CTLA-4 x OX40 bispecific antibody ATOR-1015 induces anti-tumor effects through tumor-directed immune activation

Anne Månsson Kvarnhammar, Niina Veitonmäki, Karin Hägerbrand, Mia Thagesson, Doreen Werchau, Kristine Smedenfors, Anna Rosén, Maria Johansson, Per Norlén, Christina Furebring and Peter Ellmark

Alligator Bioscience AB, Lund, Sweden

Summary & Conclusions

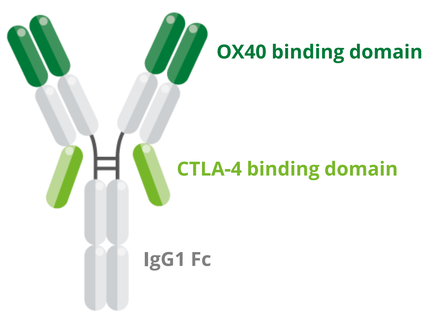
ATOR-1015 is a human IgG1 CTLA-4 x OX40 bispecific antibody. It consists of a CTLA-4 binding domain, generated by FIND® optimization of the CTLA-4 ligand CD86, fused to an agonistic OX40 antibody generated from the ALLIGATOR GOLD® library. ATOR-1015 is a next generation CTLA-4 antibody designed for improved benefit-risk profile. It will enter clinical phase I in H2 2018.

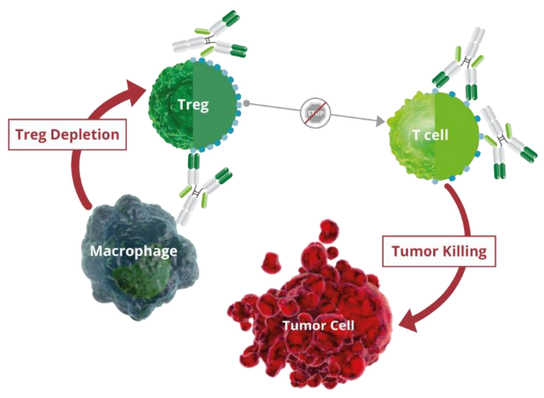
Mode of action

- Tumor-directed immune activation through dual targeting of OX40 and CTLA-4 on tumor infiltrating regulatory T cells (Tregs)
- Depletion of Tregs and activation of effector T cells (Teffs)

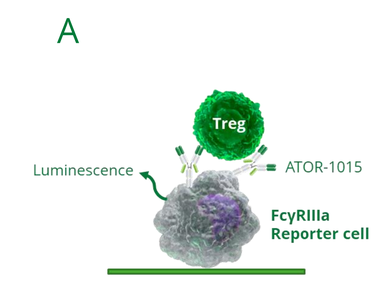
Results and conclusions

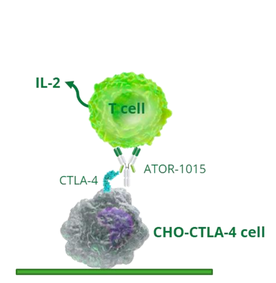
- ATOR-1015 induces superior depletion of Tregs and activation of Teffs compared to the combination of monotargeting antibodies (Figure 1)
- ATOR-1015 localizes to the tumor (Figure 2)
- ATOR-1015 increases the Teff/Treg ratio in the tumor, but not in lymphatic organs (spleen), by promoting depletion of Tregs and infiltration and expansion of Teffs (Figure 3)
- ATOR-1015 mediates anti-tumor effects and immunological memory (Figure 4)
- ATOR-1015 enhances the anti-tumor effects of αPD-1 treatment (Figure 5)

