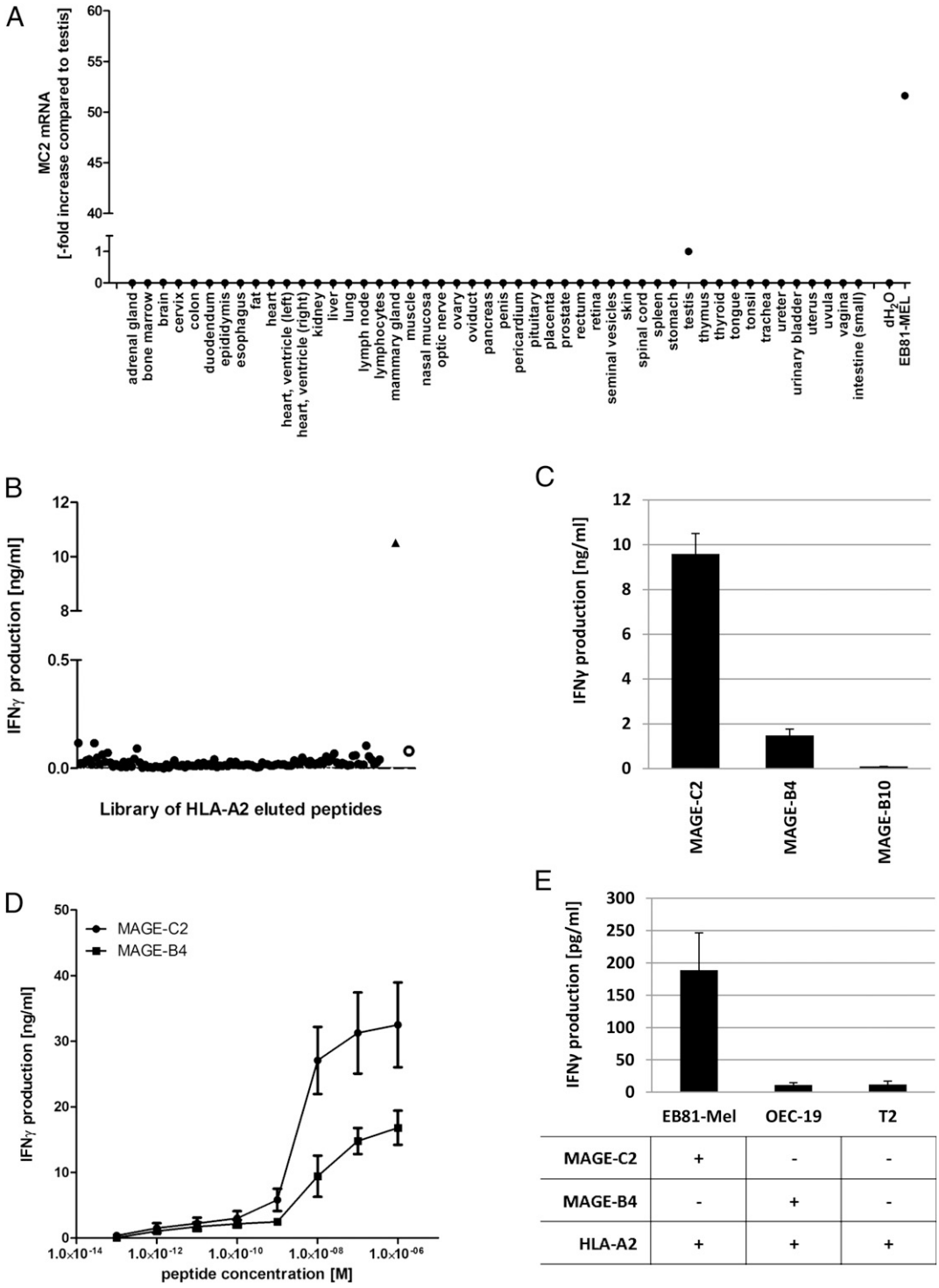


FIGURE 7. MC2 expression in healthy tissue is restricted to testis, and TCR6 shows no reactivity to other, including highly similar, epitopes. (**A**) MC2-specific qPCR was performed using a cDNA library of 48 healthy, human tissue samples. MC2 mRNA levels are expressed as fold increase compared with testis, the only healthy sample positive for MC2 mRNA. Fold increase was calculated based on the ΔΔCt method and normalized for GAPDH expression. Patient-derived MC2+ melanoma cell line EB81-MEL served as positive control. (**B**) TCR6 T cells were cocultured with T2 cells loaded with 10 μM of 114 different HLA-A2–restricted peptides for 24 h. IFN-γ levels in culture supernatants were measured by ELISA and are displayed as mean ($n = 3$). IFN-γ response against the MC2 LLF peptide (positive control) is depicted as a triangle, and against no peptide (T2 cells only; negative control) is depicted as a hollow circle. (**C**) TCR6 T cells were cocultured with T2 cells loaded with 100 nM of highly similar peptides of MB4 (LVFGLALKEV) and MB10 (LIFGLDLKEV) (amino acids different from the MC2 LLF peptide being underlined). IFN-γ levels in culture supernatants were measured by ELISA and are displayed as mean ± SEM ($n = 4$). IFN-γ response against the MC2 LLF peptide served as a positive control. (**D**) Again, TCR6 T cells were cocultured with T2 cells but now with titrated amounts of MB4 and MC2 LLF peptide for 24 h. IFN-γ levels in culture supernatants were measured by ELISA and are displayed as mean ± SEM ($n = 4$). Curves and EC_{50} values were analyzed using GraphPad Prism (EC_{50} for MC2: 3.1×10^{-9} M; EC_{50} for MB4: 7.15×10^{-9} M). (**E**) Finally, TCR6 T cells were cocultured with EB81-MEL ($MC2^+/MB4^-/A2^+$), OEC-19 ($MC2^-/MB4^+/A2^+$), or T2 ($MC2^-/MB4^-/A2^+$) tumor cells. Expression of MC2, MB4, and HLA-A2 was verified or ruled out via RT-PCR. IFN-γ levels in culture supernatants were measured after 24 h by ELISA and are displayed as mean ± SEM ($n = 4$). IFN-γ production by TCR6 T cells was corrected for IFN-γ production by mock T cells; IFN-γ levels produced by mock T cells ranged from 5 to 13 pg/ml.

