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Double-blind, randomised, placebo-controlled Phase 3 study to assess the efficacy of the MAGE-A3 immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive Stage III melanoma (DERMA)

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Abstract (290 words max 300)

Background: Even with newly approved treatments, metastatic melanoma remains a life-threatening condition. We conducted a worldwide multicentre Phase 3 trial to evaluate the efficacy of the MAGE-A3 immunotherapeutic in patients with Stage IIIB or IIIC melanoma in the adjuvant setting.

Methods: DERMA was a randomised, double-blind, placebo-controlled trial in patients aged at least 18 years with MAGE-A3-positive histologically proven, completely resected Stage IIIB or IIIC cutaneous melanoma with macroscopic lymph node involvement. Patients were to have a performance score of 2 or less. Patients were randomly assigned (2:1) to receive up to 13 intramuscular injections of recombinant MAGE-A3 with AS15 immunostimulant (MAGE-A3 immunotherapeutic) or placebo over a 27-month period: 5 doses at 3-weekly intervals, followed by 8 doses at 12-weekly intervals. Randomisation and treatment allocation at the investigator site was done centrally via internet with stratification for the presence of a predictive gene signature versus no gene signature. Participants, investigators, and those assessing outcomes were masked to group assignment. A minimisation algorithm accounted for disease stage, nodal stage, stage of the primary tumour, extra-capsular extension of the lymph node, study centre and prior treatment with interferon and/or anti-CTLA4 drugs. The co-primary objectives were: efficacy in terms of disease-free survival (DFS) in the overall population, and DFS in patients with a potentially predictive gene signature (GS+) identified previously. The gene signature was defined and prospectively validated using an adaptive signature design. The final analyses included the total treated population (all patients who had received at least one treatment dose). This trial is registered with ClinicalTrials.gov, number NCT00796445.

Findings: Of 3,914 patients screened, 2,092 had a MAGE-A3-positive tumour, 1345 were randomized and started treatment. At the final analysis (median follow-up 28·0 months [interquartile range, IQR, 23·3-35·5] in the MAGE-A3 group and 28·1 months (IQR 23·7-36·9) in the placebo group), median DFS was 11·0 months (95% CI 10·0-11·9) in the

MAGE-A3 group and 11·2 months (95% CI 8·6-14·1) in the Placebo group (Hazard Ratio [HR] 1·01, 95% CI 0·88-1·17, p=0·86). Median DFS in the GS+ population was 9·9 (95% CI 5·7-17·6) and 11·6 months (95% CI 5·6-22·3) in the respective treatment groups (HR 1·11, 95%CI 0·83-1·49, p=0·48). Within 31 days of treatment, adverse events (AEs) ≥grade 3 were reported by 14% of patients in the MAGE-A3 group and 12% in the Placebo group; treatment-related AEs ≥grade 3 by 4% versus 1%, respectively; and ≥1 serious AEs by 14% of patients in both groups. The most frequently reported grade 3 or higher adverse events were neoplasms (33 [4%] in the MAGE-A3 group and 17 [4%] in the Placebo group), general disorders and administration site conditions (25 [3%] versus 4 [<1%]) and infections and infestations (17 [2%) versus 7 [2%]). There were no treatment-related deaths.

Interpretation: An antigen specific immunotherapeutic alone was not efficacious in this clinical setting. Based on these results, development of the MAGE-A3 immunotherapeutic for use in melanoma has been stopped.

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Introduction (4483, max 4500 words)

Melanoma is the most aggressive form of skin cancer and 5-year overall survival (OS) in patients with stage IIIB/IIIC disease is 35%-60%.[1] Treatment is complete surgical resection, but patients with stage IIIB disease (macroscopic involvement of lymph nodes [LN]) remain at high risk of relapse, that increases with the number of invaded LN and capsular extension. Adjuvant therapies such as interferon-alpha and pegylated interferon prolong relapse-free survival but do not appear to influence OS significantly, with discordant clinical results according to the dose, duration and targeted population.[2,3] Adjuvant ipilimumab has been shown to improve relapse-free survival and OS, but more than half of patients experience grade III-IV toxicity, and some die.[4] Two recent studies have shifted the landscape in adjuvant melanoma treatment. First, Nivolumab as adjuvant treatment among patients with resected stage IIIB, IIIC and IV melanoma resulted in significantly longer recurrence-free survival with lower rates of severe toxicity compared to Ipilimumab.[5] Second, the combination of the BRAF inhibitor dabrafenib plus the MEK inhibitor trametinib significantly lowered the risk of recurrence in patients with stage III melanoma with BRAF V600E or V600K mutation when compared to placebo.[6] The safety profile of dabrafenib plus trametinib was consistent with that observed with this combination in patients with metastatic disease.

The MAGE-A3 cancer-testis tumour antigen is expressed in up to 76% of melanomas, but the gene is silent in all normal human tissues except placenta and testis.[7,8] The MAGE-A3 immunotherapeutic (GSK) comprises a recombinant MAGE-A3 protein (recMAGE-A3) administered with the GSK proprietary immunostimulant AS15, and was designed to enhance both humoral and cell-mediated responses against MAGE-A3-expressing tumours.[9] In a Phase 2 proof-of-concept study in patients with early progressive metastatic melanoma, an objective clinical response was observed in 5/72 patients treated, and another 10 patients showed disease stabilisation.[10] The Phase 2 study evaluated recMAGE-A3 combined with two different immunostimulants, $AS02_B$ or AS15. Both immunostimulants had a similar safety profile, and four of the five objective responses were in the 37 patients who received AS15.

Based on these results and those of previous preclinical and clinical studies,[11-13] AS15 was selected for further development. These results, along with encouraging results in a randomized Phase 2 study of the MAGE-A3 immunotherapeutic in patients with non-small-cell lung cancer (NSCLC),[14] were considered sufficient to commence a worldwide, multicentre, Phase III study to evaluate the clinical efficacy of the MAGE-A3 immunotherapeutic in patients with stage III melanoma with macroscopic LN involvement. An immune-related gene signature associated with clinical benefit following immunisation with the MAGE-A3 immunotherapeutic was identified in the Phase 2 proof-of-concept melanoma study, and retrospectively validated in the Phase 2 NSCLC study.[10,14,15] Therefore, the DERMA study also sought to optimise and prospectively validate this candidate predictive gene signature using an adaptive signature design.[16,17]

Methods

Study design and participants

In this double-blind, randomised, placebo-controlled Phase 3 study conducted in 31 countries, we recruited patients aged ≥18 years who had histologically proven, completely resected stage IIIB or IIIC cutaneous melanoma with macroscopic LN involvement defined according to the TNM staging system (sixth edition). A summary of the protocol is located at https://www.gsk-clinicalstudyregister.com/search/?study_ids=111482.

Eligible patients had to have been surgically rendered disease-free no more than 9 weeks before randomisation. Patients with unknown primaries were also eligible (TxN1b-N2b-N3 M0). For patients undergoing elective regional LN dissection followed by lymphadenectomy, the date of the radical lymphadenectomy was considered the day the patient was disease-free. Quantitative MAGE-A3 gene expression was determined by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis on formalin-fixed paraffin-embedded tissue. At the time of randomisation, patients had to have adequate renal and hepatic function and bone-marrow reserve, and an Eastern Cooperative Oncology Group performance score <2.

All patients gave written informed consent for MAGE-A3 expression screening and gene expression profiling, and for study participation. Prior systemic treatment with an immunomodulator (i.e., interferon and/or anti-CTL-A4) following a previous surgery was allowed, provided that a wash-out period of 30 days before randomisation was respected. Previous radiotherapy was allowed, provided that the treatment had been completed before the lymphadenectomy that qualified the patient for study participation.

