A phase IB study on intravenous synthetic mRNA electroporated dendritic cell immunotherapy in pretreated advanced melanoma patients

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Background: Autologous monocyte-derived dendritic cells (DCs) electroporated with synthetic messenger RNA (mRNA) encoding a CD40 ligand, a constitutively active Toll-like receptor 4 and CD70, together with mRNA encoding fusion proteins of a human leukocyte antigen (HLA)-class II targeting signal (DC-LAMP) and a melanoma-associated antigen (MAA); either MAGE-A3, MAGE-C2, tyrosinase or gp100 (TriMixDC-MEL) are superiorly immunogenic.

Patients and methods: In this phase IB clinical trial, 24 million viable DCs were administered by four biweekly combined intradermal (id) and intravenous (iv) administrations, and a fifth administration on week 16. The number of iv-administered DCs was escalated in four sequentially treated cohorts. Immune responses were assessed by analysis of antigen specificity of blood-derived T-cells and skin infiltrating lymphocytes (SKILs).

Results: Fifteen patients with pretreated advanced melanoma tolerated administration of TriMixDC-MEL well. Two patients achieved a complete response and two patients a partial response. All objective responders are progression-free after a follow-up of, respectively, 24+, 28+, 33+, and 34+ months. Post-therapy antigen-specific SKILs were documented in 6 of 12 patients, and antigen-specific CD8+ T-cells were detected in the blood of 4 of 5 patients.

Conclusions: Cellular immunotherapy with TriMixDC-MEL is safe and immunogenic. Antitumor activity with durable disease control is observed across the investigated iv-dose levels.

ClinicalTrials.gov Identifier: NCT01066390.

Key words: TriMixDC-MEL, dendritic cells, immunotherapy, melanoma

Introduction

The molecular characterization of several immunogenic melanoma-associated antigens (MAAs) has led to the design of antigen-specific immunotherapeutic strategies for this most lethal type of skin cancer [1]. Therapeutic protein or peptide vaccines (e.g. against MAGE-A3 or gp100) have demonstrated limited activity as a single agent in patients with advanced melanoma [2–4]. Following the administration of a therapeutic vaccine, the antigen will need to be taken up, processed, and presented to the effector cells of the immune system by so-called ‘professional antigen presenting cells’ (APCs) [5]. Dendritic cells (DCs) are APCs that play an essential role in the initiation, expansion, and memory function of both innate and adaptive immune responses to foreign and self-antigens, including immune responses to cancer cells [6]. Over the past decade, clinical trials on DC-based cellular immunotherapy have met with limited clinical efficacy [5, 7]. Moreover, first generation DC-formula failed to improve the results obtained with non-cellular tumor vaccines in two randomized phase II trials [8, 9]. This underscores the need for further optimization of cellular immunotherapies such as the type of DCs and its maturation status, the process of antigen loading and presentation, dose and administration regimen [7, 10].

We previously reported a superior T-cell stimulatory capacity by co-electroporation of DCs with synthetic messenger RNA (mRNA) encoding CD40 ligand (CD40L), CD70, and a constitutively active Toll-like receptor 4 (caTLR4) (TriMix-DC) [11, 12]. Effective antigen loading was achieved by co-electroporation of TriMixDC with a full-length MAA-encoding mRNA, allowing for the presentation of the full range of antigenic peptides resulting in a broader MAA-specific T-cell response [13, 14]. Additionally, enhanced MAA presentation in both human leukocyte antigen (HLA) classes I and II is achieved by fusion of the MAA-encoding sequence with an HLA class II targeting signal [15]. Intradermal (id) administration of autologous TriMixDCs expressing the
MAGE-A3, MAGE-C2, tyrosinase and gp100 antigens (referred to as TriMixDC-MEL) was found to be feasible, safe, effectively stimulating CD8+ and CD4+ T-cell responses, but did not result in objective tumor responses [16]. Preclinical insights into the organ-specific trafficking of vaccine-induced T-cell populations indicated that combination of different routes of administration may be beneficial to target different tumor locations [17,18]. In this prospective phase IB clinical trial we investigate the clinical activity of escalating doses of intravenously administered TriMixDC-MEL.

