ORIGINAL RESEARCH

Effect of vaccination with N-glycolyl GM3/VSSP vaccine by subcutaneous injection in patients with advanced cutaneous melanoma

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Abstract: NeuGc-containing gangliosides have been described in melanoma cells and are an attractive target for cancer immunotherapy because they are minimally or not expressed in normal human tissues. Melanoma patients treated with a vaccine based on N-glycolyl gangliosides have shown benefit in progression free survival and overall survival. We conducted a multicenter Phase I/II clinical trial in patients with metastatic cutaneous melanoma treated with the N-gycolyl GM3/very-small-size proteoliposomes vaccine by the subcutaneous route. Selecting the optimal biological dose of the vaccine was the principal objective based on immunogenicity, efficacy, and safety results. Six dose levels were studied and the treatment schedule consisted of five doses administered every 2 weeks and then monthly until 15 doses had been given. Dose levels evaluated were 150, 300, 600, 900, 1200, and 1500 µg with five patients included in each dose level except the 900 µg dose (n = 10). Immunogenicity was determined by antibody titers generated in patients after vaccination. Antitumor effect was measured by response criteria of evaluation in solid tumors and safety was evaluated by common toxicity criteria of adverse events. The vaccine was safe and immunogenic at all doses levels. The most frequent adverse events related to vaccination were mild to moderate injection site reactions and flu-like symptoms. Vaccination induced specific anti-NeuGcGM3 immunoglobulin M and immunoglobulin G antibody responses in all patients. Disease control (objective response or stable disease) was obtained in 38.46% of patients. Global median overall survival was 20.20 months. Two patients achieved overall survival duration of about 4 and 5 years, respectively. The 900 µg dose resulted in overall survival duration of 19.40 months and was selected as the biological optimal dose.

Keywords: melanoma, clinical trial, therapeutic vaccine, ganglioside, N-glycolyl GM3

Introduction

Surgery is the only intervention that has achieved clinical benefit in melanoma patients, thus melanoma continues to be a difficult tumor to treat.[1] Other adjuvant treatments include chemotherapy, radiotherapy, and high-dose interferon but none of these has had an impact on overall survival. Currently, several agents are undergoing research and development in the treatment of melanoma. Immunotherapy is a newer alternative treatment under investigation, and is based on the very large number of antigens that have been studied in melanoma cells and on the immunogenicity of this tumor.[2,3]

The N-glycosylated gangliosides are a very attractive option for cancer immunotherapy because they are overexpressed in tumor cells and minimally or unexpressed in normal human tissue. Melanoma is one of the tumors that overexpress N-glycolyl gangliosides, specifically the N-glycolyl GM3 (NGcGM3) gangliosides.[4,5] Other tissues with similar behavior are breast and ovarian tumors.[6,7] The Center of

Molecular Immunology in Havana, Cuba, developed a vaccine based on this ganglioside which has been used in clinical trials in melanoma and breast cancer patients and shown a very good toxicity profile and some evidence of efficacy.

A Phase I/II clinical trial of intramuscular NGcGM3/very-small-size proteoliposomes (VSSP) vaccine using Montanide ISA 51 as an adjuvant was conducted in melanoma patients.[8] The vaccine was safe and immunogenic and some patients achieved an overall survival duration superior to other reports in the literature of melanoma patients.[9,10] However, local reactions related to the adjuvant were observed. For this reason we decided to evaluate the effect of the vaccine given by the subcutaneous route without the adjuvant in patients with melanoma. A Phase I/II clinical trial was designed to study dose levels of the vaccine based on the doses used by the intramuscular route. The primary objective was to select the biological optimal dose based on the results of safety and efficacy obtained after vaccine administration.

