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Drug repurposing through nonhypothesis driven phenotypic screening

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The tremendous biological complexity associated with living systems results in significant limitations on the reductionist or target-based drug discovery approach. Increasingly it is being recognized that allowing for more serendipity to enter drug discovery vis-à-vis phenotypic screening provides for more cost-effective drug discovery with higher productivity. Several compelling studies and examples help establish this point of view.

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Introduction

The productivity crisis in the pharmaceutical industry continues to emerge as a transformative event that will reshape the industry, as we know it for many years to come. Simply said, too few NCEs are being approved at too high a cost and with too little net revenue generated to sustain the 'big pharma' business model. One of the take-home messages from this failing productivity scenario is that, despite tremendous scientific advances over the past three decades, the pharmaceutical industry collectively is no 'smarter' at predicting what targets to pursue, what candidate to develop, or what risks remain hidden. On the surface, it would appear that drug repositioning is a strategy that can address several major productivity issues including cost, time to market and risk. Since 2000, several companies have emerged with drug repositioning central to their business model and yet none have truly left a mark on the industry. In this article it is suggested that crucial weakness of the majority of drug repositioning efforts has been the continuation of hypothesis-based (e.g. target-based) approaches. These approaches have arguably been a core weakness of the modern day pharma R&D paradigm. A drug-repositioning tactic that is independent of the collective knowledge base most certainly would be a more successful strategy. The successful cases of repositioned drugs support this idea.

The crisis

The productivity crisis in pharmaceutical drug discovery, or 'innovation gap' as it has been coined, is well recognized. It is the subject of much debate in the industry, and has been a dark cloud over this industry for more than a decade. In 2010 only 21 NCEs were approved in the U.S., down from 25 in 2009 and 24 in 2008. Meanwhile industry R&D spending in 2010 was at an all time high: $127.4 B, up from $124.5 B in 2009. This trend of fewer NCE approvals each year coupled with ever increasing pharma R&D spending has persisted now for 15 years [1]. Couple this sobering statistic with the fact that the average fully loaded cost of NCEs now far exceeds $1B [2]. Finally, to add insult to injury, between 2009 and 2014, $92B in industry revenue is scheduled to go off patent, a figure representing a full one-third of all total pharma revenue. It is no longer a contested statement to say that the

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pharma R&D model is unsustainable in its current form. This is consistent with the massive reduction in industry market capitalization in recent years. Between December 2000 and December 2010, the industry has shed over $500 billion in market capitalization [1].

Not by choice, but by necessity, the industry will increasingly be forced toward a paradigm shift regarding how drugs are discovered and developed.

The limitation of the 'target-based medicinal chemistry' paradigm

In studying the productivity of the industry over the past several decades it is useful to consider how the drug discovery paradigm evolved during this period. Within the past 20 years the drug discovery model has increasingly embraced a central dogma for discovery that is recognized today as 'target-based medicinal chemistry'. Central to this model are the well-recognized and structured stages of early drug discovery, including target identification, high throughput screening and lead optimization efforts. Before this period, the industry discovery paradigm was more intuitive and relied far more on *in vivo* observation and on serendipity. Contrary to widely held expectations, the modern paradigm has delivered the lowest rate of new drug approvals in generations. To be fair, the approval rate is influenced by other exogenous factors, such as an evolving regulatory environment; it nonetheless behooves those of us in the industry to question the modern-day industry model of drug discovery [3].

Today's pharma industry drug discovery model employs a hypothesis-based approach. The hypotheses have their origins in an existing collective knowledge base that we must acknowledge is significantly incomplete. For this reason, the modern-day industry model of drug discovery suffers from a bias limitation: attention is focused on familiar areas, looking for one's keys underneath the proverbial street light. Although the past two decades have heralded the discovery of a plethora of new molecular targets, there remains a very poor understanding of the spectrum of biology influenced by any given target. For any given protein receptor, for example, descriptions of the biochemical pathways that are impacted does not begin to describe the complex interactions in that pathway, the biologically relevance of the target or the target's influence on intersecting pathways. This statement is certainly true in a simple cell-based system. The complexity of these interactions rises exponentially when we consider biological influence in a fully assembled organism [4–6].

