Design and Generation of Bispecific Antibodies for Immuno-Oncology

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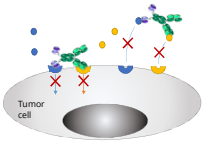
Step 1 - Design: Choosing a format compatible with the aimed MoA is essential

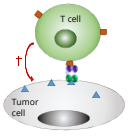
Examples of what can be achieved with bsAbs and challenges when choosing format

Increased effect due to dual targeting
Ex: Simultaneous blockade of two signaling pathways[1]



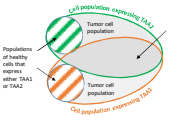
Challenges when choosing format:
- Tetravalent formats will have high retention to cell membranes which can lead to internalization and degradation.

New cell-cell biology
Ex: T cell redirection[4]



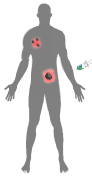
Challenges when choosing format:
- Length of bsAb binding arms may affect strength of cell-cell synapse.

Improved specificity by dual targeting
Ex: targeting of tumors with 2 TAA[3].



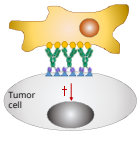
Challenges when choosing format:
- Formats that result in strong binding due to avidity may not gain tumor specificity.

Directed activity
Ex: Immuno-activation contained to tumors[1, 2]



Challenges when choosing format:
- Binding strength/ binding valency to TAA vs immuno-activator

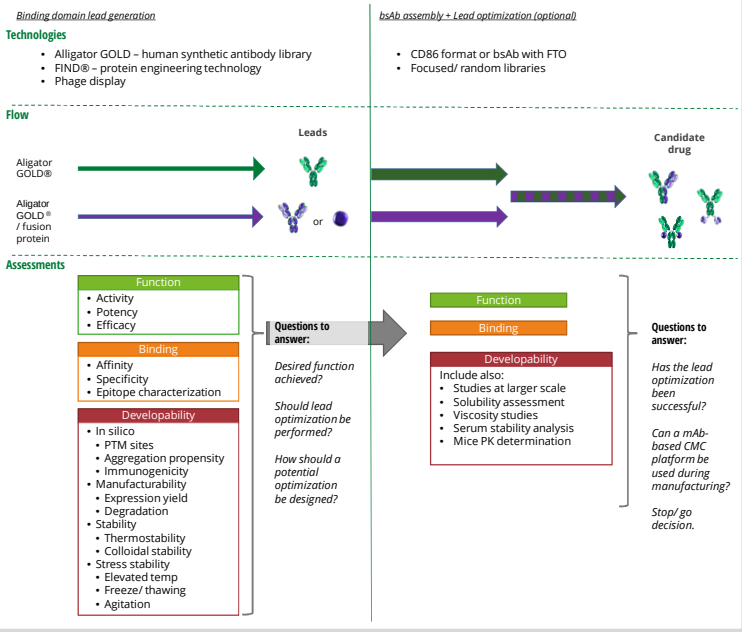
Signaling by clustering/ dimerization
Ex: Induction of apoptosis by cross-linking[5]



Challenges when choosing format:
- Clustering using monovalent bsAb will require high affinity that may lead to increased risk for internalization and degradation.

Step 2 - Generation: Ensuring not only function but good developability is crucial

Platform for integrated functional and developability screening

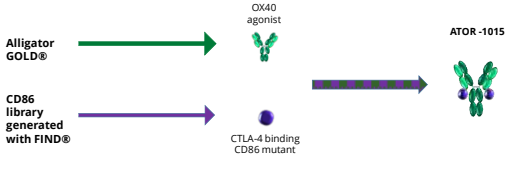


Case study: ATOR-1015, a next generation CTLA-4 targeting therapy

Design - Bivalent targeting of both OX40 and CTLA-4 achieves:

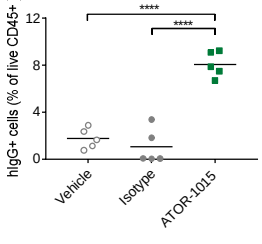
- **A) Tumor localization.** OX40 and CTLA-4 are highly expressed on TILs in the tumor area
- **B) Improved specificity.** More selective targeting of Tregs that express both CTLA-4 and OX40
- **C) Signaling by clustering/ dimerization.** CTLA-4 dependent activation of OX40
- **D) Increased immune effect.** Targeting of two T cell mechanisms leads to improved immune activation

Generation – OX40 mAb fused to CD86 mutant:

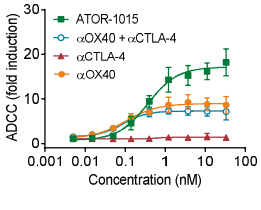


Developability assessment results
- *In silico* ✓
- Manufacturability ✓
- Stability ✓
- Stress stability ✓
- Solubility ✓
- Viscosity ✓
- Serum stability ✓

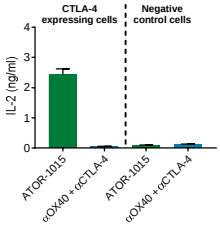
A) Tumor localization



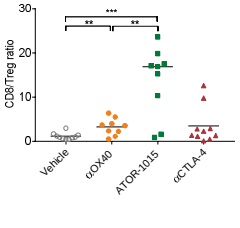
B) Improved Treg targeting



C) Signaling by dimerization



D) Increased immune effect



(A) 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 (not shown) 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. **(B) Superior depletion of Tregs by ATOR-1015 compared to combination.** *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). **(C) OX40 activation of Teffs through CTLA-4 crosslinking.** CD4$^+$ T cells were incubated *in vitro* with CTLA-4- or mock-transfected cells with suboptimal αCD3 and antibodies. After 72 h, IL-2 was measured by ELISA (n=8). **(D) 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 (not shown) were done 24 h after last the treatment.

References

1. Fisher et al. 2015 Arthritis Rheumatol
2. Öffner et al. 2006 Mol Immunol
3. Mazor et al. 2017 Scientific Reports
4. Dahlén et al. 2018 Ther Adv Vacc ImmunoTher
5. Wanders et al. 2018 J Nucl Med
6. Brünker et al. 2016 Mol Cancer Ther

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Summary

Bispecific antibodies can provide new biology that cannot be achieved with combination therapies.

The aimed MoA should guide the design of the bsAb molecule, in particular the valency of targeting and target binding kinetics

Developability should be tested early with a battery of analyses that view the molecules from different perspectives.

Case study:

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.