Association of homogeneous inflamed gene signature with a better outcome in patients with metastatic melanoma treated with MAGE-A3 immunotherapeutic

Jean-François Baurain,1 Caroline Robert,2 Laurent Mortier,3 Bart Neyns,4 Florent Grange,5 Céleste Lebbe,6 Fernando Ulloa-Montoya,7 Pedro Miguel De Sousa Alves,8 Marc Gillet,7 Jamila Louahed,7 Silvija Jarnjak,7 Frédéric F Lehmann9

ABSTRACT

Purpose This study assessed clinical activity, safety and immunogenicity of MAGE-A3 immunotherapeutic in patients with MAGE-A3-positive metastatic melanoma.

Patients and methods In this open-label, multicentre, uncontrolled, Phase II study (ClinicalTrials.gov NCT00896480), patients received ≤24 doses of MAGE-A3 immunotherapeutic (4-cycle schedule). At screening, two skin lesions were biopsied for MAGE-A3 expression analysis and presence/absence of a previously identified gene signature (GS) associated with favourable clinical outcome. Clinical activity was assessed in terms of clinical response, time-to-treatment failure (TTF) and progression-free survival (PFS). Adverse events (AEs) and serious AEs (SAEs) were recorded. MAGE-A3-specific immune responses were assessed. Clinical activity and immunogenicity were analysed overall and separately in patients with 2/2 (GS++/+), 1/2 (GS+/-) or 0/2 (GS-/-) biopsies presenting GS.

Results Of 49 screened patients, 32 had MAGE-A3-positive tumours; 24 (8 GS++/+, 8 GS+/-, 8 GS-/-) were treated. Two complete (GS++/+ patients) and two partial responses (one GS++/+, one GS+/-) were reported; of note, one of the two complete responses was unlikely to be related to the study treatment. Median TTF and PFS were 14.8 and 7.2 months for GS++/+, 2.3 and 2.8 months for GS+/- and 2.4 and 2.9 months for GS-/- patients. Three grade 3 AEs and two SAEs unrelated to treatment were reported. All patients were seropositive for MAGE-A3 antibodies on vaccination with no differences between the different GS profiles. MAGE-A3-specific CD4+ and CD8+ T cell immunogenicity was detected; 12/16 (75.0%) of patients presented CD4+ T cell responses.

Conclusion Treatment with MAGE-A3 immunotherapeutic showed signs of clinical activity in GS++/+ patients. Treatment was well tolerated and immunogenic. No differences in immune responses according to GS status were observed.

Trial registration number NCT00896480 (Results).

Key questions

What is already known about this subject?
► Immuno-oncology therapeutics are based on the function of the immune system to fight cancer. Immune-checkpoint inhibitors are drugs that reactivate the immune response against tumour cells. The introduction of immune-checkpoint inhibitors has changed the treatment landscape of metastatic melanoma and shown long-term efficacy in subset of patients. Cancer vaccines targeting tumour antigens are also considered a promising treatment approach; however, they have fallen short of expectations with respect to their clinical efficacy. A pretreatment gene signature (GS) associated with a clinical response to the tumour antigen MAGE-A3 immunotherapeutic has been previously described in a Phase II study (JCO, 2013).

What does this study add?
► We assessed tumour MAGE-A3 expression and GS heterogeneity by collecting two biopsies prior treatment. Administration of MAGE-A3 vaccine showed clinical activity in patients presenting the GS in both biopsies and was associated with a good MAGE-A3-specific CD4+ and weak CD8+ postvaccination response.

How might this impact on clinical practice?
► MAGE-A3 vaccine may be a candidate for immune-oncology combination therapy in patients with few mutated or viral antigens.