Methods

Thirty-five patients who had metastatic cutaneous melanoma participated in the study and were recruited at two research sites. The characteristics of the patients are shown in Table 1. The study protocol was performed according to the principles of the Declaration of Helsinki, accepted by the institutional ethics committee, and approved by the Cuban Regulatory Agency. The protocol was carefully explained to the patients, all of whom gave their written consent to participate in the study. Inclusion criteria were a histological diagnosis of malignant melanoma, good performance status (grade 1 or 2 according to criteria of the World Health Organization (WHO)), age older

Table 1 Patients' characteristics

Eligible patients (n)	35
Evaluable patients (n)	34
Age (median, range) (years)	51 (26–81)
Performance status (WHO) (n)	
0–1	32
>1	2
Metastatic site	
Skin + node + subcutaneous tissue	18
Lung	11
Visceral + bone	5
Prior treatment (n)	
Surgery	32
Chemotherapy	8
Radiotherapy	3
Interferon	1
Treatment doses (n)	
<5	4
5–10	14
>10	16

Abbreviation: WHO, World Health Organization.

than 18 years, life expectancy more than 6 months, and normal laboratory parameters. Exclusion criteria were contraindications such as pregnancy, decompensated chronic disease, brain metastases, or active infections.

This study was designed as an open-label trial, evaluating six doses levels of NGcGM3/VSSP vaccine in six cohorts of five patients each except the 900 μg level which included ten patients. The principal objective of the study was to select the biological optimal dose based on immunogenicity, safety, and efficacy of the vaccine.

Treatment with the vaccine was initiated about 4 or 6 weeks after oncospecific treatment finished. Patients received 15 subcutaneous doses of the vaccine. The first five doses were given every 2 weeks and subsequent doses every 4 weeks, until one year of treatment. Additional immunizations were allowed at the discretion of the physician. Blood samples were collected prior to and during treatment for evaluation of hematological and biochemical parameters and for determining antibody titers. Safety was evaluated by analysis of the frequency and intensity of adverse events and their relation to the vaccine. Adverse events were classified according to the Common Toxicity Criteria for Adverse Events (CTCAE; version 3.0).[11] Antitumoral activity was evaluated by response evaluation criteria in solid tumors version 1.0.[12] Tumor size was evaluated by imagenology before starting treatment and at months 3, 6, 9, and 12. Additionally, delayed type hypersensitivity (DTH) responses were evaluated when NeuGcGM3/VSSP vaccine was injected intradermally. Dermal indurations were measured 48 hours after NeuGcGM3/VSSP injection and considered positive if the diameter was higher than 5 mm.

Overall survival was determined as the time between randomization and death; overall survival was analyzed by Kaplan–Meier methods.

Results

Analysis was performed by intention to treat.

Patient population

The mean age of patients was 54.23 years. The majority of patients (97.06%) had received treatment for metastatic disease before inclusion into the study; only one patient received the vaccine naïve of oncology treatment. The most frequent prior treatment for metastatic disease was surgery (91.4%). In some cases patients received chemotherapy, radiotherapy, and hormone therapy with high-dose interferon. Most patients (91.40%) had good performance status (0–1) according to WHO criteria. Most patients had stage IV disease (53.3%), and the rest had evolutive metastatic disease at

inclusion. The number of metastatic lesions was variable but more than 50% of patients had multiple lesions and predominantly had nonvisceral metastases (55.8%).

The distribution of all parameters was similar at all dose levels. Demographic characteristics, previous therapies, site of metastases, and total vaccination dose are show in Table 1.

Treatment compliance

A total of 338 immunizations were administered. All patients were treated with the vaccine. Complete treatment (15 immunizations) was received by 38.25% of patients while the remaining patients received more than five doses, except four patients who were not evaluable.

Twenty-one patients discontinued treatment during the study (61.76%) but no patients withdrew due to vaccine complications. The principal causes of discontinuation were schedule noncompliance, patient decision, worsening of performance status, or death.

Safety results

All patients developed grade 1 or 2 vaccine-related adverse events. The most frequent adverse events were local events related to the injection site: pain, indurations, and erythema. The most common systemic adverse events were flu-like symptoms consisting of fever, myalgias, hypotension, and headache. Other events included vomiting, high blood pressure, and nausea.

