Published Ahead of Print on February 29, 2016 as 10.1200/JCO.2015.63.4121
The latest version is at http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2015.63.4121

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Phase II Study of Autologous Monocyte-Derived mRNA Electroporated Dendritic Cells (TriMixDC-MEL) Plus Ipilimumab in Patients With Pretreated Advanced Melanoma

Sofie Wilgenhof, Jurgen Corthals, Carlo Heirman, Nicolas van Baren, Sophie Lucas, Pia Kvistborg, Kris Thielemans, and Bart Neyns

Sofie Wilgenhof, Kris Thielemans, and Bart Neyns, Universitair Ziekenhuis Brussel; Sofie Wilgenhof, Jurgen Corthals, Carlo Heirman, Kris Thielemans, and Bart Neyns, Vrije Universiteit Brussel; Nicolas van Baren, Ludwig Institute for Cancer Research; Sophie Lucas, de Duve Institute, Université Catholique de Louvain, Brussels, Belgium; and Pia Kvistborg, The Netherlands Cancer Institute, Amsterdam, the Netherlands.

Published online ahead of print at www.jco.org on February 29, 2016.

Supported by grants from the IWT-Vlaanderen, Interuniversity Attraction Poles Program–Belgian State–Belgian Science Policy, the Stichting tegen Kanker, an Integrated Project and a Network of Excellence sponsored by the European Union, the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO-Vlaanderen), and the Willy Gepts Wetenschappelijk Fonds of the Universitair Ziekenhuis Brussel.

K.T. and B.N. share senior authorship.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Corresponding author: Bart Neyns, MD, PhD, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium; e-mail: Bart.Neyns@uzbrussel.be.

© 2016 by American Society of Clinical Oncology

0732-183X/15/3499-1/$20.00

DOI: 10.1200/JCO.2015.63.4121

ABSTRACT

Purpose

Autologous monocyte-derived dendritic cells (DCs) electroporated with synthetic mRNA (TriMixDC-MEL) are immunogenic and have antitumor activity as a monotherapy in patients with pretreated advanced melanoma. Ipilimumab, an immunoglobulin G1 monoclonal antibody directed against the cytotoxic T-lymphocyte-associated protein 4 receptor that counteracts physiologic suppression of T-cell function, improves the overall survival of patients with advanced melanoma. This phase II study investigated the combination of TriMixDC-MEL and ipilimumab in patients with pretreated advanced melanoma.

Patients and Methods

Thirty-nine patients were treated with TriMixDC-MEL (4×10^6 cells administered intradermally and 20×10^6 cells administered intravenously) plus ipilimumab (10 mg/kg every 3 weeks for a total of four administrations, followed by maintenance therapy every 12 weeks in patients who remained progression free). Six-month disease control rate according to the immune-related response criteria served as the primary end point.

Results

The 6-month disease control rate was 51% (95% CI, 36% to 67%), and the overall tumor response rate was 38% (including eight complete and seven partial responses). Seven complete responses and one partial tumor response are ongoing after a median follow-up time of 36 months (range, 22 to 43 months). The most common treatment-related adverse events (all grades) consisted of local DC injection site skin reactions (100%), transient post–DC infusion chills (38%) and flu-like symptoms (84%), dermatitis (64%), hepatitis (13%), hypophysitis (15%), and diarrhea/colitis (15%). Grade 3 or 4 immune-related adverse events occurred in 36% of patients. There was no grade 5 adverse event.

Conclusion

The combination of TriMixDC-MEL and ipilimumab is tolerable and results in an encouraging rate of highly durable tumor responses in patients with pretreated advanced melanoma.

J Clin Oncol 34. © 2016 by American Society of Clinical Oncology

INTRODUCTION

Antitumor T-cell activation requires interaction of antigen-derived peptides displayed in the context of major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells (APCs) with the T-cell receptor (signal 1) and interaction of the T-cell costimulatory receptor CD28 with its ligands CD80 (B7-1) and CD86 (B7-2) on the APC (signal 2). The cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) receptor is expressed at the surface of T cells and serves to control the early stages of naive and memory T-cell activation by binding CD80 and CD86 on APCs, preventing these two ligands from engaging the CD28 costimulatory T-cell receptor.[1-3] The main physiologic function of CTLA-4 is to downmodulate $CD4^+$ T-helper cell activity and increase regulatory T-cell (Treg)–mediated immunosuppression.[4]

Monoclonal antibodies that inhibit signaling through CTLA-4 (ipilimumab and tremelimumab) have antitumor activity in patients with advanced melanoma.[5-8] Ipilimumab significantly improved the overall survival (OS) of patients with advanced melanoma, either as a monotherapy when compared with a gp100 peptide vaccine or when added to dacarbazine in comparison with dacarbazine monotherapy.[6,9]

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.
Copyright 2016 by American Society of Clinical Oncology

A long-term incremental OS benefit is achieved in approximately 10% to 15% of patients, with a plateau in the survival curve appearing 2 to 3 years after the first dose of ipilimumab.[6,9-11]

The combination of a CTLA-4–blocking monoclonal antibody and a dendritic cell (DC) vaccine or granulocyte-macrophage colony-stimulating factor–producing cellular vaccine (Gvax) demonstrated enhanced activity in mouse models.[12,13] In a phase I clinical trial on 16 patients with melanoma treated with the combination of MART-1 peptide-pulsed DCs and tremelimumab, a higher rate of durable objective tumor responses was observed than what was expected from each agent alone.[14] In addition, patients with melanoma who developed anti–NY-ESO-1 immune responses before or during ipilimumab therapy were found to be more responsive to CTLA-4 blockade.[15]

DCs are professional APCs that play a crucial role in the initiation and regulation of the adaptive immune system.[16] We previously demonstrated that the immune stimulatory capacity of autologous monocyte-derived DCs can be optimized by coelectroporation with mRNA encoding CD40 ligand (CD40L), CD70, and constitutively activated TLR4 (caTLR4; so-called TriMix-DC).[17] Administration of TriMixDC-MEL (a mixture of TriMix-DC coelectroporated with one out of four melanoma-associated antigens fused to an HLA class II targeting signal [gp100, tyrosinase, MAGE-A3, or MAGE-C2 fused to DC.LAMP]) is safe and immunogenic in patients with melanoma.[18-20] In a phase IB study, durable tumor responses were observed in four of 15 patients with pretreated advanced melanoma when administrating TriMixDC-MEL both by the intravenous and intradermal routes.[19] In vitro, TriMix-DCs retain their capacity to mature and secrete IL-12p70 in the presence of ipilimumab. Moreover, ipilimumab significantly increased interleukin (IL) -2 secretion of $CD4^+CD25^-$ T cells upon polyclonal stimulation with TriMix-DC and anti-CD3 beads (Data Supplement). We here report the results of a phase II study investigating the combination of TriMixDC-MEL and ipilimumab in patients with pretreated advanced melanoma.

