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ORIGINAL RESEARCH

# NGlycolylGM3/VSSP Vaccine in Metastatic Breast Cancer Patients: Results of Phase I/IIa Clinical Trial

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**Abstract:** Patients treated with vaccines based on NGlycolil gangliosides have showed benefit in progression free survival and overall survival. These molecules, which have been observed in breast cancer cells, are minimally or not expressed in normal human tissue and have been considered as antigen tumor-specific. For this reason they are very attractive to immunotherapy. A phase I/II clinical trial was carried out in metastatic breast cancer patients with the NGlycolylGM3/VSSP vaccine administered by subcutaneous route. Selecting the optimal biological doses of the vaccine in these patients was the principal objective based on the immunogenicity, efficacy and safety results. Six levels of doses of vaccine were studied. Treatment schedule consisted of five doses every two weeks and then monthly until reaching a fifteenth doses. Doses levels studied were 150, 300, 600, 900, 1200 and 1500 µg. Five patients in each level were included except at the 900 µg dose, in which ten patients were included. Immunogenicity was determined by levels of antibodies generated in patients after vaccination. The response criteria of evaluation in solid tumors (RECIST) was used to evaluate antitumoral effect. Safety was evaluated by Common Toxicity Criteria of Adverse Event (CTCAE). The vaccine administration was safe and immunogenic in all does levels. Most frequent adverse events related to vaccination were mild or moderate and were related to injection site reactions and "flu-like" symptoms. Vaccination induced specific anti-NeuGcGM3 IgM and IgG antibodies responses in all patients. Disease control (objective response or stable disease) was obtained in 72.7% of evaluated patients. Median overall survival was 15.9 months. Two patients of two different dose levels achieved overall survival values of about six years. The dose of 900 µg was selected as biological optimal dose in which overall survival was 28.5 months.

**Keywords:** metastatic breast cancer, clinical trial, therapeutic vaccine, ganglioside, NGcGM3

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## Introduction

Breast cancer is the most common malignancy and second leading cause of cancer death in females.[1] In Cuba, breast cancer was the leading cause of incidence cancer cases (2981) in 2006. It is the second cause of cancer mortality in Cuban women (1414 cases in the year 2009) (Data National Cancer Registry, 2009).[2]

Most deaths related to breast cancer are the result of complications from metastatic or recurrent disease. In initial presentation, metastatic breast cancer is rare, existing only between 6% to 10% of patients with metastases at diagnosis. Despite advances in cancer treatment, 20% to 85% of patients subsequently develop distant metastases during the first 5 years after initial diagnosis.[3]

Currently, metastatic breast cancer is considered incurable and treatment goals are generally palliative. Current treatment options for metastatic breast cancer consist of schemes based on combined chemotherapy of doxorubicin or taxanes.[4] The response to first-line chemotherapy for metastatic disease may last between 8 and 14 months.[5] Once metastasis is detected, the median survival time is within the range of 18 to 24 months.[6]

The progression of the disease is inevitable and responses in subsequent therapies were progressively lower. The benefit of second-line chemotherapy is more controversial, particularly in terms of survival. Chemotherapy beyond the first-line is associated with obtaining responses in few patients and there are no consistent or discernible effect on median survival. The effectiveness of second and subsequent lines of chemotherapy is limited to responses in the range of 20% and median survival is usually less than 10 months, in the range of 6 to 12 months. This is a stimulus for the development of more effective new drugs and new therapeutic strategies.[7,8]

The gangliosides NGlycosylated gangliosides are very attractive options in immunotherapy as they are over-expressed in tumor cells and minimally or unexpressed in normal human tissue.[9–11] Breast cancer is one tumor that over-expresses NGlycolyl gangliosides, specifically the NGlycolylGM3 gangliosides.[12,13] Others tissues with similar behavior are melanoma and ovarian cancer. The Center of Molecular Immunology had developed a vaccine based on this ganglioside which has been used in clinical trials in breast cancer patients with a very good toxicity profile and some efficacy evidence.

