Discovery and Characterization of Covalent BET Bromodomain Inhibitors

Olesya A. Kharenko, Reena G. Patel, David Brown, Peter R. Young, Ravi Jahagirdar, Eric Campeau, Sarah Attwell, Emily M. Gesner, Karen Norek, Cyrus Calosing, Laura Tsujikawa, Sanjay Lakhotia, Henrik C. Hansen

Zenith Epigenetics Corp

BET bromodomain proteins are epigenetic readers which interact with the acetylated lysine (AcLys) residues of histones and transcription factors. Recently it has been shown that disruption of this interaction with small molecule BET bromodomain inhibitors is a promising approach to treat various disease states including cancer, inflammation, autoimmune and cardiovascular diseases.

Covalent inhibitors and covalent drugs offer several advantages including increased duration of action, more durable target modulation, and potential pharmacological effect at lower concentrations. To demonstrate that covalent inhibition is a promising approach to target BET proteins, we rationally designed and developed a series of covalent BET inhibitors. Using biochemical AlphaScreen assay we demonstrate that covalent BET inhibitors potently and selectively disrupt the interaction of BET bromodomains with AcLys histone peptides. Additionally, we show that the covalent interaction with BET bromodomains is time-dependent and can be monitored by thermodenaturation assay as a distinct peak from the non-covalent inhibitors. The covalent bond formation with BRD4 was further confirmed by high resolution crystal structure and MALDI spectrometry. In cellular assays, the covalent inhibitors potently inhibit proliferation and induce apoptosis in cancer cell lines. Importantly, the covalent inhibition of the BET proteins exhibits a sustained pharmacodynamic effect in washout experiments as demonstrated by enhanced inhibition of proliferation, decreased mRNA expression of several BET-dependent genes compared to reversible BET bromodomain inhibitors.

In summary, the first generation of the covalent inhibitors demonstrated promising durable target modulation which further prompted the development of a second generation of covalent inhibitors with good drug-like properties for future oncology applications.