ATOR-4066, a Neo-X-Prime bispecific antibody targeting CD40 and CEACAM5, induces strong myeloid and T cell dependent tumor immunity and synergizes with PD-1 blockade

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INTRODUCTION

ATOR-4066 is a preclinical stage bispecific antibody targeting CD40 and CEACAM5, developed using Alligator Bioscience's novel Neo-X-Prime platform

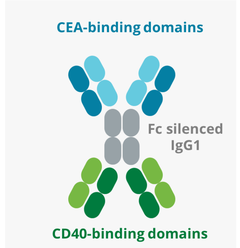
We have previously shown that ATOR-4066 induces strong, CEACAM5-conditional activation of CD40 expressing cells *in vitro* which translates into potent anti-tumor activity *in vivo*

SUMMARY

We demonstrate that ATOTR-4066 induce strong anti-tumor activity that depends on both myeloid cells and T cells for full activity. Further, combination with PD-1 enhances the T cell response and long-term tumor control.

The presented data shows the potential of ATOR-4066 as an anti-tumor treatment both as a stand-alone therapy but also in combination with anti-PD-1 treatment

ATOR-4066: CD40xCEACAM5



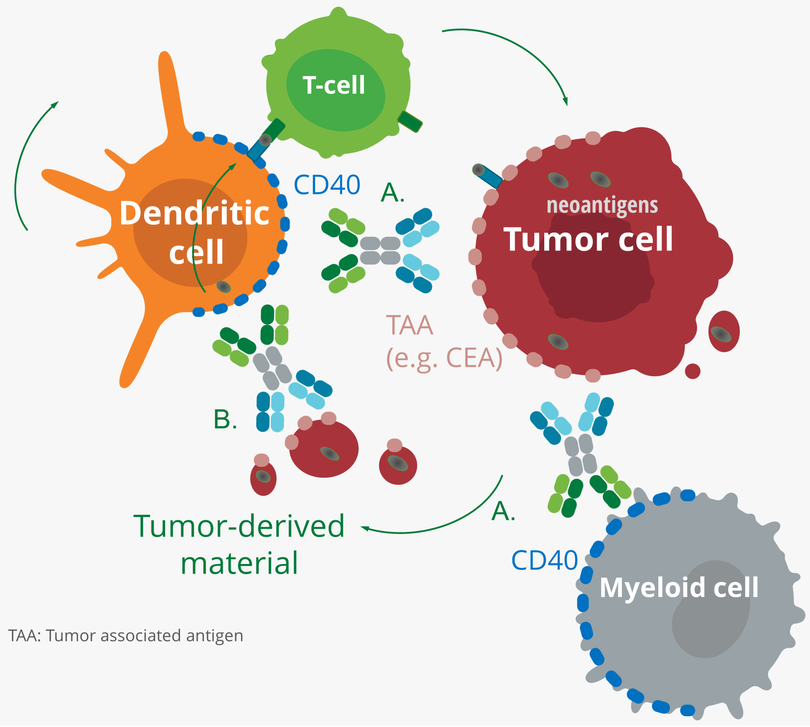
ATOR-4066 is a human Fc-silenced IgG1 in the bispecific tetravalent RUBY™ format, comprising two sets of binders targeting CD40 and the TAA carcinoembryonic antigen 5 (CEACAM5 or CEA) for immunotherapy of patients with advanced solid cancers expressing CEA

ATOR-4066 binds to both targets simultaneously and subsequently activates CD40-expressing cells only when binding to CEA-expressing tumor cells

MODE OF ACTION

MoA of Neo-X-Prime:

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



TAA: Tumor associated antigen

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.

