# ATOR-4066, a Neo-X-Prime bispecific antibody targeting CD40 and CEACAM5, induces myeloid and T-cell dependent anti-tumor activity and synergizes with PD-1 inhibition

Ida Uddbäck[1*], Hampus Andersson[1,2], Lill Ljung[1], Anneli Nilsson[1], Mona Celander[1], Amulya Krishna Shetty[1], David Gomez Jimenez[1], Malin Lindstedt[1,2], Karin Hägerbrand[1], Sara Fritzell[1] and Peter Ellmark[1,2]

[1] Alligator Bioscience AB, Medicon Village, Lund, Sweden; [2] Department of Immunotechnology, Lund University, Lund, Sweden *Presenting author

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## INTRODUCTION

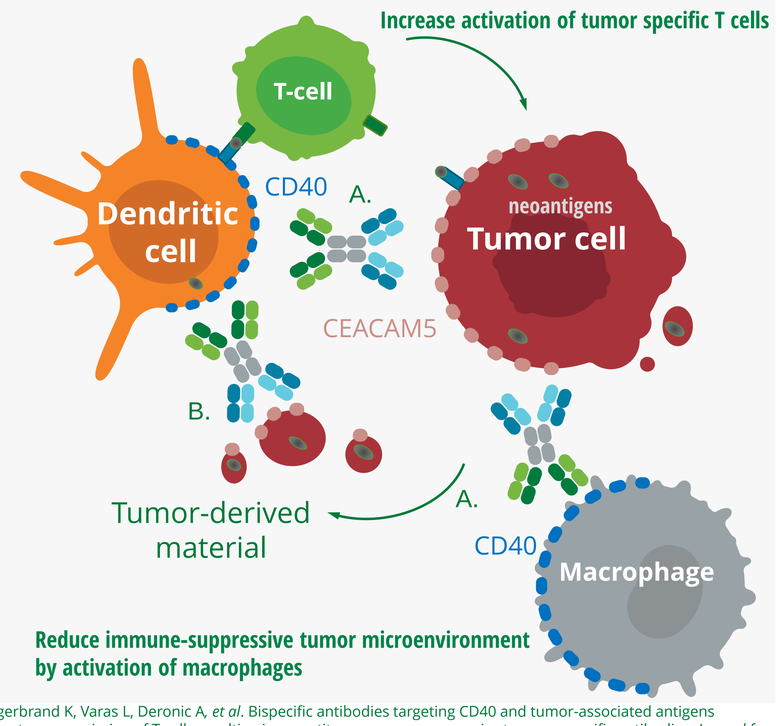
- ATOR-4066 is a preclinical stage bispecific antibody targeting CD40 and CEACAM5, developed using Alligator's novel Neo-X-Prime™ platform, which induces neoantigen specific T-cell responses by activating antigen presenting cells (APCs)
- ATOR-4066 binds to CD40 on dendritic cells (DCs) and CEACAM5, a tumor-associated antigen (TAA), expressed on tumor cells and on tumor-derived material (such as exosomes or tumor debris containing neoantigen), leading to tumor directed activation of the DCs, enhanced uptake of tumor-derived material, cross-presentation of neoantigen, priming of neoantigen-specific T cells and killing of tumor cells.
- We have previously demonstrated potent anti-tumor efficacy of ATOR-4066 treatment *in vivo*
- Moreover, ATOR-4066 induces strong activation of CD40 expressing cells *in vitro* using CEACAM5 expressing tumor material from patients
- Here we present further preclinical data strengthening 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: CD40 x CEACAM5 targeting bispecific antibody


- ATOR-4066 is a human Fc-silenced IgG1 in the bispecific tetravalent RUBY® format, comprising two sets of binders targeting CD40 and the tumor associated antigen carcinoembryonic antigen 5 (CEACAM5) developed for immunotherapy for patients with advanced solid cancers expressing CEACAM5.
- ATOR-4066 binds to both targets simultaneously and subsequently activates CD40-expressing cells only when binding to CEACAM5-expressing tumor cells. The CEACAM5-conditional activation is anticipated to limit toxicity due to systemic immune activation.

## MODE OF ACTION

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




Reduce immune-suppressive tumor microenvironment by activation of macrophages

[1]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.

