HIGH AFFINITY ANTITUMOR HLA CLASS I-RESTRICTED TCRS THAT CAN BE STAINED WITH AN HLA/PEPTIDE MONOMER COMPLEX POSSESS BROAD CROSS-REACTIVITY

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TCR gene therapy is technically feasible and a promising treatment modality for cancer immunotherapy. Safety and efficacy largely depend on the selection of a TCR that induces minimal toxicity while possessing sufficient antitumor reactivity. Many, if not all, TCRs possess unwanted cross-reactivity in addition to desired antitumor reactivity. Interestingly, some TCRs exhibit chain centricity, where recognition of MHC/peptide complexes is dominated by one of the TCR hemi-chains. The shared TCRα gene, clone SIG35α, recognizes HLA-A*02:01(A2)/MART1$_{27-35}$ when paired with various clonotypic TCRβ counter-chains. Last year, we had demonstrated that, regardless of their HLA-A2 positivity, a substantial subset of peripheral CD8$^+$ T cells transduced with SIG35α gained reactivity for A2/MART1$_{27–35}$. Interestingly, when transduced with SIG35α, peripheral CD4$^+$ T cells, from both A2$^+$ and A2$^-$ donors, also recognized A2/MART1$_{27-35}$ and expanded in an antigen-specific manner upon stimulation. The generated A2/MART1$_{27-35}$-specific T cells used various TRBV genes with a high proportion encoding TRBV2, 5-1, or 27, and the CDR3β sequences were highly diverse. Fourteen clonotypic TCRβ chains were randomly chosen and individually reconstituted along with SIG35α on human TCRαβ-deficient T cells that do or do not express the CD8 coreceptor. These TCR transfectants possessed high structural and functional avidities. Surprisingly, four out of the 14 CD8$^+$ TCR transfectants were clearly stained by an A2/MART1$_{27-35}$ monomer complex and two of the four were stained even in the absence of CD8. Importantly, these two highly avid TCR transfectants demonstrated broader cross-reactivity to A2-bound human-derived self-peptides homologous to the A2/MART1$_{27-35}$ peptide. These results suggest that affinity-matured and/or thymically unselected antitumor TCRs should be carefully selected for use in TCR gene therapy to avoid the unpredictable risk of TCR cross-reactivity.