New generation dendritic cell vaccine for immunotherapy of acute myeloid leukemia

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Abstract Dendritic cell (DC)-based immunotherapy is a promising strategy for the elimination of minimal residual disease in patients with acute myeloid leukemia (AML). Particularly, patients with a high risk of relapse who are not eligible for hematopoietic stem cell transplantation could benefit from such a therapeutic approach. Here, we review our extensive studies on the development of a protocol for the generation of DCs with improved immunogenicity and optimized for the use in cell-based immunotherapy. This new generation DC vaccine combines the production of DCs in only 3 days with Toll-like receptor-signaling-induced cell maturation. These mature DCs are then loaded with RNA encoding the leukemia-associated antigens Wilm's tumor protein 1 and preferentially expressed antigen in melanoma in order to stimulate an

AML-specific T-cell-based immune response. In vitro as well as in vivo studies demonstrated the enhanced capacity of these improved DCs for the induction of tumor-specific immune responses. Finally, a proof-of-concept Phase I/II clinical trial is discussed for post-remission AML patients with high risk for disease relapse.

Keywords AML · Cancer immunotherapy · Clinical trial · Dendritic cells · Vaccine · PIVAC 13

Abbreviations

AML	Acute myeloid leukemia
BM	Bone marrow
CTL	Cytotoxic T lymphocytes
DC	Dendritic cells
GMP	Good manufacturing practice
hCMV	Human cytomegalovirus
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
Ivt	In vitro-transcribed
LAA	Leukemia-associated antigen
LSC	Leukemic stem cell
MFC	Multiparameter flow cytometry
MRD	Minimal residual disease
NK	Natural killer
OS	Overall survival
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cells
PRAME	Preferentially expressed antigen in melanoma
RFS	Relapse-free survival
RQ-PCR	Real-time quantitative polymerase chain reaction
TAA	Tumor-associated antigen
Th1	T helper 1
TLR	Toll-like receptor

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Introduction

In adult acute myeloid leukemia (AML), intensive induction chemotherapy induces complete cytomorphological remission in up to 80 % of patients. However, most patients experience subsequent recurrence of disease. Relapse rates are substantially higher in patients over the age of 60 and vary according to cytogenetic and molecular risk groups [1]. Post-remission therapy is critical for the elimination of minimal residual disease (MRD) and is a prerequisite for achieving cure. Allogeneic hematopoietic stem cell transplantation (HSCT) was shown to provide the most potent immunological antileukemic effect, with the lowest rate of relapse and a relevant benefit for overall survival (OS) in certain age groups [2]. However, this approach is restricted to only a subset of patients due to patient-associated comorbidities, donor availability, and age. Targeted immunotherapies may provide suitable alternate therapeutic approaches to eliminate MRD in patients not eligible for HSCT [3].

Recent advances in the field of tumor immunology have resulted in the identification of a number of leukemia-associated antigens (LAAs): specific cytotoxic T-lymphocyte (CTL) responses were detected against the Wilm's tumor protein 1 (WT1), proteinase 3, preferentially expressed antigen in melanoma (PRAME), human telomerase reverse transcriptase (hTERT), and Fms-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD), to name only a few. For several of these LAAs, clinical peptide vaccination trials were initiated. So far, clinical Phase I/II trials have demonstrated immunological and molecular responses that translate into clinical efficacy in up to half of the vaccinated AML patients [4]. A major challenge in peptide vaccination, however, is the conversion of T-cell tolerance into specific CD8⁺ CTL and T helper 1 (Th1) type CD4⁺ T-cell responses directed against self-restricted LAAs. It is known that tumor immunity against AML is conferred by both these cell types and by natural killer (NK) cells.

Dendritic cells (DCs) are "nature's adjuvants" for eliciting cellular and humoral immunity, and many animal experiments have shown that the injection of tumor antigen-loaded DCs reliably induced tumor-specific CTL responses, tumor resistance, and in some cases regression of metastases. Therefore, DCs have the potential to be more successful than peptide vaccination in eliciting immunological responses needed for the eradication of MRD. RNA loading of DCs is an attractive technique to overcome the need for patient selection due to MHC restriction and allows development of multiplex immune responses mediated by both CD4⁺ and CD8⁺ T cells using all MHC molecules of the patient. In a recently updated Phase I/II trial,

the clinical efficacy of an autologous, mRNA-electroporated DC vaccination was clearly shown in 17 patients with AML [5, 6].

