Intra–Lymph Node Prime-Boost Vaccination against Melan A and Tyrosinase for the Treatment of Metastatic Melanoma: Results of a Phase 1 Clinical Trial

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Abstract

Purpose: The goal of this study was to test the safety and activity of a therapeutic vaccine, MKC1106-MT, in patients with metastatic melanoma.

Experimental Design: MKC1106-MT comprises a plasmid (pMEL-TYR) and two peptides (E-MEL and E-TYR), corresponding to Melan A and tyrosinase, administered by intra–lymph node injection in a prime-boost sequence. All 18 patients were HLA-A*0201 positive and received a fixed priming dose of plasmid and a low or a high peptide dose. Enumeration of antigen-specific T cells was done prior to and throughout the treatment. Patients who did not exhibit disease progression remained on study and could receive up to eight cycles of treatment.

Results: The MKC1106-MT regimen was well tolerated and resulted in an overall immune response rate of 50%. The treatment showed disease control, defined as stable disease that lasted for 8 weeks or more in 6 of 18 (33%) of the patients: 14% and 46% in the low and high peptide dose, respectively. Interestingly, four patients, all with tumor burden largely confined to lymph nodes and Melan A–specific T cells at baseline, showed durable disease control associated with radiologic evidence of tumor regression. There was no noticeable correlation between the expansion of antigen-specific T cells in blood and the clinical outcome; yet, there was evidence of active tumor-infiltrating lymphocytes (TIL) in two regressing lesions.

Conclusions: MKC1106-MT showed immunogenicity and evidence of disease control in a defined patient population. These findings support further development of this investigational agent and the concept of therapeutic vaccination in metastatic melanoma.

Introduction

New technologies that are simple, "off the shelf," and potent enough to elicit objective responses in measurable disease would greatly accelerate the development of next-generation active immunotherapies for cancer. One approach can be derived by following the Zinkernagel antigen localization hypothesis that antigens need to reach T-cell areas within secondary lymphoid organs to efficiently overcome the possible T-cell tolerance (1, 2). Preclinical studies showed that intra–lymph node administration of tumor antigen epitopes, together with costimulation through Toll-like receptor ligands, resulted in potent T-cell immunity and antitumor activity (3, 4). In addition, other preclinical studies showed that intranodal DNA plasmid immunization achieved effective priming of MHC class I–restricted immunity, encompassing PD-1lo CD8+ T cells, of central memory phenotype. These cells showed robust proliferative and differentiation capabilities upon peptide boosting, also being relatively resistant to negative regulatory mechanisms (5, 6). The efficacy of intra–lymph node immunization is likely due to the proximity of innate immune cells (such as antigen-presenting cells) and T cells, overcoming the modest bioavailability of DNA or peptides when administered by other routes.

Intranodal plasmid administration may be an effective priming approach for potent heterologous prime-boost vaccines. In support of this concept, sequential intra–lymph node dosing of plasmid and peptides in transgenic mice expressing a chimeric murine human HLA-A*0201 (HHD mice; ref. 7) resulted in a significantly elevated immune response as compared with subcutaneous administration or other dosing regimens (5, 6). This translated into more than 10% of CD8+ T cells being specific to he immunizing epitopes in blood and secondary lymphoid organs in this preclinical model. In addition, coinoculation...
Translational Relevance

Although it is thought that therapeutic vaccination is more applicable to minimal residual disease, control of metastatic tumors through active immunotherapy remains an important aim. The objective of this phase 1 study was to test the activity of a plasmid-prime–peptide-boost regimen against Melan A and tyrosinase, administered to clinically uninvolved lymph nodes of patients with metastatic melanoma. Immunization resulted in disease control (durable stable disease associated with radiologic evidence of tumor regression) in a patient subpopulation with tumor burden largely confined to lymph nodes and preexisting antigen-specific immunity. Although there was no apparent correlation between clinical outcome and immune responses measured in blood, the presence and phenotype of tumor infiltrating lymphocytes, in a very limited data set, were in line with an immune mechanism of action. Overall, these results support a focused, expedited evaluation of this and other novel active immunotherapies in select metastatic disease indications.

of robust immunity—in the same preclinical model—against both Melan A and tyrosinase, 2 well-recognized melanoma-associated antigens (8, 9), was also achieved by this approach (6).

