ATOR-4066, a Neo-X-Prime™ bispecific antibody targeting CD40 and CEA, activates myeloid cells in primary human tumors *in vitro* and induces anti-tumor immunity *in vivo*

Karin Hägerbrand[1], Anette Sundstedt[1], Mattias Levin[1], Laura Varas[1], Yago Pico de Coaña[1], Deniz Bölükbas[1], Lill Ljung[1], Maria Johansson[1], Anneli Nilsson[1], Mona Celander[1], Hampus Andersson[1], David Gomez Jimenez[1,2], Malin Lindstedt[1,2], Peter Ellmark[1,2]

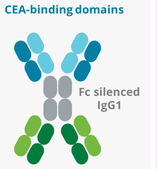
[1]Alligator Bioscience AB, Medicon Village, Lund, Sweden; [2]Department of Immunotechnology, Lund University, Lund, Sweden

Abstract 2939

INTRODUCTION

- Alligator's Neo-X-Prime™ platform consists of bispecific antibodies (bsAbs) targeting CD40 on antigen presenting cells (APCs) and a tumor-associated antigen (TAA) on tumor cells, aiming to efficiently enhance priming of tumor neoantigen-specific T cells.
- We have previously demonstrated that binding of Neo-X-Prime CD40xTAA bsAbs to CD40 on dendritic cells (DCs) and TAAs on tumor-derived material (exosomes or debris) leads to activation of the DC, uptake of tumor material, cross-presentation of tumor-derived antigen and priming of tumor antigen-specific T cells [1]. This has the potential to result in an increased quantity and/or quality of tumor-targeting T cells and enhanced anti-tumor activity.
- Here we present ATOR-4066, a CD40xTAA Neo-X-Prime bsAb with the potential to induce strong anti-tumor responses in patients with CEA-expressing tumors.

ATOR-4066: CD40xCEA

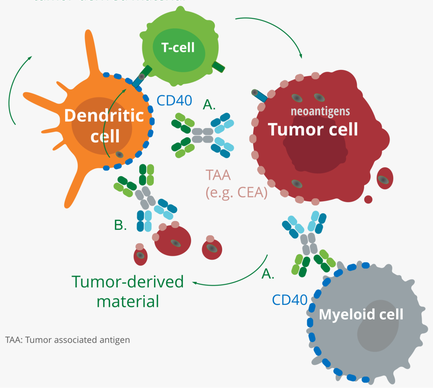


- ATOR-4066 is a human Fc-silenced IgG1 in the bispecific tetravalent RUBY™ format, comprising two sets of binders targeting CD40 and the TAA carcinoembryonic antigen 5 (CEACAM5 or CEA) for immunotherapy of patients with advanced solid cancers expressing CEA.
- ATOR-4066 binds to both targets simultaneously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

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



[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

CEA-conditional activation of primary human immune cells by ATOR-4066

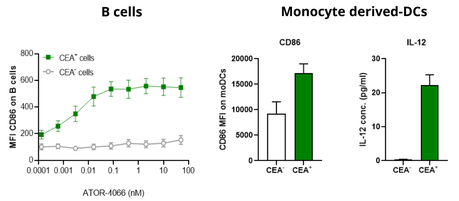


Figure 1. Primary human B cells were cultured in the presence of CEA-transfected or wildtype CHO cells and activated by ATOR-4066 for 48 hrs. The expression of CD86 was analyzed by flow cytometry (n=5; left graph). Monocyte-derived DCs were cultured in the presence of CEA-coated or uncoated beads and ATOR-4066 for 48 hrs. The expression of CD86 was analyzed by flow cytometry and IL-12p40 levels in supernatants were analyzed by ELISA (n=3; right graphs).

Activation of immune cells in human primary colorectal tumor samples by ATOR-4066

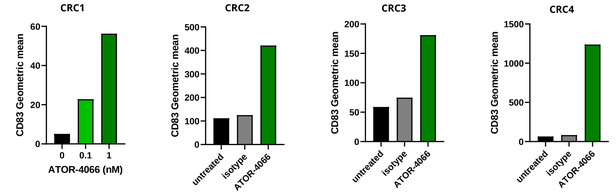


Figure 4. Dissociated cells from CEA-positive human colorectal cancer (CRC) tumors (Discovery Life Sciences, n=4) were analyzed for their ability to provide cross-linking to ATOR-4066 and induce CD83 upregulation following stimulation of the tumor-infiltrating immune cells for 48 hrs. An isotype×CD40 bsAb was used as a control. CD83 levels were determined on immune cells gated on viable CD45$^+$CD3$^-$CD56$^-$ cells by flow cytometry.

Strong anti-tumor efficacy and induction of immunological memory after ATOR-4066 treatment *in vivo*

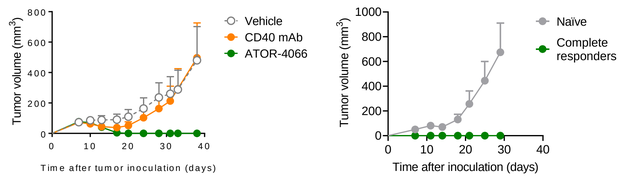


Figure 7. hCD40tg mice were inoculated with MC38-CEA$^+$ cells s.c. and 100 µg CD40 mAb or a molar equivalent dose (167 µg) of ATOR-4066 were administered i.p. on days 7, 10 and 13 (left graph). Complete responders cured from MC38-CEA$^+$ tumors by bsAb treatment were rechallenged with MC38 WT tumors (right graph). Naïve hCD40tg mice served as tumor growth control. Tumor volume mean±SEM (n=10/group) is presented.

