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NGcGM3/VSSP vaccine as treatment for melanoma patients

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Gangliosides are glycosphingolipids that are present in the plasma membranes of vertebrates and are involved in multiple cellular processes. In the Center of Molecular Immunology an NGcGM3 ganglioside based vaccine has been developed and is conceptualized as a targeted therapy in cancer. NGcGM3/VSSP vaccine had been used as treatment of metastatic melanoma patients and had showed to be safe and immunogenic. The treatment improved antitumoral response or maintain the response obtained with previous onco-specific treatment as chemotherapy. The results indicate that the vaccine improved overall survival of metastatic melanoma patients after first line-chemotherapy. The clinical trial ongoing currently will allow corroborating these results.

Keywords: metastatic melanoma, vaccine, NGlycolylated ganglioside, clinical trial

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N-glycolylated Gangliosides

Gangliosides are glycosphingolipids containing two well-defined moieties: a hydrophilic oligosaccharide with different carbohydrate residues, including at least one sialic acid molecule, and a hydrophobic ceramide portion containing primarily saturated fatty acids with 16–22 carbon atoms.[1] Gangliosides are present in the plasma membranes of vertebrates[2,3] and are involved in multiple cellular processes such as growth, differentiation, adhesion and regulation of cell death signaling pathways.[1]

As N-glycolyl neuraminic acid (NANA) is one of the most common types of sialic acids present in most mammals' tissues[2] it's not surprising that Nglycolylated gangliosides has been reported as tumor-associated antigens or tumor markers in these species.[3-5] But much more interesting is the fact that while the N-glycolylated form of GM3 ganglioside (NGcGM3) is absent from normal human tissues, its overexpression has been reported in certain human tumors. This is one of the reasons why NGcGM3 has been considered as a privileged target for cancer immunotherapy.[3,4,6]

Another reason is that losing membrane integrity is one of the tumor cell death mechanisms described for antibodies specific to NGcGM3. Roque-Navarro et al. described how the anti-NGcGM3 mAb 14F7 induced this effect in murine L1210 leukemia cells but not in mouse normal cells expressing the antigen.[7] The remaining fact is the potent immune suppressive effect exerted by this glycolipid through the down-modulation of CD4, both in human and mouse T cells.[1,8]

NGcGM3/VSSP Vaccine

In the Center of Molecular Immunology an NGcGM3 ganglioside based vaccine has been developed since the nineties of the past century. The NGcGM3/VSSP vaccine is conceptualized as a targeted therapy in cancer, sustained on the role of N-glycolylated gangliosides in disease progression, together with its unique feature of tumor-specific antigens in humans. Several pre-clinical and clinical evidences support these assertions.[9-12]

Perhaps the best-documented presence of NGcGM3 in human tumors has been achieved in breast cancer. For the first time the presence of a significant amount of this ganglioside was detected in breast tumors that were histopathologically

Table 1. Clinical trials with NGcGM3/VSSP in melanoma patients

Design	Indications	Dose / Administration route	Current state
Phase I/II, open, dose scalating	Advanced melanoma	Intramuscular 0.2 and 0.4mg	Published[20] Cancer Biology and Therapy 2008
Phase I/II, open, dose scalating	Metastatic melanoma	Subcutaneous 150, 300, 900, 1200 and 1500 μg	Published[21] Cancer Management and Research 2012
Physician led	Metastatic melanoma	Subcutaneous 900 μg	Ongoing
Phase II	Metastatic melanoma	Subcutaneous 900 μg	Ongoing
Phase II	High risk melanoma	Subcutaneous 900 μg	In design

classified as invasive ductal carcinomas by mass spectrometry.[13] Importantly, a further confirmation of this finding emerged after the "in vivo" detection of NGcGM3 in human breast primary tumors by radioimmunoscintigraphy with 99Tc labeled 14F7, a highly specific anti-GM3 (NeuGc) ganglioside monoclonal antibody.[14]

