

## Verification of the unique functionality of ATOR-1017 by 3D structure determination

### THE INDUSTRIAL CHALLENGE

Alligator Bioscience (Alligator) is a biotechnology company that develops innovative tumor-directed antibody-based immunotherapies for unmet medical needs. ATOR-1017 is an agonistic antibody targeting the co-stimulatory CD137 receptor developed to activate tumor specific T cells for immunotherapy of cancer. This specific antibody has been developed by Alligator to address the limitations of other CD137 binding antibodies that have failed in clinical trials. Urelumab from BMS was shown to be toxic and utomilumab from Pfizer suffers from poor efficacy. A remaining need has been to define the binding epitopes of the antibody ATOR-1017, as well as a second CD137-binding antibody also developed at Alligator.

### WHY USING A LARGE SCALE FACILITY

One of the most established methods to gain molecular information in atomic detail is X-ray crystallography. Since laboratory-based X-ray sources lack high enough flux beam and pixel detectors, current data collection is exclusively performed at synchrotrons. In the case of protein-protein complexes the use of a synchrotron beam is an absolute must in order to get good enough data (2-3 Å). This resolution is needed to accurately trace the amino acid sequence and model the interface between the antigen and the CDR loops of the antibody.

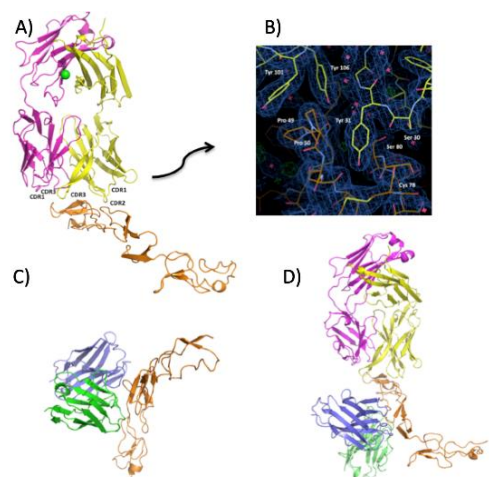
### HOW THE WORK WAS DONE

The results have been obtained in a collaboration between Alligator and SARomics Biostructures, a leading Swedish expert in the field of structural biology. The binding parts of the two antibodies and target protein CD137 were produced in mammalian or bacterial cultures. Protein complexes of antibody fragments bound to CD137 were purified and used to form crystals. Data were collected at the I03 and

I04 beamlines of Diamond Light Source, UK, and at the BioMAX beamline of MAXIV.

### THE RESULTS AND EXPECTED IMPACT

Two complexes of Fab (fr the second CD137 binding antibody) or scFv (fr ATOR-1017) fragments and CD137 were determined to 2.3 Å (fig. A and B) and 3.1 Å (fig. C) using molecular replacement. A third structure determined to 3.5 Å involved a complex of both antibody binding domains and CD137 (fig. D). The epitopes were compared with those of urelumab and utomilimab.



The structures show that two different ways of binding CD137 are possible for potent CD137 activating antibodies that are not super agonists. This shows the benefit of using crystallography when developing antibody drug candidates as the information increases the understanding on how drug candidates targeting CD137 can activate immune cells. The data also helps to differentiate Alligator's drug candidate ATOR-1017 from urelumab and utomilumab by demonstrating that ATOR-1017 binds to a unique epitope on CD137. The gained structural understanding may also aid the field in discerning the relationship between the binding site and agonistic activity of CD137-binding antibodies.



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**Vinnova's project No:** 2018-03295 **Duration:** October 2018 – March 2020

Funded by Sweden's Innovation Agency, Vinnova, in order to build competence and capacity regarding industrial utilisation of large-scale research infrastructures such as MAX IV and ESS.