Patients were excluded if they had a history of autoimmune disease (excluding vitiligo), infection with Human Immunodeficiency Virus, another confirmed or suspected immunosuppressive or immunodeficient condition, psychiatric or addictive disorders that may have compromised his/her ability to give informed consent or to comply with the study procedures, severe concurrent severe medical problems that would limit full compliance with the study or expose the patient to unacceptable risk, previous or concomitant malignancies (except effectively treated non-melanoma skin cancers, carcinoma in situ of the cervix or effectively treated malignancy that had been in remission for over 5 years and was highly likely to have been cured), or an uncontrolled bleeding disorder. Amendments to the protocol, exclusion criteria, efficacy and safety follow-up procedures and patient withdrawal information are provided in appendix (p13-15).

This study was conducted in accordance with the principles of "good clinical practice", the principles of the Declaration of Helsinki and all applicable regulatory requirements. The protocol was approved by national, regional, or investigational centre institutional review boards or ethics committees. During the course of the study, whenever potential or actual issues with regard to the conduct of the study were identified, either via site monitoring activities or brought to GSK’s attention by other oversight mechanisms, these issues were investigated and whenever possible, appropriate corrective/preventive actions were taken. An Independent Data Monitoring Committee (IDMC) monitored the study and reviewed study endpoints and safety data.

Randomisation and masking

Patients were randomised 2:1 to receive either the MAGE-A3 immunotherapeutic or placebo at the investigator site using a central internet randomisation system. The randomization and treatment number assignment was handled centrally at GSK Vaccines. The central randomization system was accessed by staff at the investigator site using internet. The 2:1 ratio was used to make a potentially active treatment available to a larger proportion of trial subjects. A minimisation algorithm (with a 10% random element) accounted for disease stage (IIIB or IIIC or IIIx (undefined stage III Tx)), nodal stage (N1 or N2 or N3), stage of the primary tumour (Tx-0 or T1-2 or T3 or T4), extra-capsular extension of the LN (yes or no), study centre and prior treatment with interferon and/or anti-CTLA4 (cytotoxic T-lymphocyte-associated antigen 4) drugs (yes or no).

Individual treatment assignment was masked at all levels except to the IDMC and the independent statistician performing 6-monthly safety assessments and efficacy analyses. The study remained blinded until the primary analysis of disease-free survival (DFS) in the GS+ population (patients with a gene signature potentially predictive of a treatment benefit), which occurred 2 years after the primary analysis of DFS in the overall population due to the development and analytical validation of the gene expression assay.

Procedures

The composition and treatment schedule of the MAGE-A3 immunotherapeutic and placebo are provided in appendix (p6). Patients were to receive up to 13 intramuscular injections of MAGE-A3 immunotherapeutic or placebo over a 27-month period: 5 doses at 3-weekly intervals, followed by 8 doses at 12-weekly intervals. No dose reductions were permitted, but doses could be skipped or delayed if the patient was acutely ill at the time of the scheduled administration, if influenza vaccine or blood products needed to be given (with at least a 7-day interval between vaccination/blood products and treatment), any other medical reason considered by the investigator that would expose the patient to an unacceptable risk. In case of postponement of study treatment administration for any reason, a visit to administer the missed treatment had to be planned as soon as possible to catch up the originally planned

schedule. The next study visit had to be planned at a time allowing a minimum of 14 days between two treatment administrations and to keep up with the schedule as based on the date of first study treatment administration.

Patients were required to discontinue treatment if any of the following criteria became applicable: evidence of disease recurrence; receipt other anti-cancer treatments or investigational products; any grade 3 or more allergic reaction following the administration of study treatment; any intolerable AE or persistence moderate AE that could be worsened by further administration of study treatment; signs or symptoms of an immune disorder (except vitiligo); any immune deficient/suppressive condition, inability of the patient to complete the study evaluations, development of other conditions for which, according to the investigator, it was in the patients best interests to withdraw, patient request, and for female patients, pregnancy or the decision to become pregnant.

Procedures for the assessment of efficacy are provided in appendix (p16). Efficacy assessments during treatment were done every 3 months, alternating chest and upper abdomen CT-scans or chest x-rays. At every visit, the investigator performed a physical examination and clinical assessment. Brain CT or MRI were done if clinically indicated. Active follow-up for survival and disease recurrence was planned to continue for at least 5 years from the first study treatment.

Due to multiple differences in assays, sample type and clinical setting between the Phase 2 and Phase 3 studies, optimisation and clinical validation of the gene signature were done using a split-sample approach based on the adaptive signature design.[16,17] In summary, the first set of patients ('training set', one-third of study patients) was used to define a predictive gene signature that could identify patients most likely to benefit from treatment, different gene signature classification models were evaluated in the training set, starting with 55 target genes measured by quantitative real-time PCR (qRT-PCR). The remaining two-thirds of patients comprised the 'test set' that was used for clinical validation of the selected gene signature after the final analysis of the first co-primary endpoint and validation of the gene

signature assay. Details of the qRT-PCR assay, steps and methods for classifier development and clinical validation of a multigene predictive gene signature are provided in appendix (from p39).

The schedule for laboratory evaluations of safety and reporting period for adverse events (AEs) is provided in appendix (p15). The investigator inquired about the occurrence of AEs, SAEs, pregnancy and autoimmune diseases at every visit/contact during the study and throughout the follow-up period. Patients were instructed to contact the investigator immediately in case of signs or symptoms they perceived as serious. AEs were recorded for 31 days (day 0-30) after each dose. Serious adverse events (SAEs) were recorded from study start until the end of the treatment phase. SAEs related to the investigational drug or any concurrent GSK drug were recorded from consent until study end. New onset of autoimmune disease and pregnancies were recorded from the first treatment for 5 years. AE intensity was graded by the investigators using Common Terminology Criteria for Adverse Events (CTCAE Version 3.0).[18] Individual AEs were coded to the Preferred Term level using the Medical Dictionary for Regulatory Activities (MedDRA). The investigator assessed potential causal relationships between the investigational product and each AE.

Safety laboratory assays assessing the haematological parameters, renal and hepatic functions were performed during screening, and at week 12, month 12, month 24 and month 30. Additional tests could be performed if clinically indicated. Using an enzyme-linked immunosorbent assay performed centrally at GSK's laboratories,[19] we measured anti-MAGE-A3-specific immunoglobulin-G antibodies at baseline, after 2, 4, 6, 7, 9 and 13 treatment administrations and one year post-treatment conclusion. Seropositivity was defined as an antibody titre ≥assay cut-off of 27 EU/ml.

Health-related quality-of-life (QoL) utility was assessed using the EuroQoL-5D (EQ-5D) questionnaire.[20] Patients self-completed the questionnaire prior to injection at treatment dose 1, 3, 5, 6, 7, 8, 12, at the first follow-up visit, and again after the patient had been informed of

a recurrence before starting a new anti-cancer treatment. QoL was re-assessed by staff via telephone on the day after injection on visits 1, 3 and 5.

Outcomes

The co-primary study objectives were to demonstrate clinical efficacy of the MAGE-A3 immunotherapeutic compared to placebo in terms of disease-free survival (DFS, defined as the interval from randomisation to either the date of first disease recurrence or death due to any cause) in the overall population (objective 1), and in the population presenting a potentially favourable predictive gene signature (objective 2). Secondary endpoints were OS (the interval from randomisation to the date of death due to any cause), DFS at 2, 3, 4 and 5 years, disease-free specific survival (DFSS: the interval from randomisation to the date of first recurrence or death due to melanoma) and distant metastasis-free survival (DMFS: the interval from randomisation to the date of first distant metastasis or date of death (any cause)). Other secondary outcomes were immunogenicity (MAGE-A3 seropositivity rates); the occurrence of AEs up to 30 days after each study dose, and of SAEs and autoimmunity up to 30 days after the last administration of study treatment; and the EQ-5D Utility Score and Visual Analog Score and change from baseline. All endpoints were calculated in the overall population, and in the populations with and without a favourable gene signature (GS-/GS+).