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

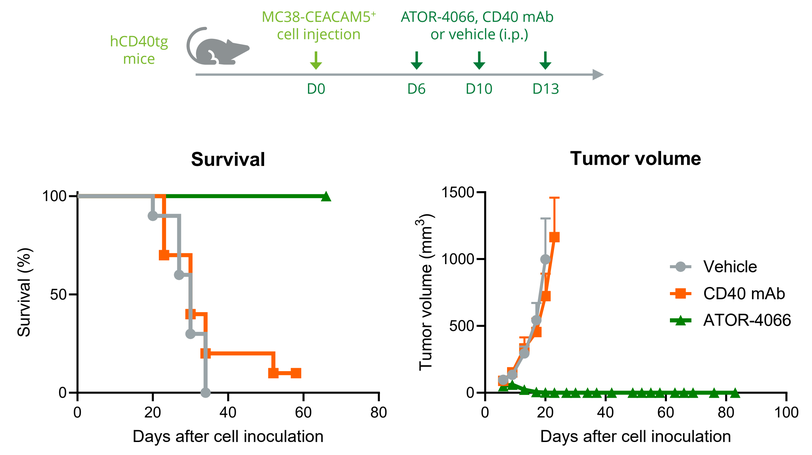


Figure 1. F1 C57BL/6xhCD40tg mice were inoculated subcutaneously (s.c.) with MC38 cells transfected with human CEACAM5 and 100 µg CD40 mAb or a molar equivalent dose (167 µg) of ATOR-4066 or vehicle control were administered intraperitoneally (i.p.) on days 6, 10 and 13 and tumor volume and survival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

ATOR-4066 increases both the intratumoral abundance of immune cells and activation of myeloid cells and T cells

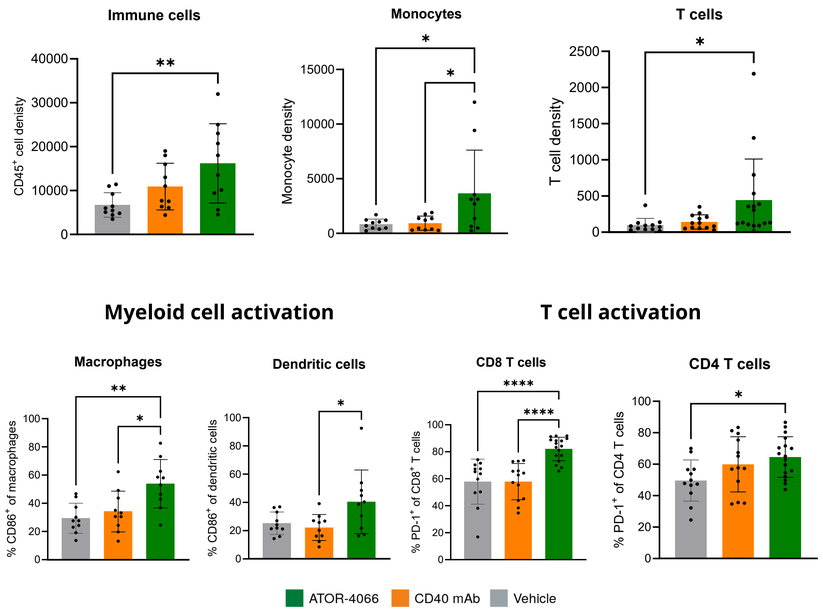


Figure 4. Flow cytometric analysis of tumors treated with ATOR-4066, CD40 mAb or vehicle. ATOR-4066 increases both the number of total immune (CD45+) cells and myeloid cells (monocytes) and T cells within tumors. Further, ATOR-4066 also activates intratumoral myeloid cells, as evidenced by increased frequency of cells expressing CD86 compared to CD40 mAb and vehicle, as well as T cells, evidenced by increased frequency of cells expressing PD-1. For analysis of myeloid cells, mice were treated on days 6 and 10 after inoculation and tumor were analyzed on day 11. For analysis of T cells, mice were treated on days 13, 17 and 20 after inoculation and tumors were analyzed on day 21.

