

Clinical benefits in patients with advanced solid tumors after long-term immunization with a VEGF therapeutic vaccine

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Abstract

CIGB-247 is cancer vaccine candidate that uses a recombinant variant of the human Vascular Endothelium Growth Factor (VEGF) isoform 121 as antigen, in combination with VSSP, a bacterially-derived adjuvant. Four years ago, CIGB-247 was studied in a phase I clinical trial (code name CENTAURO), where the vaccine was administered to thirty patients with advanced solid tumors at three antigen dose level (50, 100 or 400 µg), all combined with 200 µg of VSSP. The vaccine was administered subcutaneously once a week, for 8 weeks, with a final re-immunization on week 12. Evaluation of patients on their week 16 proved that CIGB-247 was safe, with mainly low-grade local adverse events, and elicited anti-VEGF neutralizing antibodies, and gamma IFN producing T-cells after *in vitro* stimulation with a mutated VEGF version.

Starting on week 16, surviving trial patients received supervised voluntary off-trial re-immunizations every 4 weeks, using 400 µg of antigen and 200 µg of VSSP. The present article is a case series presentation of the safety and clinical follow up of eight CENTAURO patients that have received between 62 and 66 immunizations with CIGB-247, with no other onco-specific treatment, and have shown an accumulated survival time ranging between 4.5 to 4.9 years after trial inclusion. No important adverse events have been reported during long term vaccination. Of the eight patients, two ovary cancers and one NSCLC have achieved complete response status. A patient with a duodenum adenocarcinoma with pancreas infiltration has recently relapsed after two years free of disease. An individual with a pure seminoma has a documented partial response. The other three patients (a metastatic alveolar soft-part sarcoma, a small intestine carcinoid tumor, and a metastatic pancreas neuroendocrine carcinoma) have maintained a stable disease status already for several years. These safety and clinical evolution evidences justify the continuation of the clinical development program of CIGB-247.

Abbreviations

CIGB: Center for Genetic Engineering and Biotechnology; CECMED: Cuban State Center for Medicament Control; ECOG: Eastern Cooperative Oncological Group; ELISA: enzyme linked immunosorbent assay; ELISPOT: enzyme-linked immunospot; RECIST: Response Evaluation Criteria in Solid Tumors; VEGF: vascular endothelial growth factor; VSSP: very small-sized particles; VEGFR2: VEGF receptor 2; IFN: interferon; PFS: Progression Free Survival; PFI: Progression Free Interval; NSCLC: non-small cell lung cancer

Keywords

cancer; active immunotherapy; VEGF; angiogenesis; clinical trial; therapeutic vaccine

Background

VEGF is a key molecule in physiological neo-angiogenesis and in the maintenance of normal blood vessels, but this growth factor has been also associated with the occurrence of pathological angiogenesis in a wide spectrum of human diseases [1]. In the case of cancer [2], VEGF is produced by tumor cells in response to hypoxia, stimulating vascular endothelial cells of nearby normal blood vessels, as well as their bone marrow precursors, and eventually leading to the development of tumor blood vessels that are necessary for cancer growth and metastasis. Additionally, VEGF produced by cancer cells is also a powerful inhibitor of the immune response against tumors [2].

CIGB-247 is a therapeutic cancer vaccine candidate that combines a recombinant antigen representative of human VEGF isoform 121, and VSSP, a powerful bacterially-derived adjuvant [3]. The vaccine was designed to elicit specific antibodies able to block the interaction of tumor-produced VEGF and vascular endothelial cell receptors, thus inhibiting neo-angiogenesis, and to stimulate the development of specific cytotoxic T cells that could directly kill tumor and tumor stromal VEGF-secreting cells. In mouse experimental models, CIGB-247 was shown to be immunogenic and to inhibit tumor growth and metastasis [3, 4]. The vaccine was also found to be safe in preclinical tests done in rats, rabbits, and non-human primates [5, 6].

In 2011, the Cuban regulatory authority (CECMED) approved the development of a multi-center phase I clinical trial with CIGB-247 in advanced cancer patients (RPCEC00000102 in the Cuban Public Clinical Trial Registry; code name CENTAURO). The study involved thirty patients with advanced solid tumors, most of which had received all available onco-specific therapies without response. The individuals were distributed in three similar cohorts that received either 50, 100 or 400 μ g of the antigen, combined in all cases with 200 μ g of VSSP. Immunization was done subcutaneous in a weekly fashion, for up to 8 weeks, followed by a re-immunization on week 12.