decisive factor with respect to the clinical value of this Ag for future studies. qPCR of a large series of human tissues demonstrated that there is no gene expression of MC2, with the exception of testis (Fig. 7A). These findings confirmed earlier findings (11, 61) (http://www.proteinatlas.org/ENSG00000046774-MC2/tissue and http://www.cta.lncc.br). Testis tissue is immune privileged (no MHC expression) and is expected to express cancer germline Ags. In fact, our findings are in line with MC2 protein staining by Zhuang et al. (37). Further safety tests included exposure of T cells expressing TCR6 to a peptide library, as well as peptides that were found to be highly similar to the MC2 LLF peptide. We observed that TCR6-transduced T cells demonstrated no cross-recognition of any of 114 HLA-A2–eluted peptides tested (Fig. 7B). When testing peptides that are highly homologous to the MC2 LLF peptide (i.e., the MB4 LVF and MB10 LIF peptides), TCR6 T cells showed a slight response to the MB4, but not MB10, peptide. Further experiments revealed that the MB4 peptide has a higher EC_{50} value than the MC2 peptide; however, most importantly, and in line with predicted peptide processing and MHC class I presentation (http://www.cbs.dtu.dk/services/NetCTLpan/), TCR6-transduced T cells do not recognize target cells expressing native MB4 (Fig. 7C–E). Collectively, these results provide clear evidence against risks for on- or off-target reactivities when targeting MC2 via TCR6.

Taken together, we demonstrate that T cell therapy benefits from the combination of targeting a safe, yet effective, Ag, such as MC2, and epigenetic drug-enhanced antigenicity. We are preparing a phase I/II trial with adoptive transfer of MC2 TCR T cells, in combination with administration of AZA/VPA, to treat patients with metastatic melanoma and HNSCC.

Disclosures

The authors have no financial conflicts of interest.

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