PATIENTS AND METHODS

Patients

Patients with histologically confirmed, unresectable, American Joint Committee on Cancer (AJCC) stage III or IV melanoma with measurable disease, who experienced treatment failure with at least one prior line of systemic treatment, were eligible. Other key inclusion criteria included the following: age ≥ 18 years; WHO performance status of 0, 1, or 2; normal hematologic, liver, and renal function tests; and negative serologic tests for HIV, syphilis, hepatitis B, and hepatitis C. Exclusion criteria included prior treatment with an anti–CTLA-4 antibody, history of autoimmune disease, primary uveal melanoma, untreated or symptomatic CNS metastasis, and the need for permanent therapeutic anticoagulation. This trial was approved by the institutional ethics committee (ClinicalTrials.gov identifier: NCT01302496). All patients provided signed informed consent.

TriMixDC-MEL Production

Immature DCs were generated by culturing monocytes in the presence of 1% autologous plasma, 1,000 U/mL of granulocyte-macrophage colony-stimulating factor, and 500 U/mL of IL-4. After leukapheresis, monocytes were enriched by plastic adherence. On day 6, DCs were harvested and coelectroporated with TriMix-mRNA (CD40L-, CD70-, and caTLR4-encoding mRNA) and mRNA encoding one of four melanoma-associated antigens (MAGE-A3, MAGE-C2, tyrosinase, or gp100) linked to an HLA class II targeting signal, as reported previously.[21] After electroporation, the four different TriMixDC-MEL cellular constituents (ie, DCs expressing one of the four antigens) were mixed at equal ratios and cryopreserved. DCs were thawed 2 to 3 hours before injection. An in-process quality check and quality control of the final product were carried out, as reported previously (Appendix Table A1, online only).[18]

Study Design

This clinical trial was designed as an open-label, single-arm, single-center, two-stage phase II clinical trial. The primary objective of this study was to estimate the disease control rate (DCR) at 6 months. The DCR is the proportion of patients with a confirmed complete response, partial response, or stable disease according to the immune-related response criteria (irRC).[8] A Simon two-stage design (minimax) was used to test the null hypothesis that the DCR at 6 months was ≤ 33% versus the alternative hypothesis that the DCR at 6 months was ≥ 50%. Using an α error of .05 and a β error of .20, a DCR by irRC at 6 months of greater than seven of 19 patients was needed to continue the study and recruit a final total of 39 patients.[22] If more than 17 patients achieved DCR in the total study population of 39 patients, TriMixDC-MEL plus ipilimumab would be considered worthy of further investigation.

Secondary objectives included safety, estimation of the overall response rate (ORR) by irRC, duration of response, and estimation of the median progression-free survival (PFS) and median OS according to Kaplan-Meier statistics. IBM SPSS software version 22.0 (SPSS, Chicago, IL) was used for the statistical analysis.

Correlation of outcome measurements (best overall response and survival) with baseline covariables (age; sex; performance status; lactate dehydrogenase, C-reactive protein, and absolute lymphocyte count [ALC] measurements; AJCC stage; and baseline presence of brain metastases) and surrogate covariables (development of grade 3 or 4 immune-related adverse events) was analyzed by cross-tabs statistics (two-sided Fisher's exact test) and for survival by univariate analysis (log-rank test) and multivariate analysis (Cox logistic regression).

Treatment Schedule

Eligible patients received ipilimumab intravenously at a dose of 10 mg/kg over a period of 90 minutes once every 3 weeks for four doses (induction phase of the treatment plan, weeks 1 to 24). Patients with stable disease or an objective response at the tumor response assessment at week 24 were eligible to enter a maintenance phase in which ipilimumab was continued every 12 weeks until progression, unacceptable toxicity, patient refusal to continue treatment, or maximal treatment duration of 3 years.

One hour after the end of the ipilimumab administration, TriMixDC-MEL was administered intravenously (20×10^6 DCs) and intradermally (4×10^6 DCs), as previously described.[19] In the first 18 patients, one TriMixDC-MEL administration was performed 2 weeks before combined administration with ipilimumab. Thereafter, the first TriMixDC-MEL administration was eliminated after an amendment to the protocol. The administration schedule is shown in the Data Supplement (online only).

Immunologic Assessments

Peripheral-blood mononuclear cells were collected by leukapheresis (before treatment) and by 10-mL whole blood collection in week 6 or 9 and a buffy coat in week 12. Phenotypic and functional markers of T cells were evaluated by flow cytometry. The frequency of Tregs was assessed by quantification of demethylated *FOXP3i1* sequences using a methylation-sensitive quantitative polymerase chain reaction assay on bisulfite-treated genomic DNA as previously reported.[23] Antigen-specific $CD8^+$ T cells in HLA-A*0201 patients were analyzed by peptide-MHC multimer-based monitoring as previously reported[24] (Data Supplement).

Assessment of Tumor Response and Toxicity

Tumor assessments were performed by whole-body 2-fluoro-2-deoxy-D-glucose–positron emission tomography/computed tomography

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

Table 1. Baseline Patient Demographic and Clinical Characteristics

Characteristic	No. of Patients (%)
Sex	
Female	16 (41)
Male	23 (59)
Age, years	
Mean	46
Range	24-70
WHO-PS	
0	26 (67)
1	9 (23)
2	4 (10)
Stage	
IIIc	1 (2.6)
IV-M1a	6 (15)
IV-M1b	4 (10)
IV-M1c	28 (72)
Brain metastases at baseline	7 (20)
Lactate dehydrogenase	
≤ ULN	24 (62)
> ULN	15 (38)
Absolute lymphocyte count	
≥ 1,000/μL	29 (74)
< 1,000/μL	10 (26)
C-reactive protein	
≤ ULN	22 (66)
>ULN	17 (44)
Prior treatments	
Cytotoxic chemotherapy	18 (46)
BRAF inhibitor	20 (51)
MEK inhibitor	3 (7.7)
Interferon alfa-2b	7 (18)
Dendritic cells (intradermally)	4 (10)

Abbreviations: WHO-PS, WHO performance status; ULN, upper limit of normal

at baseline and every 12 weeks thereafter. Objective response was evaluated using irRC.[8] Safety was assessed continuously throughout the study. Clinical and blood parameters were assessed every 3 weeks. Adverse events (AEs) were graded for severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0).