Statistical analysis

In order to control the two-sided type I error <5%, a Bonferroni adjustment was applied with a 2-sided 4·00% alpha assigned to objective 1 and a 2-sided 1·00% alpha assigned to objective 2. To detect a relevant increase in median DFS in the overall population with a 2-sided nominal alpha of 4·00% and a power of 80%, the study needed to randomise 1300 patients to reach 850 events at time of final analysis. This number of events was based on simulations taking into account a potential delayed treatment effect, assuming a HRof 0·90 during the first 2 months following randomisation and HR of 0·77 subsequently. The second objective was to be assessed on the test set of two-thirds of patients with a sample

available. Assuming that 50% of patients were GS+, at least 184 events were anticipated to be observed in patients allocated to the test set at the final analysis; which would provide 80% power to detect a statistically significant treatment difference in DFS at the two-sided 1·00% significance level, assuming a HR of 0·59.

The primary analysis of efficacy on the Total Treated cohort included all randomised patients who had received at least one treatment dose, using the treatment assignment as randomised. Hazard ratios (HR) were estimated using a Cox proportional hazards regression using randomisation-minimisation factors (except study centre) and ulceration status as covariates in the model.[21] The efficacy analyses in the population of test set patients presenting with the potentially favourable predictive gene signature were also adjusted for the prognostic gene signature score (a gene signature associated with a worse clinical prognosis in the placebo arm) (appendix p13), prospectively defined in the training set as the 8-gene-Th1/IFNγ gene expression signature.[22] Additional details are provided in Appendix (p17). Safety analyses were conducted on the Total Treated cohort that included all randomised patients who had received at least one treatment dose, according to the actual treatment received.

The analysis of immunogenicity was performed on the according-to-protocol immunogenicity cohort that included all eligible patients who complied with protocol-defined procedures, who had received at least first four consecutive treatment doses and for whom immunogenicity data were available. The final analysis of DFS in the overall population (first co-primary objective) was performed when at least 850 events had occurred in the overall population (database cut-off date 23 May 2013). The final analysis of DFS in the GS+ population (second co-primary objective) was done in August 2015 and was performed on the same clinical database after technical development and validation of the gene expression assay.

In the primary analysis of efficacy, groups were compared using the Likelihood Ratio test. Non-parametric estimates of median time-to-event endpoints were generated using Kaplan-Meier methodology with confidence intervals (CI) calculated using the Brookmeyer and

Crowley method. All CI were 95% two-sided nominal, and all reported p-values were two-sided. The co-variates for efficacy analyses were based on values recorded in the patient case report form, except when missing, in which case the value reported at randomization was used. An exploratory predictive factor analysis was also performed using a likelihood ratio test for interaction between the baseline covariate and treatment after including both as main effects in a Cox model. Only patients with all baseline values available were considered in the predictive analysis. A few patients with an ineligible stage were pooled with the closest category. Results for categorical variables were presented in forest plots.

Sensitivity analyses of the co-primary endpoints used different analysis methods or models. DFS was analysed using a Logrank test without stratification, and stratified by the minimization factors (except centre) and ulceration status. For these analyses, estimates of the HR and 95% CI were obtained by an unadjusted Cox model. An analysis of DFS used a Cox model adjusted for all baseline covariates. The likelihood ratio test was used to compare the groups. Sensitivity analyses were also conducted using variations of the DFS endpoint definition: taking the date of previous assessment date as date of event for patients with a recurrence, considering the start of a new therapy for a melanoma recurrence before a documented recurrence as an event, and using the GSK assessment of the recurrence date.

EuroQoL-5D (EQ-5D) health dimensions, utility values and Visual Analogue Scores and their changes from baseline were reported descriptively. Differences between the two treatment groups were compared per time point using the non-parametric Wilcoxon test. No correction for multiple testing was applied. An exploratory analysis assessed changes in mean scores over time and overall with a repeated measures analysis using a mixed effects model.

Statistical analyses were performed using SAS (version 9.2). This study is registered with www.clinicaltrials.gov, number NCT00796445.

Role of the funding source

The study was designed and interpreted by GlaxoSmithKline Biologicals SA in cooperation with an international Steering Committee. Data collection, statistical analysis and writing assistance were provided by GlaxoSmithKline Biologicals SA. BD, CD and MD had access to the raw data. All authors had access to the results and final responsibility for the analysis, interpretation and submission. The corresponding author had full access to all of the data and final responsibility for publication submission.

Results

The first patient was screened on 01 December 2008 and the last patient was randomized on 19 September 2011. There were 3914 patients screened at 263 centres; 3182/3914 (81%) patients had a valid tumour sample and 2092/3182 (66%) of these had a MAGE-A3-positive tumour (Figure 1). There were 1345 patients who were randomised and received at least one dose of treatment and contributed to the final analysis. Between the final and follow-up analyses, one patient was found to have an invalid consent form and was not included in the follow-up analysis (N=1344).

The study groups were comparable in terms of baseline characteristics (Table 1). Overall, of 1345 patients 724 (54%) patients had Stage IIIC disease, 479 (36%) had ulceration of the primary tumour, 448 (33%) had extracapsular LN extension and 202 (15%) had received prior treatment with interferon and/or anti-CTL-A4 drugs. Median time from lymphadenectomy to randomization was 7·1 weeks in both groups (interquartile range [IQR] 5·9-7·9). The percentage of patients with an unknown primary was 18·1% in the MAGE-A3 group and 16·8% in the placebo group.

There were 786/895 (88%) patients in the MAGE-A3 group and 403/450 (90%), in the Placebo group who received at least four treatment doses. The number of patients who received all 13 doses was 256/894 (29%) in the MAGE-A3 group and 133/450 (30%) in the Placebo group.

Reasons for treatment or study withdrawal were distributed similarly in each group (Figure 1). There were 4 patients (all in the MAGE-A3 group) who discontinued treatment due to an AE considered by the investigator to be related to vaccination; rash, autoimmune hepatitis, fatigue and influenza-like-illness. There were 3 patients (all in the MAGE-A3 group) who discontinued treatment due to an SAE considered by the investigator to be related to vaccination; polyneuropathy, pyrexia and blurred vision.

All 1345 patients in the Total Treated population were included in the analysis of efficacy in the overall population. The median duration of follow-up at the time of the final analysis was 28·0 months (IQR 23·3-35·5) in the MAGE-A3 group and 28·1 months (IQR 23·7-36·9) in the Placebo group (p=0·44).

At the time of the final analysis there were 856 events (recurrence or death): 572 events in 893 patients (64%) in the MAGE-A3 group and 284/452 (63%) in the Placebo group. Disease recurred in 565/893 (63%) of patients in the MAGE-A3 group and in 283/452 (63%) in the Placebo group. The additional DFS events were 8 deaths in absence of recurrence (7 in the MAGE-A3 group, 1 in the Placebo group).

Overall median DFS at the time of the final analysis was 11·0 months (95% CI 10·0-11·9) in the MAGE-A3 group and 11·2 months (95% CI 8·6-14·1) in the Placebo group (HR 1·01, 95% CI 0·88-1·17, p=0·86) (Figure 2). Exploratory subgroup analyses according to baseline demographics, tumour and treatment parameters showed that the estimated HRs CI included '1' for all parameters, except for nodal stage (Figure 3).

There were 366 patients allocated to the training set and 729 to the test set. Of the training set samples, 357 gave valid qRT-PCR results. The gene signature discovered in the Phase 2 studies, summarised by eight of the pre-selected genes (Th1/IFNγ gene signature) was identified as being associated with clinical outcome in the placebo arm of the training set (prognostic effect) [22] independent from all other pre-defined clinical prognostic factors, and was used as a covariate to adjust the final statistical analysis. Adjusting for the prognostic effect of this signature in the training set, a clinical benefit of MAGE-A3 immunotherapeutic

over placebo was observed in DFS for patients selected (GS+) with a novel potentially predictive gene signature classifier set up in the training set (appendix p44-45). As per the study protocol, the study team remained blinded to the result in the overall population. When this new predictive gene signature was applied to the remaining two thirds of the samples (test set), the median DFS in the GS+ population was 9·9 months (95% CI 5·7-17·6) in the MAGE-A3 group and 11·6 months (95% CI 5·6-22·3) in the Placebo group (HR 1·11, 95% CI 0·83-1·49, p= 0·48) (Figure 2). The number of DFS events in the test set GS+ population was 124/200 (62%) in the MAGE-A3 group and 72/116 (62%) in the Placebo group. There was no difference between the MAGE-A3 and Placebo groups in the GS+ or GS- populations in terms of DFS, OS, DFSS or DMFS, or in the assessment of DFS for each year of follow-up (appendix p18-19). Exploratory subgroup analyses of GS+ and GS- populations showed that the estimated HRs CI included '1' for all parameters (appendix p34-35).

