VERIFICATION OF THE UNIQUE FUNCTIONALITY OF ATOR-1017 BY 3D STRUCTURE DETERMINATION

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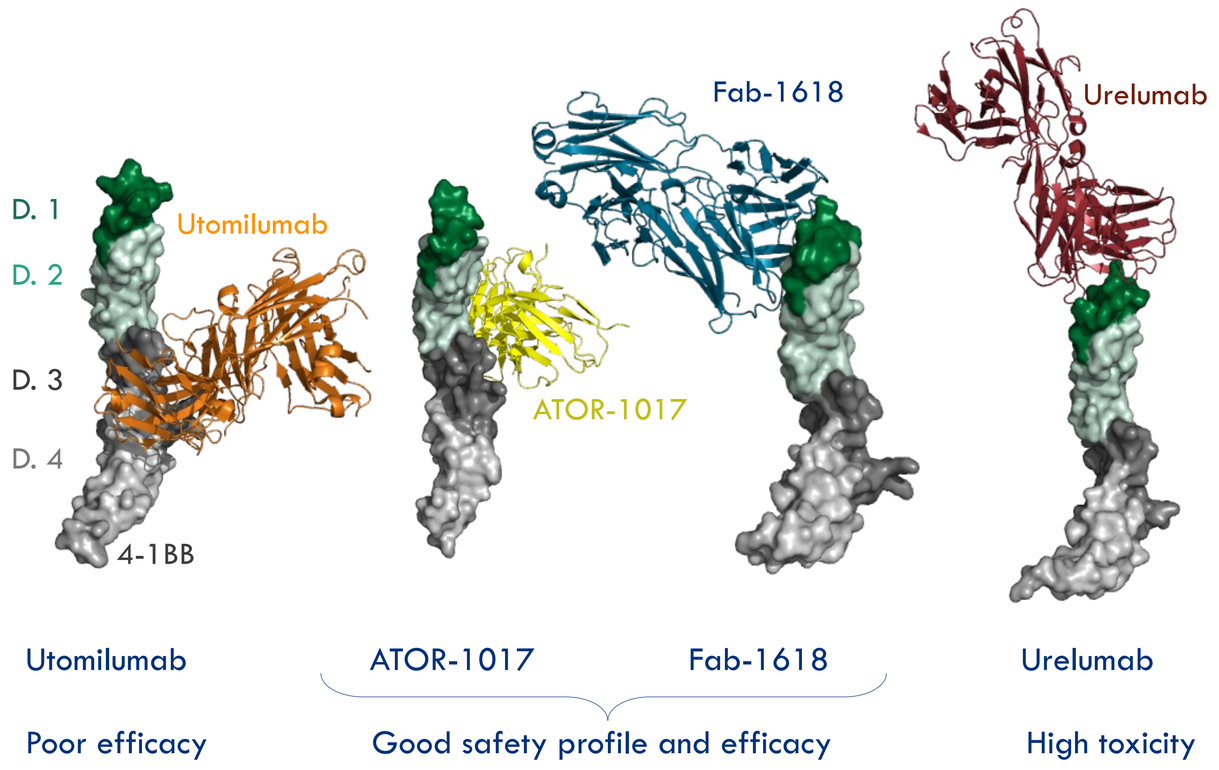
BACKGROUND

SARomics Biostructures is a contract research organization (CRO) that offer structure determination services. Alligator Bioscience is a biotechnology company that develops innovative tumor-directed antibody-based immunotherapies. SARomics and Alligator have worked together to determine the binding epitope of the antibody ATOR-1017 using X-ray crystallography.

ATOR-1017 is an agonistic antibody targeting the co-stimulatory receptor 4-1BB (CD137) developed to activate tumor specific T cells for immunotherapy of cancer. This specific antibody was designed and optimized at Alligator to address the limitations of the monospecific 4-1BB antibodies (Urelumab and Utomilumab), which have suffered from either being toxic to humans or having poor efficacy, respectively.
The main goal of the project has been to determine the binding epitope of the antibody ATOR-1017 using X-ray crystallography and to compare the epitope with those of Urelumab and Utomilumab that have very different profiles.

