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Part of a balanced strategy for accelerating drug discovery

Executive Summary

With the advent of increasingly high throughput biochemistry and molecular biology based assays in the context of the genomics era, the predominant drug discovery strategy in the pharmaceuticals industry has evolved from an empirical or phenotypic approach, to molecular or target-based discovery. Surprisingly, this shift not only failed to accelerate discovery of new first-in-class medicines, it has led to overall higher attrition rates for new compounds and biologics than before the shift. With this realization, the industry is now evaluating how to implement a more holistic approach that incorporates both discovery modes, depending on the available mechanistic information and experimental models. Technology innovations were crucial to the shift from phenotypic to target-based discovery and will again be important in enabling this newly emerging balanced strategy. We will briefly review major pharmaceutical companies that have expanded the use of phenotypic drug discovery and examine the challenges of phenotypic screening, including the remaining technology gaps and underserved model systems.

Until recently, the number of novel New Molecular Entities (NME) submitted to the FDA had been in decline since the 1970s (Center for Drug Evaluation and Research 2013). Changes in the nature of pharmaceutical research have been widely implicated as contributing to this trend.

Historically, drug discovery has employed mainly "phenotypic" approaches—often characterized by physiological observations on whole animals or organ models (Kotz 2012). Most of the first-in-class medicines, those that use a unique mechanism of action to treat a medical condition, developed before the 1980s were discovered by phenotypic drug discovery (PDD). These include many drugs that are still in use and constitute major classes of antibiotics, antihypertensive drugs, anti-cancer drugs, and pain medications.

New technologies, including easier and less expensive ELISAs in the mid 1980's led to a shift to more target-based drug discovery (TDD). With advances in molecular biology and biochemistry in the 1990s, the approach of testing defined substances in complex systems, such as living animals and isolated organs, was largely abandoned in favor of a more 'reductionist' target based approach (Terstappen 2007). High throughput screening (HTS) assays were well suited to these biochemical approaches and could be quickly scaled to explore large compound libraries and, starting in the late 1990s, combinatorial libraries.

With the sequencing of the human genome the adoption of target-based drug discovery accelerated. The ability to rapidly screen libraries for modulators of a protein target was perceived as the modern way to drive productivity.

An analysis by Swinney and Anthony in 2011 evaluated the relationship between drug discovery strategy and success (Swinney 2011). The authors questioned whether an over-reliance on genomic and target-based approaches, while de-emphasizing PDD, is the potential reason for reduced success in the discovery of first-in-class medicines. The result is an in-depth analysis of the discovery strategies and the molecular mechanism of action (MMOA) for NMEs and new biologics approved by the US FDA between 1999 and 2008 (Figure 1). The study found that, of the 259 agents that were approved, 75 were first-in-class drugs with new MMOAs and the remainder were follower drugs. The results also showed that the contribution of phenotypic screening to the discovery of first-in-class small-molecule drugs exceeded that of

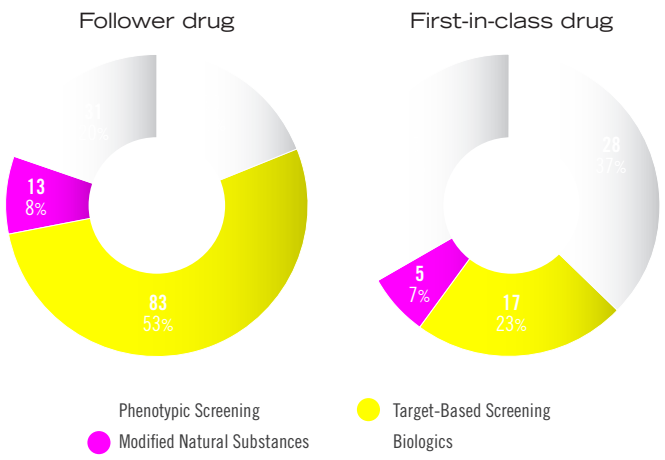

Figure 1. The distribution of new drugs discovered between 1999 and 2008, according to the discovery strategy. Adapted from Swinney and Anthony 2011. The graph illustrates the number of NMEs in each category. Phenotypic screening was the most successful approach for first-in-class drugs, whereas target-based screening was the most successful for follower drugs.

target-based approaches — with 28 and 17 of these drugs coming from the two approaches, respectively — in an era in which the major focus was on target-based approaches. (Swinney, 2013).