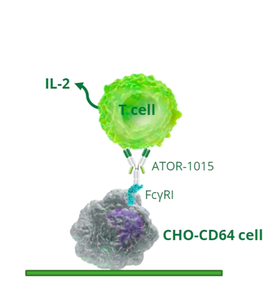


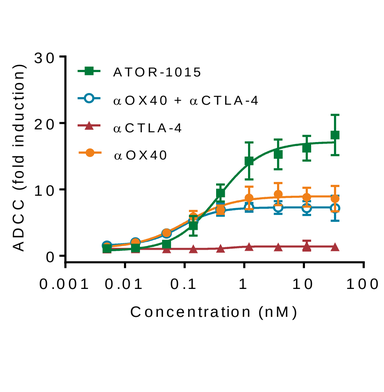


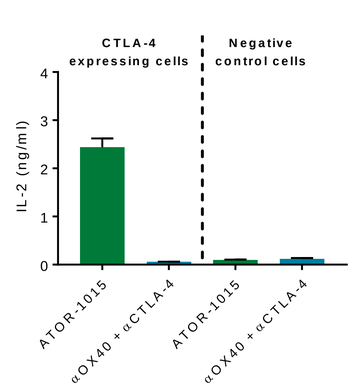
ATOR-1015 depletes human Tregs and activates Teffs *in vitro*











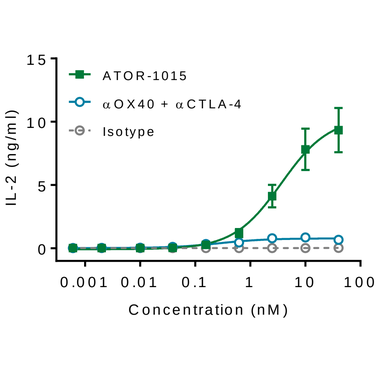
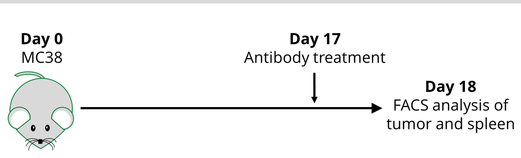


Figure 1. (A) *In vitro* activated Tregs were incubated with antibodies and depletion was measured in an ADCC reporter assay using FcγRIIIa Reporter cells as a model for Treg depletion (n=5). **(B)** CD4⁺ T cells were incubated with CTLA-4- or mock-transfected cells with suboptimal αCD3 and antibodies. After 72 h, IL-2 was measured by ELISA (n=8). **(C)** CD4⁺ T cells were incubated with CD64- (FcγRI)-transfected cells with suboptimal αCD3 and antibodies. After 72 h, IL-2 was measured by ELISA (n=8). Graphs show mean ± SEM.

ATOR-1015 localizes to the tumor



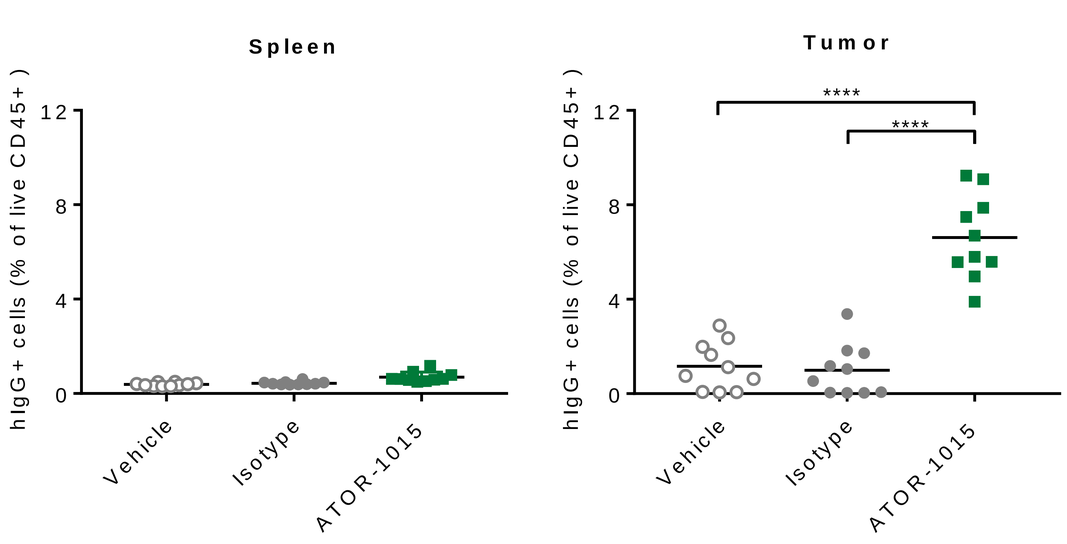
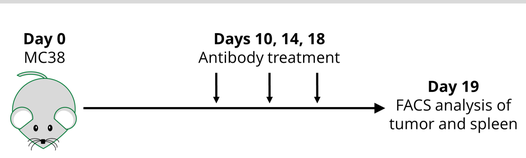