RESULTS

Patients

Between May 2011 and November 2013, 39 patients with pretreated advanced melanoma were enrolled onto the study and initiated treatment with TriMixDC-MEL plus ipilimumab. Baseline patient demographics and clinical characteristics are listed in Table 1. Thirty-two patients (82%) had metastases to visceral sites (AJCC stage IV-M1b in four patients and stage IV-M1c in 28 patients), including metastasis to the brain in seven patients (20%). Fifteen patients (38%) had an elevated lactate dehydrogenase serum level, and 10 patients (26%) had an ALC of less than 1,000 lymphocytes/μL. Eighteen patients (51%) had previously experienced disease progression on BRAF inhibitor therapy, and 18 patients (46%) had experienced progression on cytotoxic chemotherapy.

Study Treatment Disposition

Database lock occurred on June 1, 2015, after a minimum follow-up of 18 months. Twenty-four patients (62%) completed the induction phase according to the protocol. Fifteen patients (38%) discontinued treatment during the induction phase (seven patients [18%] because of progressive disease and eight patients [20%] because of toxicity). Twenty patients (51%) initiated ipilimumab administrations in the maintenance phase of the study. Sixteen patients stopped ipilimumab maintenance administrations because of progressive disease (n = 12; 31%) or toxicity (n = 4; 10%). Two additional patients ended treatment 3 years after the first dosing of ipilimumab (both were in a complete remission). Ipilimumab maintenance treatment is ongoing in two patients. The median number of ipilimumab administrations in the maintenance phase is four (range, one to 11 administrations).

Safety

Grade 2 TriMixDC-MEL dermal injection site reactions were observed in all patients. Chills (grade 1 to 2) occurring within the

Table 2. Treatment-Related AEs

Treatment-Related AE	No. of Patients (%)		
	Grade 1/2 AEs	Grade 3 AEs	Grade 4 AEs
TriMixDC-MEL related			
Injection site reaction	39 (100)	0	0
Postinfusion chills (< 1 hour after infusion)	15 (38.5)	0	0
Flu-like symptoms (< 72 hours after administration)	33 (84.6)	0	0
Immune-related adverse events			
Dermatitis	23 (59.0)	2 (5.1)	0
Colitis/diarrhea	4 (10.2)	2 (5.1)	0
Hepatitis	0	4 (10.2)	1 (2.6)
Hypophysitis, hypopituitarism	3 (7.7)	3 (7.7)	0
Pneumonitis	0	3 (7.7)*	0
Sarcoid-like lymphadenopathy	5 (12.8)†	0	0
Colonic pseudo-obstruction	0	1 (2.6)	0
Muscle pain and elevated CRP/ESR	0	1 (2.6)	0

Abbreviations: AEs, adverse events; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
*All three patients diagnosed with pneumonitis presented with fever, elevated CRP, normal to slightly elevated WBC count, and negative microbiologic cultures and serology. All patients recovered within 3 weeks on antibiotic therapy without need for corticoids or immunosuppressive therapy. However, one of these three patients experienced recurrent pneumonitis when she was retreated with ipilimumab and TriMixDC-MEL.
†Five patients were diagnosed with sarcoid-like symmetric fluorodeoxyglucose-avid hilar and mediastinal adenopathies on the positron emission tomography/computed tomography assessment in week 12. In all but two patients, there was a complete normalization on imaging by week 24.

