Moving beyond Molecular Obesity and Potency as addictions in Drug Discovery

Mike Hann
Chemical Sciences – MDR/PTS
GSK Medicines Research Centre
Stevenage, UK
Mike.M.Hann@gsk.com
Outline

– Brief recap on what we have (re)learnt and embedded
  – Physchem props PFI, solubility, dose
– What’s new in our thinking and experiments and what might just be useful in the future!
  – Cellular concentration and disposition
  – Imaging
Where have we come from & what sort of works for us?

The expanding “sciences” of Medicinal Chemistry and drug discovery
The curse of Molecular Obesity

– The tendency for drug discovery molecules to become too large and too lipophilic for their own good during lead optimisation through the quest for potency and specificity.

– It presents a high risk to the future “health” of the compound as a drug candidate.

– As with medical obesity, which is measured by Body Mass Index BMI, we now make use of indices such as Ligand Efficiency Index LE and Lipophilic Ligand Efficiency Index LLE to help identify and control the problem.

– But why do we make obese molecules?

---

The link of potency and molecular obesity - cont’d?

Typical order of events in a drug discovery screening cascade

1. Biochemical assay
2. Cellular assay
3. *in vivo* assay

– We often start with **isolated protein** in a biochemical assay with none of the environment of more phenotypic assays to help balance the physicochemical properties.

– We look for early signs of **cellular potency** in our screening cascades - this needs both some intrinsic potency and cellular penetration.

  – Both of these are very easily driven by increasing logP.
  
  – Once we get cellular activity the damage may already be done if we do not revisit our molecules to look at how we got there.
Is MW or logP the source of promiscuity?

Graph showing series of pie charts in different cLogP and MW bins for a set of approximately 2500 compounds tested in more than 490 assays. The size of each pie chart represents the average number of hits for compounds in that pie, where a hit is defined as a pXC50 value of 5 or higher. The colours indicate the proportion of compounds within each pie having particular numbers of hits (red: <5; blue: 5-15; yellow: 15-25; black: >25), where a hit is defined as activity greater than 10mM in any of the ~490 assays.

Property Forecast Index PFI – a useful overall guide to where to look for developable compounds

**TABLE 2**

Percentages of compounds achieving defined target values in the various developability assays categorised by PFI or iPFI bins

<table>
<thead>
<tr>
<th>Assay / target value</th>
<th>&lt;3</th>
<th>3-4</th>
<th>4-5</th>
<th>5-6</th>
<th>6-7</th>
<th>7-8</th>
<th>8-9</th>
<th>9-10</th>
<th>&gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility &gt;200 μM</td>
<td>89</td>
<td>83</td>
<td>72</td>
<td>58</td>
<td>33</td>
<td>13</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>%HSA &lt;95%</td>
<td>88</td>
<td>80</td>
<td>74</td>
<td>64</td>
<td>50</td>
<td>30</td>
<td>17</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>2C9 pIC50 &lt;5</td>
<td>97</td>
<td>90</td>
<td>83</td>
<td>68</td>
<td>48</td>
<td>32</td>
<td>23</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>2C19 pIC50 &lt;5</td>
<td>97</td>
<td>95</td>
<td>91</td>
<td>82</td>
<td>67</td>
<td>52</td>
<td>42</td>
<td>42</td>
<td>56</td>
</tr>
<tr>
<td>3A4 pIC50 &lt;5</td>
<td>92</td>
<td>83</td>
<td>80</td>
<td>75</td>
<td>67</td>
<td>60</td>
<td>58</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td>Clint &lt;3 ml/min/kg</td>
<td>79</td>
<td>76</td>
<td>68</td>
<td>61</td>
<td>54</td>
<td>42</td>
<td>41</td>
<td>39</td>
<td>52</td>
</tr>
<tr>
<td>Papp &gt;200 nm/s</td>
<td>20</td>
<td>30</td>
<td>46</td>
<td>65</td>
<td>74</td>
<td>77</td>
<td>65</td>
<td>50</td>
<td>33</td>
</tr>
</tbody>
</table>

iPFI = mChrom log P + #Ar

*Colouring refers to the % chance of achieving benchmark value in that PFI bin: green, >57%; yellow, 34–57%; and red, <33%.*

Sweet spot for permeability is in conflict with other desirable properties!