In the final trial evaluation made in 2012 after all patients had reached week 16, CIGB-247 was found to be safe, tolerable, and immunogenic. Positive specific IgG titers, the ability of serum to block VEGF-VEGF Receptor 2 (VEGFR2) interaction, and positivity in a gamma-IFN ELISPOT were dose dependent, and best with the higher antigen dose vaccine combination. This, and the finding of clinical benefits in some patients, led to supervised voluntary off-trial immunizations of individuals surviving after the trial time, which started on week 16, and were done subsequently every 4 weeks, with the highest antigen dose, until safety issues, patient general state or death would prevent further vaccination.

In a first paper published by our group in 2014 [7] we described the CENTAURO trial results, as well as the clinical and immunological follow-up of patients that had been submitted to off-trial re-immunizations for close to two years. The present article presents and discusses our clinical findings in eight of the CENTAURO patients that have survived between 4.5 and 4.9 years after trial inclusion, with no additional treatment than monthly re-immunizations with CIGB-247.

Results

Table 1 presents the general data of the eight patients included in this publication, starting with

their code name and followed by diagnosis at trial onset, number of immunizations received (including vaccinations within the CENTAURO trial period), accumulated survival time since trial inclusion, and response evaluation according to RECIST criteria [8] at different times after trial start. In the moment in which this article was sent for publication (August 2016), three of the patients showed complete responses, and another individual had relapsed and recently passed away due to disease progression after two years free of disease. Of the rest of the patients, one individual showed a partial response in his tumor, and three others had maintained a stable disease status for several years.

The Table also depicts a basic classification of the patients with respect to their positivity in two specific immune responses tests. In the period 2012-2016, all eight individuals have shown positive results in an *in vitro* competitive ELISA assay [9] that specifically measures the ability of antibodies in serum to block the interaction between VEGF and VEGFR2. Exception made of CQ-17, all individuals have also shown to be positive in a gamma-IFN ELISPOT test [7], after stimulation of their peripheral blood lymphocytes with a VEGF mutated antigen.

From the point of view of the safety of long term CIGB-247 vaccination, no new adverse events attributable to vaccination were documented, with respect to the local grade 1 pain and erythema at injection site, and occasional fever, reported during the CENTAURO trial and first follow-up [6]. Interestingly, injection site signs and fever events progressively disappeared with chronic vaccination, until the patients made no further reports in this sense in their routine interviews and immunization appointments.

A detailed description of the individual patient clinical evolution since their inclusion in the CENTAURO trial, is now presented.

Patient code	Diagnosis at trial onset	Total number of immunizations with CIGB-247(*)	AS (**) (months)	RECIST status (***)				Immune Response Blocking ability/ ELISPOT (****)
				Initial	2012	2013-14	2015-16	
CH-11	Peritoneal metastases from an ovarian adenocarcinoma	66	58	SD	SD	CR	CR	+ / +
CH-19	Peritoneal metastases from an adenocarcinoma of uterus-ovary	64	56	PD	SD	CR	CR	+ / +
CH-18	NSCLC with metastases in both lungs	64	56	PD	SD	CR	CR	+ / +
CH-25	Duodenum adenocarcinoma with pancreas infiltration	64	57	SD	SD	CR	CR, R,†	+ / +
CH-28	Lung and bone metastases from alveolar soft-part sarcoma	62	54	PD	SD	SD	SD	+ / +
CH-07	Liver, lymph nodes, and ovarian metastases from Small intestine carcinoid tumor	66	59	SD	SD	SD	SD	+ / +
CH-15	Pancreatic neuroendocrine carcinoma with adrenal, lymph node and spleen metastases	62	58	PD	SD	SD	SD	+ / +
CQ-17	Pure mediastinum seminoma	64	57	SD	SD	PR	PR	+ / -

Table 1: Patient Characteristics

Notes: (*)number includes the 9 vaccinations made during the 12 weeks of the CENTAURO trial; (**) accumulated survival since the beginning of immunizations with CIGB-247; (***) Response Evaluation Criteria in Solid Tumors (RECIST) classification: CR – Complete Response, PR – Partial Response, SD – Stable Disease, PD – Progressive Disease, R – Relapse, † patient dies of disease progression; (****) for tests descriptions, please check reference [9] and text; “+”sign indicates reports of positive results in tests conducted during the period 2011-2016.