A phase II clinical trial has been conducted in breast cancer patients with NGlycolylGM3/VSSP vaccine administered by intramuscular route using Montanide ISA 51 and an adjuvant.[14] This trial showed that the vaccine is safe and immunogenic and some patients achieved values of overall survival superior to reports in literature of those with non-visceral metastases. Two phase III clinical trials were conducted, one in early stage breast cancer patients and the other one in metastatic breast cancer patients.[15,16] Despite the vaccine being safe, it was observed that some local reactions may be caused by the adjuvant. For this reason, it was decided to prove the effect of the vaccine by subcutaneous route without adjuvant in similar patient types. A phase I/II clinical trial was designed to study some dose levels based on dose scaling used by intramuscular route. The main objective of this trial was to determine the biologically optimal dose based on results of safety and efficacy obtained after vaccine administration.

## Methods

### Study Participants

Thirty-five advanced breast cancer patients participated in the study recruited at "Dr. Celestino Hernandez Robau" hospital. Characteristics of patients are given in Table 1.

### Ethical considerations

The study protocol, which was performed according to the principles of the Declaration of Helsinki, accepted by the institutional ethics committee and approved by Cuban Regulatory Agency, was carefully explained to the patients, all of whom gave their written consent to participate in the study.

### Inclusion and exclusion criteria

The patients chosen for the study were required to meet criteria with regards to histological diagnostic of breast cancer and advanced disease at inclusion moment. Other criteria included good performance status (grade 1 or 2 according with WHO criteria), age older than 18 years of ages, life expectancy of more then 6 months, and normal parameters of clinical laboratory. All selected patients had no contraindications such as

**Table 1.** Patients characteristics.

| | |
|---|---|
| Eligible patients (evaluated) | 35 |
| Age (Median–range) | 57.83 (32–78) |
| Performance status (PS) (WHO) | |
| 0–1 | 29 |
| 2 | 6 |
| Clinic stage | |
| I | 7 |
| II | 11 |
| III | 13 |
| IV | 2 |
| Histological type | |
| Ductal carcinoma | 23 |
| Lobular carcinoma | 3 |
| Papillary carcinoma | 2 |
| Comedocarcinoma | 2 |
| Colloid carcinoma | 1 |
| Carcinoma | 2 |
| Metastatic site | |
| Visceral | 12 |
| Non visceral | 23 |
| Prior treatment to metastatic disease | |
| Chemotherapy | 29 |
| Chemo—radiotherapy | 5 |
| Radiotherapy | 1 |
| Treatment doses | |
| Less than 5 | 3 |
| Between 5 and 10 | 15 |
| More than 10 | 17 |

pregnancy, decompensation by chronic disease, brain metastases, or active infections

## Study design

This study was designed as an open-label trial, evaluating six dose levels of NGcGM3/VSSP vaccine in six cohorts of five patients in each on, except at the dose level of 900 µg, in which ten patients were included. The principle objective of this study was to determine the biologically optimal dose. To accomplish this objective, parameters related with immunogenicity, safety and efficacy of vaccine were evaluated. These results also allowed the evaluation of the vaccine effects in advanced breast cancer patients (Figure 1).

## Treatment schedule

Vaccine treatment was initiated about 4 or 6 weeks after patients finished oncospecific treatment. Patients received 15 vaccine doses, the first five doses being received every two weeks and subsequent doses every four weeks until one year of treatment was completed. Vaccine was administrated by subcutaneous route.

## Evaluation during study

Blood samples were collected prior and during treatment for hematological and biochemical test and for determining antibody titers. Safety was evaluated by analyzing frequency, intensity, and relationship of the adverse events with the vaccine. Common Toxicity Criteria to Evaluate Adverse Events (CTCAE) version 3.0 was used to classify according to intensity. To evaluate antitumor activity was used Response For evaluation of antitumor activity, Response Evaluation Criteria in Solid Tumor (RECIST) version 1.0 was applied. Tumor size was evaluated by imagenology before starting treatment and in months 3, 6, 9 and 12.

Additionally, overall survival (OS) of treated patients was evaluated. The overall survival was determined as time between randomization date and death date. The results about overall survival were analyzed by Kaplan-Meier methods.

# Results

Analysis was performed by intention to treat.