With increasing knowledge of how to optimize protocols for DC maturation, there is potential for the improvement of clinical benefits by DC vaccination. We developed a three-day manufacturing protocol using a cytokine cocktail containing a synthetic Toll-like receptor (TLR)7/8 agonist for the generation of monocyte-derived mature DCs with improved immunogenicity.

In this review, we discuss our approach for the development of DC vaccines, addressing the questions of DC maturation, route of vaccination as well as dose and schedule of application for post-remission therapy of patients with AML. The setup of the clinical Phase I/II trial is introduced, which is currently recruiting patients.

A three-day protocol for the rapid production of DCs

Most protocols for the generation of clinical-grade monocyte-derived DCs require approximately seven days (7d) of cell culture [7]. Thereby, these protocols are time- and labor-intensive, especially when performed in a clean room facility. In order to develop more feasible strategies for clinical translation, generation of mature DCs within 2–5 days was addressed by several groups in recent years. These fast DCs displayed mature phenotypes and had the capacity to stimulate antigen-specific T-cell responses [8, 9]. Faster generation of clinical-grade mature DCs reduces manufacturing costs, and some evidence suggests that rapid DC differentiation may better reflect the situation in vivo [10].

Our procedures allow us to differentiate and mature DCs within only 3 days (3d) of culture. Hereby, monocytes are stimulated with GM-CSF and IL-4 on the day of isolation and are further activated on day two for maturation over 24 h.

These young DCs were comparable to cells generated with a standard 7d protocol with respect to their mature surface phenotype as well as their capacities for antigen uptake and presentation and antigen-specific T-cell stimulation. Higher yields of viable DCs could be obtained using the 3d protocol. Additionally, improvements in some of the characteristics of young DCs could be observed. The relative expression of the co-stimulatory molecules CD80 and CD86 compared to inhibitory molecules, such as CD274, was higher in 3d-DCs, indicating that shortened culture is beneficial for supporting a positive stimulatory phenotype. Furthermore, 3d-DCs showed a somewhat better migratory capacity than 7d-DCs in vitro, accompanied by high expression of the chemokine receptor CCR7 [11]. These results were observed in numerous experiments using DCs generated with our 3d protocol compared to more standard

7–9d procedures, supporting the hypothesis that fast DCs will be superior in priming naïve T cells to specific antigens. This protocol could be easily transferred into a GMP cell facility and was therefore selected for further development of a DC-based vaccine study for AML patients.

A TLR7/8 agonist-containing maturation cocktail for the generation of Th1-polarizing DCs

The ability of DCs to activate effective antitumor immune responses is highly dependent on their capacity to secrete inflammatory cytokines that induce strong effector functions in T cells. IL-12p70 secretion by DCs plays a critical role in this process by polarizing CD4⁺ cells into the direction of Th1 responses [12]. CD4⁺ Th1 cells, in turn, support the activation of CD8⁺ CTLs, which have the capacity to kill tumor cells in an antigen-specific manner. In addition, IL-12p70 has an important role in the activation of NK cells, which also display cytolytic activity against tumor cells. Thus, IL-12p70 secreting DCs can orchestrate both innate and adaptive immune cells to build effective immune responses against tumors.

Various maturation stimuli have been studied for their capacity to induce IL-12p70 secretion by DCs. Activation of DCs via TLRs, which normally signal via pathogen-derived products, has been extensively analyzed. The signaling pathways activated by TLR7/8 and TLR3 stimulations were found to synergize in induction of IL-12p70 secretion [13, 14]. Recent evidence also suggests that stimulation of DCs via the TLR7/8 signaling pathway can overcome IL-12p70 production defects in patient-derived DCs [15].

We established a maturation cocktail containing the synthetic TLR7/8 agonist R848 and the TLR3 ligand poly(I:C), in combination with TNF α , IL1 β , IFN γ , and PGE2 [16]. Monocyte-derived 3d- and 7d-DCs were matured with this new cocktail or with a gold standard cocktail consisting of TNF α , IL1 β , IL-6, and PGE2 [7], which has been used extensively for the generation of DCs tested in the clinic. TLR agonist-activated 3d-DCs had a mature phenotype and displayed a positive stimulatory profile, as exhibited by increased expression of co-stimulatory molecules compared to co-inhibitory markers. Additionally, only TLR ligand-stimulated DCs could secrete high amounts of IL-12p70 upon CD40–CD40-ligand interactions. Secretion of IL-10 by TLR-activated DCs was low. This is important since IL-10 can counteract the polarizing effects of IL-12p70 [17].