© 2016 by American Society of Clinical Oncology

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

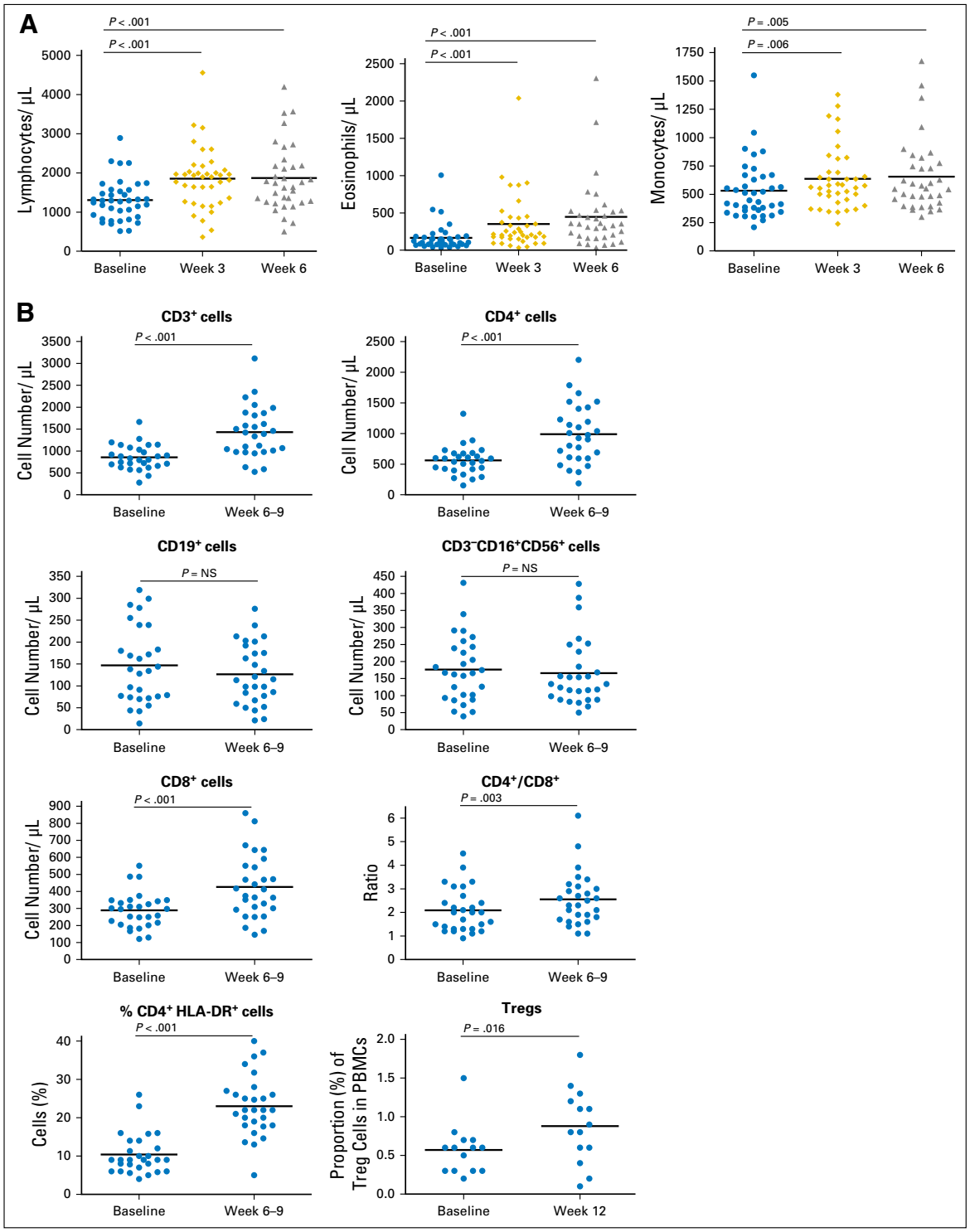

Fig 1. Absolute leukocyte counts and immunomonitoring in patients treated with TriMixDC-MEL and ipilimumab. (A) Absolute counts of lymphocytes, eosinophils, and monocytes in peripheral blood during the induction phase of the study treatment. (B) Peripheral-blood immunophenotyping. $CD3^+$ cells, $CD4^+$ cells, $CD8^+$ cells, $CD19^+$ cells, $CD3^-CD16^+CD56^+$ cells, and the percentage of $CD4^+HLA\text{-}DR^+$ cells were analyzed by flow cytometric analysis of peripheral-blood mononuclear cells (PBMCs) in 28 patients at baseline and between weeks 6 and 9 during treatment with TriMixDC-MEL and ipilimumab. Frequencies of circulating regulatory T cells (Tregs) were analyzed in 14 patients at baseline and week 12 during treatment with TriMixDC-MEL and ipilimumab by *FOXP3* methylation-specific quantitative polymerase chain reaction assay on PBMC. Statistical analysis was performed by a paired, two-sided *t* test. (C) Overview of $CD8^+$ T-cell responses detected in 10 patients with HLA-A*0201 melanoma before (pre) and after (post) treatment. Color code: blue, 0.005% to 0.099%; gold, 0.1% to 0.99% peptide–major histocompatibility complex multimer-positive $CD8^+$ of total $CD8^+$ cells. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease. (D) Immunohistochemical analysis of frozen specimens from a regressing subcutaneous melanoma metastasis. (a) Hematoxylin and eosin stain; abundant infiltration by (b) $CD3^+$ and (c) $CD8^+$ lymphocytes; (d) scarcity of residual melanoma cells stained with panMEL antibody cocktail; and residual tumor cells express cleaved caspase-3 (an apoptosis marker) at (e) low and (f) high magnification.

 © 2016 by American Society of Clinical Oncology

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

C

Patient	028		053		122		127		130		143		150		152		160		171	
Time point	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
MART-1																				
gp100																				
Trp2																				
MAGE-C2																				
Best objective response	PR		CR		PD		SD		CR		CR		PD		PR		CR		PD	
Overall survival (months)	40+		23+		11		38+		43+		33+		8		14		33+		13	

0.005%–0.099%
0.1%–0.99%

D

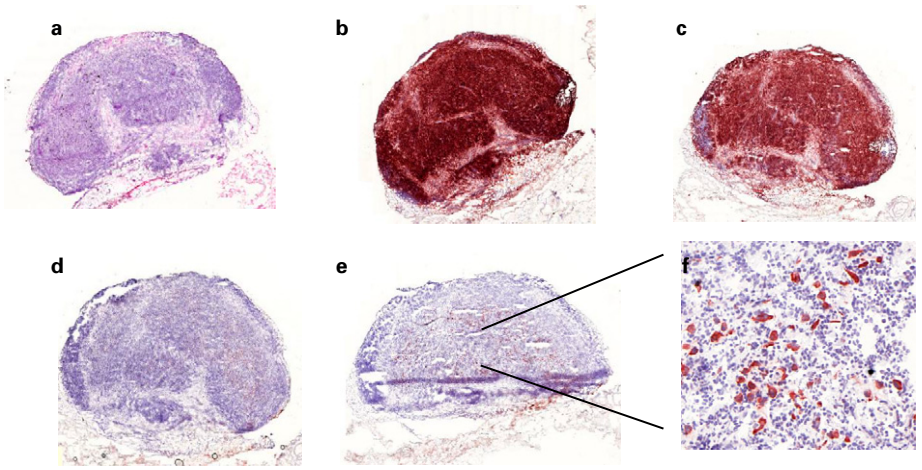

Fig 1. (continued)

first hour after intravenous infusion of TriMixDC-MEL occurred in 38% of patients, and mild flu-like symptoms (within the first 72 hours after infusion) occurred in 85% of patients. Clinically significant related AEs are listed in Table 2 and in the Data Supplement. A total of 14 patients (36%) developed treatment-related grade 3 or 4 AEs. All immune-related AEs were managed using established treatment algorithms.[25] Grade 1 to 2 dermatitis (diffuse itching, erythema) was the most frequent AE and was reported by 64% of patients. Systemic corticosteroids were required to manage immune-related AEs in 18 patients (46%). Mycophenolate mofetil was used in one patient with grade 4 hepatitis. No treatment-related deaths occurred. Treatment-related AEs were completely reversible in all patients except for hypopituitarism requiring continued hormonal substitution in all affected patients.

Peripheral-Blood Cells and Immunomonitoring

No differences in lymphocyte, eosinophil, or monocyte counts were observed 2 weeks after the first DC administration in the first 18 patients on the trial (data not shown). A significant increase in peripheral-blood lymphocytes, eosinophils, and monocytes was observed at the second and third administration of TriMixDC-MEL plus ipilimumab, compared with baseline. Flow cytometric analysis of peripheral-blood mononuclear cells was performed in 28 of the 39 patients (in 11 patients with early progressive disease, no sample could be obtained in week 12). A significant increase in $CD3^+$, $CD4^+$, and $CD8^+$ cells as well as the ratio of $CD4^+$ to $CD8^+$ cells was documented after induction treatment with TriMixDC-MEL and ipilimumab. There was a significant increase of the HLA-DR^+ activation marker on $CD4^+$ cells. No changes in the number of $CD19^+$ cells (B cells) or $CD3^+CD16^+CD56^+$ (natural killer cells) were observed. An increase of the frequency of Tregs in the peripheral blood (assessed by quantification of demethylated *FOXP3i1* sequences using a methylation-sensitive quantitative polymerase chain reaction assay on bisulfite-treated genomic DNA) was observed in 11 of the 14 patients.

Of the 10 HLA-A*0201–positive patients who could be analyzed for antigen-specific $CD8^+$ T-cell responses, one was found to have pre-existing responses against MART-1 and gp100, and these responses persisted at week 12. In two patients, novel antigen-specific $CD8^+$ T-cell responses (against MACE-C2 and trp-2) were detected. In one HLA-A*0201–negative patient, immunohistochemical analysis of a formalin-fixed paraffin-embedded regressing subcutaneous melanoma metastasis showed a dense infiltrate of $CD8^+$ T cells with sparse melanoma cells. The absolute leukocyte counts and immunomonitoring are summarized in Figure 1.