INTRODUCTION

Cutaneous melanoma is the most aggressive form of skin cancer with incidence increasing worldwide over the past 50 years, especially in fair-skinned populations; currently about 132,000 melanoma skin cancers occur globally each year.1 Patients with Stage III–IV malignant melanoma have an unfavourable
prognosis, with a 5-year survival of <15% and a median survival for patients with distant metastases of less than 1 year and only 4 months for patients with brain metastases.5-3

Melanoma is a highly heterogeneous neoplasm composed of irregular zones of actively proliferating and quiescent cells; these subpopulations of tumour cells have distinct molecular and biological phenotypes.5 7 This so-called intratumour heterogeneity poses an important challenge for predicting tumour behaviour and clinical outcome. During the development and progression to melanoma, melanocytes undergo genetic alterations, such as loss or mutation of certain tumour suppressor genes, leading to increased proliferation, disease progression and resistance to therapy.5-10 The tumour microenvironment (TME), consisting of various cell types, blood and lymphatic vascular networks and the extracellular matrix, also plays a major role in the disease progression and response to therapy.11-16

Although many cases of primary melanoma can be successfully treated with surgery, therapy of metastatic melanoma remains challenging. Treatment options for patients with metastatic melanoma include targeted therapies, such as protein kinase inhibitors, and immunotherapies, such as immune-checkpoint inhibitors.17-20 In addition, combined therapies with targeted agents and immunotherapies are currently evaluated.18 21 However, although targeted therapies and immunotherapeutic agents have shown to improve progression-free survival (PFS) and overall survival (OS), metastatic melanoma remains difficult to treat because of primary or secondary resistance that might occur, even with these new treatments. Furthermore, considering the tumour heterogeneity, monotherapies targeting one subpopulation of tumour cells might be ineffective for others. In addition, therapies can induce changes in TME which can further influence disease outcome.14 22

Cancer vaccines targeting tumour antigens have been considered as a promising approach for treatment of malignant melanoma.23 The cancer germline gene MAGEA3 is silent in normal cells except male germ cells and trophoblast cells of the placenta and is expressed in up to 76% of metastatic melanoma, making the MAGE-A3 tumour antigen a potential target for cancer immunotherapy.24-26 However, although spontaneous immune responses against tumour antigens have been observed in patients with cancer, most tumour antigens are poorly immunogenic and need to be combined with immunostimulants (adjuvants) to generate an effective immune response sufficient to eradicate tumours.27-30 MAGE-A3 antigen combined with the GSK proprietary immunostimulant AS15 (MAGE-A3 immunotherapeutic) has been tested in previous clinical trials in patients with melanoma or non-small cell lung cancer.31-34

In patients with metastatic melanoma, MAGE-A3-specific antibodies and/or T-cell responses could be measured in patients immunised with recombinant MAGE-A3 protein.31 34 35 In a Phase II study in patients with melanoma, MAGE-A3 immunotherapeutic was immunogenic and induced clinical responses, although no correlation was found between immunogenicity and clinical response.31 Therefore, in addition to further characterisation of the clinical activity, safety and immunogenicity of MAGE-A3 immunotherapeutic in patients with MAGE-A3-positive advanced melanoma, this study aimed to assess the heterogeneity of MAGE-A3 expression and TME gene expression in different lesions from one patient as well as the effects of the treatment on TME and immune-related biomarkers at the site of the tumour by taking tumour biopsies during and after treatment; however, the assessments of treatment-induced changes were not performed due to the low number of samples collected after treatment. Therefore, to assess the predictive value and heterogeneity of the gene signature (GS) in the present study, samples from two different skin lesions were collected during screening and assessed for the presence of this GS. The clinical activity and immunogenicity of MAGE-A3 immunotherapeutic were evaluated in the overall patient population and separately in patients for whom, of the two biopsied lesions, both were GS-positive (GS+/+), only one was GS-positive (GS+/−) or none were GS-positive (GS−/−).

METHODS

Study design, objective and treatment

This study was an open-label, multicentre, uncontrolled, descriptive, exploratory Phase II study with a single study group conducted between 2009 and 2014 in six centres in Belgium and France (ClinicalTrials.gov NCT00896480). Patients with MAGE-A3-positive metastatic melanoma received up to 24 doses of MAGE-A3 immunotherapeutic administered according to a 4-cycle schedule (online supplementary figure S1). The total duration of the treatment for each patient from screening to the end of cycle 4 was approximately 4 years.

Continued treatment in cycles 2, 3 and 4 depended on an adequate clinical response at the end of the respective previous cycle. Treatment response qualifying patients to receive further MAGE-A3 immunotherapeutic administrations was: objective response (ie, complete response (CR) or partial response (PR)), stable disease (SD), mixed response (MR) and slow progressive disease (SPD) (for definitions refer to online supplementary materials). SPD and MR allowed continuing the treatment even in the event of progressive disease (PD).

