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DRUG EVALUATION

Racotumomab for treating lung cancer and pediatric refractory malignancies

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ABSTRACT

Introduction: Racotumomab (originally known as 1E10 mAb) is an anti-idiotype murine IgG1 directed to membrane glycoconjugates expressed in aggressive solid tumors. It was developed as a mirror image of the idiotype of another antibody against N-glycolyl-containing molecules, such as the NeuGcGM3 ganglioside. After a successful phase II/III study, racotumomab formulated in alum was conditionally approved in Latin American countries as maintenance therapy for advanced non-small cell lung cancer.
Areas covered: This review analyzes the biology of the target antigen, summarizes preclinical studies and discusses clinical trials in adults and the pediatric experience with racotumomab.
Expert opinion: Proper patient selection and combination with chemotherapy, radiotherapy or checkpoint inhibitors appear to be critical issues to maximize the effects of racotumomab vaccination in lung cancer. In a recent phase I clinical trial in children with relapsed or resistant neuroectodermal malignancies, racotumomab was well tolerated and immunogenic, and its evaluation as immunotherapy for high-risk neuroblastoma is warranted.

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1. Introduction

Breakdown of immune tolerance and consequent reactivation of host immune system constitutes an attractive treatment option against cancer. Recent knowledge has provided new approaches for cancer immunotherapy, and a new era of treatment for advanced malignancies is definitely arriving. In non-small cell lung cancer (NSCLC), different immunotherapies have been tested, including general immune stimulants, whole-cell or peptide vaccines, and checkpoint inhibitors.[1,2] Large phase III trials testing vaccination approaches in advanced NSCLC showed no convincing clinical benefit in the overall population. The liposomal vaccine L-BLP25 (tecemotide) achieved a significant improvement in survival in a subgroup of stage IIIb patients receiving chemoradiotherapy.[3] Tecemotide targets the core peptide of mucin 1, a glycoprotein antigen commonly overexpressed in adenocarcinoma. Belagenpumatucel-L, a transforming growth factor β2 antisense-gene modified allogenic cell vaccine, was evaluated as maintenance after initial therapy in a randomized phase III trial. Although the overall survival was not improved, a subgroup of stage IIIb/IV patients who were randomized early after completion of chemotherapy or received prior radiation had a marked survival benefit with belagenpumatucel-L.[4] The melanoma-associated antigen 3 (MAGE-A3) vaccine showed promising results in completely resected MAGE-A3-expressing NSCLC in phase II. However, the MAGRIT phase III study did not achieve its primary end point of demonstrating an improvement in progression-free survival.[5,6] Recent results regarding the checkpoint inhibitors are quite remarkable, particularly in the case of nivolumab, an anti-programed cell death-1 (PD-1). Two phase III trials demonstrated superior overall survival of nivolumab as second-line therapy versus docetaxel in patients with advanced squamous and non-squamous NSCLC.[7,8] Similarly, PD-1 inhibition with pembrolizumab significantly prolonged overall survival in patients with previously treated, PD-1 ligand-positive, advanced NSCLC.[9]

Therapeutic monoclonal antibodies (mAbs) are potent and specific antitumor agents, able to activate immune effector mechanisms such as complement-mediated cytotoxicity and antibody-mediated cell-dependent cytotoxicity.[10] An original strategy to elicit effective immune responses against tumor-specific antigens consists of using anti-idiotype mAbs as antigen surrogates. The idea behind this is to obtain a second-generation mAb that is a precise mimic of the original antigen epitope. A remarkable advantage of anti-idiotype mAbs is the fact that not only the epitope contained in the hypervariable region (Fab fragment) but also the constant region (Fc fragment) serves to boost antitumor immune responses and break tolerance.[11] The IgG1 racotumomab (formerly designated as 1E10) is an anti-idiotype murine mAb to the *N*-glycolylneuraminic acid (NeuGc), a sialic acid residue found in mammalian cells as membrane glycoconjugates like the GM3 ganglioside (NeuGcGM3) (see Box 1). It was obtained as a mirror image of the idiotype of another antibody that specifically recognizes NeuGc-containing molecules and sulfated glycolipids. Thus, racotumomab mimics NeuGc antigens on tumor cell surface.[12]

2. Biology of the target antigen

Gangliosides are a broad family of glycosphingolipids present on the cell surface, implicated in cell communication, immune regulation, and metastatic progression.[13] Although the

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Box 1. Drug summary.

