ATOR-1144 is a tumor-directed CTLA-4 x GITR bispecific antibody that acts by depleting Tregs and activating effector T cells and NK cells

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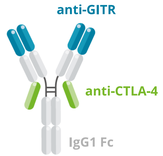
Background

Rationale

- CTLA-4 is a checkpoint receptor highly expressed on tumor-infiltrating T cells, particularly T regulatory cells (Tregs)[1, 2]
- GITR is a TNFR superfamily member highly expressed on tumor-infiltrating T cells, in particular Tregs, but also on NK cells and tumor cells[1, 3-7]
- ATOR-1144 combines targeting of CTLA-4 and GITR to achieve tumor-directed immune activation

About ATOR-1144

- ATOR-1144 is a CTLA-4 x GITR bispecific IgG1 antibody that binds to both targets with high affinity (nM range)
- ATOR-1144 was developed for treatment of solid tumors and hematological malignancies



References

1. Arce Vargas et al. Cancer Cell. 2018; 33: 1-15. 2. Montler et al. Clin Trans Immunol. 2016; 5: e70. 3. Sukumar et al. Cancer Res. 2018; 77(16): 4378-88. 4. van Beek et al. Int J Cancer. 2019; Feb 4. 5. Argast et al. Proceedings: AACR Annual Meeting 2018 (abstract #3826). 6. Wang et al. Proceedings: AACR Annual Meeting 2017 (abstract #5621). 7. Holland et al. Proceedings: AACR Annual Meeting 2018 (abstract #3813).

GITR is expressed on TILs and tumor cells

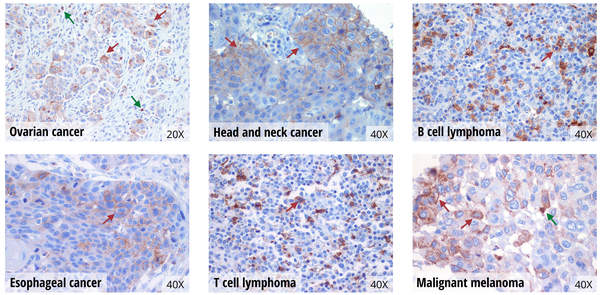


Figure 1. GITR expression (brown) was assessed in formalin-fixed, paraffin embedded tissue microarrays from various cancer indications using a rabbit anti-human GITR antibody (ab237713, Abcam). **Green** arrows indicate staining of infiltrating mononuclear cells. **Red** arrows indicate staining of neoplastic cells.

ATOR-1144 induces formation of cell complexes

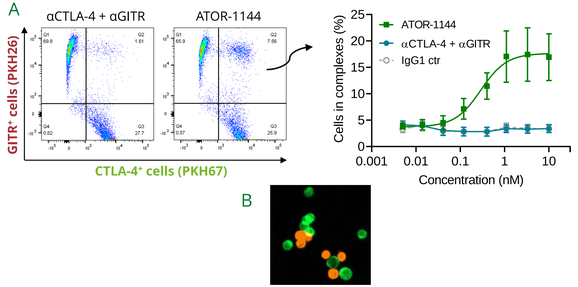


Figure 2. GITR⁺ and CTLA-4⁺ CHO cells were labelled with PKH26 (red dye) and PKH67 (green dye), respectively. Cells were mixed 1:1 and antibodies were added. **(A)** The percentage of cells in complexes was quantified by flow cytometry (n=4). **(B)** The formation of cell complexes was visualized using a Cytation 5 Cell Imaging reader.

