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MONOGRAPH

ASTUPROTIMUT-R

Therapeutic Cancer Vaccine

USAN

GSK-249553
MAGE-A3
MAGE-A3 ASCI
SB-249553

19,137,308,342,395-Penta-[*S*-carbamidomethyl] [2-aspartic acid(K^2>D) 3-proline(L^3>P)]glycerophosphoryl diester phosphodiesterase (*Haemophilus influenzae* strain 86-028NP EC 3.1.4.46)(1-127)-peptide fusion protein with [2-aspartic acid(P^2>D)]human melanoma-associated antigen 3 (MAGE-3 antigen, antigen MZ2-D, cancer/testis antigen 1.3 or CT1.3) fusion protein with glycylglycylheptahistidine

Vaccine consisting of a recombinant fusion protein composed of a partial sequence of the protein D antigen of *Haemophilus influenzae* fused to MAGE-3 protein

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SUMMARY

The melanoma-associated antigen 3 (MAGE-3) is a commonly expressed tumor-specific antigen that represents a potential candidate for the development of cancer vaccines. Astuprotimut-R (GSK-249553), a vaccine comprising a recombinant MAGE-3 epitope fused to a partial sequence of the protein D antigen of Haemophilus influenzae, is currently under clinical evaluation by GlaxoSmithKline for the treatment of malignant melanoma and non-small cell lung cancer (NSCLC). Encouraging data obtained from various phase II studies have led to the initiation of MAGRIT, a large, randomized phase III trial of the vaccine conducted in subjects with resectable NSCLC, which is expected to provide valuable insight into the potential therapeutic benefit of immune-based intervention in cancer treatment.

BACKGROUND

Active cancer immunotherapy relies on the ability of a patient's immune system to elicit a specific T-cell response that targets tumor cells, leading to their destruction, while sparing the surrounding normal tissue. A novel approach to cancer immunotherapy that is currently in clinical development involves the combination of a tumor-specific antigen with an immunological adjuvant system, thus leading to the generation of a class of agents referred to as antigen-specific cancer immunotherapeutics (ASCIs). ASCIs are expected to trigger an effective T-cell response against antigens that are present exclusively on tumor cells but not normal cells (1).

Immunotherapy against a tumor target that is exclusively present on the surface of malignant cells represents a promising option for cancer treatment. The melanoma-associated antigen 3 (MAGE-3) gene (*MAGEA3*) encodes a tumor-specific antigen that is expressed on the surface of tumor cells but not normal cells, with the exception of male germline cells, and hence it is also referred to as a cancer testis (CT) antigen; however, male germline cells do not present MAGE-3 antigens due to lack of major histocompatibility complex molecules (2). The ability of CT antigens to elicit a spontaneous immune reaction in cancer patients renders MAGE-3 a promising candidate for immune-based intervention in cancer treatment (3).

The *MAGEA3* gene family, which is located on the X chromosome, was originally reported to encode tumor-specific antigens in melanoma cells. It is now known to be expressed in a variety of tumors, including esophageal, breast, lung, bladder and gastric cancer, as well as leukemia and thyroid carcinomas (4). MAGE-3-derived peptides bind to human leukocyte antigen (HLA) class I or class II molecules and the complexes are subsequently recognized by autologous cytolytic T lymphocytes (CTLs) on the surface of tumor cells (5). MACE-3 protein has been identified as a functional integrator of heparin-binding growth factor 2 (HBGF-2) and fibronectin (FN) signaling. As such, it has been shown to promote cell migration and invasion in vitro and to support thyroid tumor growth and lung metastasis in vivo. The immunogenicity of MAGE-3 peptides has supported the use of these antigens for the development of cancer immunotherapeutics (4).

Astuprotimut-R (GSK-249553, SB-249553, MAGE-A3 ASCI) is a cancer vaccine comprising the MAGE-3 epitope fused to a partial sequence of the protein D antigen of *Haemophilus influenzae* that is currently under clinical development by GlaxoSmithKline for the treatment of non-small cell lung cancer (NSCLC) and malignant melanoma (6, 7). Astuprotimut-R received orphan drug designation in the U.S. in 2009 for the treatment of MAGE-3-positive stage IIB-IV malignant melanoma, as well as for the treatment of MAGE-3-positive NSCLC.

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CLINICAL STUDIES

Fifty-seven patients with stage III or IV measurable MAGE-3-positive metastatic malignancies, mostly melanoma, were vaccinated with escalating doses of recombinant MAGE-3 protein administered in combination with the immunological adjuvant SBAS-2 in a phase I/early phase II trial. The immunization schedule comprised four i.m. injections at 3-week intervals followed by two additional vaccinations at 6-week intervals only in those patients who exhibited tumor stabilization or regression after the initial four injections. The vaccine was described as generally well tolerated, with no treatment-related adverse events being reported. The most frequently observed toxicities included mild to moderate (grade 1-2) swelling and redness at the injection site, nausea and flu-like symptoms. Among 33 melanoma subjects who were evaluable for tumor response, there were 2 partial and 2 mixed responses, as well as 1 disease stabilization lasting for 11 months. The time to disease progression in these 5 patients varied between 4 and 29 months (5).