Drug name	Racotumomab
Phase	III
Indication	Non- small cell lung cancer Neuroblastoma
Pharmacology description	Murine monoclonal antibody
Route of administration	Intradermal
Chemical structure	IgG1, anti-idiotype to NeuGc-containing glycoconjugates
Pivotal trial	Alfonso et al. [39]

significance of the overexpression of NeuGc-containing gangliosides in human cancer is still under study, NeuGcGM3 has been consistently described as a tumor antigen in advanced NSCLC [14,15] and aggressive pediatric malignancies, such as neuroblastoma and retinoblastoma.[16,17]

Synthesis of GM3 ganglioside is conducted by GM3 synthase, a type II transmembrane enzyme present in the membrane of late Golgi apparatus that catalyze the addition of a sialic acid to LacCer glycosphingolipid. As the precursor of the a- and b series of gangliosides, it is the simplest molecule of this glycosphingolipid family composed of a single sialic acid residue. In mammals, sialic acid is found in tissues either as acetylated or glycolylated forms which result, in the case of GM3 ganglioside, in NeuAcGM3 or NeuGcGM3, respectively. In humans, the lack of the key enzyme that catalyzes *N*-glycolylation (cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase [CMAH]), results in the exclusive expression of NeuAcGM3 in most normal somatic tissues.[18]

The role of NeuAcGM3has been demonstrated in several biological processes. Early reports have shown NeuAcGM3 overexpression in high-density cultures, taking part in cell contact inhibition.[19] Glycosinapses are microdomains that contain gangliosides, mainly NeuAcGM3 and tetraspanin (CD9). In contrast to regular rafts, glycosinapses participate in cell adhesion with concurrent cell signaling. In this context, NeuAcGM3 promotes the association of tetraspanin with αIII or αV integrins, leading to inhibition of cell motility and proliferation.[20]

Although normal human cells do not express CMAH, it is widely reported the presence of NeuGcGM3 in cancer tissues. [21,22] Considering that NeuGc is present in human diet such as different meats and milk products,[23] a confirmed mechanism to explain NeuGcGM3 expression in cancer cells considers that under hypoxia it is promoted the overexpression of sialin, a plasma membrane sialic acid transporter, which in turns dramatically increases NeuGc intake thus allowing the expression of NeuGc-containing gangliosides.[24]

However, such NeuGcGM3 expression is not a mere side effect of hypoxia, but an adaptive behavior of transformed cells. Evidence from animal models support the notion that accumulation of NeuGcGM3 confers advantages to cancer cells.[25,26] Although different mouse tumor cells showed absence of CMAH expression, they could actively incorporate exogenous NeuGc from culture medium to produce NeuGcGM3 and thus increase their metastatic potential when injected *in vivo*.[25] Consistent with this, mouse tumor cells transfected with CMAH demonstrated expression of NeuGcGM3 together with increased proliferative and adhesive properties *in vitro*.[26] In addition, NeuGcGM3 expression also promoted immunosuppressive effects, downregulating CD4+ T lymphocyte infiltration in tumor microenvironment, as well as inhibited dendritic-cell activity. However, it was reported that inhibitory capacity of CD4+CD25+ T regulatory lymphocytes and their proliferation induced by interleukin 2 were not modified.[27,28]

