DC Vaccination Induces Antigen Specific Immune Responses in AML Patients: A 1-Year Interim Assessment

Judith Eckl[1], Silke Raffegerst[1], Frauke Schnorfeil[1], Petra Prinz-Schulz[1], Christiane Fingerhut[1], Yngvar Fløisand[3], Dag Josefsen[2], Iris Bigalke[2], Kai Pinkernell[1], Gunnar Kvalheim[2], Dolores J. Schendel[1], Anna Tafuri[1]

[1]Medigene Immunotherapies GmbH, Munich, Germany [2]Department of Cellular Therapy, Oslo University Hospital, Oslo, Norway [3]Department of Hematology, Oslo University Hospital, Oslo, Norway

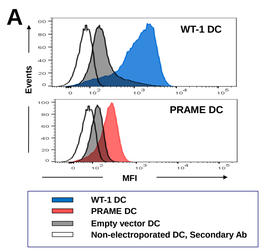
Introduction

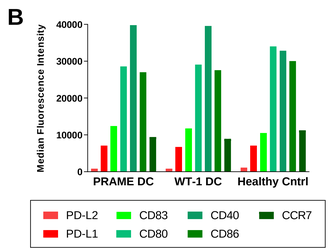
- Acute Myeloid Leukemia (AML) is the most common acute leukemia in adults. The backbone of therapy is a combination of cytarabine- and anthracycline-based regimens, followed by hematopoietic stem cell transplantation (HSCT) for eligible candidates. Unfortunately, elderly patients and patients with co-morbidities are often unable to tolerate such regimens and minimal residual disease (MRD) persists in complete response (CR), preventing long-lasting remissions.
- In animal models, dendritic cells (DCs) loaded with tumor antigens elicit both cellular and humoral immunity, and induce tumor-specific T cell responses and tumor regression. Therefore, DC-based vaccines have emerged as a promising approach to eradicate MRD in AML patients, particularly in patients not eligible for HSCT and/or at high risk of relapse.
- The Wilm's tumor protein 1 (WT-1), and the preferentially expressed antigen in melanoma (PRAME) have been identified as leukemia-associated antigens (LAAs). The importance of these antigens has been corroborated by the detection of immune response to these antigens in AML patients.
- The present first-in-human study (EUDRA CT No. 2014-003520-44) was conducted to assess the safety and feasibility of an autologous, patient-derived DC vaccine for the LAAs WT-1 and PRAME, in AML patients in CR.

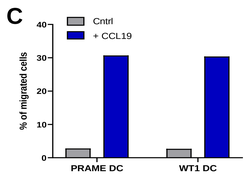
Summary and Conclusions

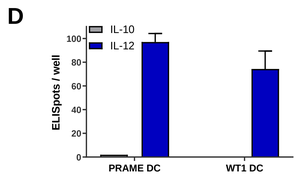
- **The Fast-DC protocol reproducibly yielded a high number of patient-derived, autologous DCs, which expressed WT-1 or PRAME, showed an immuno-stimulatory phenotype and produced IL-12. The WT-1/PRAME DC vaccine exhibited a favorable safety profile.**
- **The WT-1/PRAME DC vaccine was administered to a total of 20 AML patients in complete remission: 12/20 (60%) patients remained in stable remission, 8/20 (40%) relapsed within one year from the first vaccine administration.**
- **AML relapses were associated with an increase in the serum levels of IFN-γ, but also of IL-4, IL-5 and IL-6.**
- **Patients in remission exhibited higher levels of $CD3^+$ $HLA\text{-}DR^+$ T cells in the BM and peripheral blood; however, peripheral T cells capable of producing IFN-γ in response to *in vitro* stimulation with WT-1 or PRAME were only detected in 3/12 (20%) patients.**
- **In contrast, *in vitro* T cell production of IFN-γ in response to WT-1 or PRAME (ELISpot) was observed in 50% and 100 % of patients with early and late relapses, respectively. Such responses were associated with increased levels of PRAME and/or WT-1 mRNA in the BM.**
- **The impact of WT-1 and/or PRAME-specific T cell responses on AML remission warrant further investigation and will be further explored in the upcoming 2-year follow-up data analysis.**

Fast-DC Vaccine Characterization









Patient-derived DCs were obtained using a Fast (3-day) *ex vivo* culture protocol, after patient leukapheresis and monocyte enrichment.

DC differentiation and maturation was carried out in the presence of GM-CSF and IL-4. Mature DCs were electroporated with WT-1 or PRAME mRNA and exhibited reproducible functional and phenotypic characteristics (n=5) (A – D, *Patient 007*):

A. Electroporated DCs expressed WT-1 or PRAME, as assessed by intra-cellular flow cytometry

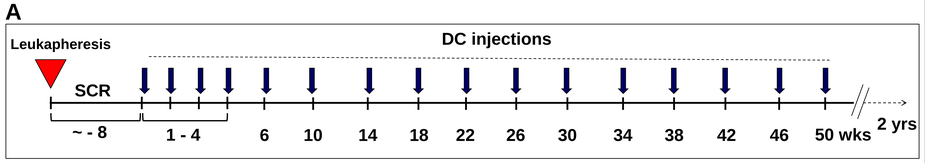
B. WT-1 and PRAME-expressing DCs showed high surface levels of costimulatory molecules (i.e. B7-1, B7-2, CD40, CD83), but low levels of immune inhibitory molecules (i.e. PD-L1 and PD-L2). Mature DCs also expressed CCR-7.

