The LifeArc Index Set: Utility for Assessing Target Tractability and Performance in a Phenotypic Screen.

Afrah Sattikar, Barbara Saxty, Kristian Birchall, Andy Merritt, Catherine A. Kettleborough
LifeArc, The Accelerator, Open Innovation Campus, Stevenage, SG1 2FX, UK

Introduction

The LifeArc compound collection comprises ~150,000 small drug-like compounds. These have been purchased over a number of years from a variety of commercial suppliers and comprise both diversity and target focussed sets, which are shared widely with academic screening groups. To meet the needs of medium through high throughput academic labs with limited automation we compiled an index set of ~12,000 compounds, representative of the full collection to permit screening of primary cells or complex assays (Figure 1). We review the hit performance for the index set screens completed to date against a broad range of target based screens and demonstrate its predictability for hit rate against the larger collection and utility for assessing target tractability using two-potassium channels as an example. Its utility for phenotypic screening using complex imaging based assays is also exemplified.

Index Set Screening Performance

The Index Set has been screened broadly across targets and cell-based screens, both internally and through library sharing with the academic community. 33 screens have been completed and hit follow up including analogue testing in dose response mode have been reported with screening performance illustrated in Figure 2.

Predicting Ligandability using the Index Set

The Index Set is a snapshot in time of our collection, which we regularly refresh and replace. We performed a retrospective analysis of our historical screens to demonstrate if screening the Index Set would be predictive of the outcome of screening our full collection.

Case Study 1: Phenotypic Screening for TDP-43

The presence of aggregates of ubiquitinated, misfolded and hyperphosphorylated transactive response DNA-binding protein of 43 kDa (TDP-43) is a hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTD) alongside implications in other neurodegenerative diseases. ASSAY: Aggregate formation was induced for 24h in HEK293 cells expressing an inducible fluorescently tagged TDP-43 construct containing 12Q/4Q repeats. Cells were then exposed to compounds from the Index Set and the Pharmacologically active set for 48h. Effects of these compounds were assessed by high content imaging on live cells looking at total number of aggregates/well, total number of cells/well and TMRM intensity (a measure of mitochondrial toxicity).

Case Study 2: Assessing Target Tractability for K2P Channels

Two-pore domain potassium channels (K2P) are characterised by their four transmembrane domain, two-pore topology. These channels carry background or shift potassium current in a variety of cell types and primarily act to maintain resting membrane potential.

K2P channels are implicated in various pathophysologies but have proven challenging to drug. ASSAY: Thalidomide flux assays to measure K2P channel function were developed using U2-OS cells transduced with Bach2m to generate cells expressing functionally active K2P channels. Using an initial representative group of channels (THIK1, TWIK1, TREK2, TASHK and TASK2) this system was used to screen the LifeArc Index Set to enable identification of functional channel activators.

Conclusions

• The Index Set (variable sizes) is designed to be representative of the complete LifeArc collection of ~150,000 small drug-like compounds.
• Retrospective analysis indicates screening of the Index set is predictive of hit rates in the larger collection.
• Case studies exemplify the value of the Index set for phenotypic screening and assessing target tractability.

REFERENCES