Only 1.7% of adverse events were severe (grade 3 according to CTC criteria). All were successfully controlled without sequelae. This trial allowed additional reimmunizations for patients having good performance status independent of clinical response. The patients reimmunized with more than 15 doses no showed evidence of cumulative toxicity. Despite continuous immunizations for 2 years, all these patients tolerated vaccinations very well; none presented with serious adverse events.

All adverse events were noted from the first immunization. The frequency and severity of adverse events was similar at all doses levels. The toxicity profile is shown in Tables 2 and 3.

Immunological response

Antibody titers against NeuGcGM3 ganglioside were obtained after vaccination in 28 of the 30 patients evaluated. Either immunoglobulin M (IgM) or immunoglobulin G (IgG) antibodies were present in the patients. IgM titers ranged between 1/80 and 1/10,240 with higher titers obtained in three patients treated with the 1500 µg dose. The best results of immunogenicity were obtained with the 1200 µg and 1500 µg doses, followed by the 900 µg dose (Table 4). When analyzed,

Table 2 Frequency of adverse events

Adverse event	Number of events
Injection site pain	132
Local erythema	72
Myalgia/arthralgia	69
Fever	57
Chills	30
Nausea	16
Headache	14
Induration	12
Vomiting	9
Hypotension	3
High blood pressure	2
Other	169
Total	585

the specific response of IgG against NeuGcGM3 was observed at titers between 1/80 and 1/5120. IgG titers were less than IgM titers and the maximum titer was obtained at a dose of 900 µg (median 1/5120). These results demonstrate that the vaccine is immunogenic at all dose levels evaluated. The most immunogenic doses were 900, 1200, and 1500 µg.

Cellular response

Induction of cellular response was evaluated in 28 patients by DTH response. Most patients (64.3%) had no DTH response prior to treatment. A trend towards a relationship between DTH response and overall survival was observed. Median overall survival of patients with a positive DTH response was 37.8 months versus 14.9 months in DTH negative patients; the difference was not statistically significant.

Efficacy analyses

Antitumor response was evaluated in 26 patients. Disease control was achieved by 34.6% of patients, including five with objective response (complete or partial) and five with stable disease (Table 5).

The best responses were obtained at the 900 µg dose level; two of three patients achieved complete response and three of eight patients achieved stable disease. Despite this success, no significant differences in antitumor response were observed between dose levels.

Table 3 Severity of adverse events

Grade	Number of events (%)
1	541 (92.5)
2	26 (4.4)
3	10 (1.7)
Not defined	8 (1.4)
Total	585 (100.0)

Table 4 Anti-NGcGM3 ganglioside antibody titers

Dose level (µg)	Median of inverse of maximum anti-NGcGM3 gangliosides antibody titers	
	IgG max	IgM max
150	1280	1280
300	1280	2560
600	640	2560
900	5120	5120
1200	2560	10,240
1500	2560	10,240

Abbreviations: Ig, immunoglobulin; max, maximum.

Prior treatment was the only prognostic factor that influenced response; patients who received oncologic treatment before vaccination obtained a better antitumor response.

Overall survival
Overall survival was evaluated in 28 patients. Global overall survival was 20.40 months (Table 6). The best overall survival duration was obtained at a dose of 600 µg followed by 900 µg (20.2 and 19.4 months, respectively). Nevertheless, no patients treated with 600 µg were alive at the time of analysis while three (30.0%) of the patients treated with 900 µg were alive at the time of analysis.

Although it was not an objective of the study, survival duration in relation to metastatic sites was analyzed. The distribution of patients according to metastatic sites across all dose levels was consistent. The results obtained and the survival values reported in the literature for patients with metastases of soft tissue, lung, or bone were compared. The survival values obtained were 37.8, 7.43, and 16.4 months, respectively. These values exceed those reported in the literature for patients with soft tissue and bone metastases (4 and 14 months, respectively).

