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Optimization and use of artificial antigen presenting cells for induction and expansion of MART-1, MUC1, NY-ESO, and WT1 specific cytotoxic T lymphocytes.

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The ultimate goal for cancer therapy is the long-term eradication of malignant cells, with limited adverse effects on healthy tissues. While surgery can often remove the bulk of solid tumors, it is not as well suited for elimination of minimal residual disease and micro metastases. In a similar vein, systemic anti-cancer therapies including chemotherapy, and radiotherapy, are limited by non-specific autotoxicity. It is therefore important to develop new systemic therapies allowing for eradication of minimal residual disease and the long-term prevention of relapse.
Oelke et al. (2003) developed an artificial antigen presenting cell (aAPC) system that is capable of generating large numbers of MART-1- and CMV-specific CTL for subsequent use in ACT. Our studies carry forward and optimize the use of this aAPC system. The aAPC used in our studies is composed of a magnetic bead, A2-Ig-dimer, and up to two costimulatory molecules (anti CD28, B7-1-Ig, or B7-2-Ig). An epitope from the TAAs MART-1, NY-ESO, WT1 and MUC1 is then bound to the A2-Ig-dimer attached to the aAPC. $CD8^+$ T cells from healthy HLA-A2 positive donors are stimulated with peptide-loaded aAPCs, and restimulated weekly. After multiple rounds of stimulation, CTL are analyzed for peptide specificity, as well as functionality, using ICS and CD107a assays. Our experiments confirmed that MART-1-specific CTL could be induced in this system. CTL induction was also achieved using other TAAs, but these showed lower growth potential and functionality. To optimize the system further we tested different compositions of aAPC by variation in the costimulatory molecules. The B7-1 and B7-2 molecules were chosen to investigate if the natural ligands have better stimulation capabilities than the anti-CD28 antibody. Assessment of three healthy donors demonstrated differences in cell growth and specificity, depending on the costimulatory molecule present on the aAPC.
Although tumor patient PBMCs may have the advantage of higher numbers of tumor-specific precursor cells, we chose healthy donors for our experiments because they represent a more readily available T cell source, have not undergone anti-cancer therapy, and do not represent a tumor-specific subset. Optimization of peptide selection is another important step affecting efficient CTL induction. Peptide-binding algorithms and assays provide a means to assess the potential binding affinity of the peptide TCR complex, but

many variables influence the actual association of a peptide to a MHC molecule. Peptides used in our study were previously shown to bind to HLA-A2, and therefore represented a large potential patient population (50% of Caucasians express the HLA-A2 molecule). Cytokines also play a role in the stimulation and differentiation of $CD8^+$ T cells, and addition of various cytokines, or cytokines in a sequential order, can change the stimulation capabilities of T cell cultures. We opted to exchange the costimulatory molecule on the aAPC as an initial step in optimization of the aAPC system, and were able to show differences in $CD8^+$ T cell specificity as well as growth. Different costimulatory molecules enhance or decrease APC-T cell interaction and provide future targets for variation in the costimulatory molecule. The development of a highly functional and dynamic aAPC in combination with other immunotherapeutic strategies like genetically modified T cells, will revolutionize the field of tumor-targeting. As one can see, the endless flexibility of the proposed aAPC system suggests great potential in future cancer immunotherapy.