ATOR-1144 activates human T cells

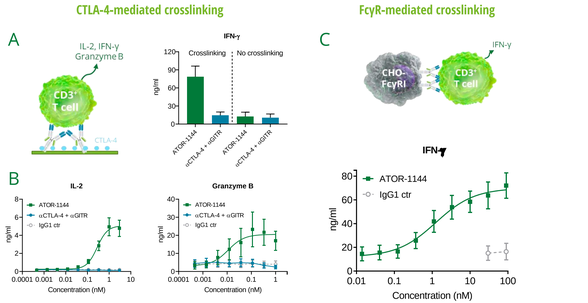


Figure 3. (A) CD3⁺ T cells cultured with or without immobilized CTLA-4, anti-CD3 and antibodies (n=2). **(B)** CD3⁺ T cells cultured with immobilized CTLA-4, anti-CD3 and antibodies (n=13). **(C)** CD3⁺ T cells cultured with irradiated CHO-FcγRI cells, anti-CD3 and antibodies (n=8). IL-2, IFN-γ and granzyme B were measured by ELISA.

ATOR-1144 activates human NK cells and enhances cytotoxicity

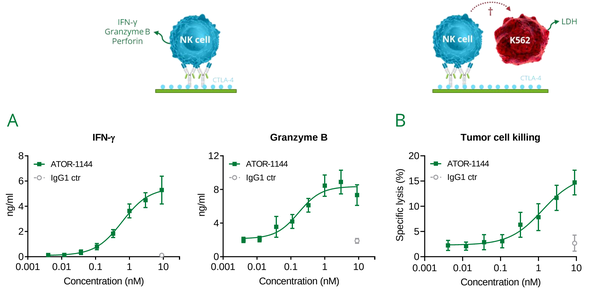


Figure 4. NK cells were cultured in plates with immobilized CTLA-4 in the presence of IL-2 and with or without ATOR-1144 and IgG1 control for 48 h. **(A)** IFN-γ and granzyme B release was measured in the supernatants by ELISA (n=8-10). **(B)** K562 cells were added, and after an additional 4-h culture period, specific lysis after background subtraction was determined as LDH release (n=10).

ATOR-1144 induces ADCC of Tregs

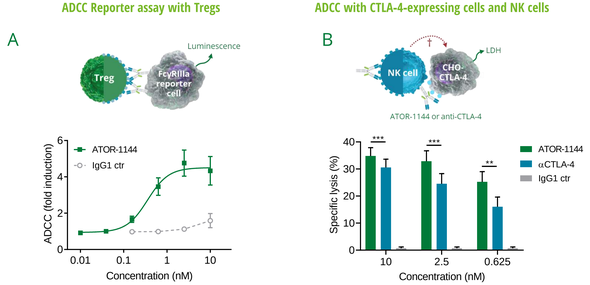


Figure 5. (A) Human in vitro activated Tregs were incubated with antibodies for 6 h. Treg depletion was measured in an ADCC (FcγRIIIa) reporter assay (n=5). **(B)** CTLA-4⁺ CHO cells were cultured together with NK cells at a 1:10 ratio in the presence of ATOR-1144, anti-CTLA-4 antibody or isotype control. Specific lysis was determined as LDH release (n=8).

ATOR-1144 induces ADCC of GITR⁺ tumor cells

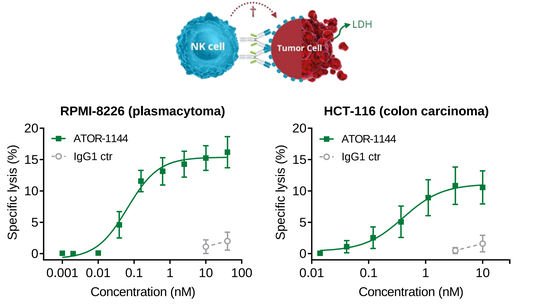


Figure 6. Tumor cells were cultured overnight with NK cells at a 1:10 ratio in the presence of ATOR-1144 or isotype control. Specific lysis was determined as LDH release (n=4-6).

Summary and Conclusions

Summary

- ATOR-1144 acts through several mechanisms:
  - Activation of effector T cells
  - Depletion of Tregs and tumor cells
  - Activation of NK cells for enhanced tumor cell killing

Conclusions

- ATOR-1144 is a next generation CTLA-4 targeting antibody with enhanced Treg depletion and direct anti-tumor activity
- Dual targeting of CTLA-4 and GITR is expected to direct the effect to the tumor area

Contact information

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