Early pharmacodynamic changes measured by RNA sequencing in peripheral blood from patients in a phase 1 study with mitazalimab, a potent CD40 agonistic IgG1 monoclonal antibody

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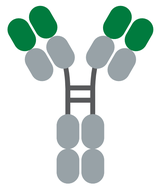
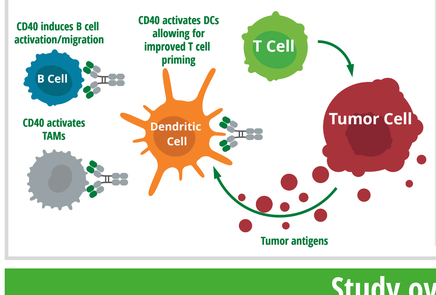
Mitazalimab – a CD40 agonist with best-in-class profile



- Mitazalimab is an FcγR crosslinking dependent CD40 agonistic antibody (IgG1) with tumor-directed immune activity [1-2]
- Mitazalimab binds with high affinity to a unique binding epitope on the CD40 receptor which allows for high efficacy and potency
- Mitazalimab has demonstrated a manageable safety profile when administered once every 2 weeks both with or without corticosteroid pre-treatment [3].

Mode of action



- Mitazalimab binds to CD40, the key activation receptor on antigen presenting cells (APCs), i.e. dendritic cells (DCs), B cells and macrophages.
- Mitazalimab activates tumor-associated macrophages (TAMs), which may reshape the tumor infiltrating myeloid microenvironment.
- Mitazalimab activates DCs, allowing priming of tumor-specific T cells and improved anti-tumor efficacy.

Study overview

The objective of the study was to evaluate pharmacodynamic changes measured by RNA sequencing of blood samples from patients treated with mitazalimab intravenously [3]. RNA from peripheral blood was collected from subjects both pre- and post-treatment in a dose escalation study of mitazalimab in patients with advanced stage solid tumors (NCT02829099) (Fig. 1A). 24-hrs post-treatment samples from cohorts receiving 75, 200, 600 and 900 µg/kg mitazalimab without corticosteroid pretreatment, and 600 µg/kg mitazalimab with corticosteroid pretreatment were analyzed. Mitazalimab treatment induced significant transcriptional activity in peripheral blood cells at 600 and 900 µg/kg dose levels without corticoid pre-treatment. In contrast, the number of significantly expressed genes in the 75 and 200 µg/kg dose groups were lower. Additionally, corticosteroid pre-treatment significantly reduced the magnitude of the mitazalimab-induced changes in gene expression in circulating immune cells in patients (Fig. 1B).

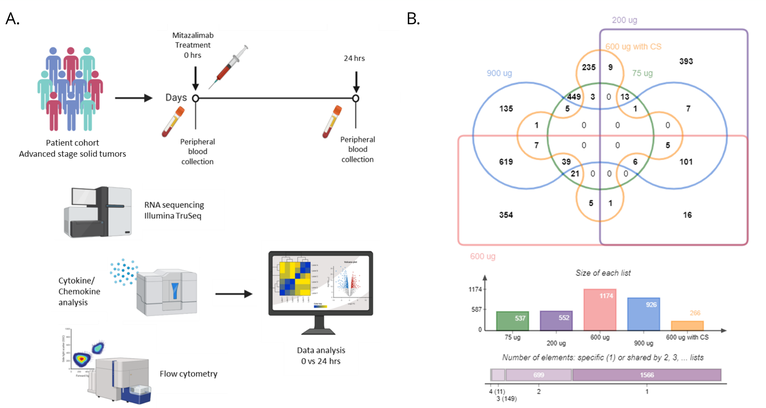


Figure 1. (A) Experimental setup and (B) Differentially expressed genes (DEGs) 24 hrs post-treatment and overlap in expression profiles between treatments.

Mitazalimab-induced transcriptional activity

- Mitazalimab treatment induce significant transcriptional activity in peripheral blood cells at 600 and 900 µg/kg dose levels without corticosteroid pre-treatment. Most of the induced transcripts were similarly expressed in the two dose groups.