At screening, two skin lesions were biopsied for MAGE-A3 expression analysis and presence or absence of a GS predicting favourable clinical outcome.36 The list of the 100 probesets (84 genes) used in the GS is shown in online supplementary table S1. Demographic and laboratory data were collected in electronic case report forms (eCRFs).

The objectives of this study included evaluation of clinical activity, safety and immunogenicity of MAGE-A3 immunotherapeutic; clinical activity and
immunogenicity were assessed according to the GS profile. MAGE-A3 immuno-therapy is composed of the MAGE-A3 protein and the AS15 immunostimulant. Patients received 0.5 mL of MAGE-A3 immuno-therapy by intramuscular injection in the deltoid or lateral regions of the thigh, alternately on the right and left side.

Inclusion and exclusion criteria

Patients aged ≥18 years with histologically proven, MAGE-A3-positive (MAGE-A3 expression in at least one of the two baseline tumour biopsies), metastatic, Stage III (in transit or unresectable) or IV M1a cutaneous melanoma, with documented PD within the 12 weeks preceding the first study treatment administration and with at least three tumour lesions of ≥5 mm diameter who signed the informed consent form were eligible for the study. Detailed exclusion criteria can be found in the online supplementary materials.

Study procedures and blood sampling

Screening was performed 4 weeks before the first MAGE-A3 immunotherapeutic administration (visit 1). At screening, two skin lesions, and at visit 7, one lesion (if still possible) were excised. Each tumour biopsy was preserved in RNAlater (Invitrogen) and used for MAGE-A3 expression analysis and gene expression profile analysis.

Expression of MAGE-A3 and gene expression profile analysis was assessed on two biopsies excised during screening. MAGE-A3 expression was analysed by quantitative real-time PCR; BRAF muta-tion testing was performed using PCR. Of note, testing of tumour biopsies for BRAF mutation was not foreseen in the study protocol; this additional translational research was performed on all remaining tumour tissue biopsied during screening. Tumour gene expression profile was analysed using Affymetrix HG-U133.Plus 2.0 (Affymetrix, Santa Clara, California, USA) microarray gene chips, the GS used was metrix HG-U133.Plus 2.0 (Affymetrix, Santa Clara, California, USA).

Assessment of clinical response variables

Only patients who presented with at least three lesions of ≥5 mm diameter at screening were eligible for the study. However, because two biopsies were taken at baseline and another at the end of cycle 1, patients who had no remaining evaluable lesions at the end of cycle 1 continued to receive the treatment until PD with the appearance of new lesions. All objective tumour response criteria used in this study are detailed in the online supplementary materials.

The best overall response was the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started).

The duration of overall response was measured from the time measurement criteria were met for CR/PR until the first date that recurrent or PD was objectively docu-mented (taking as reference for PD the smallest measure-ments recorded since the treatment started).

Time-to-treatment failure (TTF) was defined as the time from a patient’s first dose to the date of last treatment administration for patients who discontinued the treatment prematurely, regardless of the reason for study treatment discontinuation. Patients who completed their full treatment phase or who were still on treatment at the time of analysis were censored on their last study treatment administration date.

PFS was defined as the time from the date of patient first dose to either the date of PD/SPD or the date of death (regardless of the reason), whichever occurred first. Patients still alive at the time of this analysis and without any documented disease progression were censored at the date of their last tumour assessment.

Safety assessment

Routine safety assessments were performed at each study visit, and a complete assessment including disease status was performed at the end of each treatment cycle. All adverse events (AEs) (except autoimmune AEs) occurring within the 31 days following each dose administration were recorded in the patient’s eCRF. Serious adverse events (SAEs) were recorded from the date of the first study treatment dose administration until 30 days after administration of the last dose.

Severity of AEs was assessed according to the Interna-tional Common Terminology Criteria for Adverse Events (V.3.0).

Details about the safety assessment can be found in online supplementary materials.