The toxicity, tumor evolution and immunological response of a fixed dose of recombinant MAGE-3 protein administered by the s.c. and intradermal routes in the absence of immunological adjuvant were evaluated in a nonrandomized, open-label, multicenter phase I/II trial in patients with detectable metastatic melanoma. A total of 32 subjects with MAGE-3-expressing melanoma were included in the study. The vaccine was generally well tolerated, with no treatment-related adverse events being reported. The most commonly observed toxicities included mild to moderate redness and swelling at the injection site, as well as flu-like symptoms. Of 26 participants receiving at least 4 vaccinations, there was 1 partial response and 4 mixed responses, with time to progression of 3.5 to 51+ months. The clinical and immunological responses induced by the vaccine were deemed rather limited (8).

In another phase I/II trial patients with metastatic MAGE-3-positive solid tumors (mostly melanoma) were treated with the MAGE-A3 ASCI vaccine in the presence or absence of the AS02B (monophosphoryl lipid A) adjuvant and were subsequently evaluated for toxicity, as well as immune and clinical response. Treatment groups receiving the adjuvant form of the vaccine at either 30, 100 or 300 µg exhibited a significant anti-MAGE-3 IgG immune response following four vaccinations. The small number of participants in this study did not permit the detection of any correlations between the immune parameters and a clinical response (9). The vaccine was deemed to be well tolerated and was associated with clinical benefit in 6 of 39 patients who received at least 4 vaccinations. Specific T-cell responses to the antigen post-vaccination were seen in approximately 30% of evaluable subjects (10).

In a randomized, open-label phase II trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group, patients (N = 75) with measurable metastatic MAGE-3-positive cutaneous melanoma, either unresectable or in transit stage II or stage IV M1a, were randomized to receive immunization with MAGE-3 recombinant protein combined with AS15 or AS02B adjuvant as first-line treatment. Both treatment arms exhibited comparable toxicities; however, the AS15 adjuvant form of the vaccine correlated with higher specific antibody titers and a more robust induction of T-cell response (72% vs. 36% seen with the AS02B adjuvant form). Stable disease lasting for more than 16 weeks was observed in 6 and 5 patients, respectively, in the AS15 and AS02B arms (11). Microarray gene expression profiling aiming to identify markers predictive of clinical activity of the vaccine in this trial identified two gene clusters based on differential expression. A correlation between this gene expression signature and an objective response was confirmed in 22 patients, whereas additional independent validation in 30 subjects confirmed a correlation between clinical benefit and molecular signature (12). A greater immune response and more frequent clinical activity were associated with the AS15 arm of the study. The gene expression profile in metastatic melanoma was found to strongly correlate with clinical activity induced by treatment with MAGE-3 immunization (13).

Ex vivo analysis of blood T cells isolated from patients with MAGE-3-positive melanoma (N = 14) carrying the HLA-DP4 allele, who had been immunized with vaccines comprising various melanoma cell antigens in combination with the MAGE-3 (243-258) peptide presented to T cells by HLA-DP4, revealed the production of $CD4^+$ T cells able to exert regulatory T-cell function in vivo as a result of vaccination with the MAGE-3 antigen. Approximately 5% of these $CD4^+$ cells had a $CD25^+$ phenotype and displayed the ability to suppress the proliferation of another T-cell clone following peptide stimulation in vitro (14).

Adjuvant immunotherapy in NSCLC is expected to improve the 5-year survival rates, which are currently estimated not to exceed 50-60% in stage I and II operable tumors. Eligibility for adjuvant vaccination with MAGE-A3 would require the expression of both MAGE-3 protein and HLA-A1 in the primary tumors of patients. The rate of MAGE-3 expression in early-stage NSCLC was assessed in primary tumor samples derived from 204 subjects with clinical stage I or II operable NSCLC in a study conducted by German scientists and sponsored by GlaxoSmithKline. *MAGEA3* mRNA expression was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in 39.2% of the examined tumors. A significant increase in *MAGEA3* transcript expression was observed in stage II compared to stage I NSCLC samples ($P = 0.004$), which suggested a possible correlation between *MAGEA3* expression and the progression to higher stages of the disease. The high expression rate of *MAGEA3* in early-stage operable NSCLC tumors renders these malignancies promising candidates for clinical evaluation of adjuvant therapy involving MAGE-A3 ASCI immunization (2).