Tumor Response and Duration of Response

The investigator-assessed best ORR by irRC was 38%, including eight patients (20%) with a complete response and seven patients (18%) with a partial response (all responses were confirmed after an

© 2016 by American Society of Clinical Oncology

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

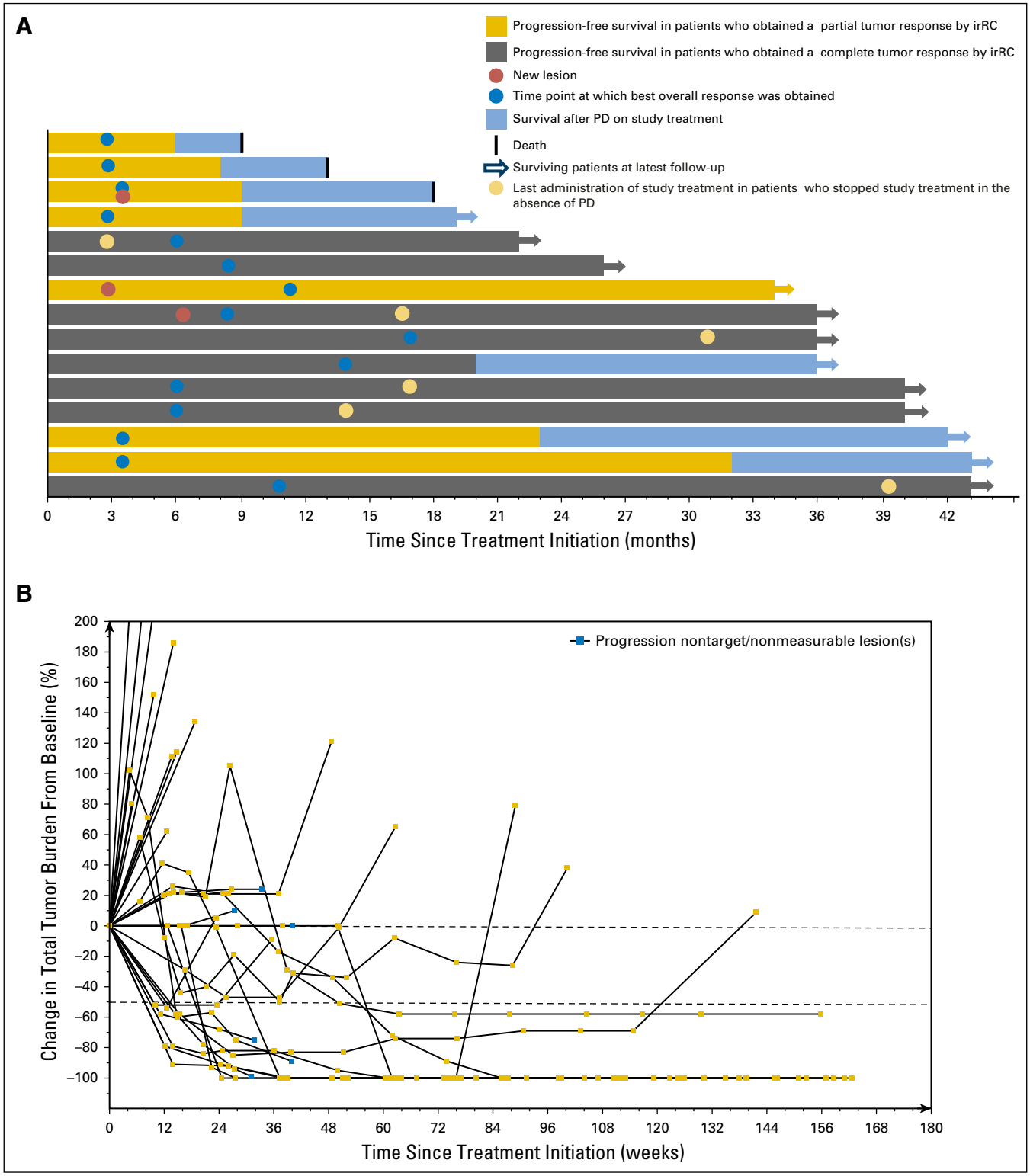

Fig 2. Tumor response characteristics. (A) Swimmer plot representing the survival, progression-free survival, and response kinetics of patients with a partial or complete response according to the immune-related response criteria (irRC). In three patients, new lesions were observed. In two patients, a new solitary brain metastasis was diagnosed after 3 and 6 months, respectively, of initiating TriMixDC-MEL plus ipilimumab and was treated successfully by stereotactic radiotherapy. In an additional patient, evaluation at week 12 showed complete remission of all baseline target lesions (lung metastases) in the presence of a new fluorodeoxyglucose-avid abdominal lymph node, which spontaneously completely regressed at the subsequent evaluation in week 24. (B) Spider plot illustrating the change in total tumor burden of 31 patients treated with TriMixDC-MEL and ipilimumab. In eight patients, rapid clinical progression occurred without scanographic evaluation. PD, progressive disease.

 © 2016 by American Society of Clinical Oncology

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

Table 3. Tumor Response by irRC and Duration of Response

Best Response by irRC	No. of Patients (%)	Duration of Response (months)*
CR	8 (20.5)	20, 22+, 26+, 36+, 36+, 40+, 41+, 43+
PR	7 (17.9)	6, 8, 10, 11, 24, 33, 34+
SD	6 (15.4)	5, 7, 8, 11, 14, 20
Progressive disease	18 (46.2)	
Best objective response rate CR + PR	15 (38.5)	
Disease control rate CR + PR + SD	21 (53.8)	

Abbreviations: CR, complete response; irRC, immune-related response criteria; PR, partial response; SD, stable disease.
*Plus signs indicate tumor response ongoing at the latest follow-up.

interval of at least 4 weeks). Seven of the eight complete responses and one of the seven partial responses were ongoing at the time of this analysis (Fig 2). Tumor responses were observed at different anatomic sites (Data Supplement). Median follow-up time for patients with an ongoing response is 36 months (range, 22 to 43 months). Response rates and the duration of response are listed in Table 3. Tumor responses occurred by an atypical pattern in four patients (10%). An additional six patients (15%) experienced stable disease that persisted for a median of 9.5 months (range, 5 to 20 months).

OS and PFS

The DCR at 6 months (by irRC) was 51% (95% CI, 36% to 67%). At the time of data analysis, a total of 31 patients (79%) had been diagnosed with progression of disease. The estimated median PFS was 27 weeks (95% CI, 9 to 44 weeks). The 1-, 2-, and 3-year PFS rates were 33% (95% CI, 18% to 48%), 22% (95% CI, 9% to 36%), and 18% (95% CI, 5% to 31%), respectively (Fig 3A).

