A DC-based therapeutic vaccine for AML patients: preclinical evaluation of a new generation of DCs expressing the leukemia-associated antigens WT1 and PRAME

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Remission induction and consolidation chemotherapy is the standard therapy for patients with acute myeloid leukemia (AML). Although, complete remission can be achieved in 60-80% of patients, the majority of these patients carries a high risk of relapse due to the presence of chemotherapy-resistant leukemic cells. For patients at relapse only few treatment options are available and the overall survival rate is low.

Cell-based immunotherapy is a promising treatment strategy for controlling or even eradicating these residual tumor cells. Therefore, we have designed a new generation of dendritic cells (DCs) with improved immunogenicity and optimized for the use in cell-based immunotherapy of cancer patients. The monocyte-derived DCs are manufactured within a three-day protocol and by using a maturation cocktail containing a synthetic TLR7/8-agonist. In order to induce a specific T cell-based anti-AML response, the DCs are loaded with RNA encoding the leukemia-associated antigens WT1 and PRAME. T cell responses primed by WT1- and PRAME-loaded DCs *in vivo* are expected to be less frequent than T cell responses activated against a foreign antigen. Therefore, our vaccine formulation also contains DCs loaded with hCMV-pp65 mRNA to provide a helper effect by activated pp65-specific T cells.

To prove the safety and feasibility of our therapeutic vaccine we plan to initiate a phase I/IIa clinical trial with high risk AML patients. Therefore, we performed a careful preclinical evaluation of our vaccine cell.

Mature DCs were generated from healthy donors according to our 3d protocol in small scale and electroporated with mRNA encoding for PRAME, WT1 or pp65. The cells were cryopreserved and their phenotype and function were analyzed subsequently after thawing. Expression of typical DC surface markers as well as antigen expression in the DCs was high and not altered by cryopreservation. Additionally, antigen expression and cryopreservation impacted neither on the migratory potential of the DCs nor on their ability to secrete the essential cytokine IL-12p70 upon CD40-CD40L interaction. DCs expressing the different antigens also showed a high capacity for stimulation of established antigen-specific T cells, demonstrating the proper processing and presentation of antigenic epitopes on the DC surface. In the autologous setting, priming experiments were performed to evaluate the capacity of our vaccine cells for *de novo* induction of antigen-specific T cell responses. WT1-, PRAME- and pp65-expressing DCs showed a high capacity for priming of naïve T cells *in vitro*. Additionally, a favourable cytokine milieu for Th1 T cell polarization was established in the priming cultures when pp65-loaded DCs were present.

These studies demonstrate the high potential of our vaccine cells for the initiation of potent anti-tumor responses in AML patients. Our manufacturing protocol, which was also successfully established for the production of GMP-grade DC in large scale, yields DCs with a robust phenotype and optimized for the use in therapeutic vaccines.