Efficacy and pharmacodynamic biomarkers of mitazalimab in combination with chemotherapy in preclinical mouse models

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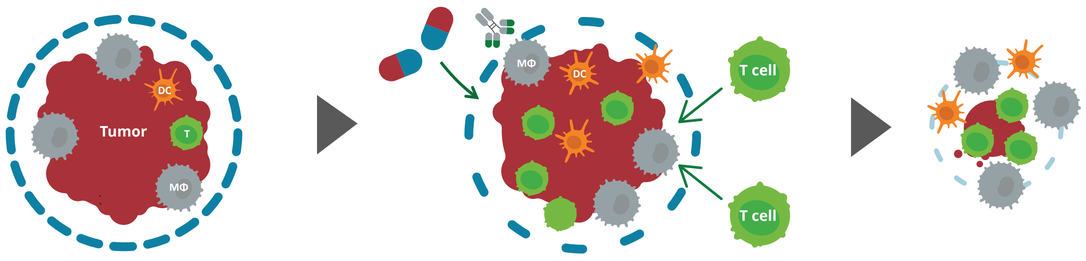
- Mitazalimab is a CD40-targeting agonistic monoclonal IgG1 antibody.
- Targeting CD40 with mitazalimab in combination with chemotherapy induces anti-tumor effects both by licensing dendritic cells (DC) to activate tumor- specific T cells, and by triggering macrophage (MΦ) polarization. Subsequently, macrophages degrade the extracellular matrix in the tumor stroma, increasing the sensitivity to chemotherapeutic agents, and favoring T-cell influx (1,2).
- Mitazalimab has been evaluated in two Phase I clinical trials (CT) (NCT02829099, NCT02379741), demonstrating a good safety profile (3).
- Currently, mitazalimab is showing promising results in a Phase 1b/2 trial (Optimize-1, NCT04888312) in combination with mFOLFIRINOX.



Objectives:

- Determine the anti-tumor efficacy and survival in tumor bearing, human CD40 transgenic (hCD40tg) mice treated with mitazalimab in combination with FOLFIRINOX.
- Identify transcriptomic changes in peripheral blood of hCD40tg tumor bearing mice that define pharmacodynamic biomarkers associated to mitazalimab, FOLFIRNIOX, and the combination treatment.

Conclusions:

- Mitazalimab in combination with FOLFIRINOX controls tumor growth and improves survival in a mouse model challenged with immunogenic bladder tumors, and non-immunogenic pancreatic tumors.
- CCL2-4, CXCL10, IFNG, and TNF-α are appropiate pharmacodynamic biomarkers to follow the effect of mitazalimab, alone and in combination with FOLFIRINOX.
- Mitazalimab and FOLFIRNOX synergize by inducing type-I interferon responses, re-organizing the extracellular matrix, and hindering DNA synthesis.

Mitazalimab controls tumor growth and improves survival

Mice treated with mitazalimab in combination with FOLFIRINOX displayed higher survival rates, as well as enhanced tumor volume control, as compared to single components and vehicle groups, and only vehicle group, respectively.