patients and methods

trial eligibility and patient inclusion criteria

Patients were considered eligible for participation in this phase IB clinical trial if they had a prior diagnosis of histologically confirmed, unresectable American Joint Committee on Cancer stage IIIC or IV melanoma. Key eligibility criteria were: age ≥18 years; World Health Organization-performance status (WHO-PS) of 0, 1 or 2; normal hematological, liver and renal function tests; and negative serological tests for HIV, syphilis, hepatitis B and hepatitis C. The exclusion criteria included primary uveal melanoma, untreated or symptomatic central nervous system metastasis and the need for permanent therapeutic anticoagulation. The study was approved by the Institutional Ethics Committee of the UZ Brussel, and a written informed consent was obtained from all patients. Clinical trials registration number is NCT01066390.

study design

The primary end point of this phase IB clinical trial was to explore the feasibility and safety of iv administration of autologous TriMixDC-MEL. Patients were allocated to four sequential cohorts receiving increasing doses of TriMixDC-MEL iv. The first cohort received 20 × 10⁶ DCs id and 4 × 10⁶ DCs iv; the second cohort 12 × 10⁶ DCs id and 12 × 10⁶ DCs iv, the third cohort 4 × 10⁶ DCs id and 20 × 10⁶ DCs iv and the fourth cohort received 24 × 10⁶ DCs iv-only. The first four TriMixDC-MEL administrations were administered at a biweekly interval with a fifth administration scheduled 10 weeks after the fourth administration (supplementary Figure S1, available at Annals of Oncology online). DCs (suspended in 15 ml of physiologic saline solution) were administered iv during a 15 min infusion by a constant flow rate in a peripheral vein. At the same time, DCs (suspended in 250 µl phosphate buffered saline containing 1% human serum albumin) were injected id at two different anatomical sites (axilla and/or inguinal region). Adverse events (AEs) were monitored continuously and graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE), version 4.0. Secondary end points included immunogenicity of the TriMixDC-MEL therapy, tumor response according to the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, progression-free survival (PFS), and overall survival (OS) [19].

TriMixDC-MEL production

Immature DCs were generated by culturing monocytes in the presence of 1% autologous plasma, 1000 U/ml GM-CSF, and 500 U/ml IL-4. Following leukapheresis, monocytes were enriched by plastic adherence. On day 6, DCs were harvested and co-electroporated with TriMix-mRNA (CD40L, CD70, and cTLR4 encoding mRNA) and mRNA encoding one of four MAAs (MAGE-A3, MAGE-C2, tyrosinase, or gp100) linked to an HLA class II targeting signal, as reported previously [12]. After electroporation, the four different TriMixDC-MEL cellular constituents (i.e. DCs expressing one of the four antigens) were mixed at equal ratios and cryopreserved. DCs were thawed 2 to 3 h before injection. An in-process, as well as quality control (QC) of the final product, was carried out, as reported previously [16].

immunomonitoring

Immunomonitoring of the peripheral blood and skin biopsies of delayed-type hypersensitivity (DTH) reactions of the patients was carried out as reported previously [20,21].

serum cytokine analysis

Ten milliliter of peripheral blood was drawn before and 30 min after the start of the intravenous TriMixDC-MEL administration. Assay of 27 different cytokines and chemokines was carried out using the BioPlex human cytokine 27-Plex A-panel in accordance with the manufacturer’s instructions (Bio-Rad, Nazareth, Belgium).

statistics

Correlations between DC immunophenotype characteristics were investigated using the Pearson correlation coefficient test. The Wilcoxon signed-rank test was used to compare changes in quantitative outcomes from pre-to post-administration cytokine and chemokine assessments. Statistical tests were two-sided and P < 0.05 was considered statistically significant. OS and PFS were estimated using the Kaplan–Meier method. All statistical tests were carried out with IBM SPSS software v19.0.