Twenty-five patients (64%) have died, all as a result of progressive disease. The median follow-up time for the 14 surviving patients was 157 weeks (range, 72 to 185 weeks). The estimated median OS was 59 weeks (95% CI, 40 to 79 weeks; Fig 3C). The 1-, 2-, and 3-year OS rates were 59% (95% CI, 43% to 74%), 38% (95% CI, 23% to 53%), and 34% (95% CI, 19% to 50%), respectively.

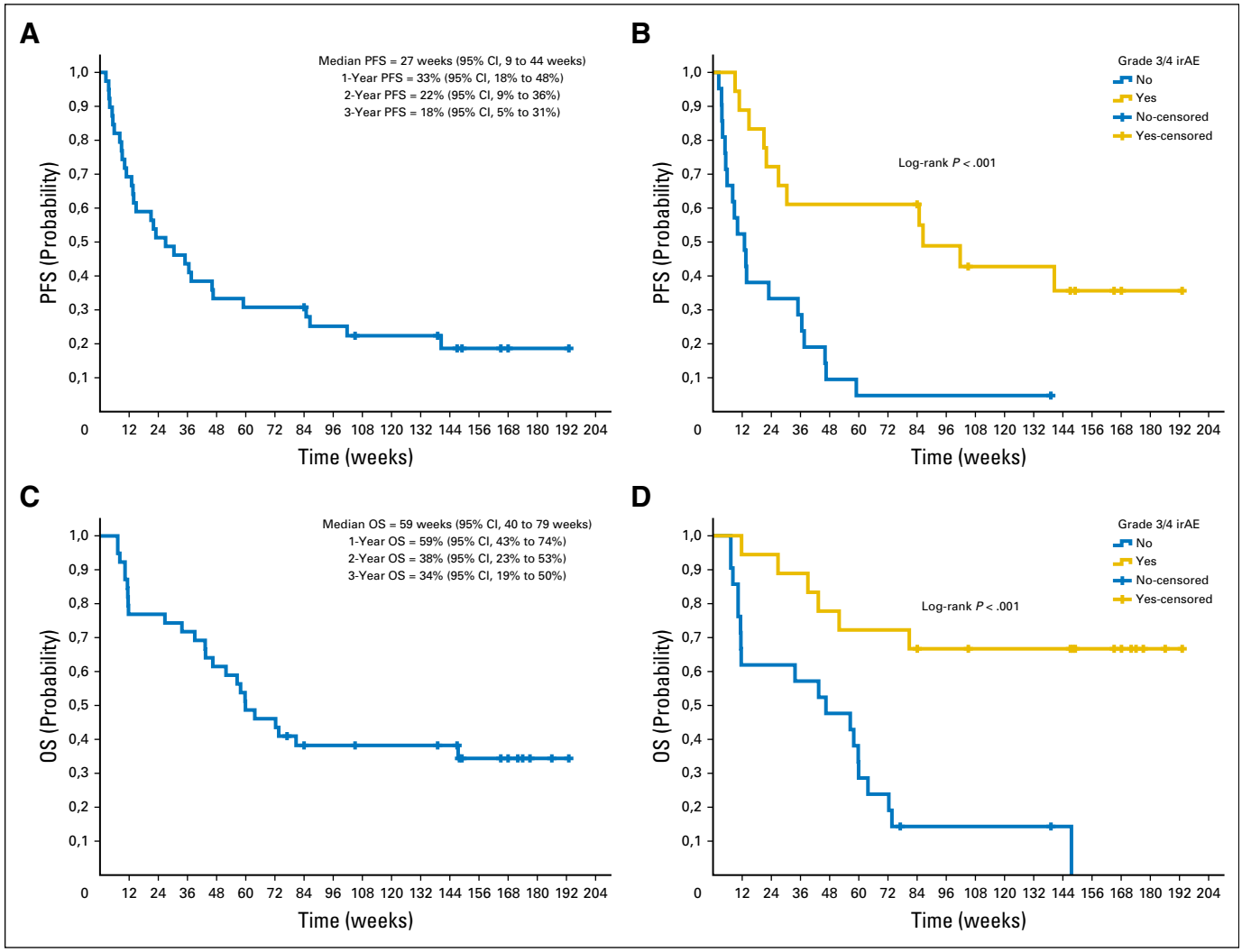

Fig 3. Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS) in patients treated with the combination of monocyte-derived autologous mRNA electroporated dendritic cells (TriMixDC-MEL) and ipilimumab. (A) PFS for the total study population (N = 39) and (B) according to the incidence of grade 3 or 4 immune-related adverse events (irAEs). (C) OS for the total study population and (D) according to the incidence of grade 3 or 4 irAEs.

© 2016 by American Society of Clinical Oncology

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

No significant correlation was observed between PFS and baseline variables. OS was significantly better in the subgroup of patients with AJCC stage III or IV-M1a-b disease (compared with patients with AJCC stage IV-M1c disease) and worse in patients with brain metastases or a baseline ALC of less than 1,000/μL (Data Supplement). Patients who experienced grade 3 or 4 immune-related AEs (and were treated with systemic corticosteroids) had a significantly better PFS and OS (Fig 3B and 3D). OS was also superior in patients with a more than 2.5-fold increase over baseline counts of eosinophils and a 1.5-fold increase of monocytes at the third administration of TriMixDC-MEL plus ipilimumab (Data Supplement).

DISCUSSION

This single-arm phase II clinical trial met its primary end point demonstrating a 51% 6-month DCR in patients with pretreated advanced melanoma who were treated with an autologous monocyte-derived synthetic mRNA electroporated DC therapy (TriMixDC-MEL) in combination with the CTLA-4–blocking monoclonal antibody ipilimumab. Of particular interest is the high rate of patients who achieved an objective tumor response (ORR, 38%; including 20% of complete responses). The observed antitumor activity compares favorably with the ORR observed with ipilimumab monotherapy in numerous prospective clinical trials in patients with pretreated advanced melanoma, and the observed frequency of complete responses is in range with the rate reported for the combination of ipilimumab and nivolumab.[6,7,11,26-28] These results were obtained despite the poor baseline prognostic factors of our study population. Unlike previous studies with ipilimumab, 20% of patients had previously been treated for brain metastases, and more than half of all patients experienced prior progression on a BRAF inhibitor therapy.