## Patient population

The mean age of patients was 57.83 years. Some patients had compensated concomitant cardiovascular and respiratory diseases. Most patients were diagnosed in an early phase but as having metastatic evolutive disease (85.7%) or locally advanced disease (8.5%). The remaining patients had visceral disease located primarily in lung and liver. 65.7% of patients had non visceral metastases (skin, lymphatic nodes and bone). The rest of them had visceral disease mostly in lung and liver. Only 2 patients were diagnosed with an advanced stage of the disease and both had metastases at non visceral sites. The number of metastatic lesions was variable but the majority of patients had one or two metastic sites. (68.6% and 20.0% respectively).

The most frequent histological type was infiltrating ductal carcinoma (69.7%). Also lobular, papillary, colloid and comedocarcinoma were present.

Every patient received treatment after initial diagnosis and metastatic diagnosis. First case treatment includes surgery, chemotherapy, radiotherapy and

hormonal therapy, and the majority of treatments include a combination of therapies. Metastatic disease treatment consists of chemotherapy alone (82.9%) and chemo-radiotherapy or radiotherapy alone at 14.3% and 2.8% respectively.

All patients included had good performance status (0–2) according to WHO criteria.

The distribution of all parameters was similar in all dose levels.

Demographic characteristics, previous therapies, site of metastases and total vaccination dose are show in Table 1.

## Treatment compliance

A total of 371 immunizations were administered. Every patient included was treated with the vaccine. The 34.2% of patients received complete treatment (15 immunizations) while the rest of patients received more than 5 doses, except of two of whom received three doses and one who received four. Seventeen patients received tamoxifen as hormonotherapy concomitant with vaccine (48.6%).

Twenty-four patients discontinued treatment during the study (68.5%) but in no case was the discontinuation caused by vaccine complications. Principal causes of discontinuation were treatment schedule noncompliance, patient decision, worsening of performance status or death. Treatment interruptions were distributed in all dose levels.

## Safety results

All safety results were analyzed. Every patient developed grade I – II vaccine-related adverse events. Only six severe adverse events were described as vaccine-related in three patients. In one patient episode, these events included fatigue, lipothymy, and sweating. In two separate patient episodes, hypotension and chills were experienced respectively. In no event was treatment interrupted, and all were successfully controlled without harm to the patient.

The most frequent adverse events observed were site-injection reactions: pain and erythema. Patients also presented systemic events but the majority was related to 'flu-like' symptoms consisting of fever, chills, nauseas, vomiting, headache, myalgias and asthenia.

Serious adverse events were not present during the trial.

All adverse effects appeared subsequently to the first immunizations. Behavior of adverse events was similar in all dose levels. Toxicity profile is shown in Tables 2 and 3.

## Immunological response

Antibody titers against NeuGcGM3 ganglioside were obtained after vaccination in 24 of the 29 patients evaluated. Both IgM and IgG antibodies were present in patients (Table 4). The IgM titer range was within 1/160 and 1/6400. Higher titers were obtained independently of dose levels, although the best median was obtained in the group treated with 900 µg of vaccine. Behavior of IgG titers was similar in all dose levels and its titers were lower than IgM's.

These results demonstrate that the formulation is immunogenic in all dose levels evaluated. The most immunogenic dose was 900 µg.

## Efficacy analyses

In twenty-two patients Antitumor response was evaluated in twenty two patients. 72.7% of patients achieved control disease; five of them achieved objective response either complete (CR) or partial (PR) and eleven patients achieved stable disease (SD) (Table 5).

Best responses were obtained at the 900µg dose level. Of the patients treated with this dose, one in three achieved CR, one in three achieved PR, and eight of eleven achieved stable disease control. In these level had not patients with progressive disease. Despite

**Table 2.** More frequent adverse events vaccination related.

| Types of events | Number of events | % |
|---|---|---|
| Local events | 79 | 23,9 |
| Reaction site injection pain | 48 | 14,5 |
| Local erythema | 31 | 9,4 |
| Systemic | 228 | 68,9 |
| Fever | 41 | 12,4 |
| Chills | 17 | 5,1 |
| Nauseas | 31 | 9,4 |
| Vomiting | 12 | 3,6 |
| Headache | 19 | 5,7 |
| Asthenia | 11 | 3,3 |
| Bone pain | 11 | 3,3 |
| Others | 86 | 26,0 |
| No classified | 24 | 7,3 |
| Total | 307 | 100 |