results

patient baseline characteristics and disposition

Between December 2009 and February 2011, 15 eligible patients with advanced pretreated melanoma were recruited (Table 1). Respectively, two and three patients were enrolled in the first and second cohort. None of them experienced unexpected treatment-related side effects. Among the first three patients enrolled in the third cohort, one patient experienced an unexpected treatment-related adverse event (chills). Therefore, this cohort was expanded with three additional patients. Recruitment to the fourth cohort ended when none of the first four patients experienced treatment-related limiting toxicity. Eight patients received all five planned TriMixDC-MEL administrations. Five patients only received the first four biweekly administrations. Two patients could not receive more than three administrations because of clinical deterioration due to progressive disease. No relationship was observed between the number of administrations and the treatment cohort.

TriMixDC-MEL cellular therapy characteristics

In all 15 patients, sufficient amounts of the TriMixDC-MEL cell therapy product could be obtained for the intended five administrations. TriMixDC-MEL preparations of all patients were characterized by a mature phenotype and IL-12p70 secretion. Higher electroporation efficiency (indicated by CD70 expression) correlated with a more mature DC phenotype (indicated by CD80 and CD83 expression) [P = 0.005 (CD80) and 0.002 (CD83)]. There was no significant difference in the maturation status of the DC preparation between the different patient study cohorts. The TriMixDC-MEL phenotype and IL-12p70 secretion are summarized in supplementary Figure S2,
Table 1. Patient baseline demographics and clinical characteristics

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<th>Primary tumor site</th>
<th>AJCC stage</th>
<th>Localization metastases</th>
<th>Prior therapy</th>
<th>LDH &gt; ULN</th>
<th>CRP &gt; ULN</th>
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<td>Nihil</td>
<td>No</td>
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<td>0</td>
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id, intradermal; iv, intravenous; m, male; f, female; AJCC, American Joint Committee on Cancer; LN, lymph nodes; GB, gall bladder; sc, subcutaneous; CNS, central nervous system; SG, surrenal gland; DTIC, dacarbazine; IFNa-2b, interferon alpha-2b; DCs, dendritic cells; ipi, ipilimumab; treme, tremelimumab; TMZ, temozolomide; C/P, carboplatin/paclitaxel; D/T, dabrafenib (GSK2118436)/trametinib (GSK1120212); LDH, lactate dehydrogenase; CRP, C-reactive protein; ALC, absolute lymphocyte count; ULN, laboratory upper limit of normal; WHO-PS, World Health Organization-performance status.
available at *Annals of Oncology* online, and supplementary Table S1, available at *Annals of Oncology* online.

**treatment-related adverse events**

TriMixDC-MEL was well tolerated, and no severe toxicity (adverse events of grade ≥3 according to the Common Terminology Criteria for Adverse Events) was encountered. All patients experienced grade 2 local skin reactions (irritation, erythema and swelling) at the id injection sites. Post-infusion grade 2 chills were observed in 3 out of 15 patients. Chills typically started about 15 min after the end of the iv-infusion of TriMixDC-MEL, and resolved spontaneously within 30 min. In addition, grade 2 flu-like symptoms and fever (38–39°C) that persisted for 2 to 3 days after the DC injection were reported by eight and three patients, respectively.

**antitumor response and survival**

The best objective response by RECIST consisted of a complete response in two patients and a partial response in two patients (for a best objective response rate of 27%). Tumor regression was evident in all responding patients at the first tumor response evaluation 8 weeks after the first TriMixDC-MEL administration (Figure 1). All four objective tumor responses were confirmed and are ongoing after a follow-up of, respectively, 24+, 28+, 33+ and 34+ months. A confirmed stable disease was documented in four additional patients (for a disease control rate of 53%). Two patients with a stable disease had a PFS of >6 months (respectively, 10 and 23 months). After a median follow-up of 28 months (range 22–34), 10 patients have died. The median PFS and OS are, respectively, 5 months [95% confidence interval (CI) 0–10] and 14 months (95% CI 5–23) (Figure 2).