ATOR-4066 induces up-regulation of genes involve in immune cell migration and inflammation within tumors

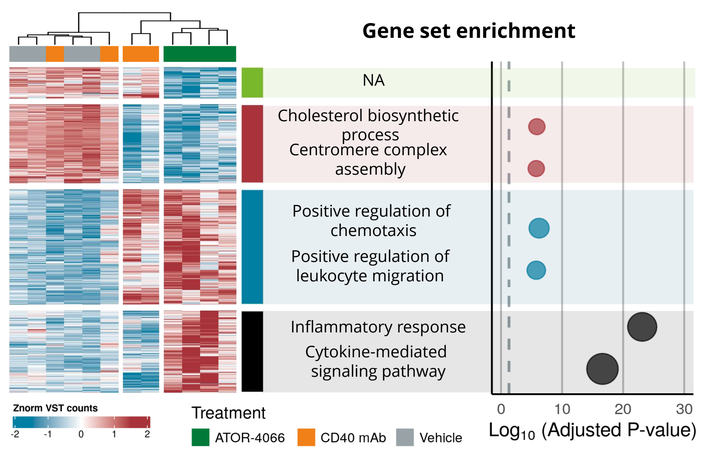


Figure 2. hCD40 mice were inoculated with MC38-CEACAM5 tumors and treated with ATOR-4066 or a CD40 mAb on day 6 and 10. At day 11, tumors were collected, and bulk RNA sequencing was performed. Heatmap displays expression levels of differentially expressed genes between treatment conditions. Gene-set enrichment of gene clusters from K-means clustering revealed increased inflammatory response in tumors treated with ATOR-4066. NA: no significant enrichment of gene sets within gene cluster.

ATOR-4066 dose-dependently alters the cytokine milieu within tumors to a pro-inflammatory state that favors tumoricidal immune effects

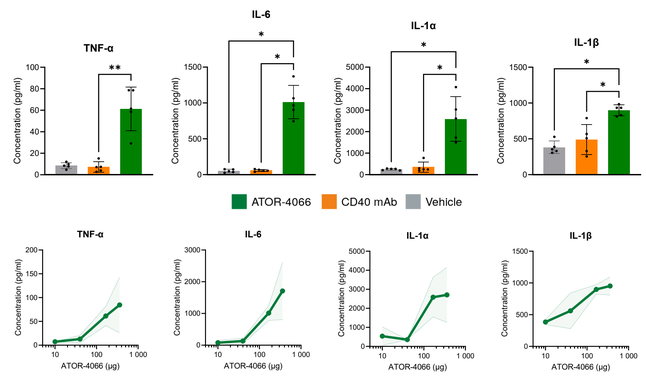


Figure 3. hCD40 mice were inoculated with MC38-CEACAM5 tumors and treated with ATOR-4066 (at 10, 40, 167 or 360 µg) or a CD40 mAb (100 µg, molar equivalent to 167 µg ATOR-4066) on day 6. At day 7, tumors were collected, lysed and analyzed for cytokine expression using a 22-plex Luminex kit. Bar plots compares molar equivalent dose of ATOR-4066 and a CD40 mAb (167 and 100 µg, respectively) and vehicle. Bottom row shows dose-response of respective cytokine following ATOR-4066 treatment. Thick line shows average, shaded area shows standard deviation.

ATOR-4066 induces initial T cell-independent tumor control, but long-term tumor control depends on T cells