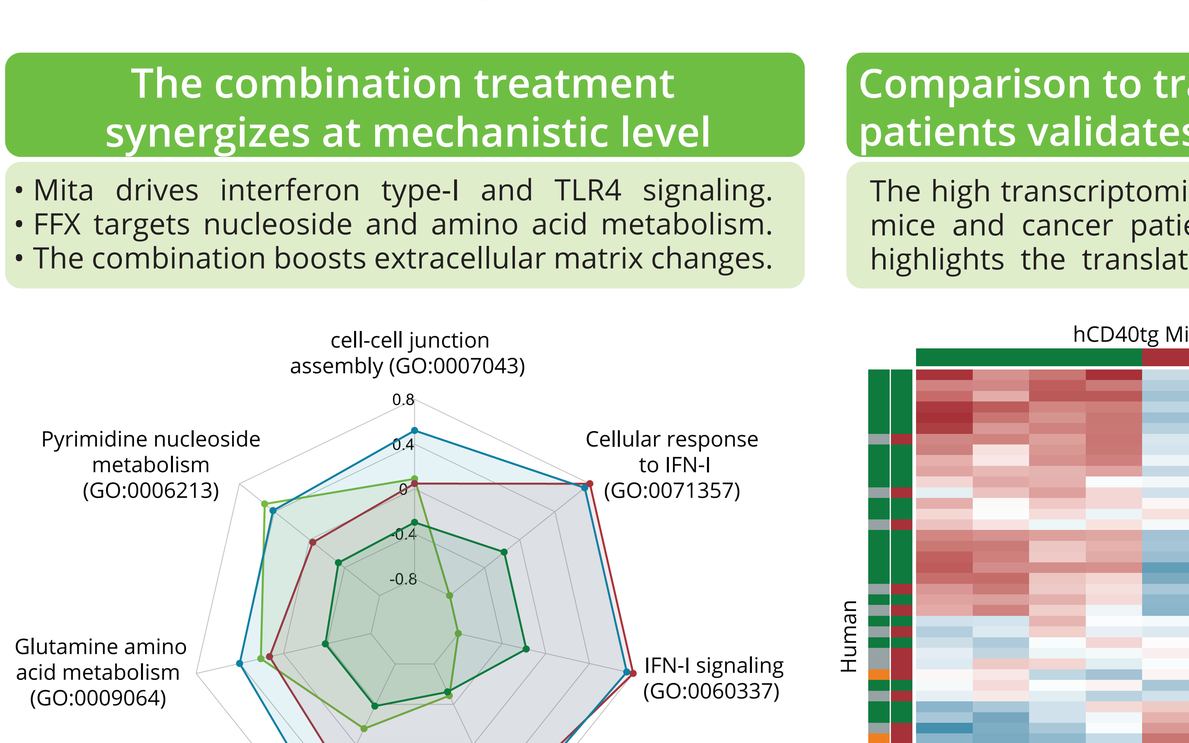


Figure 1. (A) hCD40tg mice were inoculated with MB49 tumor cells subcutaneously (n=40, 10/group). When tumors reached the target volume (50 mm^3), mice were treated with vehicle (Dextrose), FOLFIRINOX (FFX; days 7-8, 14-15 and 21-22) and/or mitazalimab (Mita; 10, 17 and 24). Blood was collected 24 hours after mizatalimab dosage, and subjected to RNA-seq. **(B)** Anti-tumnor efficacy and survival of mice treated with mita, FFX or mita + FFX. Differences in tumor volume were calculated at day 17 using Mann-whitney test.* $p < 0.05$, ** $p < 0.01$ and, *** $p < 0.005$.

Mitazalimab, alone or in combination, induces myeloid and T-cell activation changes in line with MoA

Mitazalimab harnessess anti-tumor immunity in a two-step fashion:
1. Activation of myeloid cells, which upregulate CCL2-4, and CXCL10.
2. Licensing T-cells, which in turn will upregulate IFN-γ and TNF-α.