Previous clinical evidence supported the safety and feasibility of direct intra–lymph node administration with only mild-to-moderate therapy-related adverse events (AE) such as flu-like syndrome (10–12). However, such early phase trials with individual plasmids expressing immunogenic portions of tyrosinase (11) and Melan A (12) in patients with advanced melanoma showed a modest immune response rate of approximately 20%, with no objective tumor responses or evidence of disease control. Thus, we hypothesized that heterologous plasmid priming–peptide boosting would result in enhanced biological and evidence of clinical benefit. Taking into account the Th1/Tc1-biasing activity of plasmids carrying immunostimulatory sequences (ISS; refs. 13, 14), we tested a simple boosting strategy by using peptides with no adjuvant, also delivered into nondiseased lymph nodes. To optimize the immune activity, the plasmid was previously optimized to preferentially yield immunodominant HLA-A2–restricted epitopes from Melan A and tyrosinase (15, 16). Finally, we utilized boosting peptides that had substitutions at key MHC anchor residues to increase the half-life of the MHC–peptide complexes, crucial to a higher immune activity (17).

In this phase 1 clinical trial, as in the previous ones carried out with individual plasmids (11, 12), the patients enrolled had advanced, surgically unresectable stage IIIc and IV melanoma of diverse organ localization. The results show that in a subset of patients sharing certain immune and clinical features, intranodal prime boosting with MKC1106-MT afforded durable disease control associated with radiologic evidence of tumor regression.

Materials and Methods

Study design and conduct

This phase 1 clinical trial was an open-label 2-center evaluation of a multicomponent immune-based therapy composed of plasmid and peptides, designed to induce or enhance an immune response to the tumor-associated antigens (TAA) Melan A and tyrosinase (MKC1106-MT regimen; Fig. 1). Two sequential cohorts, of at least 6 patients each, received a fixed dose of priming with plasmid, followed by a low or high dose of peptide for boosting, respectively. If 2 of the first 6 patients enrolled to either cohort experienced a dose-limiting toxicity (DLT), the cohort was to be expanded to 12 patients. If 4 or more patients enrolled in the expanded cohort experienced DLT, enrollment was to stop and safety evaluated. A total of 18 patients were enrolled in the trial to offset the potential attrition during the first 2 cycles of treatment due to rapid disease progression or possible logistical, compliance issues. A written informed consent, previously approved by the Institutional Review Board (IRB) at each study site, was obtained from each patient. The trial was conducted in accordance with local regulations, the guidelines for Good Clinical Practice, and the principles of the current version of the Declaration of Helsinki. The trial opened to accrual at 2 U.S. centers (University of California at Los Angeles, Los Angeles, CA, and Moffitt Cancer Center, Tampa, FL) was sponsored by MannKind Corporation and had the clinical trial registration number NCT00668090.

Study objectives and assessments

The primary objective was to assess the safety and tolerability of the MKC1106-MT regimen. Secondary objectives included measuring the immune response by MHC tetramer and enzymatic-linked immune spot (ELISPOT) assays in peripheral blood samples, documentation of clinical activity, evaluation of persistence of pMEL-TYR plasmid levels in the blood by PCR, and target antigen (Melan A and tyrosinase) and B2-microglobulin expression in the tumor tissue.

Study population

HLA-A*0201-positive patients older than 18 years, with histologically confirmed, surgically resectable stage IIIc and IV melanoma, performance status 0 to 1, and adequate organ function, were eligible. Eligible patients had relapsing, progressing disease, clearly documented clinically and by imaging prior to enrollment and irrespective of whether the disease was lymphatic or visceral. Patients with a history of brain metastasis were also eligible if adequately treated with surgery or radiation therapy and showed no evidence of active disease at study entry. At least 1 measurable or evaluable lesion was required. There was no restriction in terms of number of prior therapies and lactate dehydrogenase level. Patients with a history of autoimmune disease...
(other than vitiligo), immunodeficiency syndrome (including those who were HIV positive by testing), or requirement for chronic systemic immunosuppressive treatment were excluded.

