Poster # 3938

Anti-angiogenic therapy combination with hypoxia-regulating agent leads to improved tumor regression in a murine model of renal carcinoma

Imane NAFIA, Assia CHAIBI, Doriane BORTOLOTTO, Christophe REY, Manon CLEMENCEAU, Jean-Philippe GUEGAN, Alban BESSEDE

Explicyte Immuno-Oncology, Bordeaux, France
Contact: i.nafia@explicyte.com

ABSTRACT

Background: While antiangiogenic therapies have been shown effective in preclinical and clinical trials, tumors can acquire resistance conferring them the ability to survive and grow under hypoxic conditions, even, induced by antiangiogenic drugs themselves.

Methods: This study investigated the effects of an antiangiogenic strategy and hypoxia-regulating agent, each alone and in combination. Here, we have tested the synergistic activity of the anti-angiogenic agent Sunitinib and the mTOR inhibitor Everolimus in murine syngeneic renal cancer model – renal carcinoma being known as angiogenesis-dependent and hypoxia-driven malignancy. Anti-tumor effects were investigated on tumor growth and survival. Flow cytometry-based immunoprofiling was performed at the peripheral level - blood and spleen - while immunohistofluorescence and quantitative image analysis served to characterize and evaluate tumor immune cell infiltrate, tumor hypoxia, and tumor vascular density/features.

Results: While Sunitinib and more importantly Everolimus led to a significant tumor growth inhibition as single agents, their combination was more effective on tumor regression. At the peripheral level, Sunitinib, but also Everolimus, led each to declines in circulating and splenic myeloid-derived suppressor cells (MDSCs) – depicted as CD11b+/Gr1+, an event that was further optimized upon combination of both agents. In addition, tumor sections analyses showed decreased microvessel density under Sunitinib and combination treatment. Differential modulations of correlative endothelium/pericytes staining were also observed upon the different treatments.

METHODS

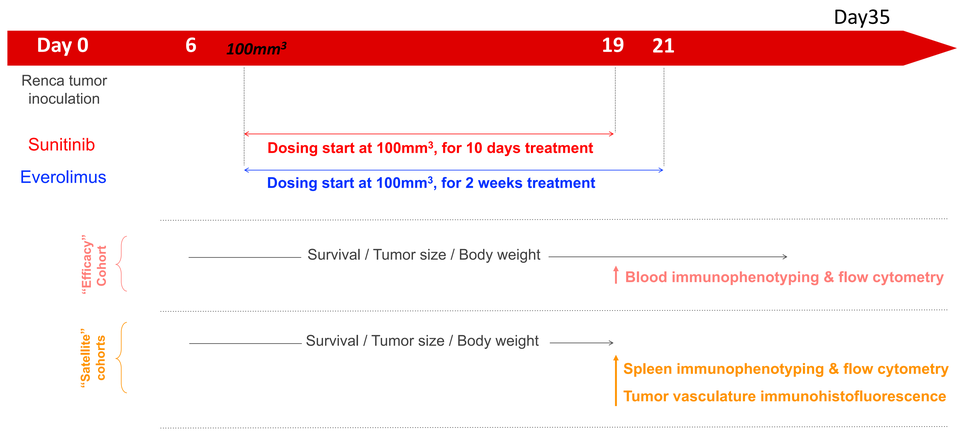

Figure 1: *After Renca tumors were inoculated in immunocompetent Balb/c mice, animals were allocated to the treatment groups and processed either for "efficacy" or as "satellites", for the respective experiments and readouts as mentioned above.*

CONCLUSIONS & PERSPECTIVES

This study on a subcutaneously-implanted Renca renal tumor mouse model confirms the preclinical efficacy of Sunitinib and Everolimus as single agents in delaying tumor progression, and further highlights the benefit from their combination on the decline of circulating MDSCs and potential improvement of peripheral T cell response, thereby translating into a better tumor growth limitation than with each drug alone. Interestingly, the tumor microvascular response induced upon Sunitinib alone and in combination with Everolimus, which sustains, at least partially, their anti-tumor effects, still supports that combination of vascular disrupting agents and hypoxia-regulating drugs is a suitable strategy that could provide conditions in which each agent will enhance the activity of the other, thereby limiting, consequently, the tumor growth progression. This combination would also deserve to be tested along with immune checkpoints inhibitors.

RESULTS

Combination of Sunitinib and Everolimus delays renal tumor growth and progression

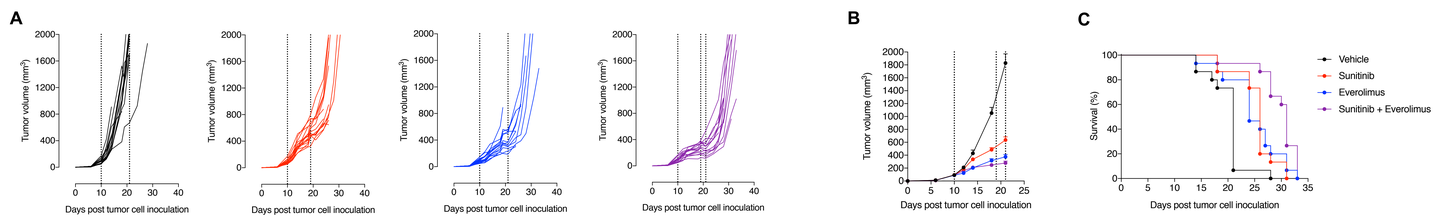

Figure 2: *Individual **(A)** and mean **(B)** tumor volume (mm³), and Kaplan-Meier plot survival **(C)** of Renca tumor-bearing mice upon vehicle, sunitinib, everolimus, or combination treatments (n=15 per group).*

