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ATOR-4066, a Neo-X-Prime™ bispecific antibody targeting CD40 and CEACAM5, induces tumor localized immune cell activation in preclinical *in vivo* tumor model

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INTRODUCTION

- ATOR-4066 is a bispecific antibody (bsAb) targeting CD40 and CEACAM5 developed using Alligator's novel Neo-X-Prime™ platform
- ATOR-4066 binds to CD40 on myeloid cells and CEACAM5, a tumor-associated antigen (TAA), expressed on tumor cells and on tumor-derived material such as exosomes or tumor debris containing tumor neoantigens
- We have previously demonstrated that ATOR-4066 has potent anti-tumor efficacy *in vivo*
- Further, we have shown that the *in vivo* results translates into strong activation of CD40 expressing cells in CEACAM5$^+$ tumor materials from patients
- Here, we present preclinical data further elucidating the mode of action of ATOR-4066 *in vivo*

ATOR-4066: CD40 x CEACAM5 bsAb in RUBY™ format

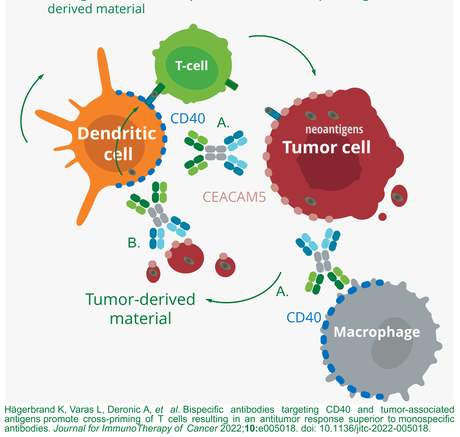
ATOR-4066 is a human Fc-silenced IgG1 in the bispecific tetravalent RUBY™ format, comprising two sets of binders targeting CD40 and the TAA carcinoembryonic antigen 5 (CEACAM5) developed for immunotherapy for patients with advanced solid cancers expressing CEACAM5.

The RUBY™ bispecific format has been developed to achieve an outstanding stability, manufacturability and functionality.

MODE OF ACTION

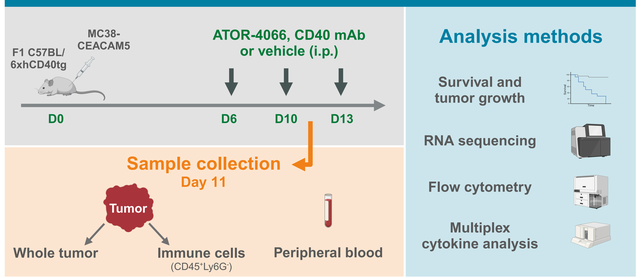
MoA of ATOR-4066:

A. CEAMCAM5-conditional CD40 activation of DCs and macrophages
B. Novel mechanism for cross-priming of neoantigen specific T cells through enhanced DC update of CEACAM5-expressing tumor-derived material



Hägerbrand K, Varas L, Deronic A, *et al*. Bispecific antibodies targeting CD40 and tumor-associated antigens promote cross-priming of T cells resulting in an antitumor response superior to monospecific antibodies. *Journal for ImmunoTherapy of Cancer* 2022;**10:**e005018. doi: 10.1136/jitc-2022-005018.

EXPERIMENTAL OUTLINE



Analysis methods

- Survival and tumor growth
- RNA sequencing
- Flow cytometry
- Multiplex cytokine analysis

ATOR-4066 induces strong anti-tumor effects

- ATOR-4066 shows superior anti-tumor efficacy as compared to vehicle or CD40 mAb in a MC38-CEACAM5$^+$ mouse model

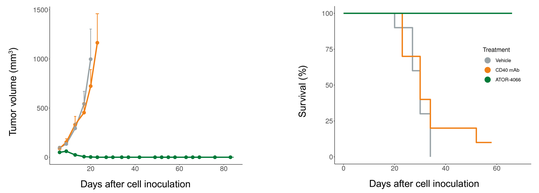


Figure 1. F1 C57BL/6xhCD40tg mice were inoculated subcutaneously (s.c.) with MC38 cells transfected with human CEACAM5* and 100 µg CD40 mAb or a molar equivalent dose (167 µg) of ATOR-4066 or vehicle control were administered intraperitoneally (i.p.) on days 6, 10 and 13 and tumor volume and survival was monitored (1).

