High-Throughput Screening (HTS) Cascade to Generate Selective and High-Value Hits for PIM Kinase.

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The proto-oncogene Proviral Integration site for Moloney murine leukemia virus 3 Kinase (PIM3K) is a protooncogene serine/threonine-protein kinase involved in numerous cancers. Identification of new PIM kinase inhibitor scaffolds is therefore highly valuable for the development of new anticancer therapies.

Kinase discovery efforts are usually particularly challenging, because of conserved active sites and high structural homology between kinases leading to safety liabilities. Here we present a comprehensive and robust screening cascade to rapidly identify selective PIM3 kinase inhibitors from our proprietary libraries. Moreover, subsequent to primary screening, novel PIM3K inhibitors can be profiled in analogous selectivity assays and confirmed in both orthogonal and cellular target engagement (TE) assays.

High quality, recombinant, active PIM3K was produced in-house to develop a robust high throughput ADP-Glo™ primary assay used to screen Eurofins Discovery's Diversity Focused and Hinge Binder libraries (73,000 compounds). High-quality data sets were obtained (Z' = 0.86 ± 0.05, n=211; SNR = 35.1 ± 2.7) with a hit rate of 1.54% enabling the selection of 1,000 hits (946 confirmed in ADP-Glo dose response curves, exhibiting pIC_{50} from 3.4 to >7.5). Those hits are in the process of being confirmed in both a PIM3 cellular NanoBRET™ TE assay (Z' > 0.6, SNR 5-6), and a mass spectrometry assay monitoring of site-specific hosphorylation events. Further triaging will take place employing Eurofins Discovery's proprietary KinaseProfiler™ kinase activity and KINOMEscan® active site-directed competition binding assay platforms to investigate their selectivity against PIM1 & PIM2 kinases (IC50 & KD) as well as the whole kinome.

Overall, a robust screening cascade has successfully been established in order to deliver highly selective and tractable kinases inhibitors in just a few short weeks. In doing so, this ensures that subsequent Hit-to-Lead and Lead optimization programs can rapidly and efficiently generate safe pre-clinical candidates.