Figure 2. Human OX40 knock-in mice bearing MC38 tumors were treated with vehicle, isotype control or ATOR-1015 (248 µg) on day 17. Tumors and spleens were collected 24 h later, stained with an anti-human IgG antibody and analyzed by flow cytometry. Data show the percentage of hIgG⁺ cells out of total live CD45⁺ cells. Statistics, Mann-Whitney, two-tailed.

ATOR-1015 depletes Tregs and activates Teffs in the tumor



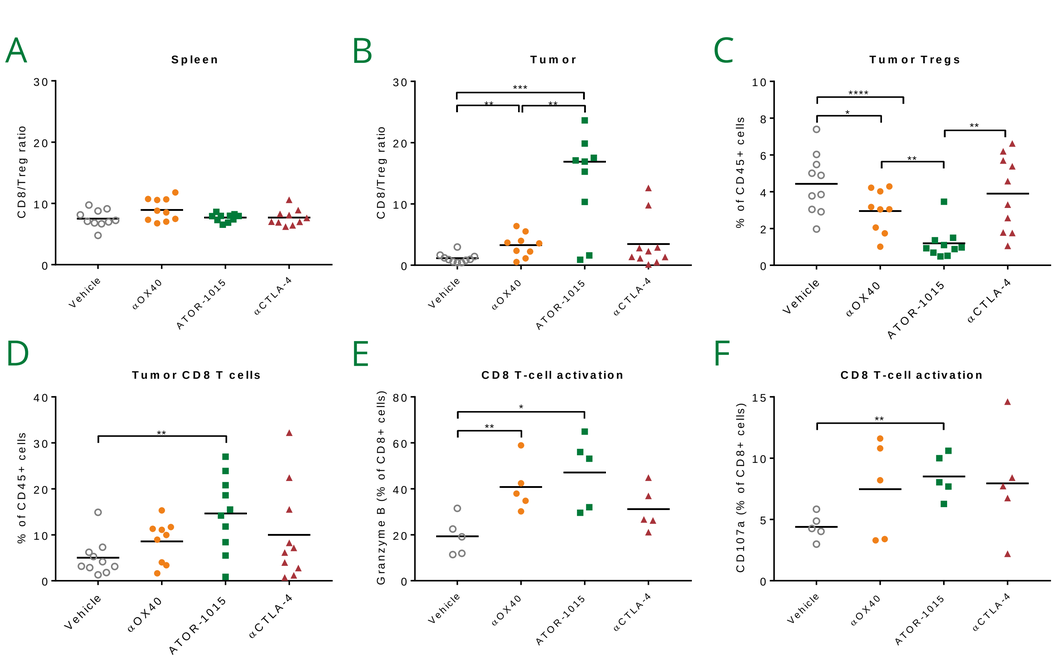
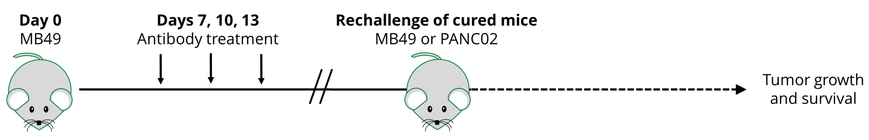