An encouraging duration of response was observed in patients obtaining a CR. Seven of eight complete responders and one patient with a partial response remained free from progression at their latest follow-up, 22 to 43 months after the initiation of study treatment. The 2- and 3-year survival rates compare favorably with the long-term outcomes of prospective clinical trials of ipilimumab monotherapy in patients with pretreated advanced melanoma.[6,7,10] In accordance with previous observations, a correlation was found between an increase in eosinophil counts and survival, but not with an increase in the ALC.[29,30] The clinical results obtained in our study may be a result of enhanced T-cell stimulation by the combination of ipilimumab and TriMixDC-MEL, with enhanced $CD4^+$ T-cells stimulation in particular, as indicated by higher level of IL-2 secretion by $CD4^+$ T cells when stimulated by TriMix-DC in vitro and the increased ALCs (both $CD8^+$ and $CD4^+$ cells) in vivo with an increased expression of the activation marker $HLA\text{-}DR^+$ on $CD4^+$ T cells. The importance of IL-2 in contributing to the antitumor effect of ipilimumab has recently been demonstrated.[31] Using a peptide-MHC multimer-based assay, no evidence was found for a higher frequency of melanoma antigen-specific T cells in the 10 HLA-A*0201 patients analyzed in our trial, as compared with historical data on patients treated with ipilimumab monotherapy.[24]

The incidence of grade ≥ 3 immune-related AEs on ipilimumab monotherapy has been approximately 20% to 30% in patients with advanced melanoma.[6,7] Recently, an incidence of 43% has been reported in an adjuvant phase III trial with ipilimumab.[32] An incidence of 36% was observed with TriMixDC-MEL and ipilimumab. No increase in AEs related to TriMixDC-MEL and no clear increase in incidence for the most common ipilimumab-related AEs were observed. All AEs could be successfully managed following established treatment algorithms. A higher incidence (13%) was observed of grade 1 to 2 sarcoid-like adenopathy, a syndrome that has been reported with ipilimumab monotherapy with an incidence of less than 1%.[33-41] The three instances of pneumonitis that occurred on this trial are suspected to be treatment-related manifestations of immune-related injury to the lung. Unlike the pneumonitis occurrences (< 1%) reported with ipilimumab or treatment with an anti-PD1 monoclonal antibody, full recovery occurred without need for immunosuppressive therapy.

Although patients with baseline AJCC stage IV-M1c or brain metastases had a worse outcome, patients experiencing grade 3 or 4 AEs had an improved survival. Such correlation has been reported previously for ipilimumab monotherapy but did not reach significance.[42] Resembling previous observations on patients treated with corticosteroids to control immune-related AEs related to ipilimumab, we suspect no detrimental effect from our observations.[43] On the basis of our observations, combinations of autologous TriMix-DC therapy and checkpoint inhibitors deserve further evaluation in prospective clinical trials.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Kris Thielemans, Bart Neyns
Financial support: Kris Thielemans, Bart Neyns
Administrative support: Kris Thielemans, Bart Neyns
Provision of study materials or patients: Kris Thielemans, Bart Neyns
Collection and assembly of data: Sofie Wilgenhof, Jurgen Corthals, Carlo Heirman, Nicolas van Baren, Sophie Lucas, Kris Thielemans, Bart Neyns
Data analysis and interpretation: Sofie Wilgenhof, Nicolas van Baren, Sophie Lucas, Pia Kvistborg, Kris Thielemans, Bart Neyns
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Krummel MF, Allison JP: CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J Exp Med 183: 2533-2540, 1996

2. Qureshi OS, Zheng Y, Nakamura K, et al: Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4. Science 332:600-603, 2011

3. Krummel MF, Allison JP: Pillars article: CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. The journal of experimental medicine. 1995. 182: 459-465. J Immunol 187: 3459-3465, 2011

4. Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12: 252-264, 2012

5. Ribas A, Kefford R, Marshall MA, et al: Phase III randomized clinical trial comparing tremelimumab

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol 31:616-622, 2013

6. Hodi FS, O'Day SJ, McDermott DF, et al: Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711-723, 2010

7. Wolchok JD, Neyns B, Linette G, et al: Ipilimumab monotherapy in patients with pretreated advanced melanoma: A randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol 11:155-164, 2010

8. Wolchok JD, Hoos A, O'Day S, et al: Guidelines for the evaluation of immune therapy activity in solid tumors: Immune-related response criteria. Clin Cancer Res 15:7412-7420, 2009

9. Robert C, Thomas L, Bondarenko I, et al: Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 364:2517-2526, 2011

10. Schadendorf D, Hodi FS, Robert C, et al: Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol 33:1889-1894, 2015

11. Postow MA, Callahan MK, Wolchok JD: Immune checkpoint blockade in cancer therapy. J Clin Oncol 33:1974-1982, 2015

12. Met O, Wang M, Pedersen AE, et al: The effect of a therapeutic dendritic cell-based cancer vaccination depends on the blockage of CTLA-4 signaling. Cancer Lett 231:247-256, 2006

13. van Elsas A, Hurwitz AA, Allison JP: Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med 190:355-366, 1999

14. Ribas A, Comin-Anduix B, Chmielowski B, et al: Dendritic cell vaccination combined with CTLA4 blockade in patients with metastatic melanoma. Clin Cancer Res 15:6267-6276, 2009

15. Yuan J, Gnjatic S, Li H, et al: CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. Proc Natl Acad Sci USA 105: 20410-20415, 2008

16. Palucka K, Banchereau J: Dendritic-cell-based therapeutic cancer vaccines. Immunity 39:38-48, 2013

17. Bonehill A, Tuyaerts S, Van Nuffel AM, et al: Enhancing the T-cell stimulatory capacity of human dendritic cells by co-electroporation with CD40L, CD70 and constitutively active TLR4 encoding mRNA. Mol Ther 16:1170-1180, 2008

18. Wilgenhof S, Van Nuffel AM, Corthals J, et al: Therapeutic vaccination with an autologous mRNA electroporated dendritic cell vaccine in patients with advanced melanoma. J Immunother 34:448-456, 2011

19. Wilgenhof S, Van Nuffel AM, Benteyn D, et al: A phase IB study on intravenous synthetic mRNA electroporated dendritic cell immunotherapy in pretreated advanced melanoma patients. Ann Oncol 24: 2686-2693, 2013

20. Van Nuffel AM, Benteyn D, Wilgenhof S, et al: Dendritic cells loaded with mRNA encoding full-length tumor antigens prime CD4+ and CD8+ T cells in melanoma patients. Mol Ther 20:1063-1074, 2012

21. Bonehill A, Van Nuffel AM, Corthals J, et al: Single-step antigen loading and activation of dendritic cells by mRNA electroporation for the purpose of therapeutic vaccination in melanoma patients. Clin Cancer Res 15:3366-3375, 2009