In a pharmaceutical R&D environment this biological complexity in conjunction with an incomplete knowledge base manifests itself in the high attrition rates associated with drug discovery and development and indeed also in the surfacing of unexpected adverse events following more widespread patient usage of drugs following approval as seen with COX-2 antagonists (e.g. Vioxx) and PPAR-gamma agonists (e.g. Avandia). It further reveals itself in the form of unexpected beneficial effects such as the analgesic effects against neuropathic pain of the gabapentinoids and the many pleiotropic effects of the statins [7].

Drug repositioning: the beginning of a solution

With the productivity crisis as a backdrop, drug repositioning is increasingly being recognized as a key strategy to surmount the innovation gap. The prospect is compelling; beginning a new drug product opportunity by starting with a compound that already has extensive human clinical safety data. This approach can largely bypass the long, risky and expensive preclinical and early clinical stages. In principal the cost, time and risk, from inception of a new drug opportunity to testing drug activity in the clinic are vastly reduced. Applying this strategy to discontinued clinical candidates can dramatically shorten the time of the drug discovery process and greatly reduce the risk of early clinical failures [8].

Since 2000, several companies have arisen with the central aim of their business model consisting of systematically repositioning or discovering new uses of existing drugs or drug candidates. Companies such as Arachnova Ltd. with their 'Virtual R&D' approach and Curidium Ltd., with their Homomatrix^{TM} platform, have in one form or another tried to do a better job at 'connecting the dots' or 'following the trail of bread crumbs' from data that exists around a drug or drug candidate to predict an alternative use. Although these efforts have been underway for more than a decade now, it is difficult to identify any great success stories, least of all any approved new drugs that have emerged from this hypothesis-based mode of identifying new uses for existing drugs or drug candidates.

Nonetheless, the literature is replete with examples of repositioned drugs telling the tale of low cost, low risk, fast to market scenarios. The vast majority of these cases have arisen from key observations, often serendipitous, made in *in vivo* biological settings. These *in vivo* settings include both pre- and postmarketing human clinical trials and preclinical experimental animal models of disease [8].

Phenotypic screening in drug repositioning: the solution to a good start

The failure of target-based drug discovery, where hypotheses are formed around ideas biased and constrained by our existing knowledge base, provides a compelling rationale for an alternative approach. While the industry collectively may be well entrenched in an era of 'target-based' or 'hypothesis-based' drug discovery, it is important to note that even within the past several years a host of approved drugs were discovered, independent of the target-based medicinal chemistry paradigm. In fact, a recent study has revealed that the contribution of phenotypic screening to first-in-class small-molecule drug discovery exceeded that of target-based

approaches [9]. In the vast majority of these instances the findings were based on serendipitous observations made in animals or in the clinic. For example sildenafil (Viagra; approved in 1998) was being studied in the clinic for the treatment of hypertension when clinical effects on erectile dysfunction were observed. While being developed as an antiepileptic, gabapentin (Neurontin) and subsequently pregabalin (Lyrica; approved in 2007) were discovered to have therapeutic activity in animal models of neuropathic pain. Ezetimibe (Zetia; approved in 2002) was identified as a blocker of cholesterol uptake and initially discovered to have this effect in hamsters although the molecular target was not clear [10]. The narcolepsy therapeutic modafinil (Provigil; approved in 1998) was discovered by serendipitous observations of wake-promoting effects in rats although the mechanism of action had been and still remains unknown [11]. There are other examples of recently approved drugs that were not discovered through a target-based route. Moreover, the mechanisms of actions of these drugs are still poorly understood. These drugs include the anticonvulsant levetiracetam (Keppra; approved 2009), the popular antidiabetic metformin (Glucophage; approved 1994), imiquimod (Zyclara, approved 2010) used to treat actinic keratoses and dalfampridine (Ampyra; approved 2010) used to help walking in MS patients and many others.