Figure 3. Human OX40 knock-in mice bearing MC38 tumors were treated with ATOR-1015 (248 µg) on days 10, 14, and 18. Flow cytometry analysis of the tumors and spleens were done 24 h after last the treatment. **(A)** Systemic Teff/Treg ratio (spleen), **(B)** Intratumoral Teff/Treg ratio, **(C)** Intratumoral Treg content, **(D)** Intratumoral Teff content, **(E)** Granzyme B expression on Teffs, and **(F)** CD107a expression on Teffs. Statistics, Mann-Whitney, two-tailed.

ATOR-1015 induces anti-tumor effects and immunological memory



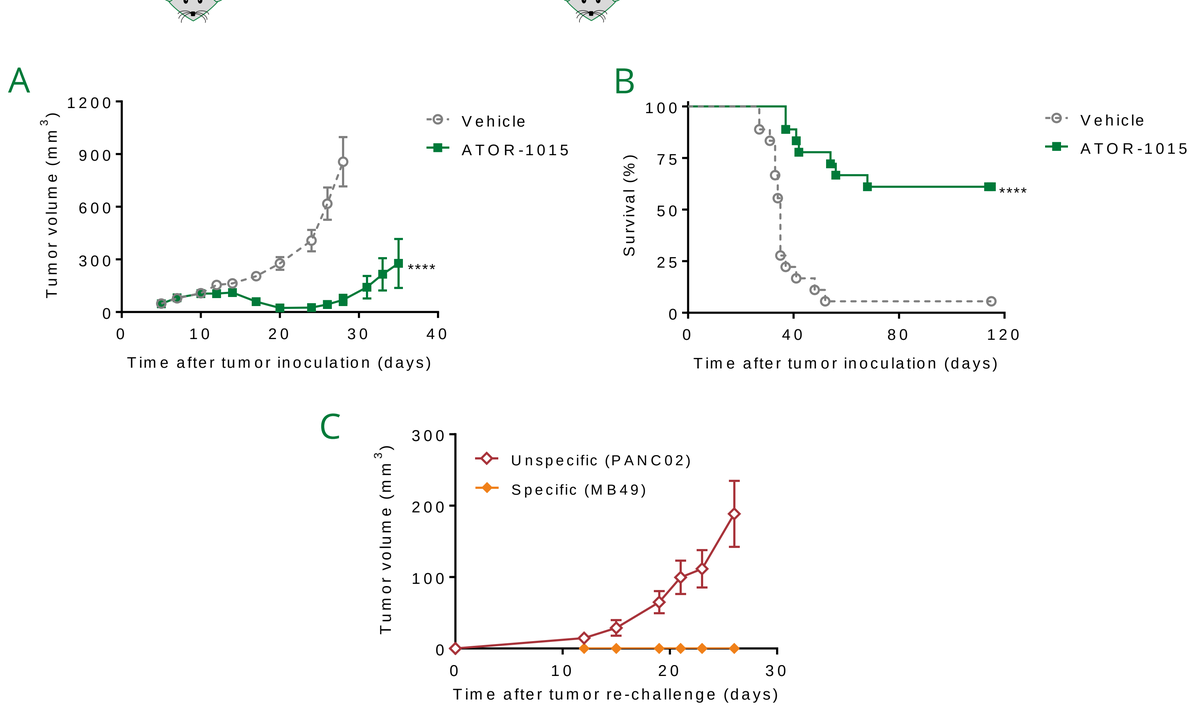
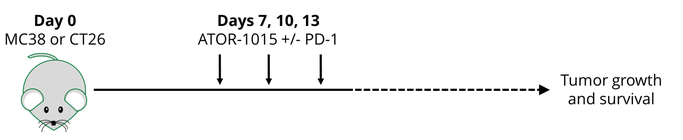


Figure 4. The effect of ATOR-1015 in MB49 bladder carcinoma in terms of **(A)** tumor growth, and **(B)** survival (n=18). Human OX40 knock-in mice were inoculated s.c. with MB49 tumor cells. ATOR-1015 (248 µg) or vehicle was administered intraperitoneally on days 7, 10, and 13. Statistics versus vehicle, Mann-Whitney, two-tailed. **(C)** Re-challenge of cured mice from (B) in a twin tumor model with a specific (MB49) or an irrelevant tumor (PANC02) demonstrating tumor-specific immunological memory.