A phase II trial conducted in 18 NSCLC patients who had undergone tumor resection evaluated the effects of MAGE-3 recombinant protein, administered either alone or in combination with the GlaxoSmithKline proprietary immunological adjuvant system AS02B, on the induction of $CD4^+$ T-cell responses. The study reported that the presence of adjuvant was required for the development of humoral and cellular responses against selected MAGE-3 peptides (3). In a subsequent study performed in 14 subjects who had received immunization with MAGE-3 in the presence or absence of adjuvant (n = 7/group) and exhibited no evidence of disease for a period of up to 3 years postimmunization, a booster vaccination with MAGE-3 combined with AS02B adjuvant correlated with distinct effects in antibody production and T-cell reactivity. In the group previously vaccinated with MAGE-3 plus AS02B adjuvant, peak antibody production was seen in six of seven subjects following a single boost injection, which also correlated with a wide spectrum of $CD4^+$ and $CD8^+$ T cells

against several new and known MAGE-3 epitopes. On the contrary, in patients who had previously received treatment with MAGE-3 protein alone, a booster vaccination with MAGE-3 plus adjuvant correlated with very limited $CD4^+$ and no $CD8^+$ T-cell reactivity and only two of seven subjects produced high titers of antibodies against MAGE-3. The data underline the importance of appropriate antigen priming using an adjuvant for the attainment of a persistent B- and T-cell memory and the generation of typical booster responses following re-immunization (15).

In an exploratory, double-blind, placebo-controlled, parallel-assignment phase II study designed to detect a clinically relevant hazard ratio, 182 patients with completely resected MAGE-3-positive stage IB or II NSCLC were randomized to receive MAGE-3 recombinant protein combined with AS02B adjuvant or placebo postoperatively. The trial aimed to assess the safety of the treatment and to evaluate the ability of the vaccine to elicit an immune response (16). Treatment was administered as five injections at 3-week intervals followed by eight injections every 3 months (17). Vaccination was initiated at ≥ 6 weeks after surgery. The primary efficacy endpoint of the trial was established as time to recurrence (18). MAGE-A3 ASCI immunization was deemed to be well tolerated overall, with only three possibly treatment-related grade 3 adverse events being reported. A positive signal for clinical activity was detected which supported additional clinical evaluation of the vaccine (19). The study confirmed the expression of MAGE-3 antigen in 35% of early-stage NSCLC cases (20). Subset analysis indicated that systematic radical mediastinal lymphadenectomy may have a positive effect on patient survival. The relative improvement in disease-free interval and disease-free survival was estimated to be 27% (21, 22). At a median follow-up period of 28 months, there were 67 cases of recurrence and 45 deaths (23). Microarray analysis (Affymetrix) was carried out in fresh-frozen tumor biopsies obtained from subjects receiving MAGE-3 or placebo (n = 108 and 51, respectively; 109 and 50 samples, respectively, from stage IB and II NSCLC tumors) in order to identify gene signatures that were possibly prognostic or predictive of MAGE-3 activity. The analysis identified a gene expression signature that was correlated with a high risk of relapse. This signature comprised immune-related genes which were associated with the pretherapeutic tumor microenvironment (24). Patients with stage IB NSCLC who did not possess this signature exhibited a low risk (< 3%) of relapse following surgery (25). Furthermore, a gene expression signature predictive of a clinical response to MAGE-A3 ASCI treatment was also identified. A twofold increase in clinical efficacy of the treatment was seen in selected patients whose tumors presented this molecular signature (26). Long-term (44-week) follow-up analysis and immunological assessments performed in this trial revealed the induction of an anti-MAGE-3 IgG immune response in more than 98% of subjects vaccinated with MAGE-A3 ASCI. A $CD4^+$ T-cell response to MAGE-3 antigen was detected in 41% of immunized participants compared to 14% of subjects in the placebo group (27).

Based on positive results obtained in this phase II study, a double-blind, randomized, placebo-controlled phase III trial (MAGRIT) was designed to investigate the efficacy of MAGE-A3 ASCI in the prevention of cancer relapse when administered following tumor resection in patients with MAGE-3-positive stage IB, II and IIIA NSCLC (28). MAGRIT has been described as the largest-ever phase III study in the adjuvant treatment of NSCLC. In this trial, MAGE-A3 ASCI will be administered as 13 i.m. injections over 27 months and participants will be followed up every 6 months for a period of 5 years. Annual follow-up assessments will be performed thereafter for up to 10 years following treatment initiation. The primary study endpoint has been determined as disease-free survival, whereas the prospective validation of a gene signature predictive of a beneficial effect from MAGE-3 therapy constitutes the secondary study endpoint. Potential treatment-related side effects will also be addressed (1). Preliminary data reported from MAGRIT revealed the expression of *MAGEA3* mRNA in 33% of formalin-fixed paraffin-embedded tumor tissue samples isolated from 2,150 patients with operable NSCLC as detected by RT-PCR. The expression profile of MAGE-3 was found to be similar across tumor stages: 34%, 35% and 31%, respectively, in stage IB, stage II and stage IIIA. Histopathological analysis indicated higher MAGE-3 expression levels in squamous cell carcinoma (46%) compared to adenocarcinoma (24%). The first 336 subjects have been randomized and the trial is currently ongoing (29). MAGRIT, which is currently recruiting participants, is expected to enroll 2,270 NSCLC patients. The estimated completion date of this study is October 2015.

SOURCE

GlaxoSmithKline plc (GB).

DISCLOSURES

The author states no conflicts of interest.

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