Ask yourself where your hits and lead optimisation compounds fit on this plot?!!
An analysis of the attrition of drug candidates from four major pharmaceutical companies

Michael J. Waring¹, John Arrowsmith², Andrew R. Leach³, Paul D. Leeson³,⁴, Sam Mandrell², Robert M. Owen⁵, Garry Pairaudeau¹, William D. Pennie⁶,⁷, Stephen D. Pickett³, Jibo Wang⁸, Owen Wallace⁸,⁹ and Alex Weir²

Abstract | The pharmaceutical industry remains under huge pressure to address the high attrition rates in drug development. Attempts to reduce the number of efficacy- and safety-related failures by analysing possible links to the physicochemical properties of small-molecule drug candidates have been inconclusive because of the limited size of data sets from individual companies. Here, we describe the compilation and analysis of combined data on the attrition of drug candidates from AstraZeneca, Eli Lilly and Company, GlaxoSmithKline and Pfizer. The analysis reaffirms that control of physicochemical properties during compound optimization is beneficial in identifying compounds of candidate drug quality and indicates for the first time a link between the physicochemical properties of compounds and clinical failure due to safety issues. The results also suggest that further control of physicochemical properties is unlikely to have a significant effect on attrition rates and that additional work is required to address safety-related failures. Further cross-company collaborations will be crucial to future progress in this area.
Cross company analysis of current status of physchem props

Figure 2 | Reasons for failure. a | Primary cause of failure for terminated compounds. b | Differences in the cause of failure for the first half (2000–2005) and second half (2006–2010) of the decade. c | Differences in the cause of failure in preclinical, Phase I and Phase II development.
candidates (mean calculated logP = 3.2) towards drugs (mean calculated logP = 2.8), it is questionable whether this would significantly reduce development attrition, based on our findings.

Conclusions
The data set collected and analysed in this study is the largest compiled so far for the purposes of linking attrition to simple molecular properties. We continue to believe that control of physicochemical properties during compound optimization is highly beneficial in identifying compounds of candidate drug quality. This study extends the analysis to their subsequent performance in development and assesses, for the first time, a link to various reasons for failure in development. The most notable finding from this data set is the difference in lipophilicity between compounds failing owing to clinical safety in Phase I and those successfully progressing to Phase II.

It is clear, however, that fully understanding and addressing the multiple causes of attrition in clinical development will require new thinking and fresh insight. This will be a significant challenge owing to the large number of potential causes of attrition and the complexities of fully understanding these causes at a mechanistic level. We can only hope to address such issues by concerted efforts across the pharmaceutical industry and beyond. That four of the largest pharmaceutical companies were able to work together to address this problem is perhaps the most significant outcome of this study, demonstrating that with an appropriate framework it is possible to tackle the key challenge of attrition by working together while still protecting the intellectual property and commercial interests of the individual organizations. We hope that this study will act as a springboard for future collaborations of a similar nature.
Three pillars and Phase II Attrition
Pfizer Data 2005-2009 (n=44 projects)


1) Does the compound get there?
2) Does the compound engage target?
3) Does it elicit the pharmacological response?
4) Does it have ‘translatability’?

Exposure & Binding
- n = 12
  - 5 tested mechanism
  - 2 phase III starts

Binding & Pharmacology
- n = 6
  - 5 tested mechanism
  - 0 phase III starts

Pharmacological confidence
- Low
- Exposure confidence
- Low

Efficacy
- High

Each pillar is necessary but not independently sufficient for efficacy.

Translatability?

you got any drug yet?
no - never seen it!

help!
The challenge of Pillar 1. *Does it get there?*

- Differences between compound activities in biochem and cellular assays often challenging to understand
  - leads to much hand waving and distress, particularly by medchemists!
- In lead optimisation indirect estimates are made based on lipophiliicity and artificial membrane permeability measurements.
- An increasing percentage of targets are intracellular
- We seek medicines with high **drug efficiency** => low dosage.
  - This requires a balance of binding affinity (ie potency) and access!!
Drug Efficiency – it’s the dose stupid!