These observations are intriguing given the heightened focus on target-based approaches prevalent at the time. It is also of interest to note that PDD was especially beneficial in central nervous system and infectious disease drug discovery.

The authors of the study concluded that an over-reliance on target-based approaches might be a root cause for high attrition rates and low R&D productivity. TDD was founded on the principle that for every disease there is a target, and that understanding the target and screening for a particular MMOA is the path to success. This is somewhat limiting when exploring new phenotypes and new areas of biology (Eggert 2013). The apparent, although not exclusive, factor contributing to lower pharma productivity may be an overdependence on a molecular target-driven drug discovery strategy and the prevalence of a molecular mind-set within contemporary business and scientific leadership (Lee, 2013).

The potential of target-based screening to deliver a steady stream of leads is essentially a reductionist approach. Although it is a reasonable approach to consider each node of a complex cellular network as a simple unit, attempting to understand the network by studying each part in isolation may not necessarily be the best approach. This is especially true for diseases that may have one-to-many or many-to-many relationships between the targets and the disease phenotype. In addition, this approach fails to take into account effects on the entire network such as immunological responses.

The realization of the limits of target-based discovery coupled with new technology developments have brought a new emphasis on phenotypic discovery as part of a successful overall drug discovery strategy (Figure 2, Terstappen 2007).

In a recent review paper Kell concluded that a strategy that "offers the opportunity of achieving a state where we can hope to predict biological processes and the effect of pharmaceutical agents upon them" requires "a return to 'function-first' or phenotypic screening" as part of a broader transformation that also includes more robust systems biology models that incorporate drug transporters, can model multiple target interactions, and also consider drug absorption, distribution, metabolism, and excretion; acceptance of the benefits of drug

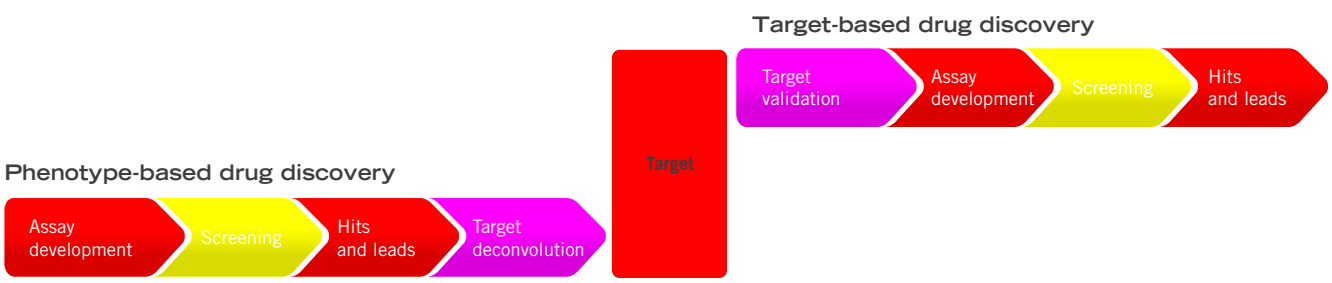

Figure 2. The different uses of the target in PDD versus TDD. Adapted from Terstappen et al. 2007

cocktails; and "novel methods for inferring modes of action by measuring the properties on system variables at all levels of the 'omes'" (Kell 2013). This more balanced approach incorporates both TDD and PDD. (Figure 3).

Phenotypic approaches, such as cellular assays, screen multiple mechanisms and targets simultaneously. Since the initial readouts are information rich and the conditions physiologically relevant, the connection between a compound's action and disease-relevant phenotypes is established earlier in the drug discovery process (Lee 2011). This introduces savings in both cost and time as unsuccessful compounds are abandoned earlier in the drug discovery process.

Despite its historical success, traditional phenotypic screening was expensive and time consuming, especially with animal models. As new technologies have been introduced that provide increased throughput and cost reductions without sacrificing biological relevance, phenotypic screening is becoming more attractive. Two major screening technologies contributed to this resurgence: high content imaging instruments and assays and, to a lesser extent, label-free detection instruments and assays.