MKC1106-MT preparation and administration

The pMEL-TYR is a recombinant plasmid–expressing fragments of 2 melanoma antigens, Melan A and tyrosinase (described previously as pSEM; Fig. 1A; ref. 12), formulated at a concentration of 4.0 mg/mL as a sterile aqueous solution. This plasmid encodes a 94-aminoacid polypeptide encompassing the 2 dominant epitopes of MART-1/Melan A (26–35 A27L) and tyrosinase (369–377), respectively, along with a larger fragment of the Melan A (31–96), plus the tyrosinase epitope 1–9. The plasmid was optimized to along with a larger fragment of the Melan A (31–96), plus

A, left, schematic representation of the plasmid (pMEL-TYR) with the location of Melan A and tyrosinase antigen fragments within the open reading frame. Right, the 2 boosting peptide analogues E-MEL and E-TYR, with the amino acid residue changes indicated. B, the immunization schema with timing of priming and boosting. CMV, cytomegalovirus; BGH, bovine growth hormone; ORI, origin of replication.

cohort) or 300 μg in 300 μL (high peptide dose cohort), were administered on cycle days 29 and 32. The peptides were administered into distinct, nondiseased lymph node groups (e.g., E-MEL in a left inguinal lymph node and E-TYR in a right inguinal lymph node). All components were administered under ultrasound guidance with a fixed 25-gauge echogenic needle (1.5-inch echogenic cannula; Ultimed). Patients were clinically evaluated after each therapeutic cycle and continued on treatment until disease progression was documented.

Safety and clinical response evaluation

All AEs were recorded at each visit during every treatment cycle and at the last visit. Patients were asked to voluntarily report any AEs to the clinical site personnel. Each AE was graded for severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE Version 3.0).

Detailed clinical evaluation of patients was carried out at baseline and after each treatment cycle, including physical examination, disease evaluation by computerized tomography (CT), other imaging as applicable, and laboratory tests. Clinical responses were categorized using the Response Evaluation Criteria in Solid Tumors Version 1.0 (RECIST 1.0; ref. 18). In face of progressing disease at enrolment, patients were assigned a stable disease if the status was maintained for a minimum of 8 weeks; or as applicable, a partial response or complete response, based on measurable changes in tumor size confirmed by repeat assessment 4 weeks later. Disease control was defined as stable disease for at least 8 weeks or better, signifying tumor growth, stabilization, or tumor reduction, and consistent with studies involving similar investigational agents in this
document the tumor size measurement, we undertook an independent, centralized review of the CT scans along with the site radiologists’ assessment.

**Immune monitoring**

A quantitative flow cytometric assay using MHC class I peptide tetramers was used to enumerate antigen-specific CD8+ T cells in peripheral blood without prior in vitro stimulation. Whole blood was collected in potassium EDTA–treated specimen collection tubes prior to immunization and on days 29 and 39 of each treatment cycle. Within 48 hours, the samples were sent to Genzyme Genetic Analytical Service, a Clinical Laboratory Improvement Amendments (CLIA)-certified independent laboratory, for processing and analysis under GLP. Briefly, peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation, incubated for 30 minutes at room temperature in dark with 7-aminoactinomycin D, anti-CD3, anti-CD8 IgGs (BD Biosciences), and tetramer reagents encompassing the peptides Melan A26–35, A27L (ELAGIGILTV), an analogue of the native epitope, and tyrosinase169–177 (YMDGTMSQV), respectively (Beckman Coulter). Cells were then washed and analyzed on a BD FACSCanto II flow cytometer by using FACSDiva Software. The analysis was focused on CD3+ cells within the FSC/SSC region corresponding to mononuclear cells of small to moderate size, after dying or dead cells were gated out. The quadrant limits were established and applied consistently, utilizing appropriate negative controls. At least 500,000 events were acquired per sample. The lower limit of detection (LLD) of this assay was established using epitope-specific T cells generated by repeat in vitro stimulation of white blood cells from healthy human donors. The LLD was 0.03% [(CD8+ tetramer+ T cells/total CD8+ T cells) × 100]. An immune response by tetramer was prospectively defined as an increase in tetramer assay value greater than 2-fold over the screening value, with levels significantly different from background. If the pretreatment tetramer value was negative (i.e., <0.03%), a tetramer response for that peptide had to be 0.06% or greater.

A direct ex vivo IFN-γ ELISPOT assay on PBMCs was run in parallel (see Discussion). The PBMCs were collected on heparin, and as an internal control, we utilized mitogen stimulation. A positive response by ELISPOT was prospectively defined as an increase in value by at least 3-fold over the screening value. If the screening value was zero, a positive response required 10 or more spots per 10⁶ cells.