DFS in the MAGE-A3 group and the Placebo group was 46·6% (95% CI 43·2-49·8) and 46·7% (95% CI 42·0-51·2), respectively at year 1, 37·2% (95% CI 34·0-40·4) and 38·8% (95% CI 34·2-43·3) at year 2, 33·2% (95% CI 29·7-36·6) and 34·9% (95% CI 30·1-39·8) at year 3 and 30·7% (95% CI 26·7-34·7) and 32·5% (95% CI 26·9-38·2) at year 4.

Overall, 467 patients died, 314/893 (35%) in the MAGE-A3 group and 153/452 (34%) in the Placebo group. Median OS was reached in the Placebo group (46·6 months, 95% CI 39·6- not reached) but not in the MAGE-A3 group (HR 1·07, 95% CI 0·88-1·29, p=0·52).

There were 850 DFSS events (566/893 [63%] in MAGE-A3 and 284/452 [63%] in Placebo) and 743 DMFS events (502/893 [56%] in MAGE-A3 and 241/452 [53%] in Placebo). The median DFSS was 11·1 months (95% CI 10·4-12·3) in the MAGE-A3 group and 11·2 (95% 8·6-14·1) in the Placebo group (HR 1·00, 95% CI 0·87-1·16; p=0·98). The median DMFS was 18·7 months (95% CI 16·3-22·1) and 23·9 months (95% CI 18·9-30·7), respectively, (HR 1·09, 95% CI 0·94-1·27; p=0·27).

The results of the follow-up analysis were consistent with the final analysis (appendix p17). All sensitivity analyses of the primary endpoints were consistent with the main conclusion of absence of treatment effect.

The final and follow-up analyses were reviewed by the IDMC. Based on their feedback and the lack of treatment effect for both co-primary endpoints, the study was terminated early, on 8 September 2015. This decision was based exclusively on the efficacy endpoints assessment and on the fact that by stopping the study, the participating patients would not be exposed to unnecessary study-related procedures. At the time of the decision, 308 patients were still on the study worldwide (follow-up phase).

AEs within 31 days of treatment administration were reported by 92% (822/894) of MAGE-A3 and 74% (334/450) of Placebo recipients (Table 2). The most frequently reported AEs were pyrexia, injection site pain and influenza-like illness, all of which were more common in the MAGE-A3 group. Most AEs reported within 31 days of treatment administration in each group were grade 1 or 2. In the MAGE-A3 group, 14% (126/894) of patients experienced AEs grade 3 or above, compared to 12% (56/450) in the Placebo group. The most frequently reported grade 3 or higher adverse events were neoplasms (33 [4%] in the MAGE-A3 group and 17 [4%] in the Placebo group), general disorders and administration site conditions (25 [3%] versus 4 [<1%]) and infections and infestations (17 [2%) versus 7 [2%]). Treatment-related grade 3 or above AEs within 31 days of treatment occurred in 4% (36/894) and 1% (6/450) of patients in the MAGE-A3 and Placebo groups, respectively. No related grade 4 or grade 5 AEs in either group was reported.

At least one SAE was reported by 14% of patients in both groups (129/894 in the MAGE-A3 group and 64/450 in the Placebo group) (appendix p24). The most frequently reported SAEs according to MedDRA System Order Class (SOC) were ‘Neoplasm benign, malignant and unspecified’ (6% [55/894] in the MAGE-A3 group and 5% [24/450] in the Placebo group), ‘Infection and Infestations’ (3% [30/894] and 3% [14/450], respectively). All other MedDRA SOCs were represented by ≤1% of patients in each group.

SAEs considered to be treatment related by the investigator were reported in <1% (8/894) of patients in the MAGE-A3 group (Pyrexia, Autoimmune thyroiditis, Polyneuropathy, Erysipelas, Wound infection, Vision blurred, Lymphadenitis, Subarachnoid haemorrhage) and <1% (4/450) in the Placebo group (Retinopathy, Thrombocytopenic purpura, Invasive lobular breast carcinoma, Pain in extremity). The same treatment-related SAE was not reported in more than one patient within the same group.

Fatal (Grade 5) SAEs that occurred at any time from randomisation until the end of study were reported in <1% of patients in both study groups (5/894 patients in the MAGE-A3 group and 1/450 in the Placebo group (Appendix p25). None of the fatal SAEs were considered treatment-related as per investigator assessment.

New onset of potential immune-mediated diseases (pIMDs) occurred in 4% (33/894) of patients in the MAGE-A3 group, and 5% (23/450) in the Placebo group. pIMDs were distributed over 11 MedDRA SOCs (Table 4). Aside from vitiligo (20 cases in the MAGE-A3 group and 13 cases in the Placebo group), only autoimmune thyroiditis (3 cases in the MAGE-A3 group) and sarcoidosis (2 cases in the Placebo group) were reported by more than one patient per group. pIMDs considered by the investigator to be treatment-related were reported by 3% of patients in both groups (26/894 in the MAGE-A3 group, 12/450 in the Placebo group). Of these, 19 in the MAGE-A3 group and 11 in the Placebo group were cases of vitiligo.

There were 14/894 (2%) patients in the MAGE-A3 group and 5/450 (1%) in the Placebo group who discontinued study treatment prematurely due to AEs. Of these, 7 patients in the MAGE-A3 and no patients in the Placebo group discontinued due to an AE assessed as treatment-related.

Results for the GS+ and GS- populations mirrored those of the overall population (appendix p26-27).

One dose was skipped by 8 patients (0·9%) in the MAGE-A3, 5 patients (1·1%) in the Placebo group, and 2 doses were skipped by one patient (0·1%) in the MAGE-A3 group. In the MAGE-A3 group, five patients had reached the maximum delay for postponing the treatment, one patient had an AE, one had an SAE and 3 doses were skipped for other reasons. In the Placebo group 3 patients had reached the maximum delay for postponing the treatment, and two were skipped for other reasons).

At least one dose was delayed in 181 patients (20·2%) in the MAGE-A3 group and by 93 patients (20·7%) in the Placebo group. Reasons for delaying doses were AEs (25/370 delayed doses, 6·8%, in the MAGE-A3 group and 10/185 delayed doses, 6·3%, in the Placebo group), SAEs (13/370, 3·5% and 1/185, 0·5%, respectively), other reasons (323/370, 87·3% and 169/185, 91·4%) and reason not known (9/370, 2·4% and 5/185, 2·7%, respectively).

Antibody geometric mean concentrations increased rapidly with MAGE-A3 immunotherapeutic treatment and remained elevated throughout the treatment period (appendix p36). Results for the GS+ and GS- populations mirrored those of the overall population (appendix p37).

Mean utility scores between 0·80 and 0·90 were observed during the treatment period. The mixed effect repeated measures analysis of change from baseline in EQ-5D utility scores over time identified a statistically significant detrimental effect of treatment on the day after the first, third and fifth treatment administrations, and at recurrence (day after visit 1 mean -0·067 standard deviation [SD] 0·011 on MAGE-A3 and 0·020 [0·016] on placebo; p<0·0001); day after visit 3 -0·129 [0·011] on MAGE-A3 and 0·019 [0·016] on placebo; p<0·0001; day after visit 5 -0.060 [0·012] on MAGE-A3 and 0·022 [0·018] on placebo; p=0·0001, at recurrence -0·100 [0·017] on MAGE-A3 and -0·029 [0·027] on placebo; p=0·026). At year 1, the difference was in favour of the MAGE-A3 group (mean at year 1 of follow-up -0·067 [SD 0·0277] on MAGE-A3 and -0·208 [0·044] on placebo; p=0·0057).