C. *In vitro* exposure of mature DCs to CCL19, resulted in efficient trans-well migration.

D. Both WT-1 and PRAME-expressing DCs predominantly produced IL-12, as assessed by double-colour ELISPOT.

Clinical Follow-Up (1-year)

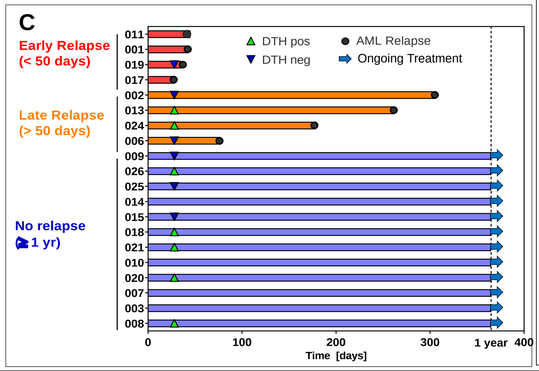
A



B

Parameter [unit]	Outcome
Number of subjects treated	20
Number of females & males n= (%)	5 (25%) & 15 (75%)
Age [years]	
• Mean ± SD	54.4 ± 14.8
• Median (min-max)	59 (24 - 73)
ECOG 0 (n= (%))	20 (100%)
AML Hovon/SAKK risk stratification (n=(%))	
• Good	13 (65%)
• Intermediate	5 (25%)
• Poor	2 (10%)
Time from start of LCLR to start of IMP treatment [months]	
• Mean ± SD	7.3 ± 3.5
• Median (min-max)	6.9 (2.0 - 14.8)
Time from first diagnosis to start of IMP treatment [months]	
• Mean ± SD	10.1 ± 3.7
• Median (min-max)	9.8 (4.5 - 17.5)
WT-1 / PRAME expression at diagnosis	
WT-1 positive (n= (%))	20 (100%)
PRAME positive (n = (%))	15 (75%)
Mutations at diagnosis	
NMP1 positive (n= (%))	3 (15%)
FLT3-ITD positive (n= (%))	0 (0%)
CEBPA positive (n= (%))	0 (0%)
MLL (n= (%))	0 (0%)

C



A total of 20 AML patients (WT-1 expressing AML blasts) in morphologic remission (CR), with or without hematological recovery (CRi) following induction chemotherapy, were treated.

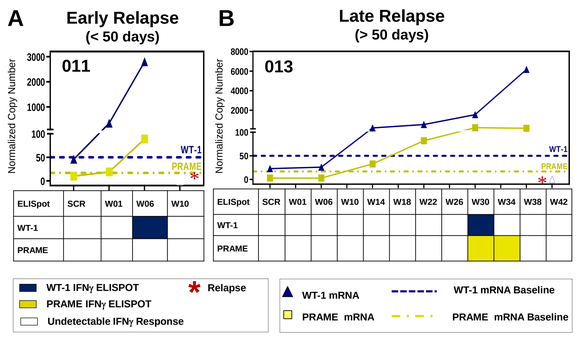
A. Patients (pts) received up to 16 DC injections in yr-1 and 12 in yr-2. At each time point, both PRAME and WT-1 DCs (2.5 – 5 x 10^6 / DC type) were administered intradermally.

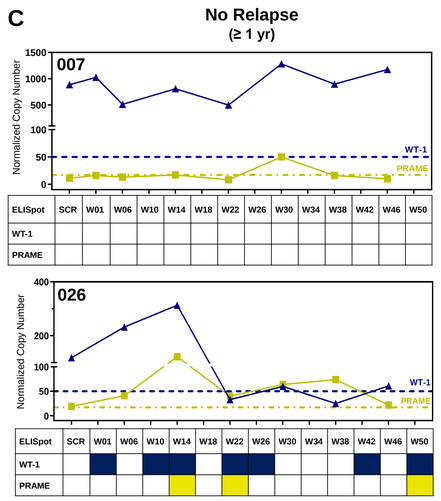
B. Baseline characteristics and demographics. WT-1 and PRAME were measured at diagnosis.

C. The clinical outcome at the end of the first year was as follows:
Early relapse: 4/20 (20%) pts
Late relapse: 4/20 (20%) pts
No relapse: 12/20 (60%) pts

DTH reactions were observed in 2/4 of patients with late relapse and 5/12 patients in remission.