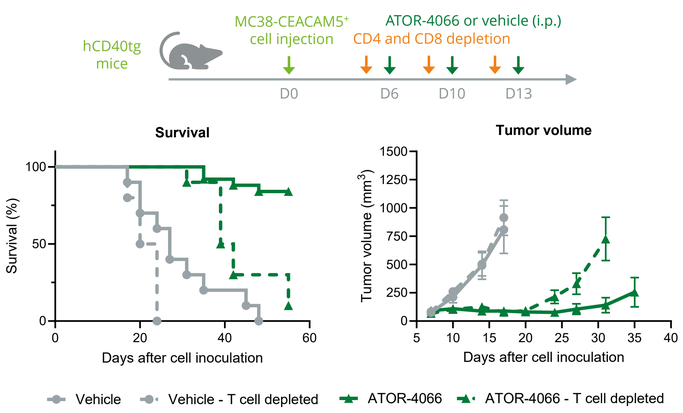


Figure 5. The importance of T cells during treatment was evaluated using anti-CD4 and anti-CD8 depleting antibodies in F1 hCD40tgxC57BL/6 mice inoculated with MC38-CEACAM5 and treated with ATOR-4066 or vehicle 6, 10 and 13 days after tumor inoculation. Tumor volume was measured and survival recorded. Tumor volume graphs shows mean +/- standard deviation, *n* = 10-20 mice per treatment condition.

Mice cured by ATOR-4066 display T-cell dependent immunological memory towards relevant cancer cells

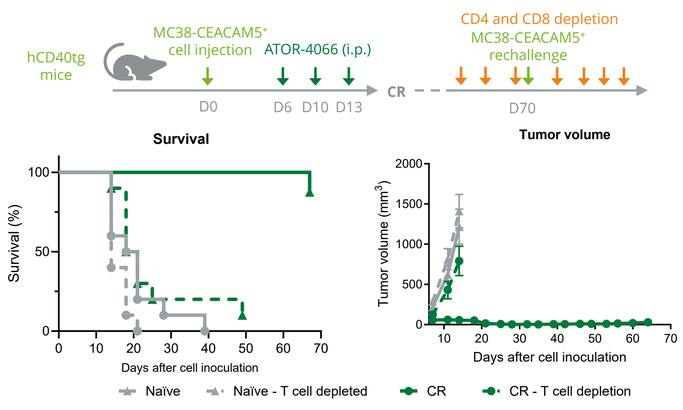


Figure 6. To study immunological memory in mice cured (CR) from MC38-CEACAM5 tumors by ATOR-4066 treatment, CR mice were rechallenged with MC38-CEACAM5 tumors. The importance of T cells in the rechallenge was investigated by depleting CD4 and CD8 T cells (dashed lines). Tumors grew in CR mice depleted of T cells, but not in CR mice with a functional T cell compartment. Tumor volume graph shows mean +/- standard deviation, *n* = 10 per treatment group.

ATOR-4066 in combination with PD-1 inhibitor enhances activation of exhausted T cells and improve anti-tumor activity

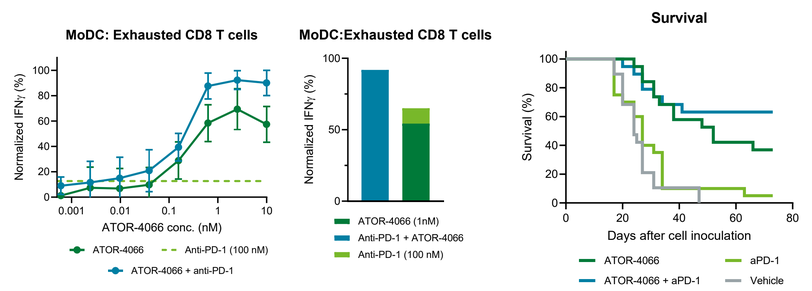


Figure 7. ATOR-4066 was evaluated in combination with anti-PD-1 antibody in mixed lymphocyte reaction (MLR) assays using allogeneic exhausted human primary CD4 or CD8 T cells and mature monocyte-derived DCs (moDCs). ATOR-4066 was titrated with a constant PD-1 concentration (100 nM) in presence of CEACAM5-beads and incubated for 7 days before supernatant was analyzed for IFNγ levels. Dots show mean +/- standard deviation, *n* = 9. Bar graphs shows mean levels at a concentration of 1 nM ATOR-4066. To study in vivo combination treatment effect, mice were inoculated with MC38-CEACAM5 and treated with 50 ug anti-PD1 on days 10 and 13 post inoculation and/or 60 ug ATOR-4066 on days 6, 10 and 13 post inoculation, *n* =18-20/group.

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