Patient CH-11:

This 24 years old woman was diagnosed in May of 2010 with ovarian adenocarcinoma. She was submitted to hysterectomy and double oophorectomy, followed by chemotherapy with 6 cycles of Paclitaxel and Carboplatin. During her first follow up after chemotherapy, she was re-diagnosed by laparoscopy and biopsy as with a stage IIIB peritoneal carcinomatosis. The patient received a second treatment line with 4 cycles of Cyclophosphamide/Adriamycin, without response, and was considered chemotherapy resistant.

Patient CH-11 was included in the CENTAURO clinical trial in September of 2011, with progressive disease and an ECOG 1 score [10], three months after her last chemotherapy treatment. After her first year of CIGB-247 vaccinations the patient was able to return to work. On the second year evaluation, the patient was reported as in stable disease status, and from her third year on and until present, in complete response, with an ECOG of 0. No lesions have been detected in CT-scans, and tumor markers CA 125 and HE4 [11] have been negative. By August 2016, CH-11 had received a total of 66 immunizations, with an overall survival of 59 months since her inclusion in the CENTAURO trial.

Patient CH-19:

CH-19 was 52 years old in October of 2010 when diagnosed of a uterus-ovary adenocarcinoma (double primary tumors). She was submitted to hysterectomy, oophorectomy, and omentectomy. Omentum metastatic lesions were found and the patient was classified as in stage IIIB. CH-19 received 6 cycles of Paclitaxel and Carboplatin. On laparoscopy evaluation after treatment, peritoneal carcinosis and liver metastatic lesions were diagnosed (stage IV). A second chemotherapy line was added (Cisplatin/Adriamycin; 6 cycles) without response.

Patient CH-19 was included in the CENTAURO clinical trial in November of 2011, 8 weeks after her last chemotherapy treatment. She was reportedly in progression and her ECOG score was 1. Similar to patient CH-11, she was reported as in stable disease after her second year evaluation, and has been in complete response since her third year evaluation. Tumor markers CA 125 and HE4 are negative. Patient CH-19 is a working university teacher, and by August 2016 she had received 64 immunizations with CIGB-247, with an overall survival of 56 months since trial inclusion.

Patient CH-18:

This female patient was 44 years old in February of 2011 when diagnosed through bronchoscopy and imaging as having a NSCLC with bilateral metastases (T4N2M0 - IIIB). She received a first line of treatment with 6 cycles of Cisplatin/Etoposide (VP-16) and radiotherapy, without response.

Patient CH-18 was included in the CENTAURO clinical trial in November of 2011, with progressive disease and an ECOG 1 score. The patient was classified as in complete response and ECOG score of 0 after

the second year evaluation and has remained since free of disease according to clinical and imaging criteria. She is an active housewife that exercises daily. By August 2016, CH-18 had received 64 immunizations with CIGB-247, with an overall accumulated survival of 56 months.

Patient CH-25:

This 58 years old male patient was diagnosed in February of 2011 by endoscopy of having a non-surgical duodenum adenocarcinoma, with pancreas infiltration (T4NxM1 – stage IV). The patient was submitted to bypass palliative surgery and received 12 cycles of 5-fluorouracil/Leucovorin.

CH-25 was included in the CENTAURO clinical trial in December of 2011, two months after the last chemotherapy cycle, with stable disease but ECOG 1 score. He maintained his stable disease status for the first two years after entering the trial, improving his ECOG score, and returning to work as a farmer in 2013. On his third year evaluation (2014), CH-25 was classified as in complete response. Abdominal CT-scan, upper endoscopy with biopsy, and tumor markers [10] CEA and CA 19-9 were negative.

On his fourth year evaluation (2015) new hepatic lesions were found in contrasted CT-scan tests, suggesting relapse. CEA was slightly over normal range, but CA 19-9 continued to be normal. An upper endoscopy was negative of cancer and the patient was asymptomatic, with an ECOG 0 score. However, the patient showed a rapid progression of his disease in the following 6 months, which eventually led to his demise. This patient had received 64 immunizations with CIGB-247, and showed an accumulated overall survival of 57 months, since his inclusion in the CENTAURO trial.