ATOR-4066 mediates clustering of CEA-expressing tumor debris with CD40-expressing APCs

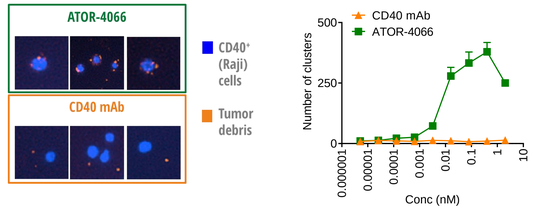


Figure 2. Fluorescently labeled CD40$^+$ Raji cells were cultured with fluorescently labeled CEA$^+$ tumor debris and different concentrations of CD40 mAb or ATOR-4066. Images were captured using a live cell imaging system and clusters of CD40$^+$ cells co-localized with tumor debris were quantified after 8 hrs of culture.

CEA-conditional activation of immune cells in human primary gastric tumor samples by ATOR-4066

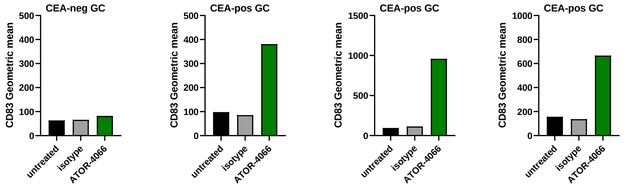


Figure 5. Dissociated cells from gastric cancer (GC) tumors (Discovery Life Sciences; 1 CEA-negative and 3 CEA-positive samples) were analyzed for their ability to provide cross-linking to ATOR-4066 and induce CD83 upregulation following stimulation of the tumor-infiltrating immune cells for 48 hrs. An isotype×CD40 bsAb served as control. CD83 levels were determined on immune cells gated on viable CD45$^+$CD3$^-$CD56$^-$ cells by flow cytometry.

CEACAM5 expression in low-passage patient-derived xenograft (PDX) models

Indication	n	% Positive samples	% Positive cells	H-Score
Colorectal	134	92	86	185
Gastric	23	91	70	168
Esophageal	11	82	68	133
Pancreatic	70	80	83	175
Cholangiocarcinoma	14	79	64	139
NSCLC	134	62	59	143
Head and Neck	70	49	12	29
Endometrial	8	38	36	71
HCC	8	38	40	74
SCLC	8	38	63	122
Breast	98	35	39	96
Cervix	3	33	30	90
Uterine	4	25	1	3
Ovarian	46	24	46	83
Prostate	11	18	2	2
Bladder	11	18	21	12
Sarcoma	9	11	1	3

Figure 8. Tissue-micro arrays of low-passage PDX models (Champions Oncology) were analyzed for CEACAM5 expression by immunochemistry. Histoscore (H-score) was calculated by a semi-quantitative assessment of both intensity of staining (graded as: 0, non-staining; 1, weak; 2, median; or 3, strong using adjacent normal mucosa as the median) and percentage of positive cells. The range of possible scores was from 0 to 300.

CEA expression on primary human colorectal cancer cells is sufficient to induce strong ATOR-4066-mediated activation of CD40-expressing cells

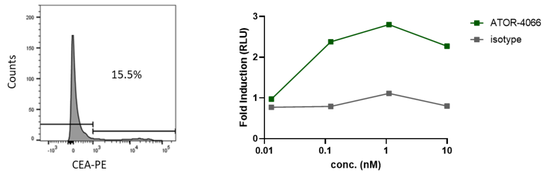


Figure 3. Dissociated cells from human colorectal cancer tumors (obtained from Discovery Life Sciences) were analyzed for the expression of CEA (gated on total viable cells) by flow cytometry (left graph) and the ability to activate CD40 reporter cells by ATOR-4066 with crosslinking provided by CEA expressed on the dissociated tumor cells during 6 hours of culture (right graph). An isotype×CD40 bsAb was used as a control. One representative sample out of 5 is depicted.

Activation of tumor-infiltrating CD40-expressing cell subsets in the presence of CEA-expressing cells

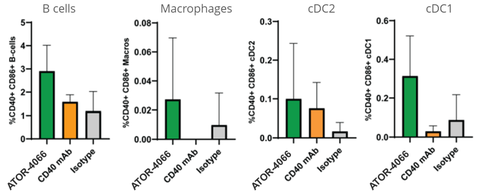


Figure 6. Human CD45$^+$HLA-DR$^+$CD3$^-$ cells from a tonsillar cancer biopsy were co-cultured with UV-irradiated CHO cells transfected with human CEA in the presence of ATOR-4066, CD40 mAb or isotype×CD40 bsAb. After 13 hrs of culture, cells were harvested and the frequencies of CD86$^+$CD40$^+$ cells were investigated using flow cytometry of CD19$^+$CD20$^+$ B cells, CD14$^+$ macrophages, CD1c$^+$cDC2s and XCR1$^+$cDC1s.

SUMMARY AND CONCLUSION

- We have demonstrated that the CD40xCEA targeting bsAb ATOR-4066 induces:
  - Efficient, CEA-conditional activation of human primary B cells and monocyte-derived DCs
  - Co-localization of CEA-expressing tumor debris and CD40-expressing APCs
  - CEA-conditional activation of tumor-infiltrating immune cells in patient-derived dissociated tumor samples from indications with high CEA expression including colorectal and gastric cancer
  - Activation of tumor-infiltrating myeloid cell subsets in the presence of CEA-expressing cells
  - Strong anti-tumor efficacy and induction of immunological memory
- Taken together, these data show the ability of ATOR-4066 to remodel the immune microenvironment and activate tumor-infiltrating immune cells in primary human tumors expressing CEA, demonstrating the promise of this new candidate drug and strongly supports further development towards the clinic.

AACR 2023
Poster 2939

Hägerbrand et al.
JITC 2022

ALLIGATOR bioscience