The TLR-activated 3d-DCs displayed several other advantageous features *in vitro*. They showed an improved capacity for Th1 polarization of both CD4⁺ and CD8⁺ T cells as well as improved capacity for antigen-specific activation of autologous T cells. A strong impact on functional activation of NK cells was also demonstrated. All these

effects were significantly weaker with DCs generated with the standard cocktail [16, 18, 19].

Since earlier studies demonstrated a synergism of TLR7/8 and TLR3 activation on IL-12p70 secretion by 7d-DCs [13, 14], we were interested to determine whether these results would also be found with 3d-DCs. A side-by-side comparison of 3d-DCs matured with cocktails containing either R848 plus poly(I:C) or R848 alone revealed no significant differences between the two different cell preparations, according to phenotype and function. Therefore, we elected to remove poly(I:C) from our maturation cocktail. This protocol was then assessed for the generation of mature 3d-DCs from monocytes of AML patients in remission and shown to result in pronounced innate and adaptive antileukemic immune responses *in vitro* [20].

In vitro-transcribed mRNA for efficient antigen loading of DCs

The specificity of an adaptive immune response is determined by the identity of the antigenic peptides presented to the CD8⁺ or CD4⁺ T cells in association with MHC class-I or class-II molecules, respectively (i.e., pMHC ligands). Tumor cells may escape immune recognition through down-regulation of single tumor-associated antigens (TAAs) [21]. Therefore, activation of T cells recognizing multiple pMHC ligands from multiple TAAs is important to guard against tumor escape variants.

To date, numerous strategies for antigen loading of DCs have been evaluated. Pulsing DCs with synthetic TAA-derived peptides has been analyzed extensively in recent years. In this case, the activated T-cell response is not only limited to the selected epitope but also restricted to a specific HLA molecule, and progressive growth of antigen-loss variants may occur [22]. Although loading of DCs with peptides derived from multiple TAAs may prevent immune escape, this approach is still limited to patients with particular MHC allotypes where knowledge of suitable peptides is available. To allow activation of T-cell responses directed against multiple TAAs, independent of the patient's genetic background, use of tumor lysates or amplified tumor-derived mRNA has been supposed to be more advantageous [23]. DCs loaded with these sources of antigens should be able to present a patient's own tumor set of known and unknown TAAs. However, these sources need to be prepared individually from each patient, and availability of tumor material might be a limiting factor.

Instead, use of *in vitro*-transcribed messenger RNA (ivt-mRNA) encoding defined antigens offers an attractive alternative for loading of DCs with message of either single or multiple TAAs. Furthermore, sufficient amounts of RNA can be produced independently of the need for access

to patient tumor material. We analyzed the capacity of DCs loaded with (1) total tumor-derived RNA; (2) amplified tumor-derived RNA, prepared from a tumor cell line; or (3) ivt-mRNA encoding a single antigen (single-species RNA), known to be present in the total tumor-derived RNA preparation, for the reactivation of antigen-specific effector-memory CTLs. Here, DCs loaded with single-species RNA showed a clear advantage in CTL activation [24]. By loading DCs with single-species RNA, high amounts of RNA molecules could be introduced into DCs, and TAA-derived epitopes could be processed and presented via MHC molecules in sufficient numbers for efficient T-cell recognition. In tumor lysates or native or amplified total tumor-derived mRNA, adequate amounts of some immunogenic antigens or antigen-encoding messenger RNA may not be sufficient for efficient antigen presentation at the DC surface, leading to inadequate activation of antigen-specific T cells [24]. Therefore, single-species ivt-mRNA as the selected form for antigen loading of DCs offers an attractive strategy to provide mature DCs with sufficient amounts of different peptides of individual or multiple TAAs.

Further, we established methods to efficiently transfect 3d and 7d mature DCs with TAA-encoding RNA by means of electroporation without changing their phenotype [11, 24]. It was clearly demonstrated that RNA-loaded DCs were able to efficiently process and present peptides derived from numerous transfected antigens, allowing activation of antigen-specific memory CTL clones as well as activation and expansion of naïve antigen-specific T cells [24–26].