Immunogenicity assessment

MAGE-A3-specific antibodies were measured by ELISA at predefined timepoints (online supplementary materials). The ELISA assay cut-off was 27 ELISA units (EU)/mL.

MAGE-A3-specific cell-mediated immune (CMI) response was assessed in terms of CD4+ and CD8+ T cells expressing interferon gamma (IFN-γ) and/or tumour necrosis factor-alpha (TNF)-α measured at prespecified timepoints (online supplementary materials), by intra-cellular cytokine staining and flow cytometry after in vitro priming peripheral blood mononuclear cells with immunocomplexed recombinant protein and restimu-lating effector cells with pools of overlapping peptides covering the full-length MAGE-A3 antigen. The clinical cut-off values for geometric mean ratio (GMR or immunogenicity score) and frequency of CD4+/CD8+ T cells expressing IFN-γ and/or TNF-α were defined using a panel of healthy donors with the same readout applied to MAGE-A3 immunogenicity assessment of patients with cancer (online supplementary table S3).
Statistical analyses
Statistical analyses were performed using the Statistical Analysis Systems V.9.2 running on Unix.

Twenty patients who received at least one dose of MAGE-A3 immunotherapeutic and at least 16 patients who completed cycle 1 were planned to be enrolled in this study. This sample size is typical for studies with a similar design and was based on general experience rather than any formal estimate or hypothesis.

The total treated population (TTP) included all patients who received at least one dose of MAGE-A3 immunotherapeutic. The according-to-protocol (ATP) population for analysis of immunogenicity included patients who met all eligibility criteria for enrolment, did not report major protocol deviations, received at least the first six doses of MAGE-A3 immunotherapeutic and had available immunogenicity results within 2 weeks postdose 6. For non-compliant patients, all data collected after protocol violation were eliminated from the ATP immunogenicity analyses.

The best overall response was analysed in terms of number and proportion of patients falling into each category, by GS result. Objective response rate was defined as the proportion of patients whose best overall response was PR or CR.

Disease control rate was defined as the proportion of patients whose best overall response was CR, PR, SD or SD/PR.

Kaplan-Meier curves were used to describe TTF and PFS by GS status and overall, and with median TTF or PFS, respectively and 95% CI.

Details on immune response analysis are provided in online supplementary materials.

RESULTS
Study patients, MAGE-A3 expression, gene signature and treatment compliance
Froty-nine patients were screened, 24 patients were recruited and included in TTP and 3 patients completed the study; nine patients (2 GS+/+, 4 GS+/- and 3 GS-/-) were excluded from ATP population (figure 1). Among the 49 screened patients, 32 (65.3%) had MAGE-A3-positive tumours; of these, 10 were GS+/+, 9 were GS+/- and 13 were GS-/- (online supplementary table S4).

Among the 24 treated patients, 8 were GS+/+, 8 were GS+/- and 8 were GS-/-.

The mean age of study patients was 65.4 years and 70.8% were female; 50.0% of the patients had Stage III and 50.0% had Stage IV melanoma (online supplementary table S5).

Figure 1 Participant flow. Of the 24 patients included in the study, 3 patients completed the study. Patients may have more than one reason for elimination from ATP population. ATP, according-to-protocol; GS+/+, patients presenting the gene signature on both biopsies; GS+/-, patients presenting the gene signature on one biopsy and not on the other one; GS-/-, patients without the gene signature; N, number of patients.
The results of the analysis of the BRAF mutational status are shown in online supplementary materials. Among the 24 treated patients, 20 (8 GS+/+, 5 GS+/- and 7 GS-/-) received six doses of MAGE-A3 immunotherapeutic and completed cycle 1, 11 patients (7 GS+/+, 3 GS+/- and 1 GS-/-) received 12 doses and completed cycle 2, 7 patients (5 GS+/+ and 2 GS+/-) received 16 doses and completed cycle 3 and 3 GS+/+ patients received 24 doses and completed cycle 4.

**Clinical activity**

**Clinical response**

Four patients (16.7%) achieved an objective response (CR or PR); two patients (GS+/+) had CR and two patients (one GS+/+ and one GS+/-) had PR (table 1).