A patient was prospectively defined as immune responder if at least 1 positive tetramer or ELISPOT response was recorded during the treatment. Preexisting immunity against either antigen, as measured by tetramer assay, was defined as T-cell frequency of 3 × LLD (0.09% of CD8+ tetramer+ T cells/CD8+ T cells) or higher.

**Tumor analysis**

The determination of Melan A, tyrosinase, and β2-microglobulin expressions was carried out by immunohistochemistry (IHC) in tumor biopsies collected prior to immunization. The staining was done with antibodies specific for β2-microglobulin, Melan A, and tyrosinase (all from Dako) at an appropriate concentration and room temperature for 60 minutes. This was followed by incubation with horseradish peroxidase polymer–labeled secondary antibody (Nemesis Polymer HRP kit; Biocare) for 25 minutes. After thorough rinse with TBST buffer, Betazoid DAB (3,3-diaminobenzidine; Biocare) substrate was added for 5 minutes. Slides were counterstained with Harris hematoxylin for 1 minute. Negative and positive tissue control samples were included in all stains. All stained slides were evaluated and analyzed by a pathologist, for intensity, pattern, and percentage of cells stained.

In 2 patients showing radiologic evidence of tumor reduction, tumor samples obtained by ultrasound-guided core biopsy were processed by standard pathology and IHC for Melan A, tyrosinase, CD8, and CD4. In addition, part of the specimen was utilized to generate a single-cell suspension for fluorescence-activated cell-sorting analysis of tumor-infiltrating lymphocytes (TIL) as previously described (20). In brief, nonadherent cells were analyzed with Melan A and tyrosinase tetramer reagents, with additional surface staining for T-cell functional markers such as CD4, CD8, CD45RA, CD27, and CD28 (20).

**Statistical analysis**

For continuous variables, data are presented as means or medians, with range and SDs. Discrete variables are expressed as frequencies or proportions; the statistical significance was calculated using the 2-tailed Fisher exact test.

**Results**

**Study patients**

During 2008–2009, 39 patients were assessed for study entry, with 18 patients enrolled (Table 1). In accordance with the prevalence of melanoma, approximately two thirds of the patients were Caucasian and male, with a mean age of 63 years. A larger percentage of patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 1 in the low-dose cohort (57%) than in the high-dose cohort (18%), with the remaining patients having an ECOG performance status of zero. Two patients had melanoma originating from choroidal or mucosal sites, whereas the majority had metastatic melanoma of cutaneous origin. All patients had surgically unresectable, progressing stage IV melanoma except for 1 patient in the high-dose cohort with stage IIIc disease. Within the stage IV group, 6 patients were categorized as lymphatic metastatic disease (M1a): 2 in the low and 4 in the high peptide dose cohort. Upon detailed retrospective analysis of the baseline imaging scans, these patients showed a few small lesions within visceral organs of unclear significance. Seven patients (39%) had received prior chemotherapy, 11 patients (61%) had received immunotherapy, and 1 (5%) had undergone radiation therapy prior to participating in this trial.
Feasibility of vaccine administration and compliance

Overall, the compliance associated with this intensive intranodal immunization protocol was high. Because of prior surgical resections or disease localization to inguinal lymph node basins, 4 patients (881-0006, 881-0010, 881-0017, and 881-0020) received IRB approval for axillary lymph node injections. Patient 881-0020 received a total of 9 cycles of treatment (1 more than the maximum of 8 cycles per protocol), at the request of the investigator, with IRB approval. One patient (881-0001) in the low-dose cohort and 2 patients (881-0006 and 881-0020) in the high-dose cohort had dose/regimen interruptions primarily due to scheduling conflicts.

Toxicities, AEs, and plasmid measurement

Drug-related AEs were generally mild, with the majority being grade 1 or 2 (Supplementary Table S2). No safety differences were observed between the low and high peptide dose cohorts. Four patients (22%) discontinued treatment due to AEs; however, only 1 was considered drug related. This patient (703-0011) in the low peptide dose cohort experienced a grade 3 fatigue during cycle 1. The patient died subsequently due to disease progression, 35 days after his last dose. Overall, 7 patients (39%) had mild, treatment-related AEs. The most frequently reported AEs in both low and high peptide dose cohorts were fatigue (39%), hypotenemia (28%), chills (22%), constipation (22%), diarrhea (22%), and hypoalbuminemia (22%). In total, 4 patients (3 in the low-dose cohort and 1 in the high-dose cohort) died while on study or within the follow-up period, all due to disease progression.