Drug Efficiency Index - a strategy towards low therapeutic dose

\[
\text{Drug Efficiency Index} = \log \left( \text{Biophase Concentration} \times 100 / \text{Dose} \right) + \text{pXC50}
\]

- Application of drug efficiency index in drug discovery: a strategy towards low therapeutic dose. Montanari, Dino; Chiarparin, Elisabetta; Gleeson, Matthew Paul; Braggio, Simone; Longhi, Raffaele; Valko, Klara; Rossi, Tino. Expert Opinion on Drug Discovery, Volume 6, Number 9, September 2011, pp. 913-920(8)
Clinical dose (mg) of marketed drugs is related to Drug efficiency and potency (DEI)

\[ Dose = \frac{Potency}{Drug_{eff}} \times 100 \]

In vivo drug efficiency shows good correlation with in vitro HPLC Drug eff max

\[
\text{In vitro HPLC log Deff max} = 2 - (0.23 \times \log k_{HSA} + 0.43 \times \log k_{IAM} - 0.72)
\]


Application of DEI within Lead Optimization

(green: known drugs, yellow: project compounds, red: candidates)

Ask yourself where your lead optimisation compounds fit on this plot?!!

Red line shows when HPLC Drug eff max = 1%
Drug Efficiency – but what does this mean at a cellular level rather than in plasma

Increasing % of our targets are intracellular

Biophase concentration is traditionally equated to plasma concentration and relies on the free drug hypothesis

Can we measure cell concentration to give an equivalent cellular drug efficiency?
Towards measuring the actual cellular concentration of compounds


Enables the concept of Cellular Drug Efficiency in early lead optimisation

Data reported as

$$p\Delta C = \log\left(\frac{[C]_{\text{intracellular}}}{[C]_{\text{extracellular}}}\right)$$
Artificial Membrane Permeability vs pDeltaC

pDeltaC of zero = equal conc inside and outside cell

PdeltaC – total associated with/in cell
logD7.4 vs pDeltaC

CHROM_LOGD_PH74_MEAN vs. CELL_CONC_MS_FCHG.PDELTA_TOTAL

Phychem_summary: Chrom LogD PH74 Mean vs. Cell Conc MS Fchg PDelta Total

Marking:
- Marking

Marker by:
- Row Number

Color by:
- Acidic
- Amphoteric
- Basic
- Neutral
- Weak Acid
- Weak Base
- Zwitterionic
- Empty

Shape by:
- None

Size by:
- All values

r² = 0.179
Immobilised artificial membrane affinity vs pDeltaC

LogkIAM vs. CELL_CONC_MS_FCHG.PDELTA_TOTAL

r^2 = 0.478

PdeltaC – total associated with/in cell
Immobilised artificial membrane affinity vs pDeltaC

PHYSCHEM_SUMMARY.LOGK_JAM vs. CELL_CONC_MS_FCHG.PDELTAC_TOTAL

PdeltaC – total associated with/in cell

acids

Non-acids

PHYSCHEM_SUMMARY.LOGK_JAM vs. CELL_CONC_MS_FCHG.PDELTAC_TOTAL

PdeltaC – total associated with/in cell
Immobilised artificial membrane affinity vs pDeltaC

Trellised by different projects
Towards understanding intracellular free conc


- Binding of compounds to cellular material measured by dialysis using a Rapid Equilibrium Dialysis RED device (Thermo Fisher Scientific Inc.)

**Figure 3.** Relationship between $f_{u,brain,predicted}$ (from HEK293 cell homogenates) and experimental $f_{u,brain}$ (from brain homogenates) (RMSE = 0.31). Negatively charged compounds at pH 7.4 are represented by triangles, neutral compounds by circles, positively charged compounds by squares, and zwitterionic compounds by diamonds. The solid line represents a perfect prediction, and the dashed lines represent a 3-fold error interval.
Towards understanding intracellular free conc

Total conc vs Free conc

- Ca. 700 compounds measured in consistent assay using HeLa cells
- Opportunity for project specific cell types – highlighting some differences
Towards a better spatial understanding of drug in early discovery

Mass Spectrum Acquired at each Position

- Laser – (MALDI)
- Ion Beam – (SIMS)

Ion Density Maps

Low Intensity | High Intensity

R.Groseclose
Towards a better spatial understanding of drug in early discovery

MALDI MSI & LESA well established at GSK

LESA (Liquid Extraction Surface Analysis)
MS/MS N-term sequence

1 hr

2 hr
Towards subcellular resolution with Secondary Ion MS = SIMS

Compound Assessment and Feasibility Study

Layout and freeze tissue

Section on Cryostat (12-16µm thickness)