The same observation was made for visual analogue scores on the day after the first and third administrations (day after visit 1 mean -7·879 [SD 1·497] on MAGE-A3 and 0·398 [2·203] on placebo; p=0·0019); day after visit 3 -8·820 [1·510] on MAGE-A3 and 0·328 [2·216] on placebo; p=0·0007). There was evidence of a group difference for the change from baseline in the overall model for utility score (adjusted mean -0·037 [SD 0·010] for MAGE-A3 and 0·002 [0·011] for placebo; p=0·0006). There was no evidence of a group difference in the change from baseline in the visual analogue scores in the overall model (adjusted mean -4·025 [SD 1·283] for MAGE-A3 and -1·268 [1·532] for placebo; p=0·0683) (see appendix p28-32). The decreased scores on MAGE-A3 treatment after the first and third treatment administrations are most likely related to the dimension pain/discomfort at the injection site. This observation from the descriptive EQ-5D analysis is consistent with the clinical safety results.

Discussion

To our knowledge, this is the largest adjuvant trial ever conducted in melanoma. Treatment with the MAGE-A3 immunotherapeutic was well-tolerated and immunogenic, inducing large increases in anti-MAGE-3 antibody levels, but without translation into clinical efficacy. The MAGRIT study, a similarly designed Phase 3 trial of adjuvant MAGE-A3 immunotherapeutic in patients with resected NSCLC that became available before study end, drew similar conclusions.[23] Despite initially encouraging, but ultimately discordant, results from Phase 2 studies, treatment with the MAGE-A3 immunotherapeutic did not improve DFS, OS or any other clinical outcome measure in the overall population, nor in subgroups according to tumour characteristics or treatment procedures. Although a gene signature potentially predictive of clinical benefit from MAGE-A3 immunotherapeutic over placebo in the GS+ population was identified in the training set, it could not be clinically validated in the test set. Of note, a Th1/IFNγ 8-gene prognostic gene signature associated with outcome in the placebo arm of the training set was validated in the test set of this study.[22] Even though the validation of the predictive gene signature using the adaptive signature design did not

succeed, we have shown it is feasible to use this approach for optimisation and validation of biomarkers for which not all parameters have been set at the start of the clinical trial.

We do not believe the study had any major limitations in design or analysis. A total of 1345 patients were randomised and started treatment and the required number of events (850) was reached at the time of the final analysis of the first co-primary objective. The study groups were well balanced and unsuspected bias or confounding factors that might have influenced the outcome are unlikely. The percentage of patients who received previous treatment with interferon and/or anti-CTLA4 was similar in both treatment groups.

Of note, the study did not have a pre-specified stopping criteria (futility analysis). This was because safety was overseen by an IDMC that reviewed study data on a 6-monthly basis throughout the study conduct and did not identify any safety concerns; no recognised alternative treatment option was available in absence of recurrence; and the implementation of a futility rule was not compatible with the search for a subgroup of subjects with a potentially more pronounced benefit from treatment (GS+), for which the assessment occurred later in the course of the study.

The reasons underlying the lack of clinical efficacy in our study are speculative, but could be related to the choice of antigen or immunostimulant, and/or the absence of the induction of T-cell responses, particularly CD8+ responses. We may have selected a target population with disease too advanced for successful vaccine immunotherapy treatment. Of note, the observed median DFS of approximately 11 months is shorter than the reference 13-month median DFS in a similar population reported by Eggermont et al.[3] Although reflecting real-world practice, there were a high percentage of subjects with unknown primaries (TxN1b-N2b-N3 M0) in the study. Although some reports suggest differences in outcome in patients with unknown primary melonoma,[24] we observed no treatment effect in this subgroup in the exploratory subgroup analyses.

MAGE-A3 was initially an attractive immunotherapeutic candidate because it is one of the most immunogenic cancer testis antigens and is human leukocyte antigen (HLA)-

independent.[8] The lack of efficacy in this trial could have been due to failure in one or multiple steps of the cancer immunity cycle,[25] including, failure to mount an appropriate anti-tumour immune response and mechanisms of immune evasion and suppression. The success of adoptively transferred and genetically modified T-cells in treating haematological malignancies and solid tumours, confirms the pivotal role of cytotoxic reactive T-cells in anti-tumour response.[26] We observed low or absent CD8+ responses in the Phase 2 study of the MAGE-A3 immunotherapeutic,[10] which seems likely to have contributed to the lack of clinical effect. Immunotherapeutics aim to induce anti-tumour T-cell responses but its effects can be inhibited by many immunosuppressive mechanisms. These include the loss of major histocompatibility complex class I, expression of ligands for inhibitory receptors (programmed death-ligand 1, CD200, HLA‑E), infiltration with suppressive cells, secretion of indoleamine 2,3‑dioxygenase, and secretion of immune-suppressive cytokines. Thus, immunotherapeutics might be more successful when used in early diseases stages when immune suppression might be less pronounced, and when combined with other treatments that can activate anti-tumour T-cell responses.[27] In the future, the most promising combination may be an immunotherapeutic with check point inhibitor for maintaining the activation of cytotoxic T cells against melanoma antigens, with the advantage of the excellent tolerance of vaccines.

The treatment of metastatic melanoma has changed dramatically in the past 5 years with the availability of BRAF/MEK inhibitors as well as multiple checkpoint inhibitors, which are immunopotentiators that are not specifically analogous to the MAGE-A3 immunotherapeutic. These drugs have changed the field of adjuvant therapy in melanoma. In the BRIM-8 trial, adjuvant vemurafenib provided substantial benefit to patients with completely resected stage IIC-IIIB BRAFV600-positive melanoma at high recurrence risk, where fewer DFS events and DMFS events were observed with vemurafenib compared to placebo. However, the benefit of this same agent was not significant in patients with resected stage IIIC melanoma, where a trend towards improved DFS was seen. The OS data are still immature for both cohorts.

The benefit of IFN in metastatic disease is well documented to be in the order of a 15% objective response, and the benefit of IFN in the adjuvant therapy of melanoma has been approximately 25-33% relapse frequency reduction (HR 0.28-0.33) in trials E1684-E1694. Ipilimumab prolongs both DFS and OS in Stage III melanoma but causes severe adverse events;[28] PD-1 inhibitors in the adjuvant setting have very recently shown significant benefit in DFS over ipilimumab although OS benefits are not yet mature.[5] Trials designed to increase the efficacy of checkpoint inhibitors in multiple combinations are ongoing. As yet, it is unknown if combinations of antigen specific immunotherapies and checkpoint inhibitors might also improve efficacy; although the combination of gp100 and the first-generation CTLA4 checkpoint inhibitor ipilimumab, provided no additional benefits in patients with advanced melanoma.[29]

Research in context

Evidence before this study

We conducted a PubMed search on 26 November 2017 using the terms “melanoma AND (vaccine OR immunotherapeutic) AND clinical trials, phase 3” without limitations on date or language. We identified eight relevant studies in patients with resected melanoma (Stages I-IV) who had received different types of immunotherapies/vaccines. None of the studies reported that treatment improved clinical outcome in the adjuvant setting.

Added value of this study

Based on the available biological and clinical information from the Phase 2 studies, it was considered that a Phase 3 study using the MAGE-A3 immunotherapeutic in patients with Stage 3b melanoma was warranted. Both MAGRIT and DERMA provide conclusive evidence of the acceptable clinical safety profile of the MAGE-A3 immunotherapeutic, but treatment did not provide clinical benefit in either patient population. The clinical development of the MAGE-A3 immunotherapeutic for these indications has therefore been stopped. Nevertheless, an acceptable safety profile of the MAGE-A3 immunotherapeutic has

been demonstrated in large populations of patients with melanoma or NSCLC, supporting the feasibility of the approach in terms of tolerability.

