| ESRF | **Experiment title:** Development of PD-1/PD-L1 interaction inhibitors | **Experiment number**: MX/2118 |
|---|---|---|
| **Beamline**: ID23-1 | **Date of experiment**: from: 30-09-2018 to: 01-10-2018 | **Date of report**: 05-12-2019 |
| **Shifts:** 3 | **Local contact(s)**: Christoph Mueller-Dieckmann | *Received at ESRF:* |

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**Report:**

**AIM**

The aim of the proposed research was to discover small-molecule antagonists which would potently antagonize the interaction between the 'so-called' immune checkpoint receptors, the programed cell death protein-1 (PD-1) and the programed cell death protein ligand-1 (PD-L1). PD-1 is expressed on T lymphocyte cells and PD-L1 on tumor cells. The binding of PD-L1 to PD-1 keeps T cells from killing tumor cells. Blocking the binding of PD-L1 to PD-1 with an (anti-PD-L1 or anti-PD-1) immune checkpoint inhibitor (ICI) allows the T cells to kill tumor cells. This new approach in the cancer treatment is called immunotherapy. Cancer immunotherapy has revolutionized cancer treatment during the last decade. This powerful strategy, of mobilizing the immune system to attack tumor cells, has shown impressive results with durable clinical antitumor responses and comprises a breakthrough in fight against cancer, even forcing formerly untreatable late stage tumors into complete remission. Immune checkpoint blockade (ICB) has been selected as a Breakthrough of the Year 2013 by Science. It is now clear that immuno-oncology has become the Fifth Pillar of cancer therapy alongside chemotherapy, surgery, radiation, and targeted treatments. Finally, in 2018 cancer immunotherapy became the basis of the highest scientific distinction, the Nobel Prize.

Current cancer immunotherapies involve the use of monoclonal antibodies (mAb) that selectively block the immune checkpoint receptors. However, monoclonal antibody therapy inherently carries a number of disadvantages including extremely high costs and the immunogenicity of mAbs. In contrast, small-molecule therapeutics can have prices, affinity and specificity features rivalling that of antibodies. Development of chemical inhibitors for the PD-1/PD-L1 pathway is far behind the antibody development (mostly because of insufficient structural information).

**ACHIEVEMENTS**

In total 110 protein crystals were tested. We had experienced a few mounting problems. 2 datasets were collected, best resolution reached 1.7 Å. Obtained data allowed to solve 1 structure with a scientific significance. Coordinates and structure factors were deposited in the Protein Data Bank under accession code PDB: 6SRU. The manuscript is in the preparation.

The achieved structure is the mouse homolog of the PD-L1 protein (mPD-L1). We decided to crystalize this protein additionally to human PD-L1 (hPD-L1)/inhibitors complexes. The differences between human and mouse structure could affect the interaction between mouse protein and small molecular inhibitors targeted to the human protein. The PD-L1 binding domain shows relatively low sequence identity (69.4%) and similarity (87.6%) between human and mouse species, indicating likely differences in the binding sites arrangement. During preparation for *in vivo* studies we decided to check cross-species activity of hPD-L1 targeted compounds and crystallized mPD-L1 in order to compare arrangement of mPD-L1 and hPD-L1 binding sites. Based on the results we discussed structural differences and their possible effect on inhibitors binding.

**REFERENCES**

manuscript in preparation:
**Working title:** Structural features of mouse and human PD-L1 determine druggability by therapeutic antibodies, peptides, and small molecules
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