Optimal biological dose
The optimal biological dose selected was 900 µg due to superior results for safety, immunogenicity, and efficacy. The volume of injection required per dose was also analyzed, because higher dose levels imply higher volume of

Table 5 Antitumor response by dose level

Type of response	Dose level						Total	%
	150	300	600	900	1200	1500		
Complete response	0	0	0	2	0	1	3	11.54
Partial response	1	1	0	0	0	0	2	7.69
Stable disease	0	1	1	1	1	1	5	19.23

Table 6 Overall survival

Dose level (µg)	Median overall survival (months)
150	6.18
300	16.40
600	20.20
900	19.40
1200	18.10
1500	Not reached
Global	20.20

administration and greater patient discomfort. The 900 µg dose allowed formulation of the vaccine in a concentration that could be administered at a single injection site.

Discussion
The use of targeted therapies is an attractive option in oncology. The molecules overexpressed in cancer cells offer a tool to manage treatment and to achieve benefits in disease progression and survival. Gangliosides have been described as associated with the membrane of tumor cells and as tumor-associated antigens.[13] These reasons support the design of molecules that bind gangliosides and downregulate signaling of tumor growth.[14]

NGcGM3 is an N-glycosylated ganglioside overexpressed in breast tumors and melanoma cells. The NGcGM3 vaccine has been administered in metastatic melanoma patients as part of Phase I/II clinical trials which have shown promising objective antitumoral responses and survival data.[8] In the present study, the biological optimal dose was evaluated among six dose levels established previously. There were no significant differences in efficacy between dose levels but in some parameters the best results were obtained with higher dose levels.

One of principal results evaluated during our study was safety. We demonstrated that the vaccine is safe at each dose level. Moreover, adverse events classified as vaccine-related were grade 1 or 2 according to CTCAE;[11] importantly, the majority of adverse events were mild (92.5%) or moderate (4.4%). On the other hand, the main adverse events observed during the study were related to injection site reactions and flu-like symptoms but these events did not lead to treatment discontinuation in any cases.

Immunogenicity data contributed to the decision to select the biological optimal dose. Despite antibody titers presenting in all patients after vaccination, higher titers were obtained in patients treated with the three highest dose levels. The antibodies present in serum were IgG and IgM isotypes; IgM titers were higher than IgG. High levels of both isotypes were obtained with the 900 µg dose level. Even though antibody titers were not high generally, the levels obtained

could be considered significant taking into account vaccine characteristics that confer poor immunogenicity.[15]

Analysis of cutaneous hypersensitivity showed that the majority of patients did not show evidence of a DTH response prior to treatment and these patients showed lower survival than patients with a DTH positive test prior to immunization. Overall survival difference was about 23 months but the difference was not statistically significant. Objective antitumor response is not commonly used in the evaluation of biological therapies. However, in this study the antitumor response was measured at four time points during the study and compared with baseline. Although the disease control rate was low some patients achieved an objective response and disease stabilization and, more importantly, a durable response.

The most striking finding was that overall survival duration was not different between dose levels. Only the patients treated with 600 µg showed a median survival much lower than other dose levels. The remaining patients survived more than 16 months, which is longer than the median overall survival reported in the literature for melanoma patients.[9,10] Three patients treated with 900 µg are currently alive after 5 years of their treatment initiation, which shows a favorable impact on survival in metastatic melanoma patients.

The results of patient survival when analyzed by different metastatic sites are interesting. Although subgroups included patients treated with different doses, the survival times obtained in patients with metastases of bone and soft tissue were more than twice that reported in the literature for melanoma patients.[16] This fact could indicate that such patients benefited more from vaccination than those with lung metastases. However, this finding should be confirmed in future studies with a larger number of patients treated with the biological optimal dose.

Finally, the biological optimal dose was selected based on all results, particularly immunogenicity and survival data. The dose selected allows administration of the vaccine at only one injection site according to volume of formulation which is important to the comfort of patients.

Conclusions

NGcGM3 vaccine is safe, immunogenic, and shows evidence of efficacy in metastatic melanoma patients. The dose

selected as the biological optimal dose by the subcutaneous route was 900 µg.

Disclosure

The authors report no conflicts of interest in this work.

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