Since the development of immune responses to multiple TAAs is advantageous, loading of DCs with pools of different ivt-mRNA species would seem warranted. We compared the stimulatory capacities of DCs transfected with either single-species ivt-mRNA or with pools of three or six RNA species, respectively. Cells transfected with a pool of three different RNAs showed an approximate 80 % capacity for CTL activation per antigen compared to single antigen-loaded DCs. Transfection with a pool of six different RNA species further reduced this capacity to 40 % [25]. Therefore, we elected to only introduce single antigens into separate aliquots of mature DCs. As a consequence of these results, in our clinical vaccination protocol, aliquots from one DC batch production are individually loaded with a selected antigen and then DCs with different antigens are administered simultaneously to the patient to allow stimulation of T-cell responses specific for multiple TAAs.

A humanized mouse model for the preclinical evaluation of DC vaccines

For preclinical evaluation of our DC-based vaccines, it was important to analyze the induction of specific T-cell

responses in an appropriate in vivo model. Such an animal model needs to bear a functional human immune system to ensure that therapeutically administered human DCs have a network of human immune cells in order to activate T cells and NK cells. The immunodeficient mouse strains NOD/SCID Il2rg^{-/-} and BALB/c-Rag2^{-/-}Il2rg^{-/-} are currently considered to be state-of-the-art models for such experiments [27, 28]. These mice can be engrafted with functional human immune cells by transplanting them with human hematopoietic stem cells (HSCs) or peripheral blood mononuclear cells (PBMCs) [29, 30].

We chose the NOD/SCID Il2rg^{-/-} (NSG) mouse model to analyze our vaccine strategy and to assess the functionality of our new generation DCs. Mice were engrafted with human PBMC and vaccinated twice with autologous human DCs on days 14 and 21 after reconstitution. Approximately one week later, the human lymphocytes were analyzed for their immunological properties. By choosing this short-time protocol, we avoided the xeno reactivity that normally occurs in longer engraftment and vaccination protocols. We compared the stimulatory capacity of standard 7d-DCs and 3d-DCs. Both DC types were matured with a standard maturation cocktail lacking a TLR ligand. Additionally, 3d-DCs were included that were matured with our TLR7/8 and TLR3 ligand-containing cocktail. MART-1 was chosen as the antigen, and DCs were transfected with ivt-mRNA encoding this antigen. Splenic-derived T cells isolated from mice vaccinated with the 3d-DCs matured with the TLR agonist-containing maturation cocktail showed the best MART-1-specific immune responses, as assessed by antigen-specific killing capacity and IFN γ secretion. In comparison, T cells isolated from mice vaccinated with standard 7d-DCs showed no or only low antigen-specific immune responses [31].

This NSG mouse model allowed a direct comparison of different DC vaccine cells, thereby the superiority of TLR-stimulated 3d-DCs in the activation of antigen-specific T-cell stimulation based on extensive in vitro experiments could be confirmed in vivo.

TLR-activated 3d-DCs for immunotherapy of AML: a proof-of-concept clinical trial

As demonstrated in the work above, our 3d, TLR-activated DCs have optimized characteristics for the induction of potent CD4⁺ and CD8⁺ Th1-polarized T-cell responses as well as NK cell responses when compared to DC vaccines used in previous clinical trials of DC vaccination in AML patients, which have already shown some promising results [5]. Therefore, we were encouraged to design a clinical trial to test this type of DCs in post-remission AML patients. We developed a GMP-compliant process for the production of

Fig. 1 Manufacturing of new generation DCs. Monocytes are differentiated to immature DCs by GM-CSF and IL-4 within 48 h. Addition of a novel TLR7/8 agonist-containing maturation cocktail for another 24 h leads to the development of mature DCs that are char-

acterized by a positive stimulatory profile and high production of bio-active IL-12p70. After electroporation with antigen-encoding RNA, these DCs are capable of polarizing Th1 responses and stimulating antigen-specific CTLs in addition to NK cell activation