3. Preclinical studies

During the preclinical phase, racotumomab had been extensively tested in different animal systems, including the use of aggressive mouse tumors. Immunization with bi-weekly intraperitoneal doses of racotumomab coupled to keyhole limpet hemocyanin in Freund's adjuvant significantly reduced primary tumor growth and delayed metastatic progression in BALB/c mice bearing the syngeneic mammary carcinoma F3II.[29] Another formulation of racotumomab emulsified in aluminum hydroxide (alum) adjuvant via the subcutaneous route was examined, showing a remarkable antitumor activity in the F3II model in combination with low doses of the chemotherapeutic agent cyclophosphamide.[30] Besides, intravenous administration of racotumomab as uncoupled mAb inhibited lung colonization by B16 melanoma cells.[29] Racotumomab demonstrated good tolerance in mice, especially with the alum-formulated vaccine. Conjugation with keyhole limpet hemocyanin produced increased cellularity in bone marrow, extramedullary hemopoiesis, and peripheral neutrophilia in tumor-bearing mice receiving several immunizations.[29,30]

Concerning to studies in lung cancer models, racotumomab was evaluated in C57BL/6-derived 3LL Lewis carcinoma, using the safe formulation in alum. Repeated subcutaneous vaccination inhibited the development of lung nodules, either with preventive or therapeutic administration schedules.[31] Interestingly, racotumomab-based immunotherapy was associated to an increase of tumor-infiltrating T lymphocytes in lung nodules, as well as a reduction of angiogenesis accompanied by induction of tumor-cell apoptosis. More recently, the antitumor activity of racotumomab–alum was investigated in combination with chemotherapy in the same 3LL lung model.[32] Vaccination exerted a comparable effect on lung tumor nodules to that of clinically relevant doses of pemetrexed. Combined chemoimmunotherapy was highly effective and well-tolerated, although a synergistic effect was not confirmed. Additionally, strong evidence on the role of NeuGc in the metastatic potential of lung carcinoma cells was obtained, and immune response in vaccinated mice was shown to be antigen-specific.[32]

The hypothesis that racotumomab acts as a surrogate of NeuGc antigen generating autologous antibodies was tested in different animal species, particularly in chickens which, like humans, do not express NeuGc-glycoconjugates in normal tissues. As expected, most chickens immunized with racotumomab developed a specific response against NeuGcGM3, as well as NeuGcGM2.[33] Surprisingly, a fraction of antibodies positive for NeuGc antigen but negative for the original

idiotype was also detected in chicken hyperimmune sera. The precise mechanism associated with the generation of these antibodies is unclear, but it seems that a natural immune network is involved.

4. Clinical trials in adults

Different phase I clinical trials and compassionate use studies with racotumomab were performed in advanced melanoma,[34] metastatic breast cancer,[35,36] small cell lung cancer,[37] and NSCLC,[38] with the main goals of evaluating safety and immunogenicity of repeated vaccination (Table 1). Patients received intradermal injections of racotumomab formulated in alum at doses of 0.5, 1, or 2 mg. The first 4 or 5 doses were administered at bi-weekly intervals (induction period) followed by monthly boosters (maintenance period) if the patient maintained a favorable clinical status. Immunization with racotumomab was safe and reasonably well-tolerated. Toxicity mainly consisted of mild local reactions at the injection site, with erythema and induration that disappeared after few days. Systemic adverse events, such as flu-like symptoms with fever, headache, and arthralgia, were also observed.

Racotumomab was shown to be highly immunogenic in humans, since a specific IgM and IgG response against NeuGc-containing gangliosides was detected in the sera of most patients.[34–37] Furthermore, NeuGcGM3-specific interferon-gamma production by peripheral blood T cells was recorded in several patients.[36]

A phase II/III randomized double-blind clinical study was conducted by Alfonso et al. [39] in 176 patients with advanced (stage IIIb/IV) NSCLC. Patients receiving racotumomab–alum at a dose of 1 mg as switch maintenance showed a significant improvement in both overall survival and progression-free survival versus placebo.[39] In addition, a significantly longer median survival time was observed in vaccinated patients who developed anti-NeuGcGM3 serum antibodies with the capacity to specifically bind and kill NeuGc-expressing tumor cells.[39] Considering such promising results in the phase II/III study, racotumomab (commercially launched as Vaxira™) was conditionally approved in Argentina and Cuba for the treatment of patients with advanced stage NSCLC. Since the compound was originally developed in Cuba, the procedure for its approval in the United States has still not prospered due to restrictions imposed by the blockade. Racotumomab is currently being explored in a multinacional phase III clinical trial in NSCLC and also evaluated for a number of cancer indications, particularly in pediatric refractory malignancies.