DERMA confirms a high rate of early disease recurrence among patients with resected Stage III melanoma. With 1345 patients studied, the study provides a large body of information on disease progression and clinical outcomes in the adjuvant setting of melanoma, and provides insights into the clinical characteristics and outcomes in patients receiving contemporary surgery for melanoma treatment. An adaptive signature design was used to optimise and validate a potentially predictive gene signature in this study using a split-sample approach. A previously reported Th1/IFNγ gene signature was not predictive of a treatment benefit from the MAGE-A3 immunotherapeutic over placebo in the GS+ population, in this setting but found to be strongly associated to clinical outcome in the placebo arm (prognostic). A novel potentially predictive gene signature found in the training set in this study failed to be validated in the test set. The feasibility of using the adaptive signature design for biomarker validation in a registration study has been demonstrated here.

Implications of all the available evidence

We and others have now shown that antigen specific immunotherapeutics alone is not efficacious in this clinical setting. The failure of these approaches might be due to inability to mount appropriate anti-tumour immune responses, and/or the need to overcome tumour immune suppressive mechanisms as shown with checkpoint inhibitors. Although considered as an immunogenic antigen, T cell repertoire specific for the characterized class I epitopes are of rather low frequencies in melanoma patients. Targeting other shared tumor antigens, together with MAGE-A3 antigen could favor the amplification of immune responses in treated patients. Ideal target antigens for vaccination purposes should combine different properties such as tumor-specific expression, and the presence of vast and high avidity specific T cell repertoire.

Author contributions

BD, TJ, EL, AT, JAT, AH, JJG, HH, FUM, BD, BS, MD, JL, PT, CD, JMK conceived and designed the study. BD, JFT, BMS, MS, TJ, RG, EL, PR, JJG, SK, KD, FG, LM, PH, AT, RC, BG, WHJK, LD, JAT, IB, JJ, GC, SP, AH, FUM, AC, BD, BS, JL, PT, CD, JMK collected or generated the data. BD, JFT, BMS, MS, TJ, RG, EL, PR, JJG, SK, KD, FG, LM, PH, IK, AT, RC, BG, WHJK, LD, JAT, IB, JJ, SP, AH, FUM, BD, BS, JL, PT, CD, JMK performed the study/project.BD, EL, JJG, KD, IK, AT, BG, JAT, IB, AH, HH, FUM, BD, BS, JL, PT, CD contributed with materials, analysis and/or reagent tools, BD, TJ, RG, EL, PR, JJG, IK, AT, RC, BG, WHJK, JAT, IB, SP, AH, JJG, HH, FUM, AC, BD, BS, MD, JL, PT, CD, JMK performed or supervised the analysis. All authors contributed substantially to the development of the manuscript and approved the final version.

Declaration of Interests

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Table 1: Demographic and disease characteristics (Total Treated population– as randomised)*

Characteristics		**MAGE-A3 N=893**	**Placebo N=452**
Age at screening (years)	Mean (SD)	56·1 (13·5)	56·1 (13·6)
	Range	18-87	20-88
	Median (IQR)	57 (44-66)	57 (44-66)
Sex n (%)	Women	344 (38·5)	189 (41·8)
	Men	549 (61·5)	263 (58·2)
Primary tumour ulceration	Yes	322 (36·1)	157 (34·7)
	No	397 (44·5)	210 (46·5)
	Unknown/Missing	174 (19·5)	85 (18·8)
Tumour stage	T1	122 (13·7)	65 (14·4)
	T2	197 (22·1)	100 (22·1)
	T3	202 (22·6)	110 (24·3)
	T4	210 (23·5)	101 (22·3)
	TX	162 (18·1)	76 (16·8)
Nodal stage	N1 a	1 (0·1)	0 (0·0)
	N1 b	356 (39·9)	181 (40·0)
	N2 a	1 (0·1)	2 (0·4)
	N2 b	272 (30·5)	137 (30·3)
	N3	263 (29·5)	132 (29·2)
Performance status n(%)	0	740 (82·9)	378 (83·6)
	1	153 (17·1)	73 (16·2)
	3	0	1 (0·2)
Stage n (%)	Stage IIIA	1 (0·1)	0
	Stage IIIB	292 (32·7)	155 (34·3)
	Stage IIIC	483 (54·1)	241 (53·3)
	Undefined Stage III (TX)	109 (12·2)	53 (11·7)
	Stage IV	8 (0·9)	3 (0·7)
Prior therapy	Interferon	130 (14·6)	67 (14·8)
	Anti-CTL-A4	3 (0·3)	2 (0·4)
	Interferon and/or anti-CTL-A4	133 (14·9)	69 (15·3)
	Radiotherapy	8 (0·9)	5 (1·1)
Number of lymph nodes invaded	1	360 (40·3)	187 (41·4)
	2	198 (22·2)	89 (19·7)
	3	81 (9·1)	51 (11·3)
	3+	223 (25·0)	110 (24·3)
	Matted	31 (3·5)	15 (3·3)
Extracapsular extension	No	591 (66·2)	304 (67·3)
	Yes	300 (33·6)	148 (32·7)
	Missing	2 (0·2)	0
Region	Europe	658 (73·7)	327 (72·3)
	International	98 (11·0)	50 (11·1)
	North America	137 (15·3)	75 (16·6)

N = total number of patients, n (%) = number/percentage of patients with the defined characteristic, SD = standard deviation; IQR = inter quartile range.
* The final analysis was performed on the population as-randomized: one patient who was randomised to the treatment group received placebo and three patients who were randomised to placebo received MAGE-A3 immunotherapeutic. This led to differences in the denominator in populations evaluated for efficacy (as-randomised) and safety (as treated).

Table 2: Summary of adverse events within 31 days of administration, by maximum Common Terminology Criteria for Adverse Events grade (Total Treated population, follow-up analysis – as treated)

	MAGE-A3 N = 894					PLACEBO N = 450				
	Grade					Grade				
	1/2	3	4	5	Unknown	1/2	3	4	5	Unknown
Preferred term (≥ 10% of patients)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Any event	696 (78%)	97 (11%)	28 (3%)	1 (<1%)	0	278 (62%)	46 (10%)	9 (2%)	1 (<1%)	0
Pyrexia	376 (42%)	5 (<1%)	0	0	0	35 (8%)	0	0	0	0
Injection site pain	324 (36%)	1 (<1%)	0	0	0	22 (5%)	0	0	0	0
Influenza like illness	261 (29%)	0	0	0	0	30 (7%)	0	0	0	0
Fatigue	202 (23%)	8 (<1%)	0	0	0	62 (14%)	1 (<1%)	0	0	0
Headache	200 (22%)	5 (<1%)	0	0	0	53 (12%)	2 (<1%)	0	0	0
Myalgia	185 (21%)	3 (<1%)	0	0	0	23 (5%)	0	0	0	0
Pain	186 (21%)	5 (<1%)	0	0	0	19 (4%)	0	0	0	0
Asthenia	140 (16%)	9 (1%)	0	0	0	43 (10%)	3 (<1%)	0	0	0
Chills	177 (20%)	2 (<1%)	0	0	0	15 (3%)	0	0	0	0
Injection site reaction	160 (18%)	0	0	0	0	6 (1%)	0	0	0	0
Nausea	123 (14%)	0	0	0	0	32 (7%)	0	0	0	0
Erythema	137 (15%)	0	0	0	1 (<1%)	10 (2%)	0	0	0	0
Pain in extremity	113 (13%)	2 (<1%)	0	0	0	25 (6%)	1 (<1%)	0	0	0
Injection site erythema	90 (10%)	0	0	0	0	3 (<1%)	0	0	0	0
Primary system organ class										
Any event	696 (78%)	97 (11%)	28 (3%)	1 (<1%)	0	278 (62%)	46 (10%)	9 (2%)	1 (<1%)	0
General disorders and administration site conditions	735 (82%)	25 (3%)	0	0	1 (<1%)	189 (42%)	4 (<1%)	0	0	0
Musculoskeletal and connective tissue disorders	365 (41%)	17 (2%)	1 (<1%)	0	0	103 (23%)	8 (2%)	0	0	0
Nervous system disorders	287 (32%)	11 (1%)	1 (<1%)	0	0	83 (18%)	6 (1%)	0	0	0
Skin and subcutaneous tissue disorders	280 (31%)	1 (<1%)	0	0	0	84 (19%)	0	0	0	0
Gastrointestinal disorders	235 (26%)	4 (<1%)	1 (<1%)	0	0	79 (18%)	2 (<1%)	0	0	0
Infections and infestations	180 (20%)	14 (2%)	3 (<1%)	0	0	91 (20%)	6 (1%)	1 (<1%)	0	0