DCs within 3 days of culture from autologous monocytes, including maturation using our TLR7/8 agonist-containing cocktail. Subsequent antigen loading is performed by electroporation of the mature cells with TAA-encoding ivt-mRNA (Fig. 1). In a head-to-head comparison, we were able to show that DCs differentiated from monocytes taken from AML patients in complete remission were comparable to DCs of healthy donors with respect to phenotype and function [20]. A proof-of-concept Phase I/II clinical trial was initiated to evaluate our new generation mature DC vaccines loaded with mRNA for WT1, PRAME, and hCMVpp65 (see below for choice of antigens) as post-remission therapy of AML patients with non-favorable prognosis (NCT01734304), and we are currently recruiting patients. As the primary end point of our trial is feasibility and safety, we adapted our DC dose and vaccination schedule to prior protocols (see below) [32, 33]. This enables us to deduce observed differences in clinical and immunological responses to the properties of the DCs rather than to DC dose or vaccination schedule. Patients are vaccinated with 5×10^6 DCs transfected with one of the three antigens, accumulating to a total of 15×10^6 DCs at each vaccination time point. The first 4 vaccinations are given weekly, followed by up to 6 vaccinations at monthly intervals. Secondary end points of our trial are immune responses and disease control with particular focus on MRD conversion as detected by real-time quantitative polymerase chain reaction (RQ-PCR) and multiparameter flow cytometry (MFC) (Fig. 2). Importantly, innate and adaptive immune responses against “self” (WT1 and PRAME) and “viral” (hCMVpp65) antigens will be closely analyzed using at

least two different immune monitoring methods according to the recommendations of the MIATA association (<http://miataprotect.org/>).

The study antigens WT1, PRAME, and hCMVpp65

In recent years, the identification of TAAs has provided many potential targets for directed immunotherapy. However, the choice of a suitable TAA for antigen-specific immunotherapy needs careful consideration in order to achieve efficient immune responses and to avoid unwanted on-target as well as off-target toxicity. In a systematic approach to the selection of the most suitable TAA, a priority-ranked list of target antigens was developed [34]. The evaluation of 75 representative cancer antigens according to predefined and preweighted criteria revealed that none had all the characteristics required to be an “ideal” cancer antigen. For our DC vaccine, we chose the LAAs WT1 and PRAME because they fulfill most of these criteria (Table 1). Importantly, these antigens were shown to be safe in prior peptide or DC vaccination studies [35–37]. Furthermore, potentially curative immunotherapy needs to target leukemic stem cells (LSCs); thus, these target antigens were selected since they are overexpressed in LSCs compared to normal hematopoietic stem cells (HSCs) [38, 39].

Based on these characteristics and its proven immunogenicity, WT1 was placed at the top of the prioritized list of potential target antigens for cancer immunotherapy [34]. Despite the fact that WT1 is a self-antigen, T cells specific

Fig. 2 Protocol for the proof-of-concept clinical trial with new generation DCs in AML. Patients with AML and non-favorable risk profile in complete remission after intensive induction therapy are able to enroll. Standard exclusion criteria apply, and patients have to be ineligible for allogeneic SCT. Patients are vaccinated intradermally

with one batch of 5×10^6 DCs for each of the three antigens (WT1, PRAME, and hCMVpp65) up to 10 times within 26 weeks. The primary end point of the trial is feasibility and toxicity; secondary end points are immune responses and disease control, with particular focus on MRD conversion

Table 1 Characteristics of the study antigens WT1 and PRAME

	WT1 Transcription factor	PRAME Cancer testis antigen
Expression AML bulk	> 85 %	> 65 %
Expression LSC	++	++
Expression HSC/others	–	–
Oncogenicity	++	++
Immunogenicity	++	++
Clinical efficacy	++	++
MRD monitoring	+++	Possible

for WT1 are detected in healthy donors and AML patients, and the immunogenicity of WT1 in an autologous setting is exemplified by the natural development of humoral [40] and cellular [41] immunity against WT1 in patients with WT1-expressing malignancies, including AML. Furthermore, naturally activated WT1-specific T cells were reported to contribute to the graft versus leukemia effect in patients after allogeneic HSCT [42]. In clinical trials,

in vivo immunological responses to WT1 peptide-based vaccines were measured in as many as 88 % of AML patients. Clinically, decreased numbers of leukemic cells and decreased levels of WT1 expression, disease stabilization, and complete hematological remission were observed in these trials. In some cases, clinical responses were at least associated, if not clearly correlated, with the observed immunological effects [36, 43, 44].

