University of Victoria | Graduate Studies

Notice of the Final Oral Examination
for the Degree of Doctor of Philosophy

of

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BSc (McGill University, 2011)

"Inhibitors of the PD1/PD-L1 Interaction: Missteps, Mechanisms and Mysteries"

Department of Chemistry

Thursday, March 1, 2018
1:30 P.M.
Elliott Building
Room 305

Supervisory Committee:
Dr. Jeremy Wulff, Department of Chemistry, University of Victoria (Co-Supervisor)
Dr. Fraser Hof, Department of Chemistry, University of Victoria (Co-Supervisor)
Dr. Alexandre Brolo, Department of Chemistry, UVic (Member)
Dr. Brad Nelson, Department of Biochemistry and Microbiology, UVic (Outside Member)

External Examiner:
Dr. David Vocadlo, Department of Chemistry, Simon Fraser University

Chair of Oral Examination:
Dr. Sally Brenton-Haden, Department of Education Psychology & Leadership Studies, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

The interactions of tumours with normal host tissue are key determinants of cancer growth and progression. The ability or inability of the patient's immune system to mount a response against the tumour is tightly correlated with prognosis. One of the ways tumours avoid detection and elimination by the immune system is by expressing programmed death ligand 1 (PD-L1). PD-L1 binds to its receptor programmed death 1 (PD1) on T cells, inhibiting T cell responsiveness to antigenic stimuli. Blockade of the PD1/PD-L1 pathway removes this negative signal and restores anti-tumour immunity. While this blockade of PD1/PD-L1 is well established through the use of antibodies, small molecule inhibitors of PD1/PD-L1 are relatively unknown.

We employed *in silico* docking in order to find small molecules capable of binding to either PD1 or PD-L1, and the highest-ranked compounds were tested in biophysical assays for their ability to inhibit PD1/PD-L1 binding. A thermal shift assay identified a pyrazole compound as a possible binding partner for PD-L1, but follow-up assays showed that it had no effect on the PD1/PD-L1 interaction and that its apparent binding was probably due to aggregation. An ELISA assay identified a tryptophan diamine compound as an apparent *stabilizer* of the PD1/PD-L1 interaction. However this compound, too, was later identified to be inactive in orthogonal assays.

We identified a family of salicylic acid derivatives that interfered with TR-FRET measurements – an unusual observation, given that TR-FRET is touted as being insensitive to most mechanisms of compound interference. This discovery should help other fragmentscreening groups identify false positives more easily.

We also probed the mechanism of inhibition of a recently-disclosed family of small molecule PD1/PD-L1 inhibitors from Bristol-Myers Squibb. Concurrently with other groups, we used protein NMR, size exclusion chromatography, and SPR to determine that the compounds were inducing homodimerization through the PD1-binding face of PD-L1. Furthermore, using cellular crosslinking and live cell imaging, we showed that these first generation inhibitors are fairly ineffective at inhibiting this interaction on the cell surface. More potent compounds will be needed to see any cellular effect from this mechanism of action.