Of note, one of the GS+/+ patients showed a rapid CR by the end of cycle 1 (visit 7). This patient had one non-target lesion disappeared when returning for the first study treatment administration, and one target lesion disappeared when returning for the second treatment administration. Thus, this CR was unlikely to be related to the study treatment.

Disease control, defined as CR, PR, SD or SD/PR, was reported for 7 patients (29.2%), and PD was reported for 17 patients; 4 of these patients presented with SPD (table 1). Of note, 4 of the 17 patients with PD (23.5%) were GS+/+.

MR was observed in five patients (20.8%), three GS+/+, one GS+/- and one GS-/- (for MR definition refer to online supplementary materials). In addition, for two patients, one of the target lesions disappeared while new lesions appeared. Although a certain degree of clinical benefit could be observed in these patients, they were not considered as mixed responders as per protocol definitions.

**Time-to-treatment failure and progression-free survival**

The median TTF was 14.8 months for GS+/+ patients, 2.3 months for GS+/- patients and 2.4 months for GS-/- patients (figure 2A).
The median PFS time was 7.2 months for GS+/+ patients, 2.8 months for GS+- patients and 2.9 months for GS-/- patients (figure 2B).

Safety
All patients reported at least one AE during the 31-day postadministration period; the most common AEs were injection site reactions (50.0%), injection site pain (50.0%), fever (42.0%), asthenia (38.0%), nausea (29.0%), headache (29.0%), fatigue (29.0%) and influenza-like illness (29.0%). Three patients (15.0%) reported at least one grade 3 AE. No grade 4 or 5 AEs were reported.

The grade 3 AEs, lymphoedema, dyspnoea and cardiac disorder (aggravated heart disease), also considered as SAE, were considered by the investigator as unrelated to the study treatment.

Treatment-related AEs as per investigator assessment were reported by 96.0% of patients, all were grade 1–2. The most common treatment-related AEs included injection site reactions (50.0%), injection site pain (50.0%), fever (42.0%), influenza-like illness (29.0%), nausea (25.0%), headache (25.0%), asthenia (21.0%) and fatigue (21.0%).

Two SAEs were reported. One patient reported severe grade 3 cardiac thrombosis 27 days after the last dose of MAGE-A3 immunotherapeutic with concurrent heart disorder. The patient was subsequently treated with clopidogrel bisulfate, furosemide, bisoprolol fumarate and heparin sodium. This SAE was assessed by the investigator as unrelated to the study treatment.

The second SAE was a case of malignant melanoma. Following administration of the last dose of the study treatment, the patient developed malignant neoplasm of cheek mucosa. This SAE was considered by the investigator as unrelated to the study treatment. The patient underwent a partial resection and was awaiting a further surgery at the time of the data lock point of this study.

One patient experienced a non-serious potentially immune-mediated disease (grade 1 vitiligo) following the administration of the fourth dose. The event was characterised only by skin discoloration, with no additional signs or symptoms. This event was assessed by the investigator as related to the study treatment.

Immunogenicity results (ATP population)

Antibody response
At baseline, among the 18 evaluated patients, three (16.7%; 1 GS+/+, 1 GS+- and 1 GS-/-) were positive for MAGE-A3-specific antibodies. Two weeks postdose 2, all patients were seropositive with a geometric mean concentration (GMC) of 1865.7 EU/mL. The antibody levels further increased up to postdose 6 timepoint (GMC 9080.5 EU/mL); thereafter, a plateau was observed (figure 3A). MAGE-A3-specific antibody profiles were similar between patients presenting a different GS status (figure 3B).

CMI response
Prior to the first MAGE-A3 immunotherapeutic administration, MAGE-A3-specific double-positive TNF-α+/IFN-γ-producing (TNF-α+/IFN-γ+) CD4+ T cells with GMR above the assay cut-off value (1.24) were found in one patient; the highest proportion of patients with these cells was observed postdose 12 (9/10 patients (90.0%); figure 4A). The highest proportion of CD4+ T cell responders was observed postdose 6 at the end of cycle 1 (8/13 patients (61.5%); figure 4B). Overall, at any timepoint assessed, MAGE-A3-specific TNF-α+/IFN-γ+ CD4+ T cell immunogenicity was found in 15/17 patients (88.2%), with 12/16 patients (75.0%) being cellular responders. The evolution of the geometric mean of MAGE-A3-specific TNF-α+/IFN-γ+ CD4+ T cells over time according to GS is shown in online supplementary figure S2.