PRAME seems to inhibit cell differentiation, growth arrest, and apoptosis by acting as a repressor of retinoic acid receptor signaling. For this reason, it is thought that PRAME may contribute to oncogenesis in general, and that it may be an important contributory factor in AML disease progression in particular [45]. PRAME has been detected in bone marrow (BM) and peripheral blood (PB) of 30–64 % of leukemia patients [46, 47]. In one study, a strong correlation between PRAME expression and unique leukemia markers was found [48], and in another one, blast counts in marrow samples were correlated with PRAME expression [47]. Furthermore, the development of PRAME-specific T cells in vivo and in vitro was reported in several studies,

underscoring the processing and immunogenicity of different PRAME-derived epitopes [49, 50].

Human cytomegalovirus (hCMV) is common in all parts of the world, with infection rates between 60 % in developed countries and up to 100 % in developing countries. The hCMV 65-kDa phosphoprotein (pp65) has been identified as a major, immunogenic and immunodominant target antigen for hCMV-specific, MHC class-I-restricted CTLs. Due to its high immunogenicity as a foreign antigen, pp65 is a promising target for immunotherapy of immunocompromised patients, such as HSCT recipients at risk of developing an hCMV infection [51].

It has been well known for the past several decades that simultaneous immunization against two different antigens may result in different outcomes of the immune response to each antigen. Classically, antigen competition has been seen by the fact that a good immune response occurs against one antigen while the immune response specific for the second antigen is suppressed. More recently, the molecular and cellular mechanisms that underlie antigen competition have been elucidated. At the molecular level, antigenic peptides from one antigen might be able to out-compete peptides from a second antigen for presentation at the surface of the DC. As a consequence, the DC will display a superior capacity to prime T cells to the dominant epitope(s) of the former antigen while being deficient in their capacity to prime T cells to poorly competing epitopes derived from the second antigen.

At the cellular level, predominance in particular pMHC complexes at the surface of the DC will allow it to attract and interact with greater numbers of T cells specific for the first antigen over time. Even if the DC expresses small amounts of pMHC from the second antigen, specific T cells for the second antigen may be unable to adequately recognize and interact with the DC due to overcrowding at the surface of the DC with T cells responding to the first antigen [52].

We completely avoid the molecular and cellular antigen competition in our DC vaccines despite the use of three different antigens, anyone of which might carry immunodominant epitope(s). This is accomplished by separating the DCs into three aliquots prior to antigen loading. Each aliquot of DCs is then electroporated separately with ivt-mRNA encoding only one of the three different antigens. This means that each of the three subfractions of DCs only needs to process and present peptides from one antigen, resulting in separate populations of DCs for each antigen.

Dosing and administration of the DC vaccine

A literature review of clinical studies of DC-based therapeutics administered intradermally to adult cancer patients

revealed that doses of up to 1×10^8 DCs per injection can be applied multiple times without the danger of patients developing high-grade toxicities, including autoimmune reactions. In general, DC vaccinations have been observed to be safe irrespective of the tumor type, DC maturation stage, antigen identity, and antigen form used to load the DCs. On average, patients received a total of 3 or 4 vaccinations at two-week intervals. Immunological responses were observed in all of these trials and were associated or even correlated with clinical responses on several occasions. Dose-limiting toxicities were not reported in any of the studies in which the dose was escalated in the $1\text{--}100 \times 10^6$ DC range [53–55]. In fact, few differences were observed among the different doses.

Scarce adverse events have been reported in the few trials with maximum DC doses ranging from 50 to 100×10^6 cells. Furthermore, toxicities higher than grade 2 (according to the National Cancer Institute's Common Toxicity Criteria) were not observed [54, 56]. In one melanoma trial, 10×10^6 DCs loaded with peptides and tumor lysates were administered up to 60 times per patient at weekly or biweekly intervals. While most subjects experienced mild and transient pain at the vaccine injection sites, in several patients, a grade 3 autosensitization dermatitis-like eruption and transient eosinophilia was observed, but none of these adverse events were considered to be severe [57]. In consideration of all the published data on DC dose and schedule, our choice to use 15×10^6 DCs per injection, ranging from 4 to 10 applications, according to availability and feasibility is judged to be acceptable for a first-in-man study.

