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Tumor-specific murine T cell receptors displace endogenous TCRs in human T cells

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A novel approach in immunotherapy of human malignancies is based on universal tumor associated antigen (TAA)-specific T cell receptor (TCR) gene delivery into human T cells. The MDM2 oncoprotein is overexpressed in a multitude of human (Hu) malignancies, counter-balancing p53 as major tumor suppressor protein. We took advantage of HuCD8xA2K^b transgenic (Tg) mice to bypass human MDM2-specific self-tolerance: the chimeric MHC-molecule of the xenogenic host enables the selection of HLA-A2.1-restricted lymphocytes thereby allowing the contribution of murine CD8 for TCR affinity maturation in the thymus and the subsequent deployment of non-tolerogenic lymphocytes into the periphery.

The TCR-encoding genes were retrovirally transduced in human T cells *in vitro*. Quantitative expression frequency of the heterodimeric TCR on a per cell basis was accomplished by introducing independent IRES selection cassettes into either TCRα/β construct so as to verify subtle phenotype/structure/function characteristics among different TCR constructs.

A major concern was to determine as to whether or not murine TCRs are able to form hybrid TCRs with the endogenous ones potentially raising the issue of autoimmunity. Based on empirical data one may hypothesize that surface expression of a certain heterodimeric TCR construct reflects it's intrinsic stability guided by it's propensity to heterodimerize and to be incorporated into the multi-step assembly of the functional TCR/CD3 complex so as to be sheltered from down-modulation and proteolytic degradation. Amino acid substitutions that affect interchain affinity will have an impact on surface expression, the formation of hybrid TCRs and functionality in terms of cytokine secretion and cytolysis in chromium release assays. For this a multitude of TCR constructs, designed as either murine and partially humanized double chain TCRs or as single chain TCRs, have been assayed on their functional outcomes and consequences for the endogenous TCRs. The expression of single murine TCR chains documents their capability to form hybrid TCRs a tendency that can be further increased by the humanization of the constant domains. Point mutations that impair pairing as deduced from protein structure reduce their surface expression and inversly accumulate in the cytoplasma.

Murine double chain TCRs as opposed to humanized TCRs are capable to compete with and to replace a significant fraction of the endogenous TCRs in an exogenous TCR (exoTCR) dose dependent fashion irrespective of the clonal TCR subfamily diversity of the particular donor. Subsets of endogenous TCR high (endoTCR^{hi} correlates with exoTCR^{lo}) and TCR low (endoTCR^{lo} correlates with exoTCR^{hi}) expressing T cells have been FACS-sorted and proceed to be verified for phenotype maintainance and functional potency. One major intriguing topic is to pinpoint to the murine TCR structure determinants that are responsible for this competing stringency in order to introduce them into tumor-specific human TCRs for their

favourable interchain pairing and subsequently their exclusively heterologous expression in human T cells.