High content imaging, pioneered by Cellomics in the 1990s, enabled highly multiplexed, multi-parameter measurements of cells at the throughput required in modern pharmaceutical and research settings (Giuliano 1997). New reporter systems and better cell models improved physiological relevancy, although using fluorescent labels, and over-expressed reporters in cultured cell lines still presents issues in terms of relevancy.

Label-free technologies, including surface plasmon resonance and impedance-based measurements, enabled screening without labels, eliminating spatial interference, autofluorescence and quenching effects. In addition, since genetically altered cell lines are not required, screens can be conducted using primary cells and endogenous receptors, which greatly improves physiological relevancy. Unfortunately however, there are concerns with generally low throughput and potentially unclear data interpretation (Hartigan 2010).

Integrating the empirical and hypothesis driven approaches of phenotypic and target-based discovery has attracted interest from some of the top pharmaceutical companies (Low 2008, Lilly 2012). Several publicly disclosed examples follow.

AstraZeneca uses phenotypic screening paradigms in lead generation and lead optimization (Isherwood 2012) in order to find novel targets for complex areas of biology where polypharmacology is likely important. They also exploit the power of multi-parametric and phenotypic panel assay screening to de-risk toxicity liability early in the drug discovery process.

Novartis AG has had a phenotypic screening program for over a decade despite the challenges of identifying which target or targets are affected by the candidate molecule when a disease-modifying effect is observed (Kotz 2012).

GlaxoSmithKline plc is also returning to phenotypic screens now that the company has built up a chemical proteomic platform that provides a complementary method to subsequently identify the targets of active molecules (Kotz 2012).

In 2011, Eli Lilly and Company launched the open-source Phenotypic Drug Discovery Initiative (PD2), whereby external research groups can submit compounds for testing in a panel of Eli Lilly's phenotypic assays. The company believes that phenotypic lead-generation strategies are complementary to target-directed strategies, but that pharmaceutical compound collections may not be diverse enough to leverage a target-agnostic approach (Lee 2011).

Despite increased adoption, researchers interested in implementing PDD and phenotypic screening assays face potential obstacles. A recent market survey indicated that target deconvolution and understanding the biological significance of results are the two largest hurdles to adopting phenotypic screening assays (Comley 2013, HTStec 2013).

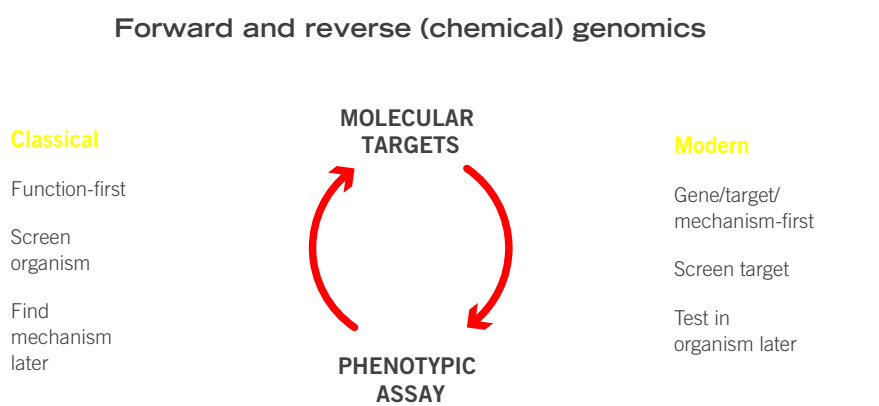

Figure 3. A contrast between function first "forward" chemical discovery with target-first 'reverse' strategy. It is suggested that a reversion to the more classical phenotypic screening approach is likely to prove beneficial from a systems point of view. Adapted from Kell 2013.

Access to relevant cell models presents the third largest obstacle. Human primary cells are ranked as the most relevant model for phenotypic screening studies, yet the most commonly available primary cells, peripheral blood leukocytes, are refractory to analysis by the technology most closely associated with phenotypic screening, high content imaging (HCI). Adherent cells are stationary and flat, making them ideal for imaging technologies. Suspension cells, however, are not amenable to imaging and this has left the development of therapeutics related to diseases of the immune system underserved by phenotypic screening. Technological innovation that addresses this need has great potential in drug discovery.

