Consistent high-quality dendritic cell vaccines produced post-chemotherapy in patients with acute myeloid leukemia for use in a Phase I/II trial

Frauke Schnorrfell1, Christiane Geiger1, Iris Bigalke2, Dag Josefson3, Yngvar Flioland2, Gunnar Kvalheim2, Dolores J. Schendel1, Anna Tafri2, Kai Pinkernell2
1Medigene Immunotherapies GmbH, Planegg/Martinsried, Germany, 2Oulo University Hospital, Department of Cellular Therapy, Immunology, Hematology, Norway, 3Medigene AG, Planegg/Martinsried, Germany

Contact: c.geiger@medigene.com

Abstract

A Phase I/II dendritic cell (DC) vaccine trial was completed in 20 patients with acute myeloid leukemia (AML) in complete remission or CR after chemotherapy who were ineligible for hematopoietic stem cell transplantation (HCT/HSCT/MDSCT). The DC vaccines were designed to delay disease progression by mobilizing natural killer (NK) cells through secretion of l-12p30(7) and activating T cells by stimulation with WT1 and PRAME; two prominent antigens in AML. DC vaccination was carried out in weeks 1, 2, 3, 4, 6, and monthly thereafter for 2 years. Two questions were prominent at the trial start. First, could mature DCs be efficiently prepared to activate the vaccine recipients, including use of exogenous DCs/fractions for each antigen. Second, could suitable quality DC vaccines be generated from patients with myeloid disease, since all had received intensive chemotherapy, including hematopoiesis, that several patients showed extended times for monocyte recovery in peripheral blood before being able to undergo apieo for production. Immune monitoring tools were used to assess DC vaccines: multi-color flow cytometry for surface and intracellular protein staining, dual-color ELISPOT for secretion of IFN-γ/L-12p15, and chemokine-directed transwell migration. Adequate regeneration of monocytes occurred post-chemotherapy in all patients, allowing production of sufficient numbers of cryopreserved vaccines (2.5 × 10^6) (DCs/antigen/mouse) to be completed. In 15/20 patients one batch was sufficient to cover all vaccinations, with 1-2 additional vaccines produced as an addition. Phenotypic and functional parameters of patient DC vaccines were compared to cells of a healthy control (HC). Patient DCs expressed CD40, CD86, CD80, CD83 and HLA-DR at frequencies levels comparable to the HC. Both DC-fractions displayed intracellular protein antigen expression in most cells. Polycationic cation of l-12p30(7) with l-6 was seen with few exceptions. Upon stimulation, DCs showed directed migration. Detection of delayed type hypersensitivity responses post-vaccination at six weeks indicated the DC vaccines were active in vivo in all patients. DC production feasibility was clearly fulfilled and high quality DCs were generated for every patient. Quantity and quality of DC vaccines did not correlate with the patient groups that relapsed or remained in remission, nor in patients who succumbed to disease during the trial. DC vaccines were remarkably consistent, although some variation was seen from patients differing in age, AML subtypes and receiving varied amounts of standard chemotherapy regimens.

Study antigens WT-1 and PRAME are expressed in patient mDCs (signal 1)

DC vaccines were produced with a fast protocol

DC vaccines showed a high expression of the maturation marker CD83 and expressed a strong B7-costimulatory profile (signal 2)

DC vaccines display chemokine-directed migratory capacity

Summary

• Thorough product characterization was performed of autologous DC vaccines manufactured from heavily pretreated patients with AML, included in a single center clinical phase I/II trial.

• DC vaccines were generated using a fast protocol and tailored to activate T cell responses against the leukemia-associated antigens WT1 and PRAME.

• Loading of mDCs with mRNA encoding full-length study antigens proved to result in well detectable overexpression of WT1 and PRAME in most cases allowing for antigen presentation by the vaccine cells.

• Surface marker analysis revealed that the DC maturation marker CD83 was clearly upregulated on the vaccine DCs subfractions when compared to corresponding immature DCs. Additionally, predominance of positive co-stimulatory molecules, important for T cell DC interactions, was consistently demonstrated for the vaccine preparations.

• Following stimulation via CD40 most patient vaccine cell preparates demonstrated capacity for secretion of the critical cytokine IL-12p70, which is important for induction of NK cells and a promoter for Th1 differentiation of T cells. Capacity for IL-10 secretion, counteracting IL-12p70, was generally low or not detectable in the DCs.

• Vaccine cells also showed expression of the homing receptor CCR7 and directed migratory capacity toward CCL19, involved in the regulation of DC migration towards lymphoid tissues.

Conclusion: Medigene’s production process clearly proved to be feasible for AML patients and to yield high quality DC vaccine cells equipped with characteristics fostering T cell activation, survival and differentiation (3 Signal concept) as well as mobilization of natural killer cells.

