

Oral presentation

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Reversible HLA multimers (streptamers) for isolation of human cytotoxic T lymphocytes functionally active against tumor- and virus-derived antigens

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Attempts to treat patients with tumor-reactive or viral-specific cytotoxic T lymphocytes (CTLs) have been limited due to the difficulty of isolating and expanding functionally active T cells present in low numbers in the peripheral blood. Recently developed MHC/peptide multimers mimic T cell receptor (TCR) ligands and, therefore, allow visualization and isolation of antigen-specific CTLs. However, the persistence of multimers leads to prolonged TCR signaling and subsequently to overstimulation and cell death. We have generated a new type of MHC/peptide multimers, termed streptamers, which can be dissociated from the TCR. In the mouse model, the dissociation of streptamers from the TCR, prevents T cells from multimer-induced cell death (Knabel *et al. Nature Med* 2002, **8**:631). In this study, we investigate the efficacy of reversible HLA/peptide multimers for isolation of human antigen-specific T cells. Melan-A and CMV have been chosen as representative tumor-associated and viral antigen (Ag), respectively. Specificity and reversibility of A2/CMV and A2/Melan-A streptamers was documented by staining of Ag-specific T cell clones and loss of staining after streptamer removal. Streptamer-stained Ag-specific T cells remained functionally active following dissociation, whereas lytic function of T cells was impaired in the presence of non-reversible multimers (tetramers). Furthermore, CMVpp65(495–503)-specific T cells were streptamer- or tetramer-sorted from HLA-A2-positive, CMV-seropositive donors either directly out of the blood or following repet-

itive peptide stimulations *in vitro*. Both attempts successfully led to the isolation of CMV-specific CTLs that were cloned by limiting dilution. Clonal proliferation was superior for CMV-specific streptamer-sorted T cells compared to tetramer-sorted T cells. CMV-specific T cell clones isolated with streptamers and tetramers displayed a similar TCR repertoire and avidity. Growing CTL clones were capable of lysing CMVpp65(495–503)-pulsed as well as CMVpp65-transfected HLA-A2-positive target cells. For isolation of melanoma-reactive CTLs, the modified decapeptide Melan-A(26–35)A27L was chosen to construct streptamers respective tetramers. Again, streptamer-sorted Melan-A-specific CTL clones proliferated better than tetramer-sorted CTL clones. The isolated Melan-A-specific CTL clones displayed different TCR motifs, which can be explained by the broad repertoire of Melan-A-specific T cells physiologically present *in vivo*. All Melan-A(26–35)A27L-specific CTL clones crossreacted with the naturally processed peptide Melan-A(27–35), but only some CTL clones lysed HLA-A2-matched, Melan-A-expressing melanoma cells. Of note, tumor recognition by some streptamer-sorted CTL clones was superior to tumor lysis by tetramer-sorted CTL clones. Our current experiments focus on the isolation of T cells using reversible multimers coupled with microbeads allowing us to sort antigen-specific T cells under the guidelines of good manufacturing practice. Clinical goal is the adoptive transfer of

antigen-specific T lymphocytes for treatment of patients with cancer or infectious diseases.

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