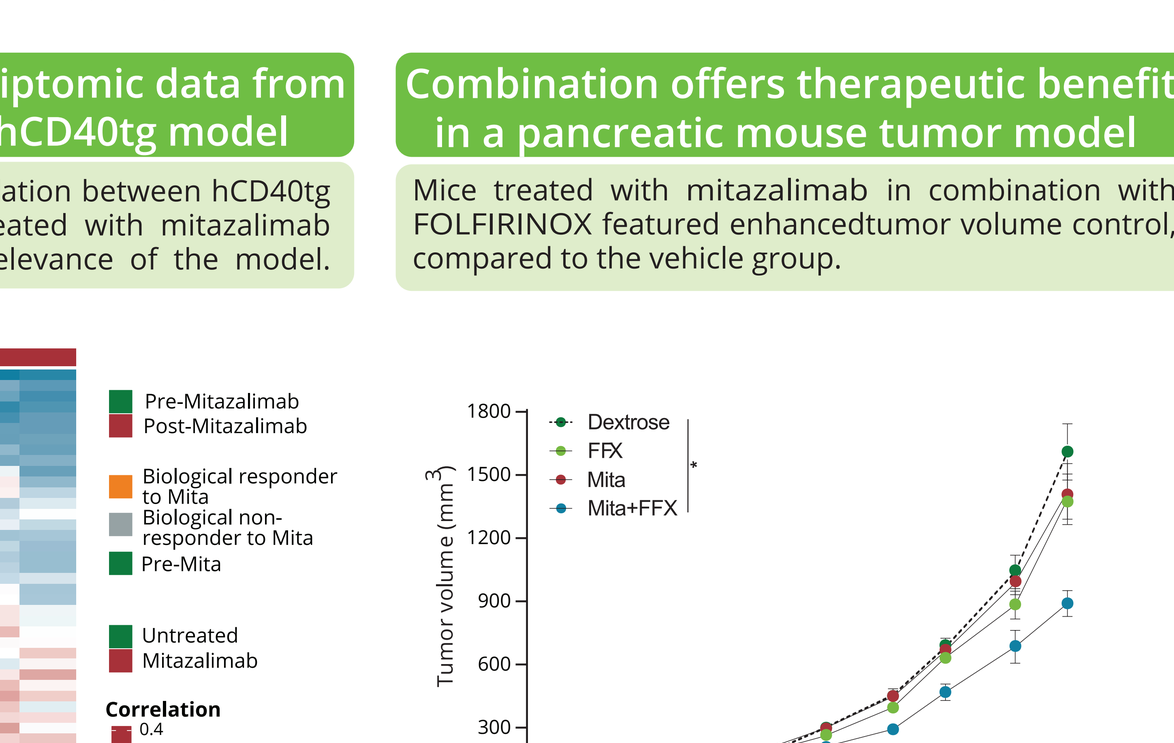


Figure 2. (A) PCA plot displaying transcriptomic changes induced by treatment in peripheral blood. PC1 separates groups that received mitazalimab, while PC2 separates groups receiving FOLFIRINOX. **(B)** Differential gene expression analysis revealed 3356 DEGs between the treatment groups. Compared to an independent cancer patient cohort receiving mitazalimab (Left, NCT02829099) , the highest ortholog gene overlap was observed in the mice groups treated with mitazalimab. **(C)** Box plot displaying key DEGs related to mitazalimab's mode of action. CT: Clinical trial.

The combination treatment synergizes at mechanistic level

- Mita drives interferon type-I and TLR4 signaling.
- FFX targets nucleoside and amino acid metabolism.
- The combination boosts extracellular matrix changes.

Figure 3. Pathway enrichment analysis based on the 3356 DEGs. Relative scores were calculated and averaged using the gene set variation method. Mita: Mitazalimab; FFX: Folfirinox.

Comparison to transcriptomic data from patients validates the hCD40tg model

The high transcriptomic correlation between hCD40tg mice and cancer patients treated with mitazalimab highlights the translational relevance of the model.

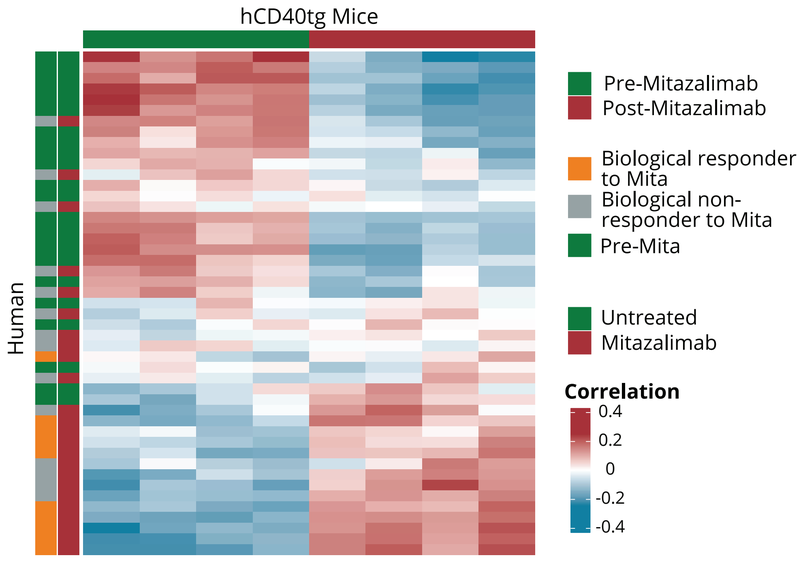


Figure 4. Correlation between hCD40tg mice and human patients treated with mitzaliamb as stand alone agent. Correlation was based on the common top 2000 highly variable genes.

Combination offers therapeutic benefit in a pancreatic mouse tumor model

Mice treated with mitazalimab in combination with FOLFIRINOX featured enhancedtumor volume control, compared to the vehicle group.

Figure 5. hCD40tg mice were inoculated with KPCY pancreatic mouse tumor cells subcutaneously (n=40, 10/group), and followed the dosing regime in Figure 1A. Mita: Mitazalimab; FFX: FOLFIRINOX.

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