To evaluate the accumulation, clearance, and/or persistence of the DNA component (pMEL-TYR) of the study regimen, a standard quantitative PCR analysis using specific primers was done on blood samples at screening, on day 18 of each cycle and at the final visit. As expected, no detectable levels of pMEL-TYR were present at screening. Plasmid levels in the peripheral blood were low or not detectable in most patients over the course of treatment. One patient (881-0006) had transient high plasmid levels during cycles 2 and 4, with the highest level reported at 1.96 million copies per microgram of genomic DNA. However, at the end of the study, there was no evidence of plasmid in blood. Only 1 patient (881-0021) had detectable levels of pMEL-TYR plasmid at the end of the trial, 1 week after the completion of the immunization regimen.

Clinical evaluation

Among the 18 patients treated (7 in the low-dose cohort and 11 in the high-dose cohort, respectively), 6 patients (1 in the low-dose cohort and 5 in the high-dose cohort) met the criteria for disease control (stable disease ≥8 weeks or better). Thus, the overall disease control rate was 33% (14% and 46% in the low and high peptide dose groups, respectively). These patients had a stable disease ranging from 12 weeks to more than 96 weeks. To corroborate the tumor size measurements and interpretation, we undertook an independent, centralized review of CT scans to
complement the clinical sites’ assessment. The best overall response according to RECIST criteria, based on the independent, centralized assessment of CT scans, is shown in Table 2.

Four patients had, in addition to a durable disease control (stable disease for 6 months or better), radiologic evidence of tumor regression as shown by evaluation of CT scans both at the sites and by the independent, centralized assessment (Fig. 2 and Supplementary Fig. S1): 1 in the low peptide dose group and 3 in the high-dose group. These patients, although falling short of meeting partial response under RECIST criteria, underwent the full 8 cycles of treatment (1 year) contemplated by the protocol and 1 received a 9th cycle upon the request of the investigator and with the IRB consent. Two of these 4 patients are still progression free at 2 years after treatment initiation (as of November 2010), whereas the other 2 progressed after the completion of the trial. Strikingly, all tumor regressions involved lymph node metastases and all patients in this category, with durable disease control, had metastatic disease largely confined to the lymphatic system.

Immunological response assessment

Fourteen patients (78%) were evaluable immunologically. The other 4 were not evaluable, as they failed to complete the first cycle of treatment. Among these 4 patients, 3 experienced rapid disease progression during the first treatment cycle and the other patient had grade 3 fatigue and was withdrawn from the protocol.

Overall, 9 patients (50%) had an immune response to either Melan A or tyrosinase, according to prospectively set immune response criteria (Table 2 and Methods). Tetramer analysis identified 2 categories of patients: immune responding and nonresponding patients (Supplementary Fig. S2). In the low peptide dose cohort, 3 of 4 evaluable patients had an immune response. All 3 patients mounted a response to Melan A, whereas 1 had a response to tyrosinase as well. In the high peptide dose cohort, 6 of 10 evaluable patients had an immune response, 4 patients had a response to Melan A, and 5 had a response to tyrosinase. One and 3 patients in the low and high peptide dose cohorts, respectively, mounted a response against both antigens. Only 1 patient in this study had an ELISPOT response: patient (881-0006) in the high-dose cohort, who had a response to Melan A and showed radiologic evidence of tumor regression as well (Supplementary Figs. S1 and S2).

Correlation between immunity and clinical evolution

Of the 4 patients with durable disease control and radiologic evidence of tumor regression, only 2 patients (881-0006 and 881-0020) showed an immune response manifested by an expansion of antigen-specific T cells in blood against both immunizing antigens (Fig. 2, Supplementary Figs. S1 and S4). Of the remaining 14 patients, only 2 developed immunity against both immunizing antigens. Three other patients in this latter group showed an immune response to either Melan A or tyrosinase, and 4 patients were not evaluable.