Patient CH-28:

This 34 years old male patient was included in the CENTAURO clinical trial in December of 2011. He had been originally diagnosed with a deep alveolar soft-parts sarcoma of his right arm, and submitted to surgery in November 2007. The tumor had a diameter smaller than 5 cm, and negative margins (T1bN0M0 - Grade II). CH-28 received postsurgical adjuvant treatment with 6 cycles of MAID (Mesna, Doxorubicin, Ifosfamide, Dacarbazine), and radiotherapy. The patient was followed up every 3 months and in June 2011, large and numerous metastatic pulmonary lesions were detected. He then was submitted to Dacarbazine/Carboplatin/Actinomycin D chemotherapy without response. The patient entered the CENTAURO clinical trial in progression and with an ECOG 1 score, 4 weeks after the last chemotherapy cycle. CH-28 has been in stable disease status and ECOG 0 score since October 2012, with no evidences of new metastatic lesions. He returned to work two years ago. By August 2016, CH-28 had received 62 immunizations with CIGB-247.

Patient CH-07:

This 60 year-old female was included in the CENTAURO clinical trial in August of 2011. She had been diagnosed in 1999 with a small intestine carcinoid tumor, which was surgically resected, and submitted subsequently to 6 cycles of chemotherapy (5-fluorouracil, DTIC, Doxorubicin), and recombinant alpha IFN for 12 weeks. CH-07 evolved well until 2010, when upper right abdominal pain appeared and was found to have multiple liver, lymph node, and ovarian metastasis. A biopsy by laparotomy confirmed metastatic disease from a carcinoid tumor. She was submitted to 6 cycles of chemotherapy with Cisplatin/Etoposide (VP-16), and another 12 weeks of recombinant alpha IFN. She

was included in the CENTAURO clinical trial 10 months after chemotherapy ended, classified as in stable disease, but with liver, lymph node, and ovarian metastasis, and a ECOG score of 1.

After one year of treatment with CIGB-247, CH-07 improved her ECOG score and returned back to work. She has maintained a stable disease status. By August 2016, CH-07 had received 66 immunizations with CIGB-247, and showed 59 months of accumulated survival, since entering the clinical trial.

Patient CH-15:

This 63 years old male patient was originally diagnosed as with a pancreatic neuroendocrine carcinoma and metastatic disease. Left suprarenal gland laparotomy showed a lesion involving the portal vein, and intra-abdominal lymph nodes. He was classified as T4N1M1, and in stage IV, with a non-functional tumor. CH-15 received 6 cycles of Adriamycin/5-fluorouracil. Sixteen months after the end of chemotherapy, the patient was in disease progression with adrenal, lymph node and spleen metastases, and ECOG score of 1. He was finally included in the CENTAURO trial in October of 2011. One year later, the patient showed an improved RECIST status and was classified as with stable disease. Since then, the patient has been stable and completely asymptomatic, with an ECOG score of 0. CH-15 returned back to work in 2014. By August 2016, the patient had received 62 immunizations with CIGB-247, and showed 58 months of accumulated survival after trial inclusion.

Patient CQ-17:

This 37 years old male was diagnosed in February of 2010 as having a pure mediastinum seminoma, based on a direct biopsy taken during an unsuccessful tumor surgery. The tumor was not removed because of infiltration and involvement of main blood vessels. The patient started with chemotherapy (Bleomycin/Etoposide/Cisplatin) but treatment had to be stopped in June of 2010 because of toxicity. The patient voluntarily declined any other possibilities of oncological treatment. CQ-17 was included in the CENTAURO clinical trial in November of 2011, with an ECOG score of 1 and classified as in stable disease.

After remaining stable for 2 years, imaging evidences of a reduction in tumor size were found by late 2013. He was then, and has been thereafter, classified as with a partial response. A reduction of tumor mass of more than 50% has been already documented. HCG in serum is negative and LDH levels are in the normal range. Symptoms (lack of breath after effort) described at the time of his inclusion in the CENTAURO trial have disappeared and his present ECOG score is 0. The patient was back to work in 2015, and does regular physical workouts. By August 2016, this patient had received 64 immunizations with CIGB-247.

Discussion

One of the most relevant findings in this off-trial re-immunization follow up study is the long-term safety profile of CIGB-247 vaccination. We had already reported no new adverse events in any of the surviving patients, with respect to the local low-grade ones found during the trial protocol, after the first two years of systematic off-trial CIGB-247 re-immunization [7]. Now, after 4.5 to 4.9 years of monthly repeated vaccination, the occurrence of injection site events and occasional fever have progressively disappeared in all treated patients. Because of the bacterial origin of VSSP, and the existence of previous

reports of local adverse events produced in humans by VSSP [13, 14], we had originally hypothesized that some of the local adverse events seen with CIGB-247 were probably due to the use of this adjuvant in our vaccine composition [7]. We think that the local effects of VSSP have become less frequent, and/or can be physiologically adjusted better by the patients, during the off-trial vaccination period, because immunizations proceed every four weeks, instead of the weekly regime used during the CENTAURO trial. In this sense, it has been shown that the administration frequency of other subcutaneous adjuvants has to do with the amount and intensity of local adverse events [15].

