Targeting of the vascular endothelial growth factor (VEGF) is known for going beyond mere modification of angiogenesis, and extends to the systemic and intra-tumoral regulation of immune cells activation state. In this study, we aim to explore the effect of a VEGF active immunotherapy based on a recombinant antigen named CIGB-247, in the systemic and tumor compartments in preclinical settings for mice, and in a subset of 23 patients with advanced gastrointestinal cancer (GIC) from phase I clinical trials (RPCECO0000102 and RPCECO0000155).

**IMMUNE RESPONSE SYSTEMIC / INTRA-PERITUMORAL IN PRECLINICAL SETTINGS**

Mice were euthanized when control group tumors reached diameters over 10 mm. Leukocytes infiltrates in whole blood, spleen, tumor, contralateral, and draining lymph nodes, and their phenotypes were evaluated using multiparametric flow cytometry after magnetic enrichment of CD45-positive cells particularly for tumor tissue. The functionality of the cells and overall effects of the treatments on the tumor microenvironment and in the systemic compartment were assessed using multiplexing systems for cytokines and growth factors and ELISpot-based evaluations of clones secreting interferon-gamma. VEGF-specific antibody titers and neutralization were evaluated in serum and tumor lysates as well. A total of 3 studies were performed and representative results are shown.

CIGB-247 effects on tumor growth and on the infiltrating leukocyte populations concentrations and phenotype: A significant reduction in tumor weight was associated with CIGB-247 treatment [A]; such effect significantly correlates with the increment of the concentrations of CD3+, CD4+, and CD8+ cells [B]. A significant decrease in PDL1 expression was confirmed for CD4+ and CD8+ cells [C]. A trend to the reduction in suppressive MDSCs [D], Tregs [E], and CD68 expressing macrophages [F].

**CIGB-247-induced Immunomodulatory effects on the specific immune response and soluble mediators of the angiogenic and immune response.** Humoral immune response specific for VEGF in the animal sera [A, B] and on individual tumor lysates [D, E]. The use of CIGB-247 significantly increased VEGF-specific antibody titers in both control and tumor-bearing mice. Of interest, the quality of the response was preserved within the tumor tissue as evidenced by the upregulation of VEGF binding to VEGF receptor 2 (VEGFR2). Evaluation of the cellular response using IFN-γ ELISpot indicates a significant increase in antigen-specific T cell clones at both contralateral and draining lymph nodes even in the presence of the CT26 tumor (C, F).

**CT-26 Tumor tissues lysates were analyzed using luminex-based assays (MCMAAG-48K, and MAGMAP MAG-24K). Data from the angiogenesis/growth factor panel are shown in panel (A) and CD8-related cytokines in panel (B).** In agreement with the antitumoral effects observed and the recovery of tumor leukocyte peritumoral infiltration a significant reduction was observed for VEGF-A, CXCL1, GMCSF, and PIGF while IFNγ, IL-2 and GrB were significantly increased in Sulfas-treated animals as compared to the PLACEBO group. Angiogenesis/growth.

**ACKNOWLEDGMENTS**

**EVALUATION OF A SUBSET OF PATIENTS WITH ADVANCED GASTROINTESTINAL TUMORS TREATED WITH CIGB-247 WITHIN PHASE I/IB CLINICAL TRIALS**

**CENTAUR II:** RPCECO0000155, Available at: http://registroidx.clinic.gob.cu/clinicaltrials/ RPCECO0000155-En. Cuban Public Clinical Trial Registry, WHO accepted Primary Registry.

**CENTAUR III:** RPCECO0000102. Available at: https://registroidx.clinic.gob.cu/clinicaltrials/ RPCECO0000102-En. Cuban Public Clinical Trial Registry, WHO accepted Primary Registry.

CIGB-247 Phase I Clinical Trials. Open / Controlled / Blinded (Advanced Solid tumors. Enrolled a total of 30 and 50 patients each in 10 dose per group), to evaluate the safety and immunogenicity of three dose levels of the CIGB-247 V vaccine and later of CIGB247 and VSSP (Alum PHA) alone or combined. All cohorts received eight administrations of the indicated dose in a weekly schedule and a booster a month later. Humoral and Cellular response follow-up was planned before vaccination started (S0) and a week after immunization (9 (S13), 12 (S25), and 18 (S49)). The four graph illustrates the distribution of solid tumor types found in included patients (data of the primary tumor).

CIGB-247 administration in the cohort of GIC patients results in a reduction or stabilization of circulating VEGF for 19 out of the 22 cases with available samples. Such reduction was related to increased survival (A). As a result of the immunomodulatory nature of the VEGF treatment, a significant increase in survival was observed (B). A fact that was also related to improved survival (B).

Furthermore, and beyond the antangiogenic effect, advanced GIC patients treated with the vaccine develop a VEGF-specific cellular response in terms of secretion of IFN gamma that after stratification indicates a potential relationship between the establishment of such response and the overall survival. Gathered results indicate the potentiality of actively targeting VEGF in GIC patients to improve the current systemic and cellular response with a potential relationship with the overall survival.

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