DC vaccines for AML use WT-1 and PRAME as ideal target antigens

Most vaccines demonstrated capacity for secretion of bioactive cytokine IL-12p70 (signal 3)

DC vaccines were produced with a fast protocol

DC vaccines showed a high expression of the maturation marker CD83 and expressed a strong B7-costimulatory profile (signal 2)

Figure 1: DC vaccines were produced with a fast protocol within four to five days. Monocytes isolated from patient apheresis material were differentiated into mature DCs utilizing a synthetic TLR9 agonist containing muramyl dipeptide and subsequently loaded with mRNA encoding full-length antigen PRAME or WT1. For each patient two subcultures of vaccine cells were prepared, with one subculture containing mDCs loaded with PRAME-mRNA and the other subculture containing mDCs loaded with WT1-mRNA. For further application, the two subcultures were conrowned in multiple aliquotes containing 2.5 × 10^6 mDCs/antigen. The manufacturing process is designed to yield young mDCs displaying improved characteristics for mobilization of natural killer cells and T cell activation.

Figure 2: Medigene’s DC vaccines are tailored to provide three signals to lymphocytes to achieve optimal immune activation.

Signal 1: Activation

Using mRNA encoding full-length protein as antigen source allows for rapid and well controlled loading of antigen into DCs. It allows for display of multiple epitopes both on MHC class I and II molecules and bypasses the need for patient HLA-selection.

Signal 2: Survival

mDCs display strong positive B7-costimulatory profile, while inhibitory molecules are expressed at a lower density, supporting T cell survival.

Signal 3: Differentiation

mDCs were loaded with mRNA encoding full-length antigens. In vitro polyclonal DC subsets were analyzed for the critical cytokine IL-12p70 but no or only low IL-10 guiding differentiation of T cells into a Th1 phenotype. Additionally, secretion of IL-12p70 could also positively influence the activation of natural killer cells.

Figure 3: The vaccine cells were tailored to present the leukemia- associated antigens WT1 and PRAME, and were used in a short label clinical phase I/II trial with AML patients. We highlight safety in earlier vaccine studies. Additionally, both antigens were also determined to be expressed in leukemic disease.

Figure 4: Treatment scheme for Medigene’s DC trial.

The Medigene DC trial was a single-center, open label clinical phase I/II trial with AML patients. Phase I of the trial included 6 patients and phase II 14 patients. The IMP was administered weekly until week 4, then at weeks 6 and 10 and every 4 weeks for a total period of 2 years. For vaccination, the IMP was administered at a dose of 2.5 × 10^6 mDCs/antigen.

Figure 5: Intraclonal overexpression of PRAME (lower red histogram) and WT-1 (lower blue histogram) in the respective mDC subbatch was analyzed after cryopreservation by flow cytometry using rabbit-rabies antibodies. Stained grey orange control remained darkly stained. While detection levels of PRAME and WT-1 varied for the different patients, intracellular expression of the two leukemia-associates antigens could be detected in all vaccine cell preparations available for evaluation. A representative selection of 10 patients is depicted.

Figure 6: Surface phenotype analysis was performed on multicolor flow cytometry using antibodies directed against the surface marker depicted. A. The DC maturation marker CD83 was analyzed both in immature (white) and mature (blue) subcultures. B. Expression of HLA-DR on DC vaccine cells was also shown to be highly consistent for any particular patient. C. The predominance of positive co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) on the vaccine cells could be clearly demonstrated. D. Both analysis were shown to have the potential for all vaccine cells preparations. For all analyses a representative selection of 10 patients is depicted.

Figure 7: The capacity of mDCs to secrete IL-12p70 and IL-10 was analyzed in a dual-color ELISPOT following stimulation of vaccine cells via CD40/CD40L interaction. Stimulation DC - T cell encounter, and once used in a synthetic TLR9 agonist. A. For most preparations, vaccine cells showed high capacity for secretion of the critical cytokine IL-12p70 while no or only low secretion of IL-10. Only mDCs from one patient (pt 022) showed a higher capacity for secretion of IL-10 compared to IL-12p70. Expression of the different study antigens had no significant impact on the cytokine secretion capacity. (A representative selection of 10 patients is shown). B. It has to be pointed out that DCs secreted either IL-12p70 (left row) or IL-10 (middle row). Concurrent secretion of cytokines was rarely detected (right row) as depicted for patient 022 shown as an example. C. The number of T cells secreting IL-12p70 or IL-10 with or without stimulation is depicted for all vaccine preparations analyzed.

Clinical data will be presented in an oral presentation at the 23rd Annual Meeting & Exposition, December 3-4, 2020

Figure 8: Migratory capacity of mDCs was measured in a transwell-migration assay.

A, B, C, D: Staining of migration assay. Cells are shown in the upper compartment of the transwell. Via the microporous membrane cells can migrate towards a chemotactic factor. Cells are shown in the lower compartment of the transwell. mDCs loaded with either WT-1 or PRAME mRNA and cryopreserved showed only a low capacity for spontaneous migration (gray line), whereas they had a high migratory potential towards the chemokine (blue line). Expression of the two leukemia-associates antigens could be detected in all vaccine cell preparations evaluated. A correlation between expression density of CCR7 on the DC surface and the migratory capacity of the DCs could not be concluded.