**assessment of T-cell responses**

A DTH skin biopsy was obtained from 13 patients 1 week after the fourth DC administration. In 10 patients, sufficient T-cells were obtained for assessment of the antigen specificity of the skin infiltrating T-cells (SKILs). In four patients, CD8⁺ T-cells were found with specificity for the treatment antigens (Table 2). In two additional patients with insufficient SKILs for direct monitoring, specific CD8⁺ SKILs could be detected after in vitro restimulation. A T-cell repertoire with specificity for more than one MAA was found in four patients, and all four MAAs were recognized by the SKILs from two patients (Table 2). Treatment-specific CD4⁺ T-cells could be detected in 5 out of 12 (42%) patients.

Before TriMixDC-MEL treatment, MAGE-C2-specific CD8⁺ T-cells were found in the blood of three out of five patients tested. Following treatment with TriMixDC-MEL, circulating CD8⁺ T-cells recognizing MAGE-A3, MAGE-C2, tyrosinase

![Figure 1](image-url). Illustration of a clinical response following TriMixDC-MEL treatment. Melanoma metastases are indicated with arrows. (A) 2-Fluoro-2-deoxy-D-glucose-positron emission tomography/ computed tomography (FDG-PET/CT) images of patient 102 before and 5 months after TriMixDC-MEL therapy, illustrating a complete response of mediastinal lymph node metastases. (B) FDG-PET images of patient 106 before and 17 months after TriMixDC-MEL therapy, illustrating a durable partial response of peri-iliacal and retroperitoneal lymph node metastases.
and gp100 were found in four out of the same five patients tested (Table 2, Figure 3).

### Serum Cytokine Analysis

Cytokine and chemokine serum levels were measured in a total of 28 paired samples obtained pre- and 30 min post-DC administration in eight patients (five patients treated in cohort 3 and three patients treated in cohort 4). Significantly higher serum concentrations of IL-1β and IL-6 were found after administration when compared with the pre-administration concentrations [Figure 4A, $P = 0.0078$ (IL-1β) and $P = 0.0156$ (IL-6)]. In addition, in four pre/post-paired measurements (obtained at the occasion of the first DC administration in patients 67, 114 and 125, and also at the second administration in patient 67), a marked increase in serum IL-1β and IL-6 was detected.

### Table 2. Immunological and clinical outcomes

<table>
<thead>
<tr>
<th>Cohort</th>
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<th>Patient code</th>
<th>No. of admin.</th>
<th>CD8⁺ T-cell responses</th>
<th>SKILs post</th>
<th>CD4⁺ T-cell responses</th>
<th>SKILs post</th>
<th>BOR</th>
<th>PFS (months)</th>
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<td>M3, MC2, T, gp100</td>
<td>MC2, gp100</td>
<td>T, MC2</td>
<td>CR</td>
<td>33.6+</td>
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<td>12/12</td>
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<td>5</td>
<td>NT, NT</td>
<td>T⁺</td>
<td>–</td>
<td>–</td>
<td>PR</td>
<td>34.1+</td>
<td>34.1+</td>
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<tr>
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<td>77</td>
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<td>SD</td>
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<td>58</td>
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<td>ND</td>
<td>PD</td>
<td>0.9</td>
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id, intradermal; iv, intravenous; admin., administrations; BOR, best objective response; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; OS, overall survival; NT, not tested; ND, not determined due to too low numbers of SKILs derived from the DTH biopsies; sig, signal peptide of the murine LAMP-1 protein; M3, MAGE.A3; MC2, MAGE.C2; T, tyrosinase.