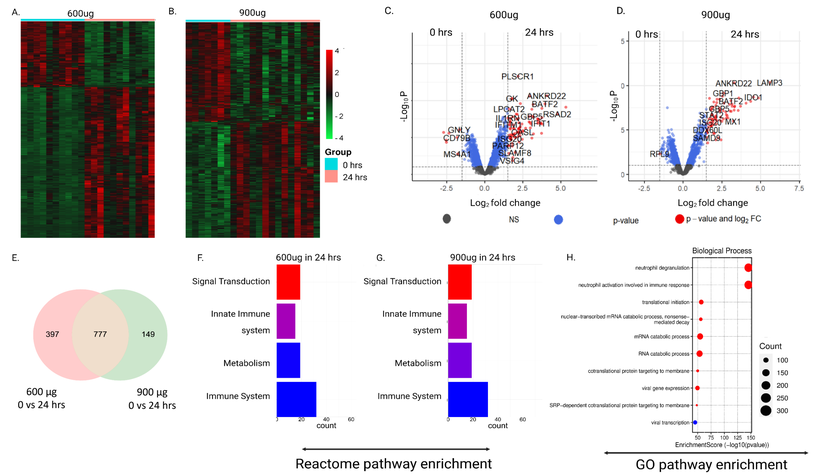


Figure 2. mRNA expression analysis of the 600 and 900 µg/kg without corticosteroids dose groups. (A, B) Heatmap and (C, D) volcano plots depicting DEGs within the two groups. (E) Venn diagram showing 777 overlapping DEGs in the 600 and 900 ug/kg groups. (F, G). Top 4 REACTOME pathways associated with DEGs in the 24 hrs compared to 0 hrs of treatment administration. (H) Top 10 GO biological pathways associated with the overlapping genes within the two doses (limma and voom correction, t-test, adjusted p-value<0.05)

Regulation of immune-related and IFN-associated transcripts

- Decreased expression levels of T cell and MHC class II transcripts, as well as increased expression of FcγR and MHC class I transcripts, were observed post treatment.
- Several IFN-regulated genes were upregulated after 24 hrs of treatment, and similarly regulated for the 600 and 900 µg/kg dose levels.

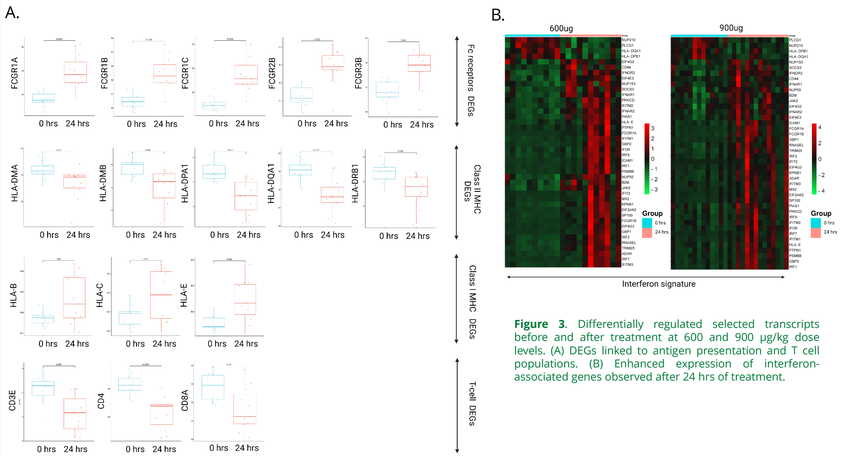


Figure 3. Differentially regulated selected transcripts before and after treatment at 600 and 900 µg/kg dose levels. (A) DEGs linked to antigen presentation and T cell populations. (B) Enhanced expression of interferon-associated genes observed after 24 hrs of treatment.