Photograph tissue

Ship samples to NPL*

Scan Optical Image

Jejunum section

Thaw mount tissue onto MALDI slide for SIMS and adjacent section on histology slide for IHC

IHC section ~6-10µm thick

SIMS section ~ 12-14µm thick

IHC section ~6-10µm thick

* In collaboration with Prof Ian Gilmore et al at the National Physical Laboratory, Teddington, UK
Secondary ion mass spectrometry, SIMS

Compound Disposition from Tissue to Cells - Developing Mass Spectrometry Imaging

In collaboration with the National Physical Laboratory, Teddington, UK

Dime: rat jejunum (~4.5 mm)

SIMS - 3D reconstruction depth profile through single villus

Dosed Compound signal green

MALDI MS Imaging

SIMS Imaging

Spatial resolution 30 µm

Spatial resolution 2.5 µm

Lamina Propria

Epithelial cells

SIMS: Dosed animal

Sum of all ions in MS (TIC)

Compound specific fragment

2.5 \(\mu m\) pixel size
SIMS surface Image

Rat jejunum - 5 day oral dose @ 100 mg/kg, 4 Hr post dose

4.3 x 4.7 mm²

All ions

Compound

Gut wall

Villi

Depth Profile

Lumen

“Feed” particle

optical

2.5 μm pixel size
Depth profile through individual villus

Lamina Propria
Epithelial cells (enterocytes)

Lipid fragment
184 m/z
Phosphocholine

Compound specific fragment

total integrated SIMS signal from the 3D image
400 x 400 μm²

1.5 μm pixel size
Rat Alveolar macrophage cell line incubated with Amiodarone

Combined image

Cytoplasm
Nucleus
Smaller Macrophage cells, less compound accumulation

Riboce
Marker nucleus

Amiodarone

Lipid fragment
Marker cell

Next generation 3D nano-SIMS

New analyser

20 nm LMIS Argon cluster

Designed for laser post-ionisation

D20 Orbitrap
What sort of works for us?

What might work for us in the future?

The expanding “sciences” of Medicinal Chemistry and drug discovery
Known knowns, known unknowns, unknown unknowns and ……..

The part that Donald Rumsfeld forgot

*Unknown knowns*

– Those things that are known but we don’t know ourselves
– Those things that are known but we have forgotten
– Those things that are known but we choose to ignore

– Lets try not to ignore the medchem knowledge that has been gained at very considerable expense over many years!

---

**Passive Permeation of Organic Compounds through Biological Tissue: a Non-Steady-State Theory**

*John T. Penniston, Laurel Beckett, Donald L. Bentley, and Corwin Hansch*

*Departments of Chemistry and Mathematics, Pomona College, Claremont, California 91711*

(Received March 8, 1969)

**SUMMARY**

A theoretical analysis of the penetration of drug molecules to their sites of action in terms of their lipophilic character is presented. This analysis justifies the parabolic equations which have been used empirically to describe the relationship between effectiveness of drug and its lipophilic character.

---

**DISCUSSION**

It is evident from the above analysis that the theoretical curve for dependence of penetration on partition coefficient can be very closely approximated by Eq. 12. This equation is identical with that postulated (15) to describe the dependence of biological response on partition coefficient. Equation 14 gives the analogous form of the equation for biological response.

\[
\log \frac{1}{C} = -k (\log P)^2 + k' \log P + k'' \quad (14)
\]
Acknowledgements

Many at GSK and beyond:

– Andrew Leach, Darren Green, Ian Churcher, James Butler, Rob Young, Martin Bayliss;
– Gareth Wayne, Bill Leavens, Morven Evans, Laurie Gordon, Klara Valko, Simon Readshaw et al
– Andy West, Peter Marshall, Carla Newman, Sir Colin Dollery, et al
– Gitte Neubauer, Gerard Drewes, Friedrich Reinhard et al
– Paul Leeson
– Simone Braggio, Dino Montanari et al
– George Keseru – Budapest
– Andre Mateus, Per Artursson – Uppsala.
– Par Nordlund – Karolinska Inst
– Ian Gilmore et al. NPL

Summary

you got any drug yet?

Yes!! 300uM total & 10uM free

Wow!