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203: Lymphocytes, Lymphocyte Activation, and Immunodeficiency, Including HIV and Other Infections

### **Improving Efficacy of Dendritic Cell Vaccination in AML: Optimization of the DC Generation Protocol and Maximization of T Cell Responses by Immune Checkpoint Blockade.**

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Dendritic cell (DC) vaccination is considered a promising immunotherapeutic strategy for AML patients with minimal residual disease (MRD). We have developed a three-day manufacturing protocol containing a synthetic TLR7/8 agonist to generate DCs with a positive costimulatory profile and high IL-12p70 secretion (TLR-DCs). These proved to be superior with respect to type 1 polarization of T cells and activation of NK cells compared to conventional monocyte-derived DCs.

In an attempt to further optimize the maturation protocol, we compared the use of standard pooled human serum (PHS) with autologous human serum (AHS) from healthy donors (HDs, n=16) and AML patients (n=6) within the generation process of TLR-DCs. Surprisingly, we found that AHS-DCs from HDs were significantly inferior to PHS-DCs concerning viability (81% vs. 91%, p=0.002) and recovery (3.8% vs. 7.1%, p=0.037) after the maturation process. A similar effect was seen in AHS-DCs from AML patients. In a functional analysis, IL-12p70 secretion was reduced and migratory capacity of the DCs to CCL19 was impaired. We are currently working on the identification of immunomodulatory components within the AHS that are responsible for these detrimental effects. In a preliminary cytokine analysis, we found that pro-inflammatory (TNF- $\alpha$ ) as well as anti-inflammatory (IL-10) cytokines were significantly increased in the AHS samples.

PHS is therefore highly recommendable for the maturation process and was used in all subsequent experiments. With this protocol, we were able to show that TLR-DCs can also be generated from monocytes of AML patients. No differences in phenotype and function were found compared to samples from healthy blood donors. Efficient and controllable expression of leukemia-associated antigens was induced by RNA electroporation. These cells are capable of both priming naïve T cells and reactivating antigen-specific pre-primed effector cells *in vitro* and are therefore highly suitable for DC vaccination strategies in the setting of postremission AML.

In order to further enhance the immunostimulatory capacity of TLR-DCs, we are currently evaluating combinatorial approaches with inhibitors of receptor-ligand immune checkpoints. Their high therapeutic potential has recently been demonstrated for various tumor entities. In a comprehensive analysis of immune checkpoint molecules, it was revealed that TLR-DCs, albeit showing a preferentially positive costimulatory profile, express significant amounts of immunoinhibitory molecules from different families (PD-L1, B7-H3, HVEM, ILT3, ILT4, 4-1BBL). We have set up an *ex vivo* coculture system for activation and expansion of T cells by autologous DCs. Using blocking antibodies against PD-1, we could demonstrate a significant increase of IFN- $\gamma$  secretion by T cells in samples of healthy donors and HIV patients as well as AML patients. The ultimate goal is to figure out which combination of immune checkpoint inhibitors is the most appropriate to increase the effect of a DC-based immunotherapy in the clinical setting of AML immunotherapy.