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

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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 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-pore potassium channels as an example. Its utility for phenotypic screening using complex imaging based assays is also exemplified.

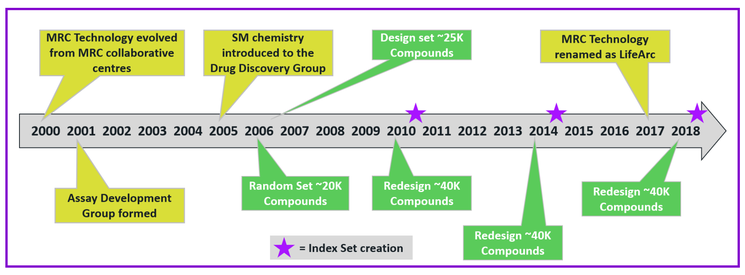

Figure 1. Timeline for Building the LifeArc Compound Collection. The diversity set comprises the largest proportion of the LifeArc compound collection. It is periodically updated to capture novel regions of commercially available chemical space as indicated in the timeline. The Index set was created as a representative set from the LifeArc collection to enable rapid hit follow up and SAR expansion following screening.[1] The first iteration of the Index set (~9,000 compounds) was built following the 2010 collection enhancement; a subsequent rebuild following the 2014 diversity collection development increased this set to around 12,000 compounds.

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.

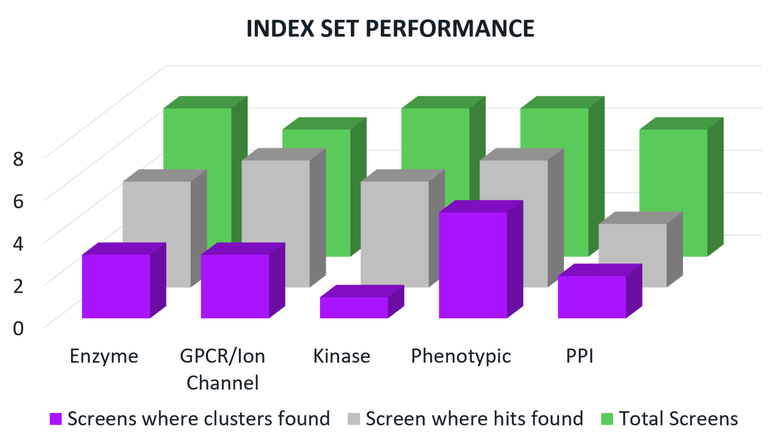

Figure 2. Index Set Performance in LifeArc and Collaborator Screens. Data have been loosely categorized across 5 target classes. Good tractability of confirmed hits and preliminary SAR were found when the Index set was screened in cell-based assays (GPCR/ ion channels) and phenotypic screens.

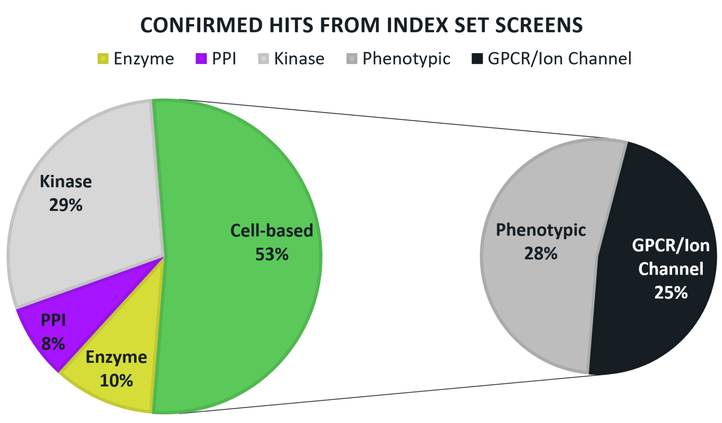

Figure 3. Confirmed Hits from Index Set Screens. In total 476 compounds (~5% of the Index set) have been reported as confirmed actives in one or more screens across the 5 target classes. 53% of confirmed actives have come from cell-based assays & phenotypic screens.

- Hits were found across all target classes screened. Following hit expansion a third are found to fall into active clusters that demonstrate the potential for further optimisation.
- The Index set has been most productive in cell-based & phenotypic screens. The hits found have provided chemical starting points from which chemistry programs have been initiated and progressed.