Although disease control did not correlate with expansion of antigen-specific T cells in blood, there was evidence for a relationship with the presence of Melan A-specific T cells at baseline (Table 3). Eight patients showed substantial levels (higher than 3 × LLD) of Melan A tetramer-positive T cells at screening. Of these, 4 patients (881-0001, 881-0006, 881-0020, and 881-0029) showed durable disease control. Other 2 patients with Melan A–specific T cells at baseline progressed too rapidly to be evaluable from an immune response standpoint, and the other 2 completed 1 immunization cycle but showed PD as well. In contrast, none of the other 10 patients, devoid of Melan A–specific T cells at baseline, showed durable disease control or evidence of tumor regression. A retrospective analysis identified the correlation between the presence of Melan A–specific T cells at baseline and durable disease control and radiologic evidence of tumor regression was statistically significant ($P = 0.0229$ by 2-tailed Fisher’s exact test). In line with this observation and because all patients who had a positive clinical outcome (disease control and evidence of tumor regression) shared a similar disease stage, there was also a positive association between disease localization at the level of lymphatic system and preexisting Melan A–specific T cells. In contrast, there was no significant correlation between the presence and number of tyrosinase-specific T cells at baseline and disease localization or clinical outcome, respectively (Supplementary Fig. S4B).

### Table 2. Immune response summary

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<tr>
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<th>Low-dose cohort</th>
<th>High-dose cohort</th>
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<tr>
<td>Immune response</td>
<td>3 (42.9%)</td>
<td>6 (54.5%)</td>
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<tr>
<td>No immune response</td>
<td>1 (14.3%)</td>
<td>4 (36.4%)</td>
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<tr>
<td>Not evaluable</td>
<td>3 (42.9%)</td>
<td>1 (9.1%)</td>
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<tr>
<td>Immune response: Melan A</td>
<td>3 (42.9%)</td>
<td>4 (36.4%)</td>
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<td>Immune response</td>
<td>1 (14.3%)</td>
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<td>3 (42.9%)</td>
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<tr>
<td>Not evaluable</td>
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<td>No immunity or not evaluable</td>
<td>3 (42.9%)</td>
<td>5 (45%)</td>
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aImmune responders were defined on the basis of prospectively defined criteria: increase in the frequency of antigen-specific T cells in peripheral blood (as per Methods).

b Patients progressed very rapidly and were taken off treatment before completing 1 cycle.
regression, mounted a quite sustained response against tyrosinase, as measured by induction and expansion of tyrosinase-specific T-cell population in blood.

Analysis of T cells in tumor biopsies

Two core biopsies from regressing lesions of 2 patients (881-0006 and 881-0029) were obtained after several immunization cycles. Tumor analysis by IHC and flow cytometry showed that both CD8⁺ and CD4⁺ T cells were present within the TIL population (Supplementary Fig. S5), with the former infiltrating diffusely the tumor masses that showed clear evidence of preserved target antigen expression. Detailed multicolor flow cytometric analysis of TILs showed the presence of both Melan A- and tyrosinase-specific T cells and a predominance of antigen-experienced T effector/memory cells (CD27⁺CD28−CD45RA−; Supplementary Fig. S5).

Discussion

The major finding of this study was that this immune intervention cotargeting Melan A and tyrosinase resulted in long-term disease control in patients with metastatic disease largely confined to the lymphatic system. This observation of disease control associated with radiologic evidence of tumor regression in several patients sharing clinical and immunologic characteristics was rather unexpected in light of other cancer vaccine trials in the past. All 4 patients in this category remained on treatment for at least 8 cycles, or 1 year from the time of treatment initiation. Two of these 4 patients remained free of disease progression at 2 years after the first dose, whereas the others progressed after 1 year of treatment.

Another finding was that the regimen was immunogenic, with 50% of all patients showing an immune response on
treatment. Despite the lack of correlation between the expansion of specific T-cell populations in blood and the clinical outcome, there was an association between preexisting Melan A–specific T cells, disease localization—predominantly to lymph nodes—and long-term disease control with radiologic evidence of tumor regression, respectively. This is in line with prior observations, suggesting a role for Melan A–specific T-cell immunity in determining tumor progression or response and in support of Melan A as a target antigen (21, 22). Nevertheless, we cannot exclude a role for the tyrosinase component of this investigational agent, as 2 patients with evidence of tumor regression showed a coelevation of immunity against both Melan A and tyrosinase. In addition, there was evidence for tumor infiltration with antigen-experienced memory/effector CD8\(^+\) T cells specific for both Melan A and tyrosinase in 2 patients with radiologic evidence of tumor regression. This supports an immune-mediated mechanism encompassing a standoff between immunity and tumoral process, tilted in favor of the former by the vaccine.

