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

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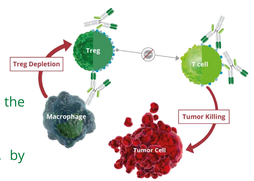
Summary & Conclusions

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 risk-benefit. It is planned to enter clinical phase I in H2 2018.

Mode of action

- OX40 and CTLA-4 are highly expressed on tumor infiltrating regulatory T cells (Tregs)
- ATOR-1015 depletes Tregs and activates effector T cells (Teffs)
- Dual targeting directs the effects to the tumor area



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 the 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*

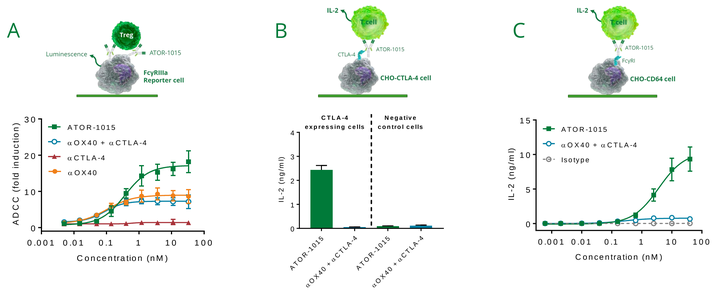


Figure 1. Superior depletion of Tregs and activation of Teffs by ATOR-1015 compared to combination. (A) *In vitro* activated Tregs were incubated with antibodies and ADCC was measured in an ADCC reporter assay using FcγRIIIa Reporter cells as a model for Treg depletion (n=5). **(B)** CD4+ T cells were 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

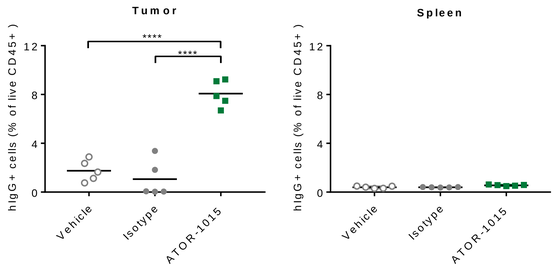


Figure 2. ATOR-1015 localizes to the tumor. Human OX40 transgenic mice bearing MC38 tumors were treated with vehicle, isotype control or ATOR-1015 on day 17. Twenty-four hours later, tumors and spleens were collected and the level of hIgG+ cells were quantified by flow cytometry. Data show the percentage of hIgG+ cells out of live CD45+ cells. Statistics, Mann-Whitney, two-tailed.

ATOR-1015 depletes Tregs and activates Teffs in the tumor environment *in vivo*

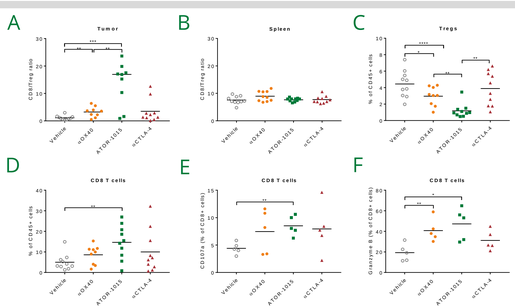


Figure 3. Increased Teff/Treg ratio in the tumor following ATOR-1015 treatment. Human OX40 transgenic mice bearing MC38 tumors were treated with ATOR-1015 on days 10, 14, and 18. Flow cytometry analysis of the tumors and spleens were done 24 h after last the treatment. **(A)** Intratumoral Teff/Treg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

ATOR-1015 induces tumor-specific immunological memory

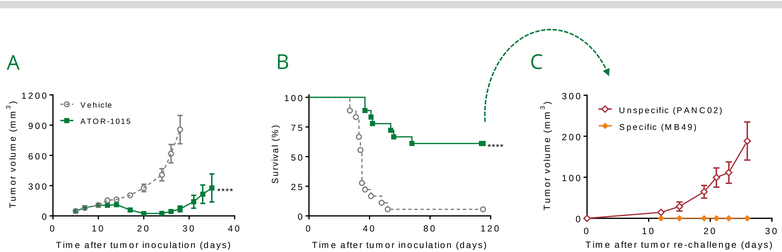


Figure 4. ATOR-1015 induces anti-tumor effects and immunological memory. The effect of ATOR-1015 in MB49 bladder carcinoma in terms of **(A)** tumor growth, and **(B)** survival (n=18). Human OX40 transgenic mice were inoculated s.c. with MB49 tumor cells. ATOR-1015 or vehicle was administered intraperitoneally on days 7, 10, and 13. Statistics, 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

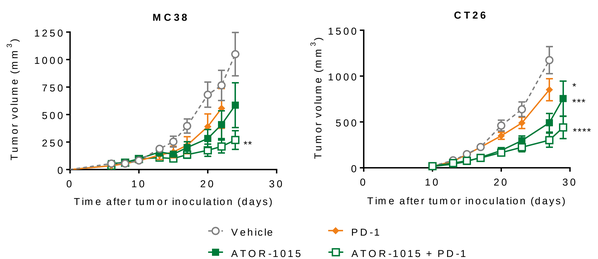


Figure 5. Combinatorial effect of ATOR-1015 and αPD-1. Anti-tumor effects of ATOR-1015 with an αPD-1 antibody (RPM1-14) in MC38 colon carcinoma (n=10) and CT26 colon carcinoma models (n=18). Tumor cells were implanted s.c. in human OX40 transgenic mice. ATOR-1015 with or without αPD-1 antibody was administered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

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