Two 4-1BB binders developed by Alligator were studied at SARomics using X-ray crystallography; ATOR-1017 now in clinical trials phase I as well as Fab-1618 which has a different binding profile.

BINDING COMPARISON



COMPLEX PREPARATIONS

Expression and purification of 4-1BB
4-1BB was expressed in mammalian cells with an Fc fusion tag. The protein was purified on protein A column before cleavage of the Fc-tag.

Preparation of Fab-1618
mAb 1618 was produced in mammalian cells. To prepare the Fab the mAb was cleaved with papain and purified on protein A column followed by a size exclusion chromatography step.

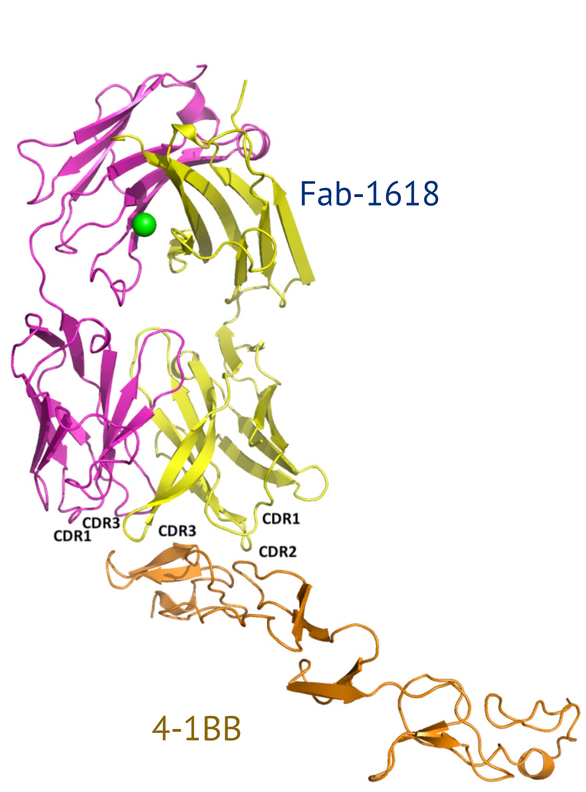
Preparation of ATOR-1017 scFv
ATOR-1017 scFv expressed in the periplasm of bacteria was purified on protein A column followed by a size exclusion chromatography.

Preparation of 4-1BB: fab complexes for structure determination
For complex formation 4-1BB was mixed with either

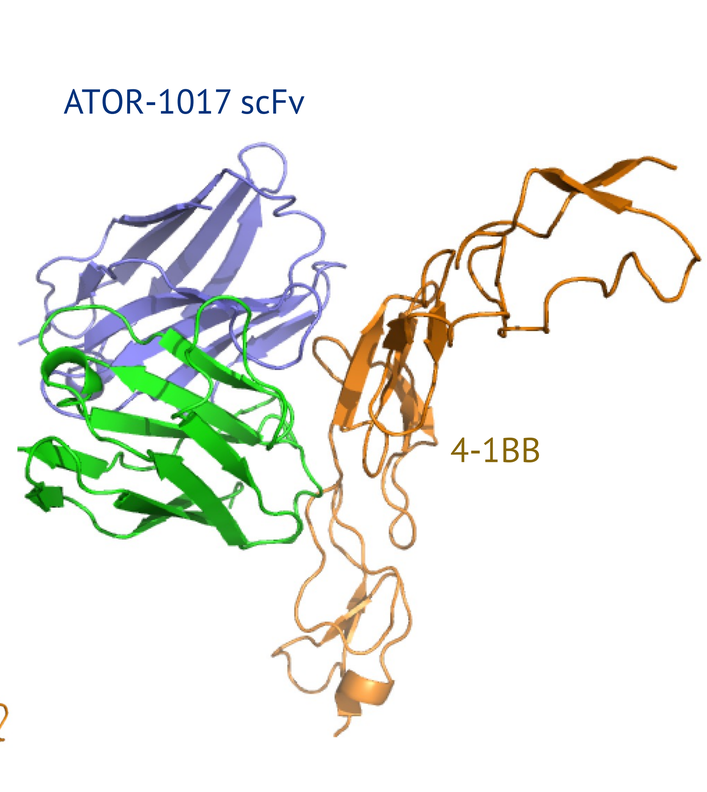
- Fab-1618 : Fab-1618 : 4-1BB
- ATOR-1017 scFv : ATOR-1017 scFv : 4-1BB
- Fab-1618 and ATOR-1017 scFv : Fab-1618 : 4-1BB : ATOR-1017scFv.