The presence of this target has been checked in other localizations such as digestive system tumors, genitourinary system tumors, neuroectodermal tumors and others. As a common fact while different levels of NGcGM3 was detected in these tumor types, an almost total absence of this glycolipid was observed in the corresponding normal tissues.[15-17]

Because of the poor immunogenicity of glycolipids, NGcGM3 was incorporated into the outer membrane protein complex (OMPC) of Neisseria meningitides with the aid of anionic detergents, forming very small size proteoliposomes (VSSP). VSSP overcame the natural tolerance to gangliosides.[18]

Melanoma and N-Glycolylated Ganglioside

In skin cancer, specifically cutaneous malignant melanoma, the results showed a overexpression of N-glycolylated gangliosides in tumor cells. The analysis of the recognition pattern of 14F7 mAb in several kinds of tumors showed that 14F7 mAb was strongly recognized by expressed antigen in fresh tissues of melanoma tumors by immunohistochemical techniques. The tumors of patients included in this study showed recognition of 14F7 mAb in all of cases while corresponding normal skin no recognized the mAb.[2]

Regarding this issue, the immunohistochemical reactivity of the 14F7MAb raised against N-GlycolylGM3 ganglioside in some benign and malignant skin neoplasms have been studied in another experiments. In the study of Blanco et al. the main results were no immunorecognition of 14F7 mAb in normal melanocytes and keratinocytes (0/10) and in benign and dysplastic melanocytic nevi the 14F7 immunostaining was observed in less than 20% of samples. On the other hand in malignant lesions derived from human skin, immunorecognition of 14F7 mAb was different regarding the kind of tumor. In non-melanoma skin tumors the recognition was less than 50% in mostly while in cutaneous malignant melanoma and melanoma lymph node metastases the recognition was 100 and 85.7% respectively. These results suggest a possible relationship between the 14F7 reactivity with the more aggressive behavior of malignant tumor of melanocytes.[19]

Melanoma and NGcGM3/VSSP Vaccine—Clinical Trials

The NGcGM3/VSSP vaccine had been evaluated in clinical trials as treatment to cutaneous melanoma patients (**Table 1**).

Two clinical trials in melanoma patients have finished; both of them were phase I/II dose escalating. In both trials positives results that indicate improve in overall survival were obtained.

In first trial the vaccine was administered by intramuscular route with Montanide ISA 51 VG20. In this trial doses of 0.2 and 0.4 mg were evaluated and 22 patients were included; 12 in the 0.2 mg dose level and 10 in the 0.4 mg dose level. The clinical trial results repeated the behavior related to the expression of ganglioside on melanoma tumors because primary tumors analyzed showed intense membrane and cytoplasmic immunostaining as well as several subcutaneous metastases and metastatic lymph nodes. In other hand no expression of gangliosides in corresponding normal skin was observed.

Regarding safety all patient development vaccine-related adverse events grade I-II. The main adverse events observed were related to local reactions as pain, induration and erythema and systemic adverse events as flu-like symptoms consisted in fever, myalgia, chills, headaches and skin changes. Only 6 patients experimented adverse events grade III, and no case provoked treatment discontinuation. Several patients received more than 15 immunizations and there was no evidence of cumulative toxicity.

Immunological response was evaluated in 18 patients. Vaccination induced specific anti-NeuGcGM3 IgM, IgG and IgA antibodies responses. Titers of IgM were greater for the highest vaccine doses. Vaccination also elicited DTH response in 45.5% of patients in the lower doses and 77.8% in the higher doses. Interestingly, 3 patients developed vitiligo although the nominal antigen NeuGcGM3 is not present in melanocytes.[20]

In second trial the vaccine was administered by subcutaneous route without adjuvant and six doses were evaluated (range between 150 and 1500 μg).[20] In this trial immunogenicity, safety and anti-tumoral response were evaluated, all to select the optimal biological doses by subcutaneous route. Also the survival was analyzed despite it was not a goal of this trial and interesting findings results of this analysis.[21]

The results about immunogenicity and safety were similar to the first trial. The 93% of patients showed antibody response against NGcGM3 and the titers were high taking into account that the immunogenicity of this kind of vaccine is relatively lower. Mostly adverse events were mild and moderate, even in patients that received treatment by two years. The adverse events coincide with events observed in previous trial.