## RESULTS

### ATOR-4066 treatment induces a strong anti-tumor effect also in larger tumors with heterogenous CEACAM5 expression

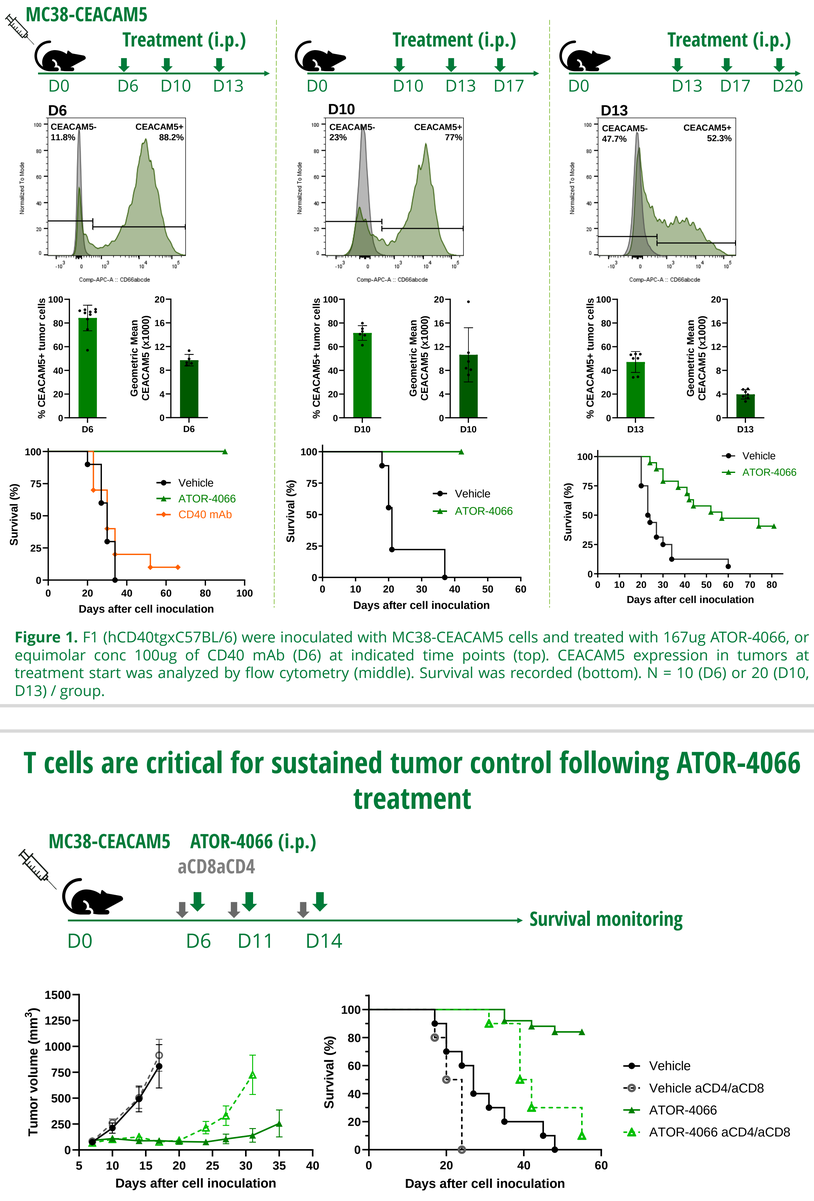



**Figure 1.** F1 (hCD40tgxC57BL/6) were inoculated with MC38-CEACAM5 cells and treated with 167ug ATOR-4066, or equimolar conc 100ug of CD40 mAb (D6) at indicated time points (top). CEACAM5 expression in tumors at treatment start was analyzed by flow cytometry (middle). Survival was recorded (bottom). N = 10 (D6) or 20 (D10, D13) / group.

### T cells are critical for sustained tumor control following ATOR-4066 treatment




**Figure 2.** F1 (hCD40tgxC57BL/6) were inoculated with MC38-CEACAM5 cells and treated with 167ug ATOR-4066 at indicated time points. 24H prior to each treatment mice were administered αCD8 (clone 2.43) and αCD4 (clone GK1.5) depleting antibodies. Depletion of CD4 and CD8 T-cells was confirmed by flow cytometry in blood (not shown). Tumor volume was measured (left) and survival recorded (middle). N=10-25/group, tumor volume mean±SEM.

