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**Public Oral Examination**

**For the Degree of Doctor of Philosophy**

**Atsunori Kaneshige**

***“Development of a first-in-class STAT5 PROTAC degrader”***

**Thursday, April 21, 2022 at 9:00am**

**NCRC Research Auditorium**

**Committee Members:**

Dr. Shaomeng Wang (Chair)

Dr. Nouri Neamati

Dr. Jolanta Grembecka

Dr. Pavel Nagorny

**Abstract:**

In the last two decades, STAT5, (signal transducer and activator of transcription 5) has become an attractive therapeutic target due to its consecutive activation in a variety of cancers including chronic myeloid leukemia (CML) for which STAT5 activation is the most frequently reported.

STAT family proteins, namely STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6, contain common domains from N-terminus to C-terminus: the N-terminal domain, coiled-coil domain, src homology-2 (SH2) domain, linker domain, DNA-binding domain, and a transactivation domain. Upon phosphorylation at the C-terminus tyrosine residue on STAT5 by activated janus-activated kinase (JAK), phosphorylated STAT5 proteins reciprocally homo- or hetero-dimerize through the phosphorylated tyrosine residue and SH2 domain, followed by the translocation of STAT5 dimers to the nucleus to activate target genes.

It has been hypothesized that STAT proteins including STAT5 could be targeted by occupying the SH2 domain. In fact, intensive efforts to target STAT5 have been made especially in the past 20 years. Despite these efforts however, unfortunately only a handful inhibitors targeting STAT5 SH2 domain all with weak target-binding and elusive selectivity have been reported in the literature, underscoring the tremendous challenge in targeting STAT5 potently and selectively.

In this dissertation, I have begun to make a systematic effort to develop our own STAT5 inhibitors. Due to the lack of reported STAT5 inhibitors with high binding affinity and selectivity as potentially good lead compounds, I started with a STAT6 inhibitor reported by McMurray group on the basis of our hypothesis that STAT6 inhibitors may be able to bind STAT5, since STAT6 and STAT5 proteins have 44% sequence identity. Even though our initial idea compound had only a very weak binding affinity to STAT5 (Ki: >10 µM) and a moderate binding affinity to STAT6 (Ki: 5.4 µM), with the help of computational modeling and many idea trials in chemistry, we were able to obtain a set of inhibitors with much improved binding affinities to STAT5 (Ki: 0.7 to 2 µM) and better binding affinity to STAT6 (0.1 to 0.5 µM). However, selectivity turned out to be particularly challenging to achieve toward STAT5 over STAT6 because of their structural similarity in the SH2 domain. Furthermore, our best STAT5 inhibitor failed to show cell growth-inhibitory activities in representative CML cells with activated STAT5, which was expected given that previously reported high affinity STAT3 inhibitors had only a minimal effect on tumor cell growth. Nonetheless and gratifyingly, we solved the first co-crystals of our STAT5 inhibitors in complex with STAT5A showing detailed interactions between inhibitors and surface residues. This success gave us a solid basis to develop STAT5 PROTAC degraders.

In this dissertation, I report first-in-class, selective, and efficacious STAT5 degraders using our own STAT5 inhibitors and cereblon ligand, with AK-2292 being the best among those tested. PROTAC technology transformed our non-selective and ineffective STAT5 inhibitors into a selective and effective STAT5 degrader *in vitro* and *in vivo*. Unbiased proteomics analysis and RNA-seq analysis with representative CML cells point to AK-2292’s excellent selectivity in proteome and transcriptome. AK-2292 shows strong cell growth-inhibitory activities in CML cells with activated STAT5 and significantly inhibited tumor growth in multiple tumor xenograft models where AK-2229 potently depleted STAT5 as well as phosphorylated STAT5 in tissues.

This research provides a first-in-class selective and efficacious STAT5 degrader, AK-2292, which serves as an excellent tool compound in the study of STAT5. Importantly, this research provides a firm starting point for the development of therapeutics for diseases where STAT5 plays a critical role in their pathogenesis and beyond.

#### Manuscript Under Review:

“A Potent and Selective Small-Molecule STAT5 PROTAC Degrader Capable of Achieving Tumor Regression *In Vivo*” **Atsunori Kaneshige**,Longchuan Bai,Mi Wang, Donna McEachern, Jennifer Lynn Meagher, Renqi Xu, Wei Jiang, Yu Wang, Hoda Metwally, Paul Kirchhoff, Hui Jiang, Bo Wen, Duxin Sun, Jeanne Stuckey, and Shaomeng Wang

**Manuscript Ready to be Submitted:**

“Structure-Guided Discovery of a Potent, Selective, and Efficacious STAT5 PROTAC Degrader” **Atsunori Kaneshige**, Longchuan Bai, Mi Wang, Donna McEachern, Jennifer Lynn Meagher, Renqi Xu, Wei Jiang, Yu Wang, Hoda Metwally, Paul Kirchhoff, Bo Wen, Duxin Sun, Jeanne Stuckey, and Shaomeng Wang

**Patents Filed:**

1) STAT5 and STAT6 inhibitors and uses thereof

2) STAT5 and STAT6 degraders and uses thereof

**Future Plans:**

Nori will continue to work on his other major projects in Dr. Shaomeng Wang’s lab as a postdoctoral researcher.