Table 1. Main clinical trials involving racotumomab in adult cancer.

Indication	Phase	*N*	Reference
Melanoma	I	20	Alfonso et al. [34]
Breast cancer	I	10	Diaz et al. [35]
Breast cancer	I	20	Guthmann et al. [36]
Small cell lung cancer	I	9	Neninger et al. [37]
Non-small cell lung cancer	CUS*	71	Alfonso et al. [38]
Non-small cell lung cancer	II/III	176	Alfonso et al. [39]
Non-small cell lung cancer	III (ongoing)	1082	NCT01460472

*Compassionate use study.

5. Pediatric experience

NeuGcGM3 is expressed consistently in a variety of pediatric malignancies, especially in those from neuroectodermal origin. It is expressed in more than 85% of neuroblastoma primary tumor specimens, including those with MYCN oncogene amplification,[16] and in virtually every case of retinoblastoma including primary and metastatic tumors as well as in commercial cell lines.[17] Its expression does not seem to be influenced by previous use of chemotherapy and radiotherapy.[10] Other tumors like nephroblastoma and Ewing sarcoma also show high NeuGcGM3 expression, but relatively less cases have been evaluated.[16,40]

The clinical relevance of these results is pertinent mostly to the potential use of racotumomab in neuroblastoma, a tumor where a role of immunotherapy has been reported.[41] However, while detailed data of the immunogenicity and safety of racotumomab in adults are available,[42] there is little information in pediatrics. In children with cancer, immunotherapy may pose specific challenges for generating an adequate immune response related to age, prior therapy, or other factors. Racotumomab is particularly interesting for its use in developing countries where neuroblastoma has a high mortality, because of its potentially higher safety.[43] For this tumor, treatments directed to eradicate minimally disseminated disease such as immunotherapy become promising options in the past decade.[44] Among these, murine or humanized (chimeric) mAbs directed against GD2, a disialoganglioside, in combination with interleukin 2 and granulocyte-macrophage colony-stimulating factor were associated to a significantly increased 2-year event-free survival in high-risk neuroblastoma,[38] recently obtaining FDA approval. This combination is delivered to patients after consolidation with high-dose chemotherapy and stem-cell rescue concomitantly with cis-retinoic acid for 6 months after transplant.[45] However, this is a complex regimen that needs sophisticated infrastructure, which may not be available or would result in unacceptable toxicity, even when anti-GD2 mAbs are used alone,[46] when used in settings with limited resources. This combination is given intravenously and, because its toxicity, it may only be given for a relatively short period of time after transplant. After the discontinuation of its use, no residual effect is detected so no long-term immune effects are evident.

Durable immune modulation may be achieved with vaccination strategies potentially replicating the antitumor effect seen with exogenously administered anti-ganglioside mAbs. A promising study of the long-term use of a bivalent GD2–GD3 gangliosides vaccine in combination with beta glucan (an immunostimulant needed to overcome the relatively low immunogenicity of ganglioside antigens) showed a mild-toxicity profile and triggered specific antibody response in high-risk neuroblastoma.[47]

A recent phase I study by Cacciavillano et al. [48] showed that racotumomab–alum is safe and immunogenic in a population of patients with advanced and refractory pediatric tumors, including high-risk neuroblastoma (Table 2). No dose-limiting toxicity occurred, so the maximum tolerated dose was not reached after the use of intradermal injections of racotumomab escalated from 0.15 to 0.4 mg. Racotumomab was injected bi-weekly for

Table 2. Clinical trials involving racotumomab in pediatric cancer.