### T-cell memory is induced after ATOR-4066 treatment

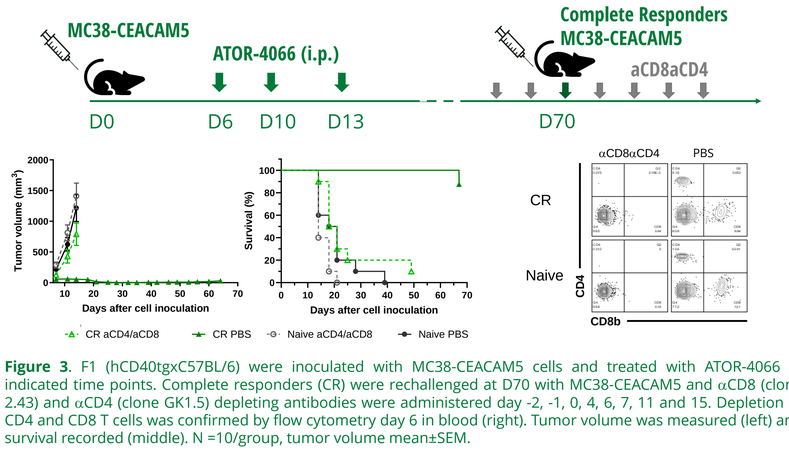



**Figure 3**. F1 (hCD40tgxC57BL/6) were inoculated with MC38-CEACAM5 cells and treated with ATOR-4066 at indicated time points. Complete responders (CR) were rechallenged at D70 with MC38-CEACAM5 and αCD8 (clone 2.43) and αCD4 (clone GK1.5) depleting antibodies were administered day -2, -1, 0, 4, 6, 7, 11 and 15. Depletion of CD4 and CD8 T cells was confirmed by flow cytometry day 6 in blood (right). Tumor volume was measured (left) and survival recorded (middle). N =10/group, tumor volume mean±SEM.

### ATOR-4066 treatment results in increased myeloid cell activation and tumor T-cell infiltration and activation

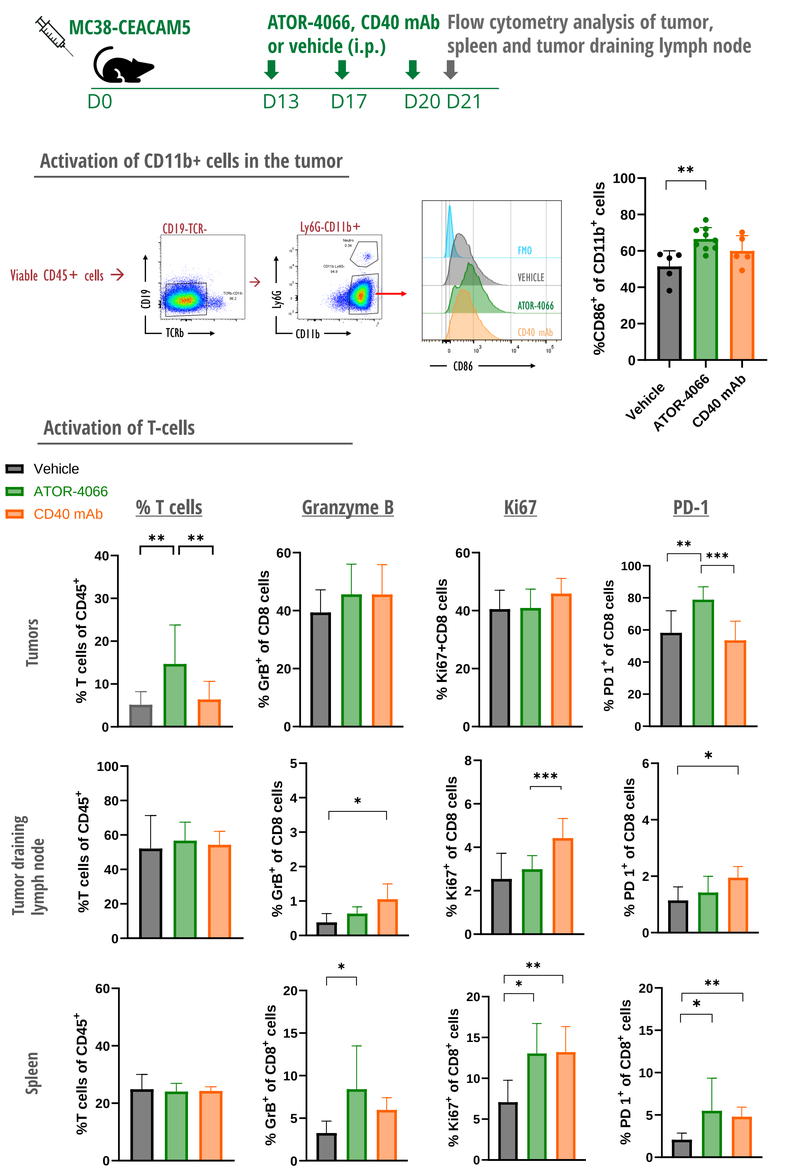



**Figure 4.** F1 (hCD40tgxC57BL/6) mice were inoculated with MC38-CEACAM5 cells s.c. and 167 µg of ATOR-4066, equimolar conc (100ug) of CD40 mAb or vehicle control were administered i.p. on days 13, 17 and 20. Tumor, tumor draining lymph node, and spleen were isolated day 21. Tissues were dissociated and single cell suspension was stained with fluorescent antibodies and analysed by flow cytometry. N = 5-9/group. Bars represent mean+SD. Statistical significancy was done using ANOVA and Wilcoxon Rank test. *:p<0.05, **:p<0.01, ***:p<0.001.