A systematic drug discovery approach that is both complementary to a target-based approach and effectively embraces the type of serendipity that accounts for all of the drugs just listed, is phenotypic screening. Broadly speaking, this entails interrogating drug candidates in one or more disease models in a manner wholly or largely agnostic of the molecular target the candidate was designed to modulate. In this way a phenotypic screening approach does not bear the knowledge base bias limitation of target-based screening.

There are several examples of instances where drug screens have been conducted on a library of existing drugs using *in vivo* disease models to identify new activities. These screens started with existing drugs as their substrate, and therefore these approaches can be considered to be drug-repositioning efforts.

This approach was used effectively in identifying a potential antimalarial compound from a library of compounds [12]. In this example, 2700 approved drugs or development-stage drug candidates were screened for inhibition of *Plasmodium falciparum* growth. This screening effort led to identification of the nonsedating anti-histamine astemizole and its primary human metabolite, desmethylastemizole as submicromolar inhibitors of three-different *P. falciparum* strains with potent oral activity in two mouse parasite suppression tests [13]. Although this method satisfies an early drug discovery approach as a high capacity and high throughput approach, it is limited to a single therapeutic area and is probable to produce infrequent hits.

Using the above example of screening through a single model, it follows that evaluating a large number of approved or development-stage drugs through multiple predictive models should yield a far larger number of repositioned drug candidates.

Several estimates suggest that the frequency of identifying potentially beneficial therapeutic potential for existing therapeutics is on the order of 30%. This 'hit rate' was described for approved drugs in a limited *in vivo* model system, and furthermore is consistent with off-label prescription rates [4,14].

One study has attempted to identify the potential success rate of such an approach. In that study, a small number of approved drugs were evaluated in several *in vivo* and cell based models for their effects on several diverse cell pathways. The drugs tested in that study included statins, glitazones, salicylates, retinoids and calcium channel blockers. The set of *in vivo* and cell-based models included insulin-mediated glucose utilization, hippocampal neurogenesis, liver collagen synthesis, lymphocyte proliferation and microglial proliferation. In this limited evaluation it was found that nearly every tested drug showed an effect in at least one of the models that was not predicted based on the original indication or known molecular target interactions [4,5]. These findings confirm that drugs with at least one identified biological activity can frequently elicit additional biological responses. Importantly, this study highlights the benefits of examining drugs for completely unrelated indications.

The approach of testing a series of compounds in an array of independent models with the aim of identifying a novel activity among one or more of the tested models fulfills the strategic requirements needed for effective drug repositioning. The relatively low throughput/high cost per compound is offset by the fact that the substrate is very high quality; drug-like compounds that are known to be well-tolerated in humans and have completed all aspects of preclinical and at least early stage clinical development.

Bringing the science back to the 'nonhypothesis' driven approach

A nonhypothesis driven phenotypic screening approach would seem to disregard good scientific method. Yet most instances in which new uses for existing compounds are identified and are in fact scenarios of 'on-target' effects. The unexpected new biology that was uncovered was the result of the compounds modulation of a molecular target that the compound was known to modulate, and not a case of the compound acting at another molecular target (off-target effect). For example, sildenafil was developed as a phosphodiesterase 5 (PDE-5) inhibitor because of the role of PDE-5 in PAH. Although unappreciated at the time, PDE-5 also has an important role in corpus cavernosum physiology and thereby ED [15]. This supports the thesis that biology is incredibly

complex and poorly understood with regard to most of the molecular targets being studied for pharmaceutical potential. Thus nonhypothesis driven phenotypic screening can indeed advance our understanding of biology around drug targets by taking us out from underneath the street light.

Conclusion

The pharmaceutical industry must radically evolve its drug discovery tactics from the current unsustainable hypothesis-directed model. Drug repositioning has widely been identified and embraced as a means of addressing the major productivity issues of cost, time to market and risk. Although the 'rational' target-based discovery approach has been applied to drug repositioning to improve its success rate, nevertheless the majority of successful drug repositioning cases have primarily been the result of unexpected findings in clinical and preclinical *in vivo* settings. We propose here that the most efficient, cost-effective and comprehensive way to reposition drugs is to find new indications in preclinical animal models of disease. Paradoxically, this seemingly unscientific approach has achieved scientific breakthroughs in areas that have been intransigent up to now.

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