The evolution of the GMR of MAGE-A3-specific TNF-α+ or IFN-γ+ CD8+ T cells over time according to GS is shown in online supplementary figure S3.

At baseline, MAGE-A3-specific TNF-α+ or TNF-α+/IFN-γ+ CD8+ T cells with immunogenicity above the assay cut-off value were found in one patient. The highest proportion of patients presenting these cells was observed postdose 12 at the end of cycle 2 (2/10 patients (20.0%); data not shown). At any timepoint assessed, these cells were observed in 3/17 patients (17.6%). No CMI responses were observed for MAGE-A3-specific CD8+ T cells. None of the two GS+/+ CR patients had levels of double expression TNF-α+/IFN-γ+ CD8+ T cells above the cut-off. One of the two patients had single-positive TNF-α+/IFN-γ- CD8+ T cells at one timepoint (postdose 16).

Single-positive MAGE-A3-specific TNF-α+/IFN-β+ and TNF-α+/IFN-α+ CD8+ T cells with immunogenicity above the assay cut-off values were observed in 1/17 patient (5.9%) at one timepoint of assessment (postdose 20) and at baseline in two patients, respectively. The highest proportion of patients with MAGE-A3-specific TNF-α+/IFN-α+ CD8+ T cells was observed postdose 6 (6/14 patients, 42.9%); overall, at any timepoint assessed, TNF-α+/IFN-α+ CD8+ T cells were found in 9/17 patients (52.9%). More details about the CMI response can be found in online supplementary materials.

DISCUSSION
This study evaluated the clinical activity, safety and immunogenicity of MAGE-A3 immunotherapeutic in patients with Stage III in transit or unresectable and Stage IV M1a cutaneous melanoma. Two previous Phase II studies, EORTC and PREDICT studies, targeted the same patient population. In addition, in this study, we assessed the heterogeneity of MAGE-A3 and the expression of a TME GS identified in the EORTC study, where it was found to be predictive of a favourable clinical outcome following treatment with MAGE-A3 immunotherapeutic. This prediction was not confirmed in the PREDICT study.
Figure 3  MAGE-A3-specific geometric mean titres (GMCs) (ATP population for immunogenicity). In the overall population, 2 weeks postdose 2, all patients were seropositive for MAGE-A3-specific antibodies (GMC 1865.7 EU/mL). The antibody levels further increased up to postdose 6 timepoint (GMC 9080.5 EU/mL); thereafter, a plateau was observed (A). There were no marked differences in MAGE-A3-specific antibody profiles between patients presenting the different gene signature on the two biopsies (B). The error bars represent 95% CI. ATP, according-to-protocol; EU, ELISA units; GMC, geometric mean concentration; GS++/+, patients presenting the gene signature on both biopsies; GS+/-, patients presenting the gene signature on one biopsy and not on the other one; GS-/-, patients without the gene signature; Pre, baseline; Postn, postdose number indicated by Arabic numerals.
MAGE-A3 expression analysis on pretreatment biopsies revealed that the majority of patients (65.3%) had MAGE-A3-positive tumours. This MAGE-A3 expression rate is similar to that observed previously in the EORTC study (59.0%) and slightly higher than that in the PREDICT study (52.1%).

Among the patients who were MAGE-A3-positive, 19 patients (59.4%) were GS-positive in at least one of the two assessed lesions (10 GS+/+ and 9 GS+/-) and only one-third of all patients presented discordant profiles of GS status (+/-), giving useful information on the heterogeneity of tumours and its association to patient’s response to MAGE-A3 immunotherapeutics. These results suggest that even distant biopsies present a similar GS profile in most patients, despite what could be assumed as potentially more heterogeneous. This might suggest some degree of clonality, but as well, similar antigenic and immunogenic potential and TME composition.