The complexes were purified on size exclusion and crystallization experiments were set up.

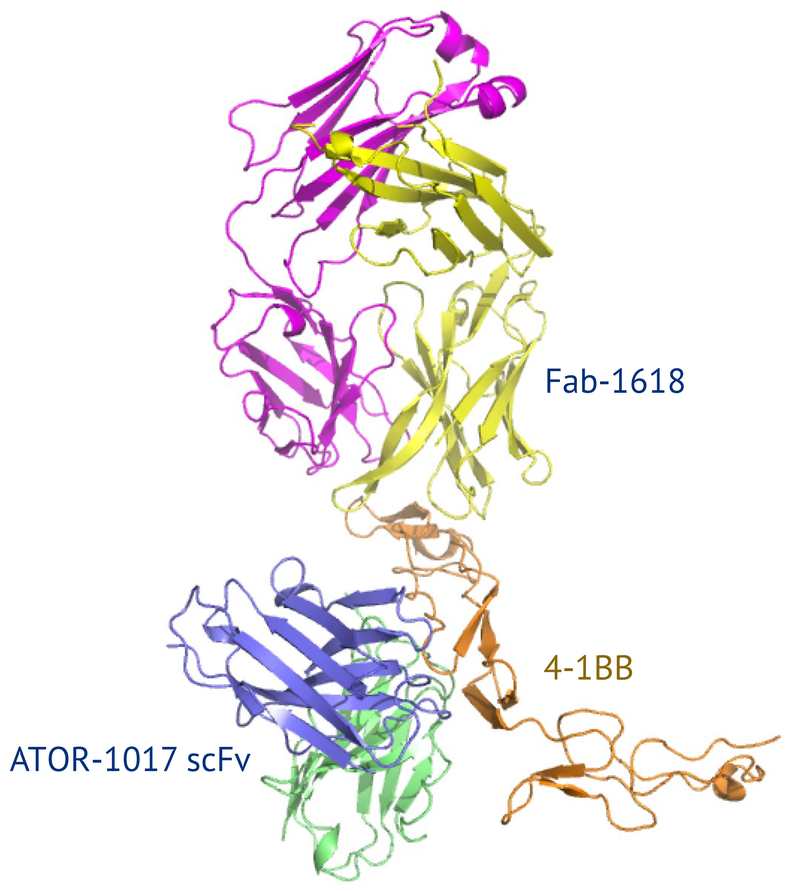
STRUCTURES



Fab-1618 : 4-1BB[1]



ATOR-1017 : 4-1BB[2]



ATOR-1017 : 4-1BB: Fab-1618

CONCLUSION

➢ Successful crystallization and structure determination of :
- Fab-1618 : 4-1BB at 2.3 Å
- ATOR-1017 scFv : 4-1BB at 3.1 Å
- Fab-1618 : 4-1BB : ATOR-1017scFv at 3.4 Å

➢ The obtained results:
- confirmed the binding of ATOR-1017 to a unique epitope on 4-1BB
- supported the advancement of ATOR-1017 in clinical studies.

References

[1] Nelson MH, et al. The Bispecific Tumor Antigen-Conditional 4-1BB x 5T4 Agonist, ALG.APV-527, Mediates Strong T-Cell Activation and Potent Antitumor Activity in Preclinical Studies. Mol Cancer Ther. 2023;22(1):89-101. doi:10.1158/1535-7163.MCT-22-0395

[2] Smith KE, et al. ATOR-1017, an Fc-gamma receptor conditional 4-1BB agonist designed for optimal safety and efficacy, activates exhausted T cells in combination with anti-PD-. Cancer Immunol Immunother (Submitted)

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