Fragment-Based Screening Approach Reveals Non-Orthosteric Pockets in The Search for Allosteric Inhibitors Of Tau-Tubulin Kinase 1

Robert P Hayes[1], Edward DiNunzio[2], Mahdieh Yazdani[3], Justyna Sikorska[2], Yili Chen[2], Sriram Tyagarajan, Younghee Park[2], Amy Lee[3], Cesar Reyes[3], Daniel Burschowsky[4], Matthias Zebisch[4], Yangsi Ou[3], Marina Bukhtiyarova[3], Shahriar Niroomand[3], Yuan Tian[3], Shawn Stachel[3], Hua Su[3], Jacqueline D Hicks[2], Daniel F Wyss[2]

[1]Merck, Boston, [2]Merck, Kenilworth, [3]Merck, West Point, [4]Evotec, Abingdon

robert.hayes@merck.com

Allosteric ligands of kinase drug targets have promise in exhibiting higher selectivity than ATP-site-directed inhibitors. Here, we employed a fragment-based screening strategy that leveraged biophysical, computational, and crystallographic approaches, in parallel, to identify a series of allosteric pockets for tau tubulin kinase 1 (TTBK1). Initial X-ray screening of ~100 promiscuous or brominated fragments capable of generating strong anomalous peaks were utilized to probe the ligandability of the TTBK1-kinase domain and revealed several allosteric pockets of interest. Encouraged by these results, screening of an in-house 2,000 fragment library (molecular weight < 230) was conducted by surface plasmon resonance (SPR) in either the absence or presence of a known TTBK1 orthosteric kinase inhibitor, yielding 183 potential allosteric fragment hits. Subsequently, a broader secondary X-ray screen was conducted on the 183 SPR assay hits, supplemented with compounds from virtual screening efforts, on pockets identified in the primary X-ray screen. Of the 431 fragments investigated in X-ray, Pan-Dataset Density Analysis (PanDDA) identified 181 events of interest across 15 different binding sites, with increased confidence towards those fragments binding at or near the orthosteric site. Manual inspection with detailed analysis prioritized 27 X-ray hits to 10 allosteric sites for biophysical and biochemical evaluation, only 2 of which had been predicted through computational pocket analysis. 5 of those identified allosteric pockets are discussed in terms of their respective potential to provide kinome selectivity for TTBK1 or isoform selectivity over TTBK2. Coupling biophysical screens with high-throughput structural biology methods that leverage automated data collection and fragment identification by PanDDA may serve as a useful strategy to identify allosteric ligands to other targets across the kinome.