MRD diagnostics in AML

Monitoring of MRD in patients with AML has become an important diagnostic tool. Improved MRD techniques and new sensitive markers are used for the assessment of response to therapy and to monitor the individual course of the disease in each patient. Reflecting initial chemosensitivity, MRD levels constitute a prognostic marker that combines various biological properties of an individual AML. Available methods to determine the levels of MRD from BM samples are RQ-PCR ("molecular MRD") and MFC ("flow MRD"). RQ-PCR offers very high sensitivity (up to 1 cell in 10,000 to 1 cell in 100,000). The high predictive value of NPM1 mutation MRD has been demonstrated in several retrospective studies. An increase over 200 NPM1 copies per 10^4 copies of the housekeeping gene ABL1 was associated with disease recurrence after a median time of 3 months [58]. WT1 is a ubiquitously expressed target antigen available for molecular MRD assessment. Measurable WT1 levels have been shown to correlate with the clinical course of disease in over 85 % of the cases [59, 60].

Therefore, WT1 is a suitable surrogate marker to monitor the success of a therapeutic approach. However, WT1 detection is less suitable to trigger an intervention, because an increase in WT1 levels is associated with a rather rapid relapse after a median of only 38 days (range 8–180 days), if monitored in >4 week intervals [61].

The use of MFC for the detection of MRD is applicable in the majority of patients, offering a sensitivity of up to 1 cell in 10.000 cells, depending on the number of events acquired. Initial studies revealed high prognostic value of flow MRD assessment for relapse risk, relapse-free survival (RFS), and OS after induction and consolidation therapy [62, 63]. The prognostic impact of flow MRD at these time points was subsequently confirmed in several other studies [64, 65]. In conclusion, molecular MRD allows the prediction of relapse over the course of the disease, with variable sensitivity (NPM1 > WT1) and different time intervals. Flow MRD at early time points of disease appears to be most suitable for improved risk stratification and identification of patients at high risk of relapse [66]. Combining the strategies of molecular and flow MRD enables risk-adapted therapy to be made while using a surrogate marker for clinical efficacy that is critical for AML monitoring in Phase I/II trials during post-remission therapy.

Concluding remarks

Within the last decade, we have covered the long distance from bench to bedside in the development of a GMP-compatible protocol for a new generation of DCs with optimized characteristics for cancer immunotherapy. We established a time- and labor-efficient protocol for DC generation within only 3 days. In combination with a novel maturation cocktail, including a synthetic TLR7/8 ligand, this protocol yields DCs with several highly advantageous properties for application in cancer immunotherapy when compared to other more conventional monocyte-derived DCs. Specifically, these DCs display a predominant positive stimulatory profile and high secretion of IL-12p70 upon interaction with T cells. Both of these signals are responsible for the superior capacity of the DCs to polarize CD4⁺ T cells toward a Th1-dominant phenotype and to activate both CTLs and NK cells. Antigen loading via electroporation with ivt-mRNA encoding LAAs was established and shown to be efficient for the induction of antigen-specific T-cell immune responses. This was demonstrated not only in vitro, but also in a humanized mouse model. Based on this extensive preclinical data, we developed a GMP-compliant process to produce these new generation DCs from leukapheresis products and have initiated a clinical Phase I/II trial to test their application in AML patients.

Acute myeloid leukemia was chosen as the first clinical entity for the assessment of these new generation DCs for several reasons: After intensive induction therapy, patients have low disease burdens, rendering post-remission the ideal time point for immunotherapy. Leukemic blasts in the BM and PB can be more easily accessed by the immune system than stroma-rich solid tumors. MRD measurements provide an excellent means to determine the efficacy of our treatment approach. Finally, the identification of molecular characteristics and immunogenic epitopes in AML is advanced. We chose WT1 and PRAME as antigens for the stimulation of T cells, accompanied by the very immunogenic viral antigen hCMVpp65, both as an adjuvant and as an internal immune monitoring control. According to the protocol of our clinical trial that is actively recruiting study participants, AML patients with non-favorable prognosis are vaccinated 4–10 times with 5×10^6 DCs for each of these antigens. We believe that this DC-based vaccine has the potential to significantly improve the clinical outcome of AML patients with a non-favorable genetic risk profile. Furthermore, by selecting appropriate TAAs, our platform technology for DC generation allows us to develop vaccine formulations specific for any type of malignancy.

Parts of this work have been presented and published in abstract books of the following scientific meetings

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Conflict of interest Dolores J. Schendel is Managing Director of Trianta Immunotherapies GmbH and Chief Scientific Officer of Medigene AG. Christiane Geiger is Associate Director of DC Vaccine Development of Trianta Immunotherapies GmbH. All other authors declare that they have no conflicts of interest.

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