Predicting Ligandability using the Index Set

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

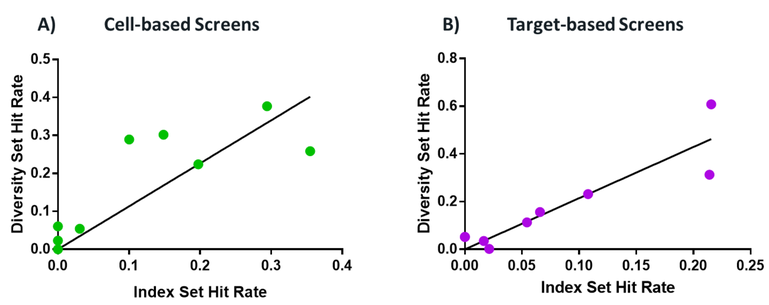

Figure 4. Retrospective analysis of Index Set Screens. 18 HTS campaigns were investigated: 9 cell-based (receptor or ion channel) and 9 target-based (enzyme or PPI). Confirmed hit rates from full screens and subset of Index compounds present in the screening data were compared. Confirmed hits from the Index set were found in 14/18 screens.

- 1/18 full HTS screens failed to find any confirmed hits and several protein target based screens had very low hit rates indicative of poor ligandability.
- Hit rates from both screen types correlated, validating the selection of Index compounds as representative of the active chemical space and a good predictor of ligandability from the full diversity set.
- We propose screening of the Index set is predictive of hit rates in the larger collection and can be used as a measure of tractability of a target.

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 (FTLD) along with 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/N 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).[2]

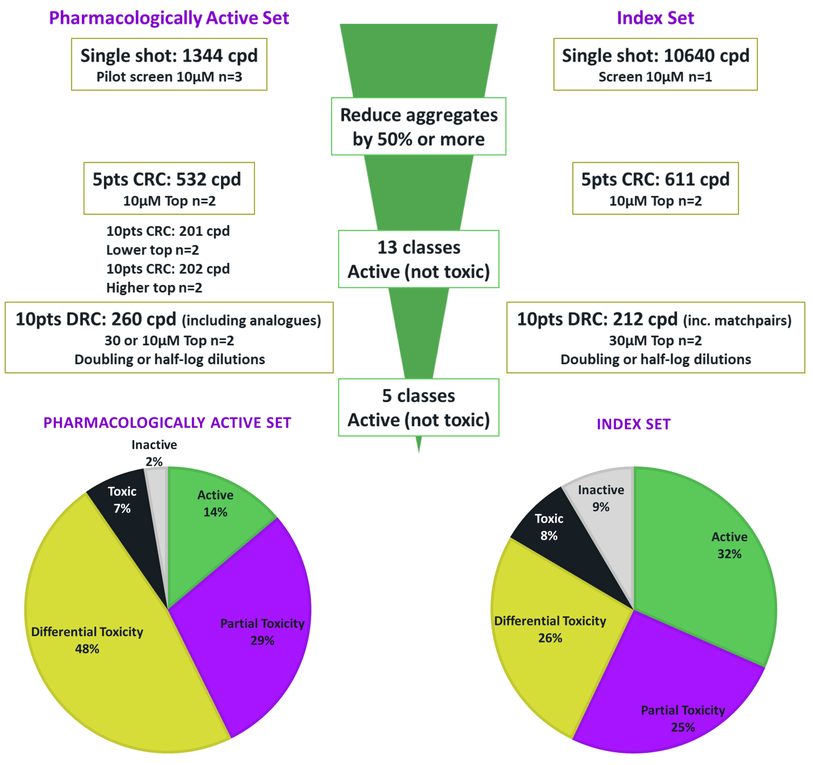

Figure 5. Screening & Hit confirmation for the TDP-43 Phenotypic Screen. Following screening and hit confirmation, five classes of compounds were identified from the Index set and from the pharmacologically active set.

- The Index set identified a higher proportion of active compounds (32%) which induced aggregate clearance in a dose dependent manner without affecting other cell physiology parameters.
- Subsequent work investigating restoration of TDP-43 function as part of the screening cascade increased confidence that the Index set hits identified are target specific and exemplify the utility of using the Index set for phenotypic screens.