Several results represent a departure from the prior preclinical experience: first, a relatively modest magnitude of immune response noted in these patients compared with preclinical data generated in rodents (5, 6), and, second, an apparent lack of peptide boosting effect in many patients, as monitored by enumeration of specific T cells in the blood. For the former, there could be species-related differences in immune responsiveness to plasmid and peptides, relatively impaired immunoreactivity of patients with melanoma (23), or iatrogenic immunosuppression due to prior treatments. It is also possible that the dosing regimen needs optimization or addition of adjuvants. Nevertheless, interestingly, the proportion of patients who showed an immune response to tyrosinase was higher in the high peptide dose cohort versus the low-dose cohort (5 of 6 vs. 1 of 3 tyrosinase responders/number of evaluable patients who developed immune responses).

This prime-boost regimen also resulted in an at least 2-fold higher immune response rate than prior trials utilizing the intra–lymph node plasmid immunization (12). Because there was no significant correlation between the expansion of total TAA-specific T cells in the blood and disease control, we hypothesized that in humans, this immune intervention may work by stimulating appropriately equipped memory/effector T cells within lymph nodes, followed by their recruitment into tumor lesions where they remain operational despite an immunosuppressive environment. It is also quite possible that intranodal immunization stimulates T cells expressing select chemokine receptors that recruit them preferentially to lymph nodes (24), thereby more effectively treating lymph node metastasis. This hypothesis is further supported by

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Abbreviations: SD, stable disease; PD, progressive disease.
\(^a\)Patients who showed durable disease control (SD of +12 months) associated with radiologic evidence of tumor regression were highlighted.
\(^b\)Progressive disease within or on completion of 1 cycle of treatment (39 days).
the increased T-cell infiltrates in the tumor biopsies, as compared with blood levels, from the 2 patients evaluated with radiologic evidence of tumor regression. Nevertheless, a baseline comparator was not built into this trial and in addition, TILs have been variably documented in nonimmunized melanoma patients (25, 26). Besides the tumor-infiltrative and antigen-experienced nature of the CD8\(^+\) TILs in this very limited data set, the expression of CD27 on the majority of specific T cells indicates functional capability. As reported previously, the frequency of such CD27\(^+\) CD8\(^+\) T cells within adoptively transferred cells was highly associated with subsequent tumor regression (27). Furthermore, these support the value of optimized multi-parametric immune monitoring techniques in conjunction with evaluating intratumoral immunity prior to and after vaccine administration (28), along with immune monitoring in peripheral blood.

The striking association of preexisting Melan A–specific T cells, disease stage, and localization with disease control has both theoretical and practical implications. First, it suggests that MKC1106-MT may act on a pre-existing TAA-specific repertoire by yielding highly functional effector T cells. Second, an effect on metastatic lymph node lesions suggests a specific immune environment within the lymphoid system, contrasting to that within large, vascularized tumors (29, 30). Third, this observation may open a path for an accelerated clinical development of immune interventions similar to MKC1106 in a well-defined subset of patients with metastatic melanoma. This is different from the traditional paradigm of developing cancer vaccines in minimal residual disease or adjuvant setting, which relies on lengthy trials, large patient populations, and select immune monitoring methods, of unclear informational value. This is also supported by the results of another recently concluded trial, involving immunization of melanoma patients with autologous tumor cell lysate-pulsed dendritic cells, that showed tumor regression strictly in patients with lymphatic metastatic disease (19).

In conclusion, the results of this clinical trial provide support for a continued and focused clinical evaluation of MKC1106-MT in patients with metastatic melanoma largely confined to lymph nodes. This evaluation should be accompanied by intratumoral immune monitoring by using conventional (31, 32) or new platform assays (33) to elucidate the mechanism of action and appropriate immune correlates to clinical outcome.

Disclosure of Potential Conflicts of Interest

A. Ribas is a consultant/advisory board member of MannKind Corp. The other authors disclosed no potential conflicts of interest.

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