ATOR-4066 induces pro-inflammatory gene expression profiles in the tumor microenvironment

- ATOR-4066 changes the transcriptome of CEACAM5$^+$ tumors into a more inflammatory state *in vivo*
- These effects are directed to the tumor as evidenced by lower transcriptomic alterations in the blood, as compared to a CD40 mAb
- ATOR-4066 modulates the immune landscape within tumors distinctly from CD40 mAb

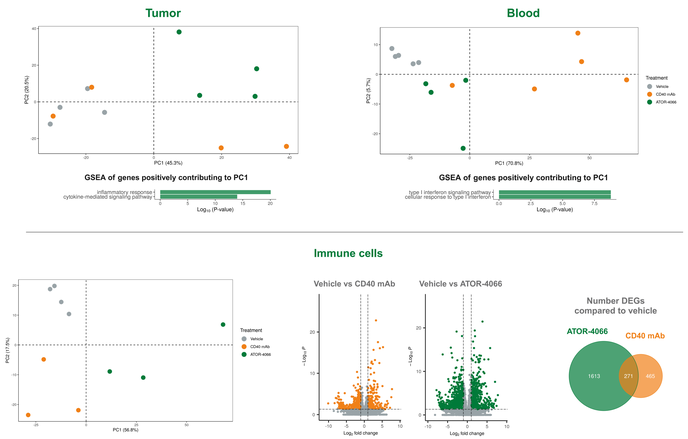


Figure 2. Principal component analysis (PCA) plots illustrating transcriptomic changes induced by treatment in tumors (top left) or peripheral blood (top right). Principal component (PC) 1 of tumor samples separates ATOR-4066 treated mice from vehicle group. The distinction is driven by an increased inflammatory response. However, ATOR-4066 induces less systemic effects as compared to a CD40 mAb. Bottom row depicts transcriptomic changes of immune cells within tumors. PCA plot display clear transcriptomic differences between treatment groups. Volcano plots, of differentially expressed genes (DEGs) of CD40 mAb or ATOR-4066 compared to vehicle, and Venn diagram illustrates that ATOR-4066 distinctively and to a larger extent than a CD40 mAb alters the immune cells within tumors. GSEA; gene-set enrichment analysis.

ATOR-4066 enhances immune trafficking to the TME and increases proliferation and activation of immune cells within tumors

- Up-regulation of genes associated with leukocyte migration were seen in tumors of all mice treated with ATOR-4066 together with a distinct up-regulation of genes associated with inflammatory response
- Immune cells in tumors treated with ATOR-4066 shows up-regulation of genes related to proliferation and T cell activation
- Together, this suggest that ATOR-4066 enhances immune cell trafficking to the TME and induces activation and proliferation of myeloid and T cells that correlates with anti-tumor activity in tumors

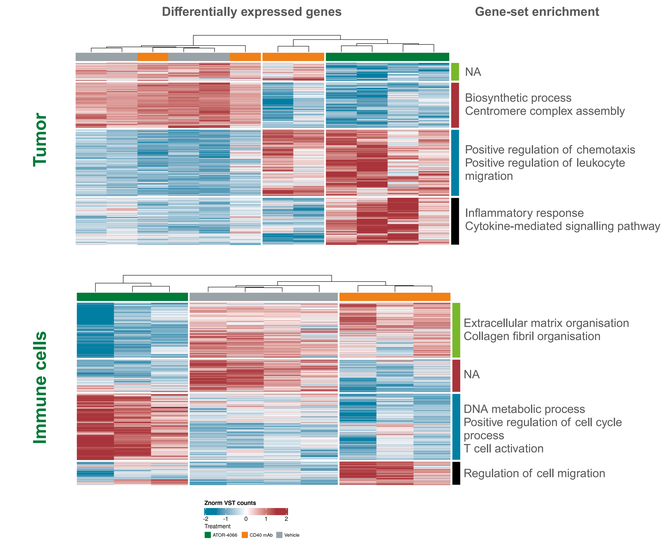


Figure 3. Heatmap displays expression levels of DEG between all conditions in whole tumor (top) and tumor immune cells (bottom). Hierarchial clustering of tumor samples shows that all ATOR-4066 treated tumors, but not all CD40 mAb tumors, cluster together indicating a homogenous response to treatment. Gene-set enrichment of gene clusters from K-means clustering revealed increased inflammatory response in tumors treated with ATOR-4066. Further, the gene expression of immune cells from tumors of ATOR-4066 treated mice showed increased proliferation and T cell activation. NA; no significant enrichment of gene sets within gene cluster.