Case Study 2: Assessing Target Tractability for K2P Channels

Two-pore domain potassium channels (K2Ps) are characterised by their four transmembrane domain, two-pore topology. These channels carry background (or leak) potassium current in a variety of cell types and primarily act to maintain resting membrane potential.

K2P channels are implicated in various pathophysiologies but have proved a difficult target class to modulate with small molecules.

ASSAY: Thallium flux assays to measure K2P channel function were developed using U2-OS cells transduced with BacMam to generate cells expressing functionally active K2P channels. Using an initial representative group of channels (THIK1, TWIK1, TREK2, TASK3 and TASK2) this system was used to screen the LifeArc Index set to enable identification of channel activators.[3]

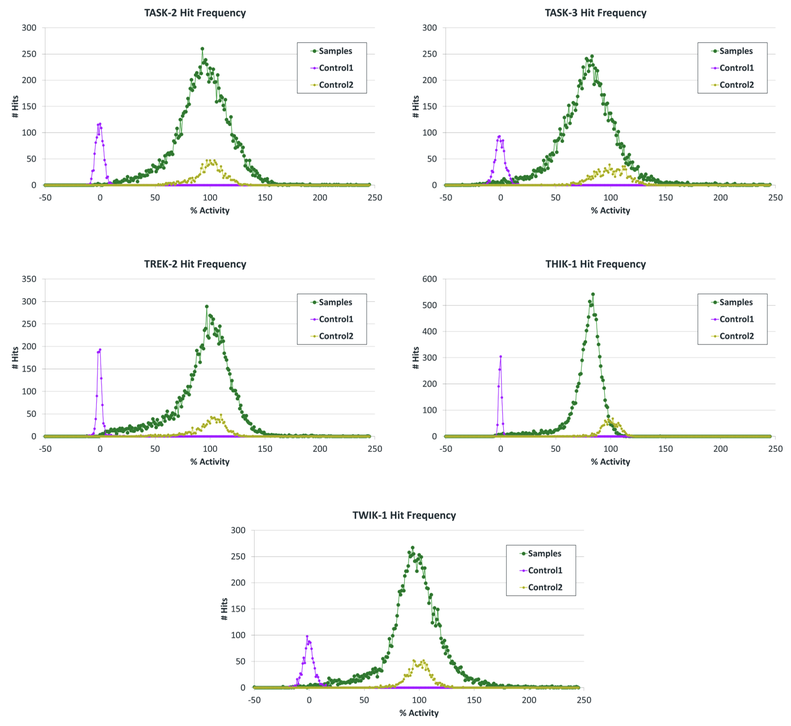

Figure 6. Summary of K2P Thallium Flux Screening Data. Graphs show number of compounds at each % Activity. 'Samples' denotes 11k compounds from LifeArc Index set. 'Control1' is low control (inhibitor), 'Control2' is high control (DMSO). All responses calculated relative to low (0)% and high (100%) controls.

K2P	Activity Cut-Off	#Hits	Hit Confirmation
TASK-2	149%	113	43
TASK-3	160%	111	80
TWIK-1	169%	114	79
TREK-2	220%	120	85
THIK-1	135%	6	2

Table 1. Summary of LifeArc Index Set Screen Against a Panel of K2P channels. For each screen the number of compounds for progression was set at ~1%, giving a variable activity cut-off. Hit confirmation n=2.

- 4/5 classes of K2P channels screened generated hit matter with confirmed activity. The low hit rate for THIK-1 gives an indication of low tractability for this class of K2P channels.
- Novel K2P activators were found and further profiling of hits demonstrated that these activators showed selectivity at K2P channels (data not shown).
- Efforts are underway to generate more novel hit matter by screening the entire compound collection against the four tractable classes.

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

1. Birchall, K. et al. (2018) 'Design of the LifeArc Index Set and Retrospective Review of Its Performance: A Collection for Sharing', SLAS DISCOVERY: Advancing Life Sciences R&D. [epub]
2. Bureau, Emilie A. et al. (2017) 'Phenotypic High-Content Screen Measuring Clearance of TDP-43 Aggregates', Alzheimer's & Dementia, Volume 13, Issue 7, Supplement, 2017, Page P292.
3. McCoull D., Ococks E., Wright P D. (2018) 'Development of a Novel Screening System to Identify Activators of Two-Pore Domain Potassium Channels (K2Ps)', Poster session presented at SLAS2018 International Conference and Exhibition, San Diego.

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