Four of the 24 treated patients (16.7%) achieved objective responses (two CR and two PR); however, for one patient showing a rapid CR at the end of the cycle 1, the two patient’s lesions disappeared before the first and the second administration, respectively. Thus, although the patient fitted the clinical response criteria, this CR was considered not related to the administration of the study treatment. In the EORTC study, recMAGE-A3 combined with immunostimulants AS15 or AS02b showed signs of clinical activity, with four (three CR and one PR) of the five objective responses observed in patients who received recMAGE-A3 combined with AS15. Without this patient, the objective clinical response rate was 12.5% and was similar to that previously reported in the EORTC study (11.1%). In contrast, the objective clinical response rate was lower in the PREDICT study (three patients: one CR and two PR; 2.4%), which was likely associated with an early treatment discontinuation as observed in this study.

Indeed, tumour response to cancer immunotherapy may have a delayed onset. Among the four patients who showed an objective response, three were GS+/+; two of these patients had CR and one had PR. In the previous retrospective analysis, the identified GS correlated with a survival advantage for patients treated with MAGE-A3 immunotherapeutic (HR for OS 0.37 (95% CI 0.13 to 1.05), p=0.06). In contrast, in the PREDICT study, no difference in outcomes between GS+ and GS- populations were observed, with a similar OS rate at 1 year and similar clinical outcomes. Although OS was not assessed in our study, the rate of objective clinical response was higher than in the PREDICT study, where only one GS- patient had CR and two GS+ patients had PR. In addition, we observed a longer TTF (14.8 months) for GS+/+ patients compared with 2.3 months for GS+-/- patients and 2.4 months for GS-/- patients, whereas in the PREDICT study, TTF was similar in GS+ and GS- populations (2.7 vs 2.4 months, respectively). Similarly, the PFS was longer for GS+/+ patients (7.2 months) than for...
GS+/- and GS-/- patients (2.8 and 2.9 months, respectively), while in the PREDICT study, the PFS was similar for both patient populations (2.8 months). Similar to the EORTC study, some patients with PD presented GS-positive biopsies; among the 17 patients with PD, four were GS+/-, suggesting that the TME features characterised by the GS might be necessary but not sufficient for disease control.

MAGE-A3-specific antibody and T-cell responses did not reveal any correlation between immune response and clinical benefit. Similarly, as previously reported in the PREDICT study, there were no differences in terms of humoral immune responses to the MAGE-A3 immunotherapeutic between the GS+/+ and GS-/- patients. Of note, in this study, we observed CD4+ T-cell responses skewing towards single cytokine-positive responses in late timepoints of vaccination, suggesting hypothetical exhaustion profile or T-cell memory differentiation, as function of disease evolution or therapeutic impact.

Tumour heterogeneity has been shown to be an important feature in different cancers; furthermore, it has been proposed to be a cause of treatment failure. In this study, we found that the expression of MAGE-A3 is highly homogeneous between the two biopsies. We also observed that the immune feature of the TME is somewhat more heterogeneous, although about two-thirds of the patients showed concordance in the GS status in the two biopsies assessed. Of note, we observed that the GS+/- status seemed to be associated with signs of clinical activity. Given the lack of placebo arm in this study, it was not possible to determine if this is purely predictive for example, associated to treatment (vs natural course of the disease, prognostic). Thus, melanoma tumours represent a complex and dynamic system where tumour development and progression is driven by heterogeneity although some features seem to be conserved when evaluated in two biopsies.

MAGE-A3 immunotherapeutic was well tolerated and there were no safety concerns. The safety profile was consistent with previous reports.

Potential limitations of this study include a small sample size, and lack of the patients’ follow-up. In addition, CD8+ T cell responses could not be detected in the peripheral blood of the study patients, which could explain the low clinical efficacy of MAGE-A3 immunotherapeutic.

In conclusion, among patients who were MAGE-A3-positive, tumour heterogeneity in terms of GS expression was observed, with approximately one-third of the patients presenting discordant profiles of GS status (+/-). MAGE-A3 immunotherapeutic was associated with signs of clinical activity, longer TTF and PFS in the patients with metastatic melanoma who were GS-positive in both assessed tumour biopsies, even though the overall response rate and durations were low. Treatment with MAGE-A3 immunotherapeutic was well tolerated and induced specific immune responses in all patients irrespective of the GS status.

REFERENCES