22. Simon R: Optimal two-stage designs for phase II clinical trials. Control Clin Trials 10:1-10, 1989

23. de Vries IJ, Castelli C, Huygens C, et al: Frequency of circulating Tregs with demethylated FOXP3 intron 1 in melanoma patients receiving tumor vaccines and potentially Treg-depleting agents. Clin Cancer Res 17:841-848, 2011

24. Kvistborg P, Philips D, Kelderman S, et al: Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response. Sci Transl Med 6:254ra128, 2014

25. Weber JS, Kähler KC, Hauschild A: Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol 30: 2691-2697, 2012

26. O'Day SJ, Maio M, Chiarion-Sileni V, et al: Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: A multicenter single-arm phase II study. Ann Oncol 21: 1712-1717, 2010

27. Larkin J, Chiarion-Sileni V, Gonzalez R, et al: Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 373: 23-34,2015

28. Postow MA, Chesney J, Pavlick AC, et al: Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med 372:2006-2017, 2015

29. Delyon J, Mateus C, Lefeuvre D, et al: Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: An early increase in lymphocyte and eosinophil counts is associated with improved survival. Ann Oncol 24: 1697-1703, 2013

30. Ku GY, Yuan J, Page DB, et al: Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting: Lymphocyte count after 2 doses correlates with survival. Cancer 116:1767-1775, 2010

31. Hannani D, Vétizou M, Enot D, et al: Anticancer immunotherapy by CTLA-4 blockade: Obligatory contribution of IL-2 receptors and negative prognostic impact of soluble CD25. Cell Res 25: 208-224, 2015

32. Eggermont AM, Chiarion-Sileni V, Grob JJ, et al: Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): A randomised, double-blind, phase 3 trial. Lancet Oncol 16:522-530, 2015

33. Wilgenhof S, Morlion V, Seghers AC, et al: Sarcoidosis in a patient with metastatic melanoma sequentially treated with anti-CTLA-4 monoclonal antibody and selective BRAF inhibitor. Anticancer Res 32:1355-1359, 2012

34. Vogel WV, Guislain A, Kvistborg P, et al: Ipilimumab-induced sarcoidosis in a patient with metastatic melanoma undergoing complete remission. J Clin Oncol 30:e7-e10, 2012

35. Tissot C, Carsin A, Freymond N, et al: Sarcoidosis complicating anti-cytotoxic T-lymphocyte-associated antigen-4 monoclonal antibody biotherapy. Eur Respir J 41:246-247, 2013

36. Reule RB, North JP: Cutaneous and pulmonary sarcoidosis-like reaction associated with ipilimumab. J Am Acad Dermatol 69:e272-e273, 2013

37. Murphy KP, Kennedy MP, Barry JE, et al: New-onset mediastinal and central nervous system sarcoidosis in a patient with metastatic melanoma undergoing CTLA4 monoclonal antibody treatment. Oncol Res Treat 37:351-353, 2014

38. Marlier J, Cocquyt V, Brochez L, et al: Ipilimumab, not just another anti-cancer therapy: Hypophysitis as side effect illustrated by four case-reports. Endocrine 47:878-883, 2014

39. Berthod G, Lazor R, Letovanec I, et al: Pulmonary sarcoid-like granulomatosis induced by ipilimumab. J Clin Oncol 30:e156-e159, 2012

40. Andersen R, Nørgaard P, Al-Jailawi MK, et al: Late development of splenic sarcoidosis-like lesions in a patient with metastatic melanoma and long-lasting clinical response to ipilimumab. Oncoimmunology 3:e954506, 2014

41. Eckert A, Schoeffler A, Dalle S, et al: Anti-CTLA4 monoclonal antibody induced sarcoidosis in a metastatic melanoma patient. Dermatology 218: 69-70, 2009

42. Lutzky J, Wolchok J, Hamid O, et al: Association between immune-related adverse events (irAEs) and disease control or overall survival in patients (pts) with advanced melanoma treated with 10 mg/kg ipilimumab in three phase II clinical trials. J Clin Oncol 27, 2009 (suppl; abstr 9034)

43. Amin A, DePril V, Hamid O, et al: Evaluation of the effect of systemic corticosteroids for the treatment of immune-related adverse events (irAEs) on the development or maintenance of ipilimumab clinical activity. J Clin Oncol 27, 2009 (suppl; abstr 9037)

© 2016 by American Society of Clinical Oncology

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Phase II Study of Autologous Monocyte-Derived mRNA Electroporated Dendritic Cells (TriMixDC-MEL) Plus Ipilimumab in Patients With Pretreated Advanced Melanoma

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or jco.ascopubs.org/site/ifc.

Sofie Wilgenhof
No relationship to disclose

Jurgen Corthals
No relationship to disclose

Carlo Heirman
Employment: eTheRNA
Stock or Other Ownership: eTheRNA

Nicolas van Baren
Research Funding: iTeos Therapeutics (Inst)
Patents, Royalties, Other Intellectual Property: Holds patents and receives royalties from discoveries related to tumor antigens (Inst)

Sophie Lucas
Patents, Royalties, Other Intellectual Property: Patent pending in a project not related to this publication

Pia Kvistborg
Consulting or Advisory Role: Neon Therapeutics

Kris Thielemans
Stock or Other Ownership: eTheRNA
Patents, Royalties, Other Intellectual Property: The use of dendritic cells electroporated with tumor antigen mRNA and TriMix is the topic of a patent (W2009/034172) on which Kris Thielemans is filed as inventor (Inst)

Bart Neyns
Honoraria: Novartis, Bristol-Myers Squibb, Merck Sharp & Dohme, Amgen, Roche, GlaxoSmithKline
Research Funding: Pfizer (Inst), Novartis (Inst), Merck Serono (Inst), Roche (Inst)

© 2016 by American Society of Clinical Oncology

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.

Acknowledgment

We thank the patients who participated in the study and their families and caregivers; Katrien Van den Bossche and Kathleen Mooren for assisting us with the data management; and Elsy Vaeremans, Petra Roman, and Chiraz Mahmoud for their technical assistance in the preparation of the TriMixDC-MEL products.

Appendix

Table A1. Quality Control Assessments of TriMixDC-MEL

Assessment	Median	Range
Expression, %		
CD70	68.5	38.7-90.8
CD40	66.9	47.3-96.2
CD80	47.1	24.2-80.6
CD83	62.3	43.4-83.2
CCR7	43.6	26.1-87.7
CD14	6.5	1.5-18.3
Viability, %	89.4	74.5-94
Purity, %	64.9	39.1-87.7
IL-12p70 secretion 0-24 hours, pg/mL	431	8-18,870

© 2016 by American Society of Clinical Oncology

Downloaded from jco.ascopubs.org by Bart Neyns on March 1, 2016 from 144.248.22.241
Copyright © 2016 American Society of Clinical Oncology. All rights reserved.