**Table 3.** Intensity of adverse events.

| Grade | Number of events (%) |
|---|---|
| 1 | 295 |
| 2 | 30 |
| 3 | 6 |
| Total | 331 |

**Table 5.** Antitumor response by dose level.

| Type of response | Dose level | | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|
| | 150 | 300 | 600 | 900 | 1200 | 1500 | | |
| CR | 0 | 0 | 0 | 1 | 0 | 1 | 2 | |
| PR | 0 | 0 | 2 | 0 | 1 | 0 | 3 | |
| SD | 2 | 2 | 1 | 4 | 0 | 2 | 11 | |

this success, no difference in antitumor responses was observed between levels. In order to antitumor responses.

## Overall survival

Overall survival was evaluated in all patients. Median global overall survival was 15.9 months (Table 6). The best value of survival was obtained at the 900µg dose level and was 28.5 months. much superior to others levels. Also, in this level were included Additionally, five of the eleven patients treated with this dose are currently still alive. Significant differences between doses levels were not observed.

## Optimal biological dose

It was determined that the optimal biological dose was 900µg as better results for safety, immunogenicity, and efficacy were obtained. The volume of injection also was also analyzed to select the optimal dose as higher dose levels require higher volume of administration and hence greater patient discomfort. The determined optimal dose allows for formulation of the vaccine in a concentration which requires only one site of injection thereby reducing patient discomfort.

# Discussion

Currently, targeted therapies are used in the treatment of cancers. When one molecule is over-expressed in cancer cells, it can be used as a receptor to drugs which modify important signals related to tumor growth. It also can be used as efficacious therapies. Gangliosides have been associated with tumor cell membranes as well as with tumor-associated antigens. Human cell membranes do not contain gangliosides which thereby allow this molecule to be used as a target in cancer therapy. These reasons support strategies to design molecules that bind to it and down-regulate signaling of tumor growth. The NGlycolylGM3 is an NGlycosylated ganglioside which is over-expressed principally in human breast tumors and melanoma cells.

The NGlycolylGM3 vaccine has been used in breast cancer patients as part of many clinical trials, the most recent of which being a phase II clinical trial demonstrating promising results of survival. Until this point, this vaccine was administered along with the adjuvant Montanide ISA 51. Adverse events were typically observed with administration of this adjuvant. Because of this, it was decided to administer the vaccine subcutaneously without Montanide. The present study was designed to determine the optimal biological dose of the vaccine through for this administration route. Six dose levels previously established were studied. The obtained results do not show big differences among dose levels, however in certain parameters, the best results were obtained at high dose levels. Results are presented in this article and they are original.

**Table 4.** Median of inverse of maximum anti-NGcGM3 gangliosides antibodies titers.

| Dose level (µg) | IgG max | IgM max |
|---|---|---|
| 150 | 320 | 160 |
| 300 | 320 | 320 |
| 600 | 160 | 640 |
| 900 | 320 | 6400 |
| 1200 | ND | 640 |
| 1500 | 640 | 2560 |

**Table 6.** Median overall survival.

| Dose level (µg) | (months) |
|---|---|
| 150 | 6.8 |
| 300 | 11.7 |
| 600 | 8.2 |
| 900 | 28.5 |
| 1200 | 10.3 |
| 1500 | 15.9 |
| Global | 15.9 |