ATOR-4066 increases the number of intra-tumoral leukocytes and activates myeloid cells in tumors

- Flow cytometric analysis of tumors treated with ATOR-4066 reveals an increase in number of total immune cells
- ATOR-4066 both increases the number of myeloid cells (macrophages and DCs) and activates these cells within tumors

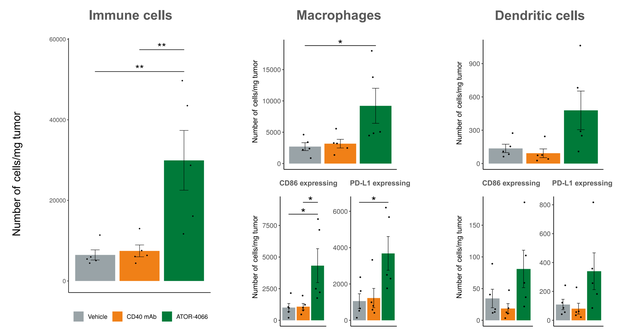


Figure 4. Flow cytometric analysis of tumors treated with ATOR-4066, CD40 mAb or vehicle. ATOR-4066 increases both the number of total immune (CD45$^+$) cells and myeloid cells within tumors. Further, ATOR-4066 also activates intra-tumoral myeloid cells, as evidenced by increased number of cells expressing CD86 and PD-L1 compared to both CD40 mAb and vehicle.

ATOR-4066 alters the intra-tumoral cytokine milieu to favor tumoricidal immune effects

- ATOR-4066 induces a distinct transcriptomic cytokine profile in tumors *in vivo*, with up-regulation of genes such as *Il1a, Il1b* and *Tnf*
- Up-regulation of pro-inflammatory genes correlates with increased cytokine levels
- This suggests that ATOR-4066 shifts the cytokine profile within tumors to a pro-inflammatory state correlating with the observed increased immune activation and anti-tumor effects

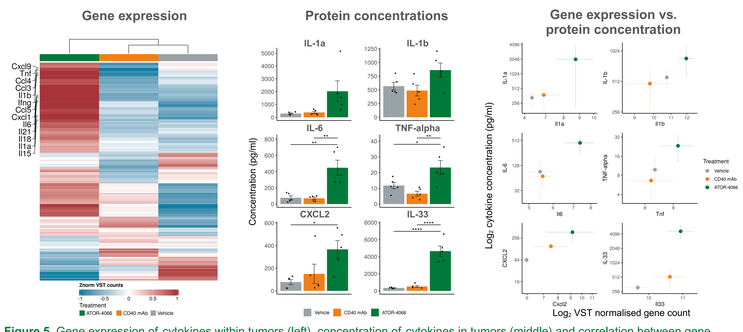


Figure 5. Gene expression of cytokines within tumors (left), concentration of cytokines in tumors (middle) and correlation between gene expression and cytokine levels (right). Heatmap shows expression of all detected genes in tumors belonging to GO:0005125 (cytokine activity). Columns are cluster by hierarchial clustering as shown by dendrogram. Cytokine levels were measured in tumor lysates using a Luminex multiplex kit. Correlation graphs show $\log_2$ of VST normalised gene expression on x-axis and $\log_2$ of cytokine concentration on y-axis. Dots represent mean, error bars show standard deviation.

CONCLUSIONS

- ATOR-4066 induces transcriptomic alterations that indicates an increased immune trafficking to the TME providing an enhanced inflammatory reponse, proliferation of immune cells and activation of T cells within tumors
- Flow cytometric and cytokine analysis confirm these findings, demonstrating that ATOR-4066 activates intratumoral myeloid cells and creates a pro-inflammatory tumor microenvironment resulting in efficient anti-tumor effects
- The biological activity is directed to the tumor microenvironment, with the majority of the pharmacodynamic activity detected in the TME
- The pharmacodynamic activity is consistent with the proposed MoA and the data supports further development and clinical testing

References

1. Uddbäck *et al*. Combination treatment with ATOR-4066, a Neo-X-Prime™ bispecific antibody targeting CD40 and CEACAM5, and anti-PD-1 reverses T cell exhaustion in vitro. SITC 2023.

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