Severe systemic adverse events have been reported after long term application of cancer therapies that target VEGF, including Bevacizumab [16, 17], the anti-VEGF humanized monoclonal antibody, commercially known as Avastin®. It is thought that outstanding suppression of cellular signalization pathways that are important in physiological microvasculature maintenance, regulation, and repair in normal tissues, underlie these adverse events, which can differ between cancers.

While our patient sample is still small, another potentially relevant finding derived from our long term CIGB-247 vaccination study is absence of events reported for other anti-angiogenic drugs. As previously mentioned by us and by others [5-7, 18-19], important pharmacological differences exist between the application of externally infused antibodies, and active immunization strategies [20]. Therapeutic vaccines lead to the production of lower, albeit sustained, levels of polyclonal antibodies, a very different scenario to that found when therapeutic monoclonal immunoglobulins are administered. In the latter, very high doses of therapeutic antibodies have to be infused in order to achieve pharmacologically active concentrations in tumors, and reported adverse effects in normal tissues are probably due to these high systemic levels of specific therapeutic immunoglobulins.

This potential very low toxicity profile opens interesting possibilities in the future use of CIGB-247 in combination with, or after, chemotherapy, radiotherapy, or even other anti-angiogenic treatments, and in the chronic application of the vaccine for long-term cancer control purposes.

The CENTAURO clinical trial was not designed to prove the potential anti-tumor effect of CIGB-247, so it is imperative to be cautious when interpreting the implications of the vaccine in the clinical findings seen after long-term follow up of surviving patients. There are however, notable evidences that suggest that some clinical benefits could be associated with vaccination. Most of the patients were symptomatic, with ECOG scores of 1, and with advanced disseminated tumors, on trial inclusion. At the time this paper was sent for publication, three of the CIGB-247 long-term immunized patients showed complete responses and were back to their normal life activities. Another individual had only recently relapsed, after being for two years free of detectable disease and asymptomatic. A fifth patient, that rejected chemotherapy due to high toxicity, had achieved a partial response status, and was back to work. Three other individuals were in stable disease, having improved their functional ECOG scores. These patients surviving the CENTAURO phase I clinical trial have received from 62 to 66 total immunizations with CIGB-247, in the absence of any other specific oncological treatment. These evidences are compelling, moreover when accumulated survival, counting time after their inclusion in the trial, ranges from 55 to 59 months, that at least duplicating the expected PFS times for these types of tumors.

Patients CH-11 and CH-19, both with advanced ovarian cancers, have shown similar positive and noteworthy evolution, and are now in complete response with negative values for related tumor markers.

The majority of patients with epithelial ovarian cancer are diagnosed instage III or IV advanced disease. After postoperative treatment, the 5-year survival rate of patients with stage III optimally de-bulked (with residual disease less than or equal to 1 cm diameter tumors) range between 20% and 30%, and these numbers decrease to less than 10% for patients with sub-optimally de-bulked stage III disease or those with stage IV tumors [21]. Most patients with recurrent ovarian cancer are destined to die of their tumors, regardless of the second-line treatment modality used [22]. Patients with a PFI<6 months are less likely to respond to second-line platinum and are often managed with an alternative agent [23]. The clinical evolution of CH-11 and CH-19 is noteworthy and has led us to consider ovarian cancer as a potential target for Phase II/III trials with CIGB-247.

Patient CH-18, with lung cancer and bilateral metastatic compromise, is definitively an unexpected case of complete response. She was admitted to the CENTAURO clinical trial in progression, and achieved stable disease after a year, and a complete response after two years of vaccination with CIGB-247. The literature describes that only 13% of lung cancer patients are expected to live 5 years. In the past 20 years, in spite of oncological treatments advances, overall survival is not improving. In the case of IIIB stages, 5 year overall survival is only 7% [24]. A phase II clinical trial that compared first line carboplatin/paclitaxel versus an arm that associated Bevacizumab to chemotherapy in patients with non-small cell lung cancer, locally advanced or metastatic, described a discreet PFS improvement of 3.2 months in the Bevacizumab arm, over the control arm [25].