Cell models that more closely mimic tissues than 2D monocultures are also ranked as highly relevant for phenotypic screening; these include 3D cell cultures and 2D co-culture models (Comley 2013). Like primary cells, these models consist of subpopulations of cells. Because they have the potential to yield the most biologically relevant results, subpopulation analysis is highly sought. It improves signal to noise for the cells or phenotypes of interest and having the ability to dissect a sample into subpopulations allows examination of complex biological systems in settings that more closely resemble the in situ state.

Subpopulation analysis is particularly useful for screens that use primary cell models because freshly isolated samples are rarely clonal, and primary cell cultures typically require the presence of multiple cell types (e.g., feeder cells) to maintain a relevant biological state. The ability to detect multiple subpopulations of cells is therefore an important criterion for phenotypic screening platforms

IntelliCyt Corporation is the first to commercialize a multiplexed, multi-parameter screening system for phenotypic assays in solution. Using the principal of flow cytometry, IntelliCyt screening systems, including its flagship iQue™ Screener, are designed to enable assays utilizing suspension cells and beads. The technology has broad applications in drug discovery screening, enabling high throughput, high content evaluation of individual cells. Antibody discovery and in vitro toxicity testing are also supported applications.

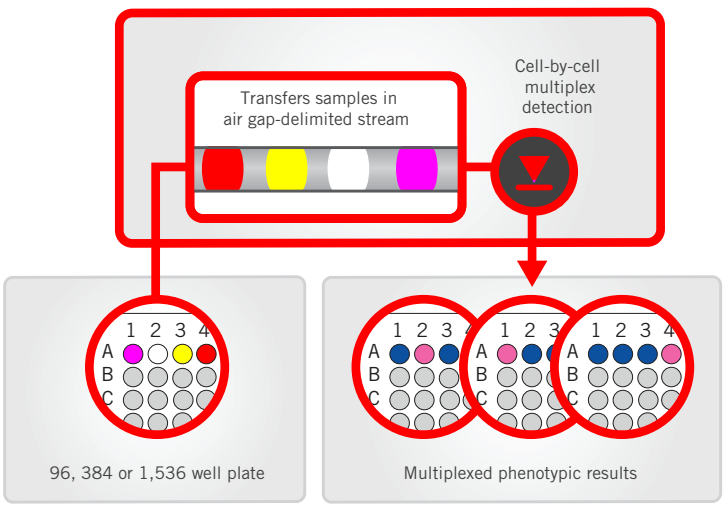

Figure 4. Schematic of IntelliCyt high content flow technology.

IntelliCyt screening systems send a continuous sample stream to the laser-based fluorescence detector, which collects multiple readouts from individual cells, including label-free evaluations of size and granularity. The system employs a unique sampling method to transfer cells or other materials from microplate wells to the detector in a continuous air gap-delimited stream. This novel method confers the following advantages:

- Typically assays a 384-well plate in 15 minutes; 1536-well plate in 60 minutes
- Samples ~10,000 objects / second
- Uses sample volumes as low as 1 µL
- Eliminates dead volume usually associated with flow cytometry
- Achieves sensitivity across a dynamic range of 6+ decades
- Detects rare events <1% (assuming that a sufficient number of cells are analyzed per well)
- Minimizes cross-contamination

Each cell or bead is individually detected as it passes through the laser-based detection system. Complex mixtures of cells can therefore be analyzed with the fluorescence signature for each subpopulation reported separately.

The system makes it straightforward to run highly multiplexed bead-based immunoassays (e.g. Luminex assays, BD CBA assays) using panels of antibody-coated beads that are uniquely identifiable by parameters measured by flow cytometry. For instance, the IntelliCyt MultiCyt™ QBeads™ Plexscreen is a panel of antibody-coated beads against cytokines and other soluble proteins. Each bead type in the panel is coated with a capture antibody specific for a single analyte. A combination of different beads is incubated with

sample or standard, plus detection antibodies conjugated to a reporter molecule. The beads are then read on an IntelliCyt system (no wash step is required). From 1 to 30 analytes can be detected simultaneously.