Respiratory, thoracic and mediastinal disorders	84 (9%)	2 (<1%)	2 (<1%)	0	0	40 (9%)	0	0	0	0
Vascular disorders	69 (8%)	11 (1%)	1 (<1%)	0	0	35 (8%)	9 (2%)	0	0	0
Psychiatric disorders	77 (9%)	0	1 (<1%)	0	1 (<1%)	31 (7%)	0	1 (<1%)	0	0
Neoplasms benign, malignant and unspecified (including cysts and polyps)	32 (4%)	16 (2%)	17 (2%)	0	0	23 (5%)	10 (2%)	7 (2%)	0	0
Investigations	62 (7%)	5 (<1%)	0	0	0	26 (6%)	1 (<1%)	0	0	0
Injury, poisoning and procedural complications	56 (6%)	4 (<1%)	0	0	0	29 (6%)	1 (<1%)	0	0	0
Metabolism and nutrition disorders	57 (6%)	3 (<1%)	0	0	0	13 (3%)	0	0	0	0
Blood and lymphatic system disorders	24 (3%)	4 (<1%)	0	0	0	17 (4%)	0	0	1 (<1%)	0
Reproductive system and breast disorders	27 (3%)	0	0	0	0	12 (3%)	1 (<1%)	0	0	0
Eye disorders	21 (2%)	0	0	0	0	13 (3%)	3 (<1%)	0	0	0
Ear and labyrinth disorders	19 (2%)	0	0	0	0	17 (4%)	0	0	0	0
Cardiac disorders	12 (1%)	5 (<1%)	1 (<1%)	1 (<1%)	0	4 (<1%)	0	0	0	0
Renal and urinary disorders	14 (2%)	4 (<1%)	0	0	0	4 (<1%)	1 (<1%)	0	0	0
Hepatobiliary disorders	5 (<1%)	1 (<1%)	0	0	0	4 (<1%)	1 (<1%)	0	0	0
Immune system disorders	4 (<1%)	0	0	0	0	7 (2%)	0	0	0	0
Endocrine disorders	4 (<1%)	0	0	0	0	2 (<1%)	0	0	0	0

N = number of patients with at least one administered dose

n/% = number/percentage of patients reporting the adverse event.

MedDRa = Medical Dictionary for Regulatory Activities

Unknown = Grade not known

Preferred terms for adverse events reported by at least 10% of patients in any group. Primary system organ class summarises all adverse events.

Table 4: Potential immune-mediated diseases determined from a predefined list of Preferred Terms and/or by the investigator (Total Treated population, follow-up analysis)

MedDRA System Organ Class	MedDRA Preferred Term	MAGE-A3 N = 894 n (%)	Placebo N = 450 n (%)
Any event	Any event	33 (4%)	23 (5%)
Blood and lymphatic system disorders	Thrombocytopenic purpura	0	1
Endocrine disorders	Autoimmune thyroiditis	3	0
	Basedow's disease	1	1
	Hypothyroidism	1	0
	Lymphocytic hypophysitis	0	1
	Polyglandular autoimmune syndrome type II	0	1
Eye disorders	Vision blurred	1	0
Gastrointestinal disorders	Colitis ulcerative	0	1
Hepatobiliary disorders	Autoimmune hepatitis	1	1
Immune system disorders	Sarcoidosis	0	2
Musculoskeletal and connective tissue disorders	Polymyalgia rheumatica	0	1
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Langerhans' cell histiocytosis	0	1
Nervous system disorders	Multiple sclerosis	1	0
	VIIth nerve paralysis	1	1
Respiratory, thoracic and mediastinal disorders	Pulmonary fibrosis	1	1
Skin and subcutaneous tissue disorders	Alopecia areata	1	0
	Psoriasis	1	1
	Skin hypopigmentation	1	0
	Vitiligo	20 (2%)	13 (3%)

N = total number of patients, n (%) = number/percentage of patients

Figure 1

Subject flow at the follow-up analysis (18 August 2015) – as treated

Footnotes:

N, n = Number of participants

1 Invalid = gDNA contamination or result out of range. Quantity not sufficient = not enough tumour tissue or insufficient RNA. Other = improper specimen. Missing = informed consent signed but no sample received.

2 Main reasons for not meeting eligibility criteria: Patient did not sign study informed consent (n=197), ineligible disease stage (106), residual disease post-surgery (280), patients unable to comply with study requirements (63), In-transit metastases (62). Note that patients could be ineligible for more than one reason.

3 The main reason for not starting treatment was ineligibility

4 Treatment completion = 13 doses administered and concluding visit attended.

The final analysis was performed on the population as-randomized: one patient who was randomised to the treatment group received placebo and three patients who were randomised to placebo received MAGE-A3 immunotherapeutic. This led to differences in the denominator in populations evaluated for efficacy (as-randomised) and safety (as treated).

Between the final analysis done on the overall population (May 2013) and the follow-up analysis in the GS+ population (August 2015), one patient was found to have an invalid study informed consent (consent form for screening signed twice in error instead of the consent form for study participation). This patient's data were included in the final analysis and in the building of the gene signature classifier (this patient was part of the training set), but were not included in the follow-up analysis.

Figure 2 Disease-free survival (DFS) and Overall survival (OS) in Total treated population and in the GS+ population (follow-up analysis, as randomised).

Figure 3 Forest plots for Disease-free survival in subgroups defined by baseline and treatment variables

HR (Hazard ratio) and 95% CI (confidence intervals) from a Cox regression model by subgroup, using Efron method to handle ties