MRD Levels and WT-1/PRAME-specific Peripheral T Cell Responses





PRAME and WT-1 mRNA levels in the bone marrow (BM) were monitored by qPCR at multiple time points to assess MRD. The diagrams depict the kinetics of PRAME and WT-1 mRNA in representative patients with early (**A**) or late (**B**) relapses, or in remission (**C**). Relapses were associated with a sharp increase in WT-1 and/or PRAME mRNA levels in the BM.

IFN-γ production was assessed *in vitro* upon stimulation of peripheral T cells with a pool of WT-1 or PRAME peptides (IFN-γ ELISpot) and used as an indicator of vaccine-induced Ag-specific T cell responses.

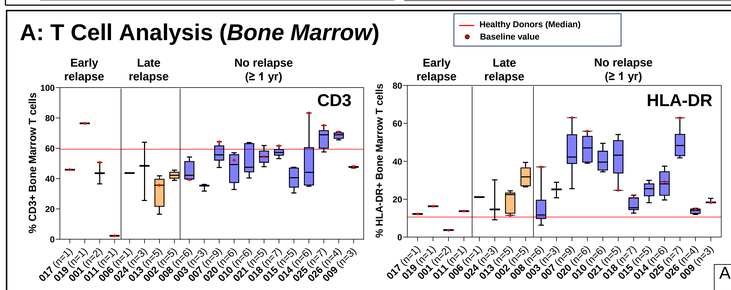
A. Early relapse : PRAME and /or WT-1-specific IFN-γ T cell responses were observed in 2/4 (50%) patients with early relapse and correlated with a sharp increase in PRAME and/or WT-1 mRNA in the BM.

B. Late relapse: PRAME and /or WT-1-specific IFN-γ T cell responses were observed in 4/4 (100%) patients and were concomitant to the increase in WT-1 and/or PRAME mRNA in the BM.

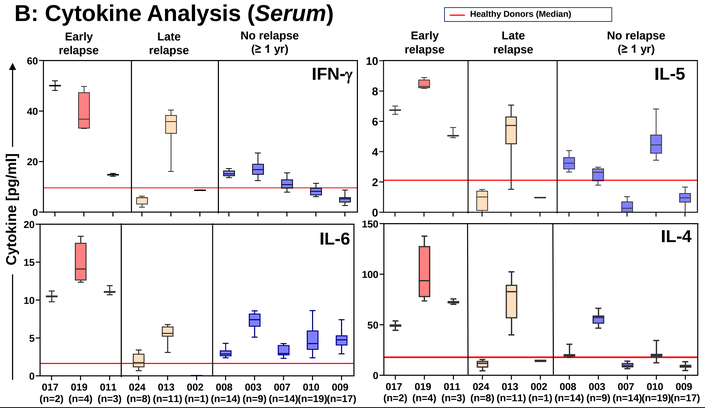
C. No relapse: PRAME and/or WT-1-specific IFN-γ T cell responses could not be detected in 9/12 patients (75%; *top*), but were observed in 3/12 (20%; *bottom*) patients in remission.

The levels of PRAME and WT-1 mRNA in the BM remained low (< 1500 copy number), and stable in a relatively narrow range around their respective clinical thresholds (PRAME: 17; WT-1: 50).

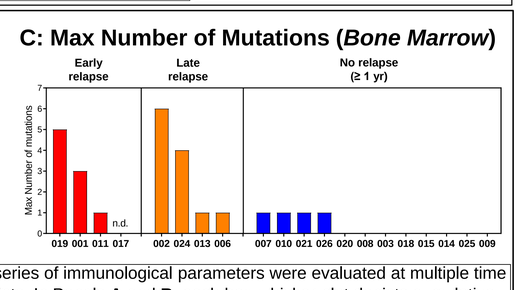
A: T Cell Analysis (*Bone Marrow*)



B: Cytokine Analysis (*Serum*)



C: Max Number of Mutations (*Bone Marrow*)



A series of immunological parameters were evaluated at multiple time points. In Panels **A** and **B**, each box-whisker plot depicts cumulative data obtained from a single patient at different time points.

A. The percentage of CD3+HLA-DR+ T cells appeared to be higher in the bone marrow and peripheral blood (*not shown*) of patients in remission than in relapsing patients.

B. The serum levels of IFN-γ, IL-4, IL-5 and IL-6 were increased in patients with early relapses, when compared to patients in stable remission.

C. Patients with early and late relapses showed an increased number of mutations, which peaked at the time of relapse. In contrast, one or no mutations (4 and 8/12 patients, respectively) were detected in patients in remission throughout yr-1, as assessed by NGS in a panel of 25 AML-related loci.

Disclosure of interest:
JE, SIR, FS, PP, CF, KP, DJS and AT are employees of Medigene Immunotherapies GmbH or Medigene AG. DJS: Equity Ownership, Honoraria, Membership on an entity's Board of Directors or advisory committees and Patents & Royalties. IB: employee of BNT GmbH. All other authors have no relationship to disclose

LIVING IMMUNO THERAPIES