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Molecular availability and accessibility - measuring and understanding cellular drug concentrations

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Introduction: Currently, 70% of drug discovery targets at GSK are intracellular, but limited ability to accurately determine intracellular concentration poses a significant challenge. We have been investigating new techniques to determine compound distribution in cells, early in drug discovery. We have developed a Cellular Concentration Assay Platform for better understanding of the differences between compound behaviour in biochemical and cellular assays, which can often be challenging.

Methods: ~$4x10^6$ HeLa cells are incubated with 20uM compound for 2.5 hours. Several cell washes cells are completed and the cells are ruptured with MPER. After centrifugation, the supernatant is split into two samples. One sample is directly analysed by RapidFire MS/MS to give the total compound concentration in the cells. The other sample is subjected to dialysis using Rapid Equilibrium Dialysis plates and then again analysed by RapidFire MS/MS to determine the free concentration. Data is represented as P**Δ**C (pDC), free concentration (Fu) and cellular drug efficiency (CDE).

Results: Correlations can be split via PFI ((Chrom log D7.4) + (# of aromatic rings)) or acid base class. There is no correlation between PAMPA (parallel artificial membrane permeability **assay**) and pDC (r^2 0.02). A positive correlation between IAM (Immobilised Artificial Membrane) assay and pDC (r^2 0.42 - 0.57). However, there is a negative correlation between IAM and Fu (r^2 0.12 - 0.8). When comparing the corrected free concentration (Fic), polar compounds demonstrate a positive correlation and lipophilic give a negative correlation on the same plot. pDCs of different cell types correlated well (r^2 0.62-0.97). The Hela pDCs were specifically correlated with MDCK cells to investigate PGP substrates (r^2 0.78-0.94).

Conclusions: Correlations of different cell types indicate Hela cells are representative of many other cell lines. Through this analysis, we have a better understanding of compound lipophilicity and cellular drug efficiency. A PFI of ~2 gives a good cellular drug efficiency. This understanding will help us optimise compound properties early in the drug discovery process and reduce attrition.

References:

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