### ATOR-4066 improves reactivation of exhausted CD4 T cells with anti-PD-L1 in MLR assays

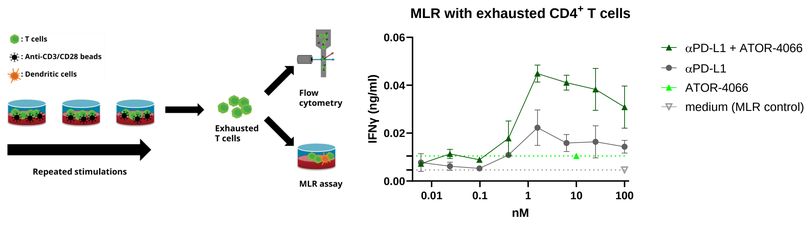



**Figure 5.** Human CD4+ T cells with an exhausted phenotype were generated by repeated (x3) stimulation with CD3/CD28 Dynabeads. After 8 days, exhausted CD4 T cells were characterized as having an increased expression of PD-1, TIM-3 and LAG-3 using flow cytometry. Human Mo-DC were generated by differentiating CD14+ monocytes from blood in GM-CSF and IL-4 for 5 days and then matured for 24 hours using IL-1β, IL-6, TNFα and PGE2. Mo-DC cells and exhausted CD4+ T cells (1:10 mix) were treated with titrating concentration of anti-PD-L1 (atezolizumab), and 10 nM of ATOR-4066 in cultures with CEACAM5-coated beads for 7 days. Supernatants were analyzed for interferon gamma (IFN-γ, Monkey IFN gamma Elisa development Kit, 3421M-1H-20, Mabtech). Mean and SD for one representative donor pair out of nine is shown.

### ATOR-4066+anti-PD1 combination synergizes in vivo resulting in decreased tumor growth and increased survival

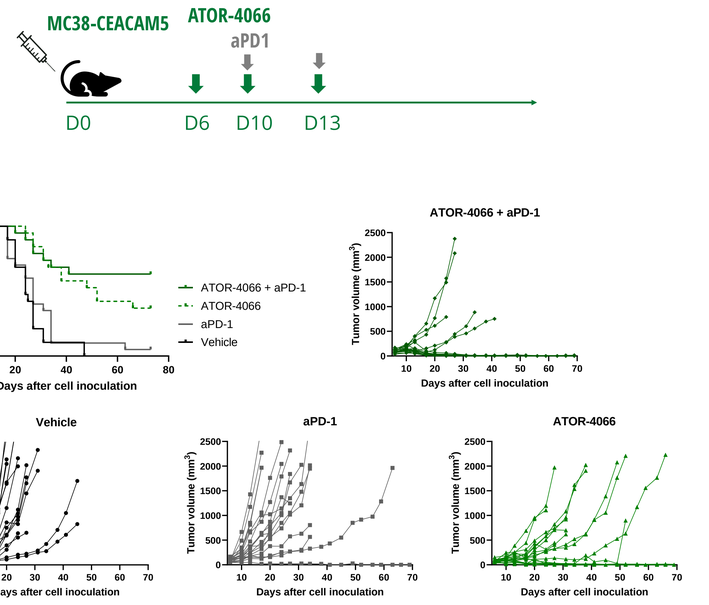



**Figure 6.** F1 (hCD40tgxC57BL/6) mice were inoculated with MC38-CEACAM5 cells s.c. and 60 µg of ATOR-4066 or vehicle control were administered i.p. on days 6, 10 and 13 and, or, 50ug aPD1 (clone RPMI-14) was administered i.p. at day 10 and 13. Tumor volume was measured and survival recorded. Tumor volume graphs show individual mice. n = 20/group.

## SUMMARY AND CONCLUSION

- We demonstrate that the CD40xCEACAM5 bispecific antibody ATOR-4066 induces:
  - » Efficient anti-tumor activity also with larger heterogenous tumors *in vivo*
  - » T-cell dependent anti-tumor activity during treatment cycle
  - » Tumor specific T-cell memory
  - » T-cell activation in the tumor and spleen
  - » Enhanced reactivation of exhausted T cells in combination with anti-PD1
  - » Synergetic anti-tumor effect with anti-PD1 *in vivo*
- Taken together, these data show the ability of ATOR-4066 to remodel the immune microenvironment by recruiting and activating tumor-infiltrating immune cells, demonstrating the promise of this new candidate drug and strongly supporting further development towards clinical testing.

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*Corresponding author
idu@alligatorbioscience.com

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