LL, UL = 95% Lower and Upper confidence limits

P-value = Likelihood ratio test for interaction with treatment

Patients with missing values for at least one baseline variable are discarded

N category = nodal stage

T category = tumour stage

Figure 1

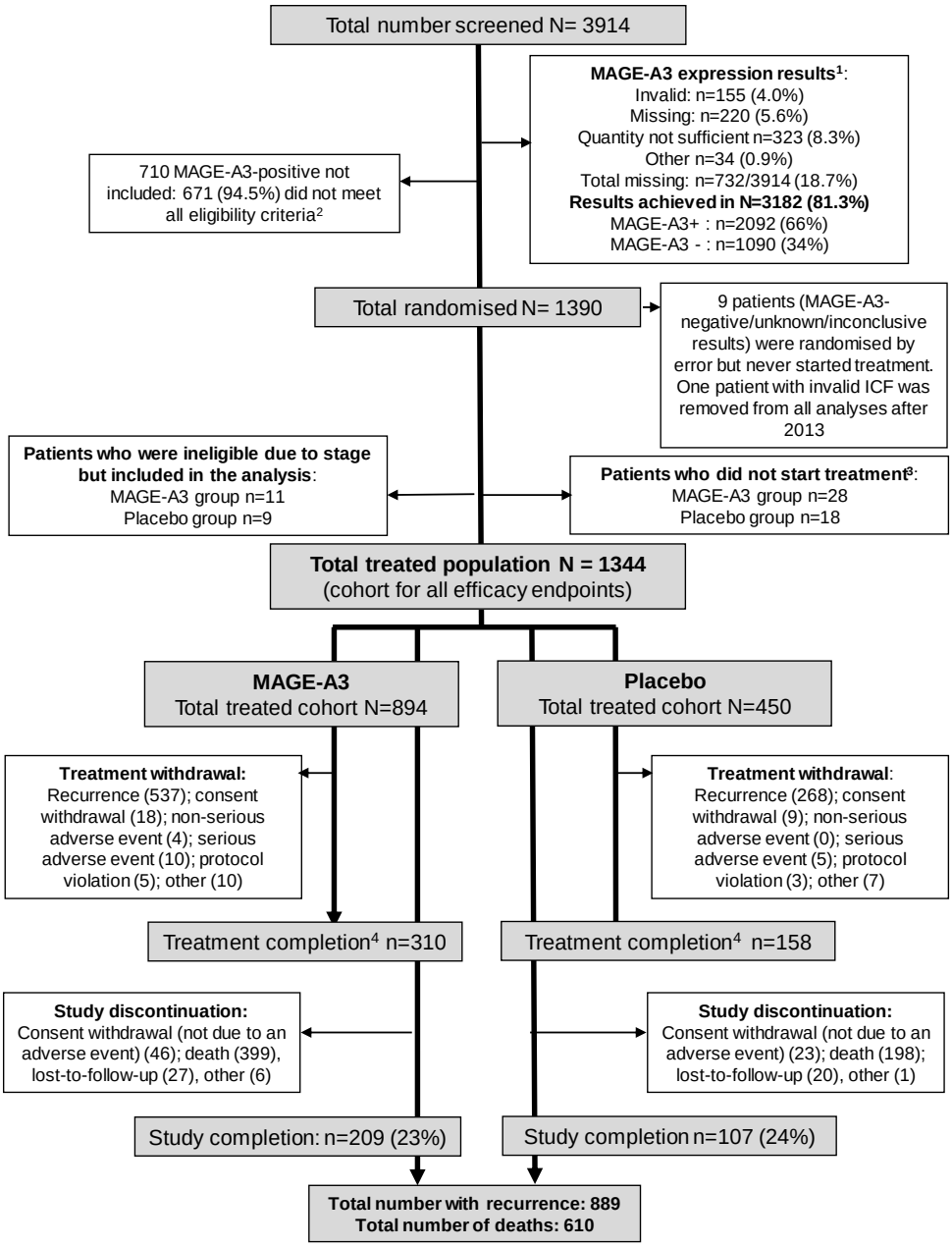
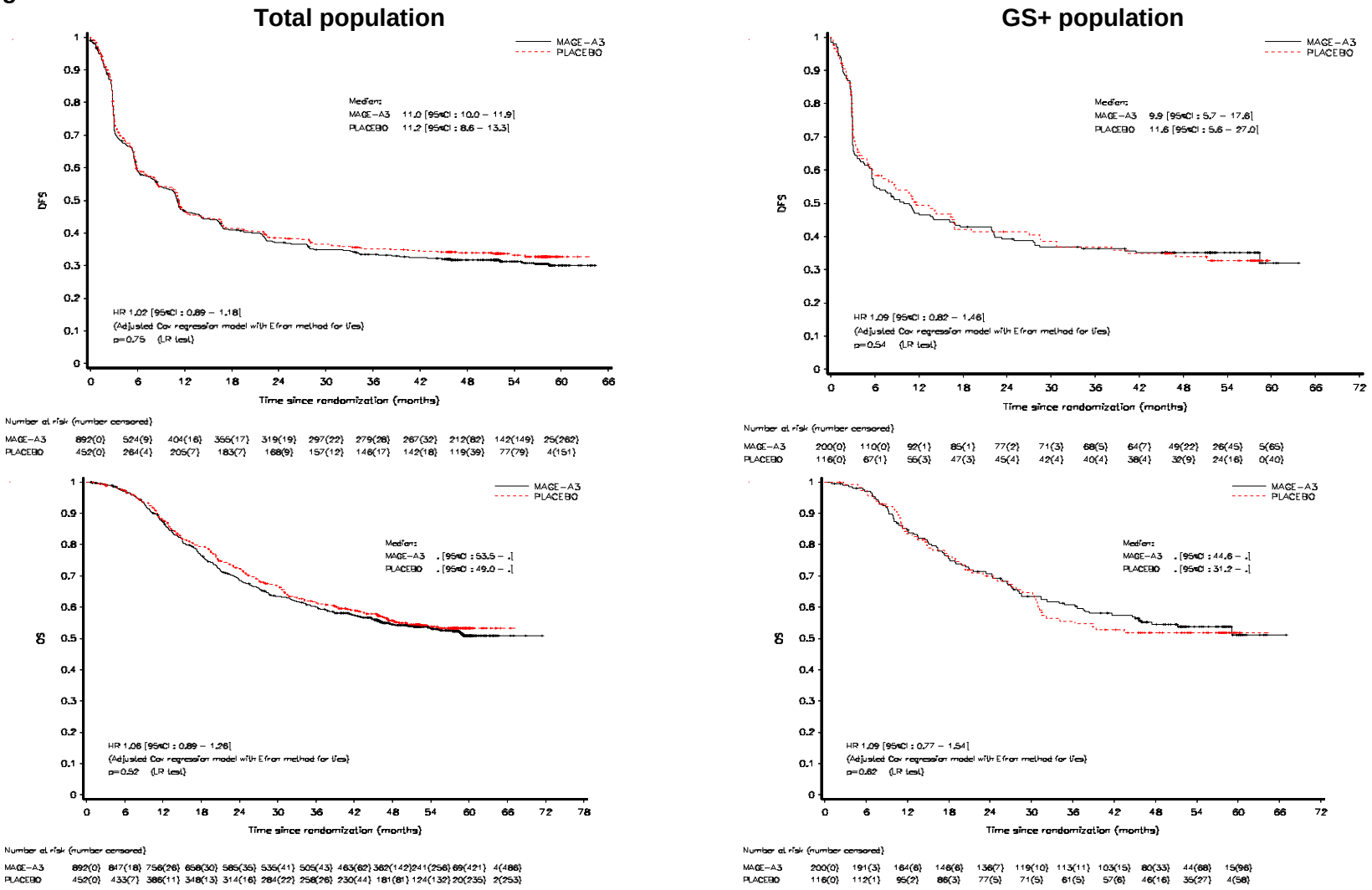

Total number screened N= 3914
MAGE-A3 expression results[1]:
Invalid: n=155 (4.0%)
Missing: n=220 (5.6%)
Quantity not sufficient n=323 (8.3%)
Other n=34 (0.9%)
Total missing: n=732/3914 (18.7%)
Results achieved in N=3182 (81.3%)
MAGE-A3+ : n=2092 (66%)
MAGE-A3 - : n=1090 (34%)
710 MAGE-A3-positive not included: 671 (94.5%) did not meet all eligibility criteria[2]
Total randomised N= 1390
9 patients (MAGE-A3-negative/unknown/inconclusive results) were randomised by error but never started treatment. One patient with invalid ICF was removed from all analyses after 2013
Patients who were ineligible due to stage but included in the analysis:
MAGE-A3 group n=11
Placebo group n=9
Patients who did not start treatment[3]:
MAGE-A3 group n=28
Placebo group n=18
Total treated population N = 1344
(cohort for all efficacy endpoints)
MAGE-A3
Total treated cohort N=894
Placebo
Total treated cohort N=450
Treatment withdrawal:
Recurrence (537); consent withdrawal (18); non-serious adverse event (4); serious adverse event (10); protocol violation (5); other (10)
Treatment withdrawal:
Recurrence (268); consent withdrawal (9); non-serious adverse event (0); serious adverse event (5); protocol violation (3); other (7)
Treatment completion[4] n=310
Treatment completion[4] n=158
Study discontinuation:
Consent withdrawal (not due to an adverse event) (46); death (399), lost-to-follow-up (27), other (6)
Study discontinuation:
Consent withdrawal (not due to an adverse event) (23); death (198); lost-to-follow-up (20), other (1)
Study completion: n=209 (23%)
Study completion n=107 (24%)
Total number with recurrence: 889
Total number of deaths: 610

Figure 2



This is the last approved revised version prior to journal acceptance. This version differs from the final version following Editorial change prior to publication.

Figure 3