Physical characteristics of cells or beads can be analyzed using forward-scatter (object size) and side scatter (granularity) measurements. Since these measurements do not rely on fluorescence labels, they provide additional sample characterization and multiplexing capabilities at no additional cost.

Like high content imaging systems, IntelliCyt technology provides multiplexed, multiparameter analysis. High content imaging is uniquely suited for morphometric analysis of adherent cells. IntelliCyt high content, flow technology excels at analysis of suspension cells. Table 1 compares the two technologies.

With the increasing adoption of technology for screening cells in solution, the advantages of flow-based methodologies now have practical application in a variety of drug discovery scenarios encompassing both phenotypic and target-based screens. These include:

- Bead-based G-protein-coupled receptor molecular assembly and receptor binding assays (Roman 2007, Simons 2003)
- Formyl peptide receptor binding assays (Edwards 2005)

Table 1. Comparison of key attributes for the iQue Screener and high-content imaging (HCI). Adapted from Black 2011.

Attribute	Intellicyt High Content Flow-Based Screening	High Content Image-based Screening
Cell throughput	Ten thousand cells per second	Tens to hundreds of cells per second
Typical 96-well plate read time	<5 min independent of the number of fluorescent parameters	5–60 min dependent on the number of fluorescent parameters
Bead assays	Optimal technique for performing multiplex bead-based assays and index labeling (barcoding)	Limited use—beads must be localized to the bottom of the plate
Label-free measurements	Forward scatter (size) and side scatter (granularity) measurements standard	Bright field microscopy is offered on some instruments
Spatial/morphological measurements	No	Yes
Cell types	Optimal for suspension cells. Adherent cells need to be detached before sampling	Optimal for adherent cells. Suspension cells need to be immobilized before analysis
Plate requirements	Standard multi-well round-, v-, or flat-bottom plates can be used	Optically clear plastic or glass bottom plates; uniform flat bottom required
Dynamic range	High dynamic range, very dim to very bright signals can be detected in the same sample	Lower dynamic range
Typical data-file size	1 to 100 MB per plate	100 to 1,000 MB per plate

- Drug efflux transporter screens (Ivnitski-Steele 2008)
- An androgen hormone receptor binding assay (Dennis 2008)
- A prostate cancer cell line screen (Haynes 2009)
- Membranome surveys using phenotypic antibody screening (Rust 2013) and toxicity profiling
- Identification of distinct apoptosis profiles (Luu 2012).

Phenotypic screening was at one time the only strategy for drug discovery. Many of the first-in-class leads of today's most common therapeutics were discovered as a result of this approach. With the flood of detailed target and pathway information that became available in the genomics era, and a new generation of molecular screening technologies, target-based drug discovery became the predominant approach. This strategy shift however, has not fulfilled expectations and increased the pace of drug discovery, and has been especially disappointing in terms of first-in-class drugs.

Many top pharmaceutical companies are implementing a return to a more balanced strategy that utilizes both phenotypic and target-based screening. Challenges remain with implementing phenotypic screening, including access to relevant cell models—especially human primary cells. High content imaging, label-free analysis, and other established technologies have enabled this renaissance in phenotypic screening, but do not address key applications that are best performed using cells or beads in solution. Assays requiring peripheral blood lymphocytes have been notably underserved by established technologies. Additionally, multiplexed assays for secreted proteins, including chemokines and cytokines, have not been available in a format geared for high throughput screening.

IntelliCyt high content, high throughput, flow-based technology addresses these underserved areas by providing a method to assay cells and beads in solution rapidly, with less sample and with multiple parameters using the company's iQue Screener instrument. IntelliCyt MultiCyt reagent kits for phenotypic screening offer a convenient, multiparameter assessment of cell health. The IntelliCyt QBeads Plexscreen, a panel of antibody-coated beads for secreted proteins, enables high throughput screens of 1–30 analytes, with no wash steps and a price compatible with high throughput screening. The Intellicyt technology suite will be of interest to research groups interested in augmenting their phenotypic screening capabilities in suspension cells, primary cells, and heterogeneous cell cultures.

Sailing the Seven C's to Better Compound Profiling

Cells:
Context:
Content:
Correlation:
Capacity:
Cost:
Confidence:

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