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POSTER ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

Discovery of First-in-Class Small Molecule Inhibitors of the IRF4-PU.1/Spi-B Interaction

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Introduction

Many transcription factors (TF) are promising therapeutic targets in hematological malignancies but have been *mislabeled* as 'undruggable' due to their dynamic structures and lack of feasible paths for bioactive small molecules. Multiple myeloma (MM), the second most common hematological malignancy, is predominantly driven by oncogenic transcription factors such as c-Myc & interferon regulatory factor 4 (IRF4). Attempts at targeting IRF4 have relied on immunomodulatory drugs (IMiDs), such as lenalidomide and pomalidomide, which target upstream TFs of IRF4 - IKZF1 & IKZF3. Unfortunately, IMiDs are highly susceptible to resistance mechanisms in the clinic and fail to provide deep and sustained downregulation of IRF4 due to theirindirect mechanism of action. Currently, no known small molecules that **directly** bind and inhibit IRF4 exist. Moreover, no probes or screening based assays have been developed for IRF4 or any member of the IRF family.IRF4 is the top ranked dependency of MM and is a validated clinical target given the success of IMiD-based regimens. Thus, orally available small molecules that can directly target IRF4 will have significant benefit for patients with MM.

Methods & Results

To identify small molecules that inhibit or bind IRF4, we first cloned, expressed, and purified numerous constructs of the interferon accessory domain (IAD - residues 238-420) and full-length IRF4 (residues 1-451). To enable traditional high-throughput screening (HTS) campaigns, we first identified peptide ligands of IRF4 based on the IRF4 - PU.1/Spi-B interaction, an oncogenic signaling node in various hematological malignancies. We profiled peptides derived from PU.1 and Spi-B and confirmed binding to the IAD domain with K_ds of 1 - 7 μM by isothermal titration calorimetry (ITC) and fluorescence polarization (FP). Encouraged by the robust signal seen with our FP assay, we further developed a TR-FRET based method using a CoraFluor labeled IAD domain which significantly boosted our signal-to-noise ratio for our HTS efforts ($Z' > 0.8$). We screened this interaction against the Chembridge2020 drug library, which consists of 50,000 lead-like small molecules. The screen afforded 68 small molecules that had inhibition > 50% by TR-FRET (hit rate of ~0.1%). We prioritized our most potent compound, **HIT-1**, which has a TR-FRET IC_{50} of 2 μM. Further binding validation including ITC, biotin-based pull-downs in human myeloma cell lines (HMCLs), and a BRET based target engagement assay confirm binding to both recombinant and full-length IRF4. Additionally, medicinal chemistry optimization led to the development of **1**, with sub-micromolar affinity to IRF4, and an inactive control **2**. Biological assessment of compounds **1** and inactive control **2** are underway in HMCLs; initial results show on-target modulation of IRF4 signaling processes. Compound **1** represents the first validated small molecule targeting the 'undruggable' IRF family and warrants its use in investigating the IRF4-PU.1/Spi-B interaction in oncology and immunology.

Conclusions

These small molecules are the first inhibitors of the IRF4-PU.1 interaction. We have developed novel biochemical assays that can be applied to other IRF family members, including IRF8. Additionally, we showcase translatable strategies to build novel biochemical assays for other transcription factors, leveraging known PPIs for probe development. These ligands represent starting points for hetero-bifunctional compounds and chemical probes for modulating IRF4 activity both *in vitro* and *in vivo*.

Disclosures Payne: *CoraFluor:* Patents & Royalties: Inventor on patent applications related to the CoraFluor TR-FRET probes. **Mazitschek:** *CoraFluor:* Patents & Royalties: Inventor on patent applications related to the CoraFluor TR-FRET probes. **Qi:**

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