* Responses were observed after in vitro stimulation of the SKILs.
accompanied by a moderate increase in the serum concentration of several additional pro-inflammatory cytokines (incl. IFN-γ, TNF-α, IL-8 and IL-17) and chemokines (incl. MIP-1α and MIP-1β) (Figure 4B).

discussion

We previously reported that the T-cell stimulatory capacity of DCs can be enhanced by co-electroporation of mRNA encoding CD40L, CD70 and cαTLR4 (TriMixDC) and that id-administration of these TriMixDC co-electroporated with MAAs (TriMixDC-MEL) is safe, feasible and immunogenic, but associated with low anti-melanoma activity [12, 16]. In this prospective phase IB clinical trial on 15 advanced and pretreated melanoma patients, we investigated the feasibility and safety of TriMixDC-MEL administration by the iv-route. In accordance with our previous study, the adverse events related to TriMixDC-MEL administrations were mild (no CTCAE grade ≥3 adverse events were observed) [16]. However, unlike our experience with the id-only administration of TriMixDC-MEL transient (15–30 min) post-infusion chills were observed. Patients experiencing chills were treated in the third (two patients) and fourth (one patient) study cohorts (receiving, respectively, 4 × 10⁶ DCs id and 20 × 10⁶ DCs iv, and 24 × 10⁶ DCs iv-only) indicative of the fact that this AE is related to the dose of DCs administered iv. Significant increases in the level of pro-inflammatory cytokines measured post-infusion in some of the patients support the hypothesis that a cytokine release phenomenon is causing the chills. Mild post-administration transient flu-like symptoms, sometimes accompanied by fever,
were reported to appear more frequently when compared with our prior experience with id-administration only and may also relate to the release of cytokines.

Notwithstanding the fact that this phase IB trial was not designed to establish the antitumor activity of TriMixDC-MEL, a total of four durable objective tumor responses (including two complete and two partial responses) were observed across the investigated iv-dose levels. All responses are ongoing after a follow-up of >2 years (24–34 months), in the absence of any other additional anti-melanoma treatment. In addition, two out of four patients with a confirmed stable disease according to RECIST had a PFS of more than 6 months. The four responding patients and the two additional patients with durable disease stabilization (PFS of >6 months) were treated in cohort 1 (two

Figure 4. Serum cytokine and chemokine analysis. (A) Analysis of 27 different cytokines and chemokines on 28 paired samples obtained pre- and 30 min post DC-administration from eight patients. IL-1β and IL-6 serum levels were significantly increased after administration. No significant difference could be detected for the other cytokines and chemokines measured. IL-2, IL-5, IL-15, and GM-CSF serum levels were below the limit of detection (data not shown). (B) Illustration of the cytokine and chemokine analysis pre- and 30 min post-DC administration in patient 114. A marked increase in serum IL-1β and IL-6 was accompanied by an increase in the serum concentration of several additional pro-inflammatory cytokines (incl. IL-6, IL-8, IL-17, IFN-γ, and TNF-α) and chemokines (incl. MIP-1α and MIP-1β).
patients), cohort 3 (three patients) and cohort 4 (one patient), indicating that all investigated dose levels of iv-administered DCs are associated with anti-melanoma activity. Because of the small size of the cohorts investigated in this phase IB trial, no definite conclusions can be drawn regarding the optimal dose level of TriMixDC-MEL.

Across the investigated id/iv-cohorts, evidence was found that TriMixDC-MEL administration was immunogenic, inducing detectable CD8\(^+\) and CD4\(^+\) T cell responses in about half of the patients tested. This percentage is comparable with our prior experience in patients receiving TriMixDC-MEL administered solely id, and no correlation was found with the administered iv-dose or the tumor response [16].

Several DC-based immunotherapeutic studies have shown encouraging immunological responses, but durable objective tumor responses in pretreated advanced melanoma patients have remained of anecdotal nature. To our knowledge, this is the first autologous DC-based clinical trial demonstrating a meaningful percentage of objective durable tumor response in pretreated advanced melanoma patients. Intravenous administration of TriMixDC-MEL, therefore, is considered to be a determinant for the observed antitumor activity.

In conclusion, this study demonstrated that iv administration of TriMixDC-MEL is safe, feasible, immunogenic and results in encouraging durable clinical responses. These phase IB data legitimate further phase II studies of iv administration of TriMixDC-MEL.

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disclosure
The authors have declared no conflicts of interest.

references