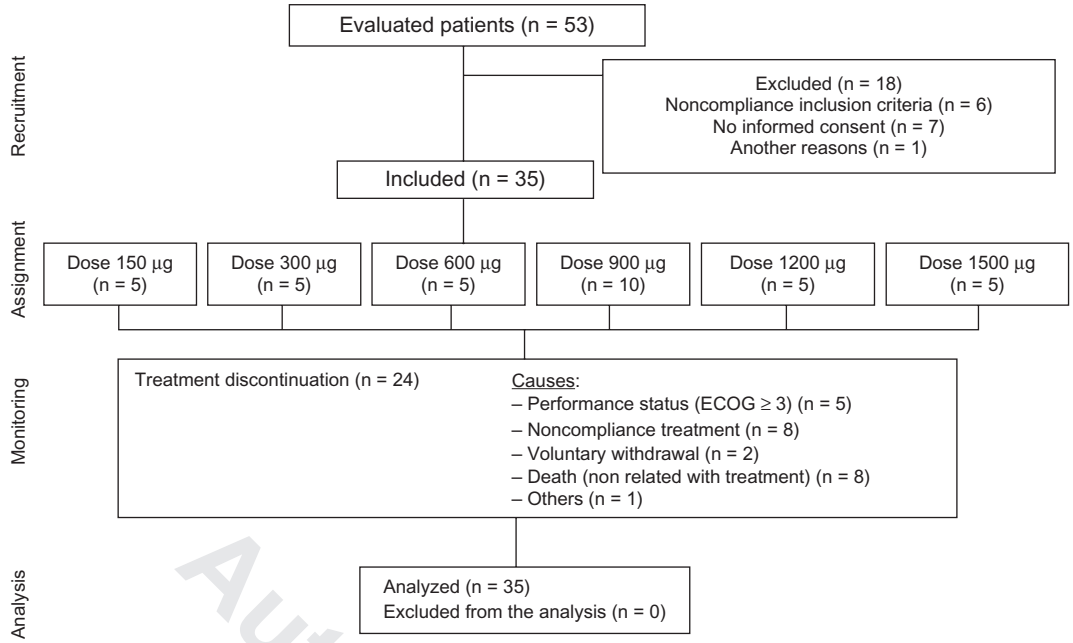


**Figure 1.** Patients involved in study stage.

Safety was one of the results evaluated during this study. Once more it was demonstrated that the vaccine is safe based on the number of adverse events observed in each dose level and the intensity of events. Vaccine-related adverse events were grade 1 or 2 according to CTCAE, and it is remarkable that that the majority of adverse events were mild or moderate. Most adverse events that were observed during the study were related to injection site reactions and flu-like symptoms and in no case led to treatment discontinuation.

Immunogenicity results also contributed to the determination of the optimal dose, despite the fact that antibody titers were obtained after vaccination at every dose level. It is important to remark that the antibodies levels are low in comparison with other titers obtained from other vaccine types. Antibody levels are expected to be low with ganglioside vaccines as it is not a protein, a characteristic which gives it a poor immunogenicity.

Antibodies present in the serum were IgG and IgM isotypes. IgM levels were higher than IgG's and high level of IgM were obtained in patients treated with 900 µg.

Objective antitumor response is not the most common form of measurement, especially in the evaluation of biological therapies. However, In this study, the antitumor response was measure at four different instances and compared to a baseline. Disease-control rate was obtained in many patients. Objective response and disease stabilization were observed in many cases and these patients showed a durable response.

Survival behavior was not significantly different among dose levels and only patients treated with a dose level of 900µg showed increased survival rate. This rate is higher than other literature values in metastatic breast cancer patients.

The biologically optimal dose was selected based on all results, especially on immunogenicity and survival. Moreover, the selected dose allows for one-site injection administration, and important factor in patient comfort.

## Conclusions

The NGlycolylGM3 vaccine is safe, immunogenic, and shows evidence of efficacy in metastatic breast cancer patients. The biological optimal dose by subcutaneous route is 900 µg.

## Author Contributions

Conceived and designed the experiments: Macias A, Saurez G, de la Torre A, Osorio M, Ortiz R, Hernandez J. Analysed the data: Santiesteban Y, Viada C, Arbolaez M, Cepeda M, Guerra PP, Garcia E. Wrote the firts draft of the manuscript: Perez K, de la Torre A. Contributed to the writing of the manuscript, results

and conclusions: Toledo D, Mulens V. Made critical revisions and approved final version: Fernandez LE, Carr A. All authors reviewed and approved of the final manuscript.

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## Competing Interest

Author(s) disclose no potential conflicts of interest.

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## Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.



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