Everolimus enhances Sunitinib-induced decline in MDSCs and peripheral T cell increase

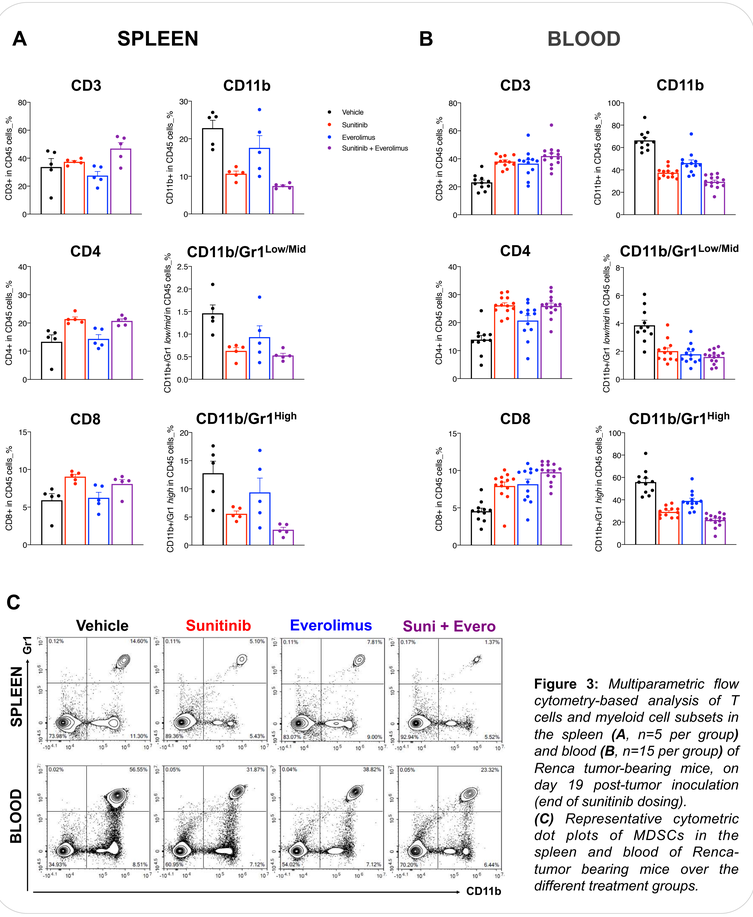

Figure 3: *Multiparametric flow cytometry-based analysis of T cells and myeloid cell subsets in the spleen **(A**, n=5 per group) and blood **(B**, n=15 per group) of Renca tumor-bearing mice, on day 19 post-tumor inoculation (end of sunitinib dosing).*
***(C)** Representative cytometric dot plots of MDSCs in the spleen and blood of Renca-tumor bearing mice over the different treatment groups.*

Sunitinib alone and in combination enhances vascular response in the tumor microenvironment

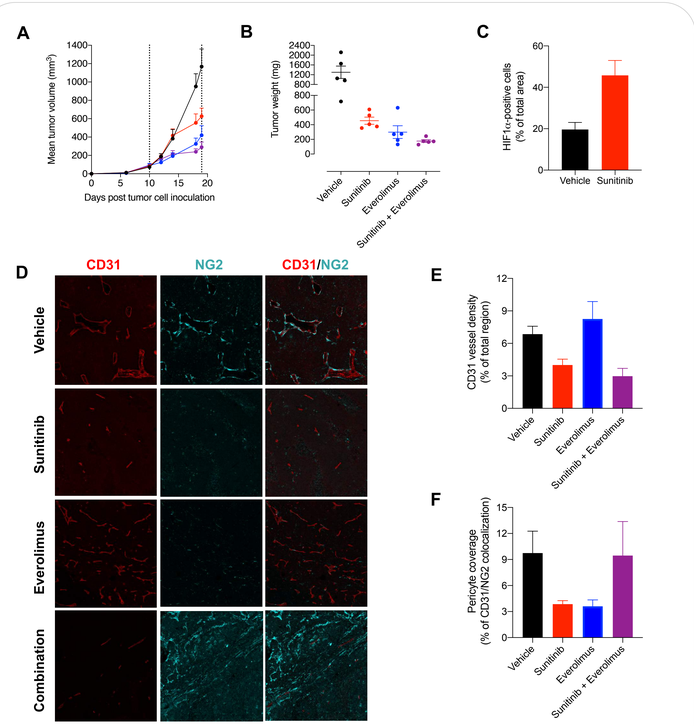

Figure 4: *Sunitinib combined with Everolimus exhibits enhanced anti-tumor effects involving tumor vessel density reduction & normalization in Renca tumor model.*
***(A)** Mean tumor growth curve (mm³) and **(B)** tumor weight (mg) on day 19 post-tumor inoculation (end of sunitinib dosing) of Renca tumors treated with vehicle, sunitinib, everolimus, or combination (n=5 per group). Tumors were collected on day 19 post-tumor inoculation and processed for HIF1α, CD31 (red, blood vessels) and NG2 (cyan, pericytes) immunohistofluorescence and image acquisition & analysis. **(C)** HIF1α signal is analyzed and presented as a percentage of HIF1α -positive cells per total sectional area. **(D)** Representative images of CD31 and NG2 immunostaining. **(E)** CD31 vessel density is analyzed and presented as a percentage per total sectional area. **(F)** Pericyte coverage is analyzed and presented as a percentage of NG2 and CD31 colocalization.*