Enhanced effect of ATOR-1015 in combination with αPD-1



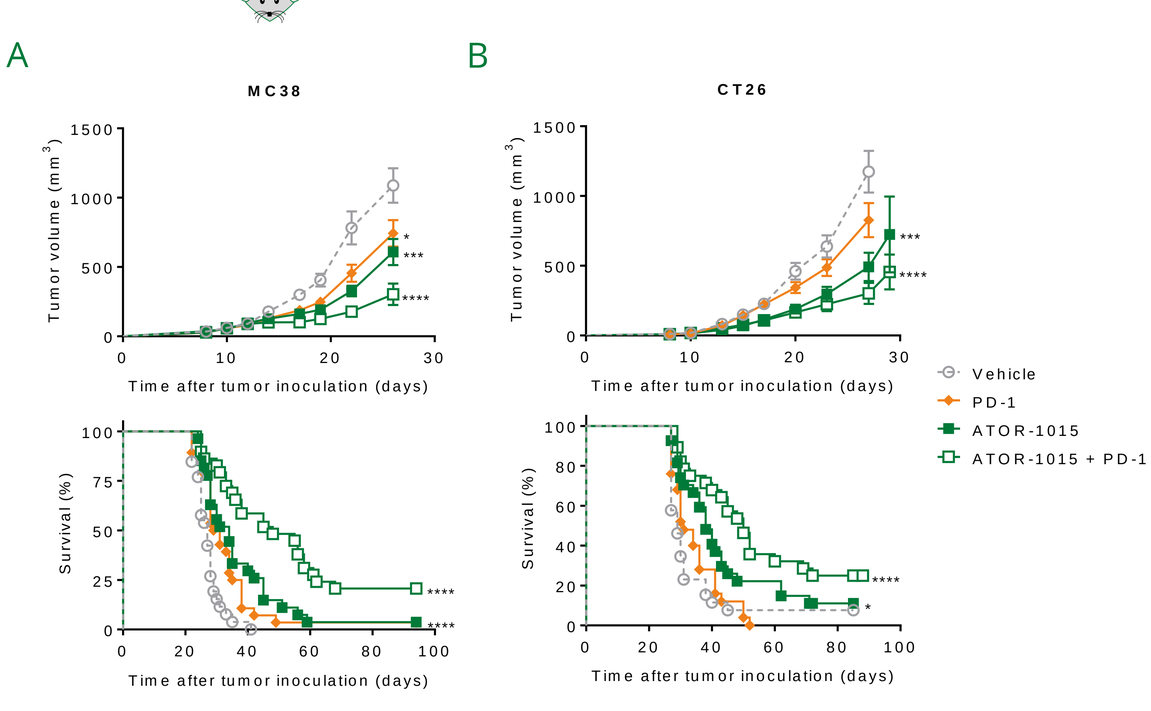


Figure 5. Anti-tumor effects of ATOR-1015 with an αPD-1 antibody (RPM1-14) in **(A)** MC38 colon carcinoma (n=10), and **(B)** CT26 colon carcinoma models (n=18). Tumor cells were implanted s.c. in human OX40 knock-in mice. ATOR-1015 (248 µg) with or without αPD-1 antibody (250 µg) was administered intraperitoneally on days 7, 10, and 13. The graphs show mean +/- SEM. Statistics versus vehicle, Mann-Whitney, two-tailed.

CIMT 2018, Poster # 222
Alligator Bioscience AB, Lund, Sweden