Patient CH-25 (duodenum adenocarcinoma, with pancreas infiltration) was classified on his third year evaluation as in complete response, only to relapse a year later, with new hepatic tumors. His accumulated overall survival at death time was of 64 months since initial tumor diagnosis. Duodenum adenocarcinoma accounts for 45% of small intestine tumors and overall survival in metastatic disease is 8.6 months [6]. As reported in a single institutional review of 217 patients, 70% of the individuals with stage III or greater small bowel cancer had 20 months as median overall survival [27].

Of the other patients of this case series, CH-28, bearing lung metastases from an alveolar soft-parts sarcoma, is an interesting case. This patient is in stable disease since late 2012, has an ECOG score of 0 and is back to work. Considering PFS is 7 months for patients with treated alveolar soft-parts sarcoma [28], further careful follow up of this patient evolution is important.

Patient CH-07, with liver, lymph node, and ovarian metastasis from a small intestine carcinoid tumor, has achieved a sustained stable disease. Her 4.83 years of accumulated survival since entering the trial is noteworthy, considering that in individuals with bowel disease and distant metastases 5-year overall survival is only 22% [29].

Patient CH-15, that entered the trial in frank progression with adrenal, lymph node, and spleen metastases of a pancreatic neuroendocrine carcinoma, is now in stable disease, and has accumulated 4.75 years of survival since trial inclusion. Median survival for patients with distant pancreatic neuroendocrine carcinoma is 25 months, and for patients 60 years or older, as in our case, prognosis is worse [30].

Finally, patient CQ-17, diagnosed with an infiltrating pure mediastinum seminoma, has been in partial response in the last 2 yearly evaluations. With an ECOG 0 score, he is back to normal life activities.



In this particular case, the patient interrupted his first line chemotherapy cycles due to high toxicity, and voluntarily declined any other treatment modalities. Chemotherapy refusal or abandonment due to side effects is not a rare phenomenon in cancer patients. Vaccination with CIGB-247 was his only potentially specific oncological therapy opportunity, due to the absence of important side-effects.

Long-term follow up in this series shows that clinical benefits in patients started to be documented after the first year of vaccination. This is in line with literature that describes the importance of chronic immunization to increase the probability of cancer patients to become good specific responders [31]. Results obtained in this investigation show the potential of active immunotherapy in eliciting specific immune responses to a self-antigen like VEGF, and all patients presented in this case series showed some evidence of responding positively to vaccination, in terms of the specific immune tests designed by us to this effect [7, 9].

Results described in this paper justify the continuation of CIGB-247 clinical development program. The vaccine candidate is now being considered for Phase II studies in specific tumors. As shown in this paper, ovarian cancer seems to be an interesting candidate. Metastatic colorectal cancer may be another relevant target tumor to study, having in mind that Bevacizumab has been approved for this specific niche [32]. Future trial designs will also take into consideration the inclusion of patients in earlier tumor stages, and the possibility of combinations with other drugs, with consequent careful vaccination timing strategies. Control groups will be part of these studies, and effect/efficacy of CIGB-247 will most probably be based on PFI or PFS, as success criteria.

Based on the results we have shown in this paper and others soon to be published, vaccination strategy will follow a similar one to that used in the CENTAURO trial [7]. i.e., an initial induction phase followed by chronic re-immunization.

New CIGB-247 trials will additionally take into account the use of validated tumor markers that are commonly employed in the follow up of treated patients with the aforementioned cancers [11, 12]. It could be thought that blood/platelet VEGF should be another potential biomarker of prognosis when using anti-angiogenic drugs. However, its use beyond investigation purposes is still in discussion because the relationship between the measurement of VEGF levels and treatment results have been inconclusive results to date, after studies done with anti-angiogenic products [33].

Lastly, we will continue monitoring the specific immune response to the vaccine in future trials, as part of our efforts to characterize the potential anti-tumor mechanisms of CIGB-247, and to define whether these tests can help in patient stratification and/or prediction of the response to vaccination, as others have done with other vaccine candidates [31, 34]. Of the three basic specific immune response tests [7, 9] that we have developed for phase I clinical trials, i.e., the measurement of anti-VEGF IgG and other immunoglobulin classes in serum, the identification of the ability of the patient's sample to block VEGF/VEGF receptors interaction, and cytokine ELISPOT tests where the patient's lymphocytes are stimulated with a mutated form of VEGF, the two first humoral response assays are most probably the ones that can be routinely employed in phase II clinical trials, with emphasis in those that could provide us with a functional characterization of the individual's specific antibody response.

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