Mitazalimab-induced effects on the peripheral immunome

- Gene signatures related to activated DCs were upregulated post treatment, confirming the CD40 agonistic activity of mitazalimab.
- Changes in frequencies of immune cell populations observed using Cibersort analysis reflects expected activation-induced migration, which also was observed by flow cytometry [3].
- Activation of B cells, as determined by upregulation of CD80 and CD86, was observed post-treatment.

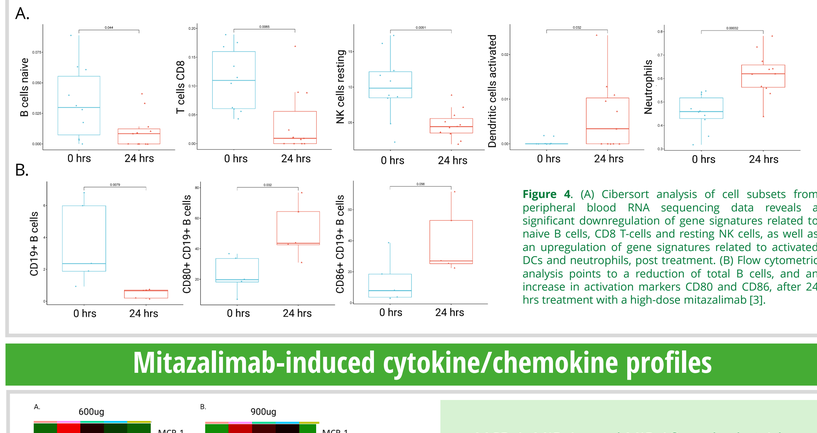
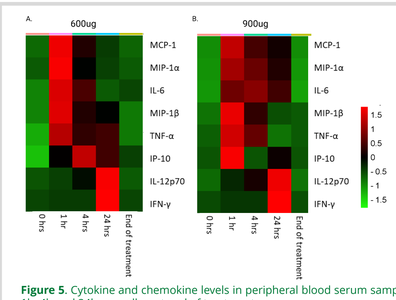


Figure 4. (A) Cibersort analysis of cell subsets from peripheral blood RNA sequencing data reveals a significant downregulation of gene signatures related to naive B cells, CD8 T-cells and resting NK cells, as well as an upregulation of gene signatures related to activated DCs and neutrophils, post treatment. (B) Flow cytometric analysis points to a reduction of total B cells, and an increase in activation markers CD80 and CD86, after 24 hrs treatment with a high-dose mitazalimab [3].

Mitazalimab-induced cytokine/chemokine profiles



- MCP-1, MIP-1α and MIP-1β peaked at 1 hr post treatment, suggesting an activation of myeloid cells.
- Prominent IFN-γ production was observed at 24 hrs, indicative of immune activation and in line with the observed IFN signature observed at transcriptional level at 24 hrs.
- TNF-α, IL-6 and IL-12p70 were also observed, but detected at lower levels.

Figure 5. Cytokine and chemokine levels in peripheral blood serum samples before and after treatment with (A) 600 µg/kg or (B) 900 µg/kg mitazalimab for 1h, 4h and 24h, as well as at end of treatment.

Summary and conclusions

- The analysis of RNAseq data obtained from whole blood clearly demonstrated that mitazalimab induces strong immune responses, e.g. activation of myeloid cells and B cells, in patients.
- Treatment with mitazalimab without corticosteroid pretreatment induced stronger inflammatory gene expression, supporting the mitazalimab schedule currently used in phase 2 development.
- The presented gene expression data confirms the biological activity of mitazalimab, further strengthening its proof of mechanism.
- An ongoing phase 2 study (OPTIMIZE-1) is currently evaluating the efficacy of mitazalimab in patients with metastatic pancreatic cancer (NCT04888312).

References

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2. Mangsbo SM, et al. The human agonistic CD40 antibody ADC-1013 eradicates bladder tumors and generates T-cell-dependent tumor immunity. Clin Cancer Res 2015;21:1115-1126.
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