Indication	Phase	*N*	Reference
Refractory malignancies	I	14	Cacciavillano et al. [48]
Neuroblastoma	II (awaiting approval)	39	ANMAT*

*National Administration of Drugs, Foods and Medical Devices, Argentina.

three doses in the initial cohort of nine patients and for six doses in a confirmation cohort of three patients. Only self-limiting mild-to-moderate local toxicities were found.[48] The study confirmed the immunogenicity of racotumomab in a cohort of children with cancer, being immunogenic in 85% of the evaluable patients.[48] These results are comparable to those reported with the use of the bivalent GD2–GD3 vaccine.[47] Most patients elicited an immune response against racotumomab, despite heavy pretreatment including stem-cell transplantation in many cases. Children receiving the lowest dosages were less likely to develop an immune response. Several immunologically responsive patients elicited anti-NeuGcGM3 antibodies (including IgG and IgM antibodies) as well. The anti-racotumomab antibodies peaked earlier, usually after the second administration and remained stable thereafter, whereas the anti-ganglioside response took up three doses to be detectable.[48]

That study included heavily pretreated and refractory children with solid tumors and its design was not intended to assess tumor response.[48] Therefore, based on these results showing a safe toxicity profile and high immunogenicity in pediatric malignancies, we are currently planning to evaluate the effect in minimally disseminated disease in children with high-risk neuroblastoma after long-term vaccination with 0.4 mg of intradermal racotumomab–alum after front-line therapy and in children with relapsed tumors.

6. Expert opinion

6.1 Advanced NSCLC

Active immunotherapy using vaccines remains mostly experimental in lung cancer.[2] The anti-idiotype mAb racotumomab was one of the first vaccine-based approaches to be approved as a maintenance therapy for advanced cancer. It obtained a conditional approval in Latin American countries in 2013, after a successful phase II/III study in stage IIIb/IV NSCLC patients.[39] Taking into account that about 20–25% of patients receiving racotumomab appeared to have great clinical benefit with longer survival, patient selection should be carefully considered in order to identify a subset of patients with a better ability to respond to vaccination. In that sense, the induction of serum anti-NeuGc antibodies with the capacity to generate cytotoxicity in at least 30% of target antigen-expressing cells appeared to be a good candidate biomarker associated with better clinical outcome of patients treated with racotumomab.[39] The identification of NSCLC patients with a favorable genetic immune profile to respond to vaccination are likely to be critical issues for the design of clinical studies for the next years.

Another relevant aspect concerns the desirable expression of the target antigen in tumors. A recent immunohistochemical study by Blanco et al. [49] confirmed that high levels of NeuGcGM3 ganglioside expression on both tumor cell membrane and cytoplasm, by means of a score integrating intensity of reaction and percentage of positive cells, correlate with a significantly poorer survival of NSCLC patients. This result supports the use of immunohistochemistry to establish prognostic factors in NSCLC and also to identify patients with increased expression of the vaccine target.

Combinatorial approaches involving chemotherapy or radiotherapy concomitant with long-term racotumomab immunization are attractive, particularly in NSCLC patients with macroscopic residual tumors. In the same line, combination of racotumomab with immune-checkpoint inhibitors may help to overcome the immunosuppressive tumor microenvironment. Suppressive mechanisms are often triggered by the same antitumor inflammatory response that immunization intends to create.[50] The checkpoints inhibitors nivolumab or pembrolizumab could thus maximize the effects of antigen-specific vaccine therapies such as racotumomab.

6.2 High-risk neuroblastoma

Despite aggressive standard multimodality therapy, most patients with high-risk neuroblastoma relapse and die from the tumor and survival remains poor.[44] In the past decade, treatments directed to eradicate minimally disseminated disease such as immunotherapy become promising options.[51] In a phase I clinical trial in pediatric patients with relapsed or resistant neuroblastoma and other refractory pediatric tumors, racotumomab vaccination had a favorable toxicity profile up to a dose of 0.4 mg, and most patients elicited an immune response.[48] Therefore, its evaluation as immunotherapy for high-risk neuroblastoma, as well as for other neuroectodermal malignancies, is warranted in further clinical trials.

Declaration of interest

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