Despite results about antitumor response were not significant, it should be noted that many patients maintain the response obtained with the onco-specific treatment with very good performance status and quality-of-life. Several patients obtained a best response during the treatment when is compared with previous response. Even patients with disease progression at the beginning of treatment and that did not achieve a best response, received many dose of vaccine and showed an unexpected survival.[21]

In none of the two trials, the survival analysis was designed, but in both, survival results observed were promissory. In first clinical trial patients achieved survival time higher than 1 y in the 40% of cases and higher than 2 y about 30%. At these times four patients included in this trial are still alive; it means that they have an overall survival between 4 and 11 y, a too uncommon survival to advanced melanoma patients. One of them had visceral metastases. Also in second trial unexpected overall survival values were obtained. The global overall survival achieved was 20 mo at the moment of final analysis. At the present time 5 patients are still alive after about 4 to 8 y of treatment with vaccine. Two of them have visceral metastases.[20,21]

Analysis at the finished of first clinical trial showed overall survival after the diagnosis by metastases sites higher than the reported to this kind of patients. At the present time this analysis was repeated and the results is showed in **Table 2**. Despite few patients still alive the results draw attention and suggest an advantage to patients.

Table 2. Overall survival after the diagnosis in melanoma patients by metastases sites

Metastases sites	OS (months)
Skin, Subcutaneous cellular tissue and nodes	25.6
Lung	19.2
Other visceral sites	21.7

Perspectives

Biomarkers. In order to find a biomarker that permit to predict the response of patient to the vaccination, several analyses using the existent data had been done. One of them was about the possible relationship between Delayed Test Hypersensitivity (DTH) response or immunological response and overall survival. Despite during the first clinical trial no relationship was observed between immunological response (serological and DTH) and the clinical outcome, in the second trial patients with positive DTH before treatment showed higher survival than patients with negative DTH (37.8 vs. 14.9 mo respectively); this result suggests it might be a relationship between these two variables.[20] Also related to immunological response some findings have been observed which make to think a possible better evolution after vaccination in patients with some previous antibody response. Another possible biomarker could be the ganglioside overexpression in tumors tissues.

All of these challenges will be faced during phase II clinical trials in metastatic melanoma patients as well as high-risk melanoma patients.

Other clinical trials. A physician-led clinical trial in 20 metastatic melanoma patients is ongoing, in which 18 patients are already included. In this trial patients whom have a disease progression after onco-specific treatment and don't have other alternative treatment are included. This kind of patient has a poor prognosis and, despite no definitive results have been obtained yet, at the present time treated patients have good performance status and quality-of-life even after 1 y of treatment.

Another clinical trial is ongoing in metastatic melanoma patients whom achieve objective response or stabilization of disease after onco-specific treatment. Eighty patients will be included and will be randomized into two groups of treatment, one treated with the vaccine and the other one with placebo. The main goal of this trial is overall survival.

Phase II clinical trial in high-risk melanoma patients is in design in order to evaluate the capacity of NGcGM3/VSSP vaccine to improve time to progression of this kind of patient.

Remarks

NGcGM3/VSSP vaccine had been used as treatment of metastatic melanoma patients and had showed to be immunogenic and very safe. The treatment improved antitumoral response or maintain the response obtained with previous onco-specific treatment. The results obtained until the present indicate that the vaccine improved overall survival of metastatic melanoma patients after first line-chemotherapy and the clinical trial ongoing will allow corroborating this results.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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