The genome as a tool for drug discovery and development

the case for embracing complexity

Conventional drug discovery and development is centred on a paradigm in which 'validated' targets are subjected to *in vitro* screens for the identification of new drug candidates. Molecular profiling using genomic approaches is becoming an important complement to this process and may eventually become a new paradigm for drug discovery that is based on complex biomarker sets, or molecular profiles, that are monitored *in vivo*.

By Dr Kenneth C. Carter

Modern reductionist approaches to the study of molecular biology have lead to a widely accepted paradigm for drug discovery that places a single 'validated' target at the centre of most commercial drug discovery and development efforts. However, despite impressive advances in the technologies for identifying, validating and *in vitro* screening of compounds for their effect on individual putative targets, it is unclear whether this has improved the rate of new drugs entering the marketplace[1]. The reasons for this are uncertain, but what is clear is that the path of drug discovery and development remains fraught with difficulty at every stage. Advances in the study and application of genomics holds promise for addressing many of the difficulties with drug discovery and may lead to a paradigm shift in which molecular profiling using genomics and other 'omics' approaches will play a central role in the discovery and development of drugs. This new paradigm would be anchored by *in vivo* monitoring of complex sets of molecular biomarkers rather than *in vitro* screening of single targets (the term *in vitro* is used throughout this article to mean a biochemical reaction carried out in a laboratory vessel using isolated reagents, as opposed to another common usage of *in vitro* that refers to cells grown in cultured conditions).

Target-centred drug discovery: promise and problems

Drug discovery efforts that focus on *in vitro* target-based screening benefit from the advantage of having a relatively simple binary assay with which to define success or failure of an individual drug candidate. Upon becoming convinced that scientific evidence has validated a given target, it is increasingly straightforward with modern techniques to design a screen that will identify compounds that affect the function of the target – at least in an *in vitro* state. This approach to drug discovery appears superficially to have the advantage of being a rational, scientific, mechanism-based method for discovering novel medicines. However, because almost all disease processes are complex and multi-factorial, drug discovery efforts that are

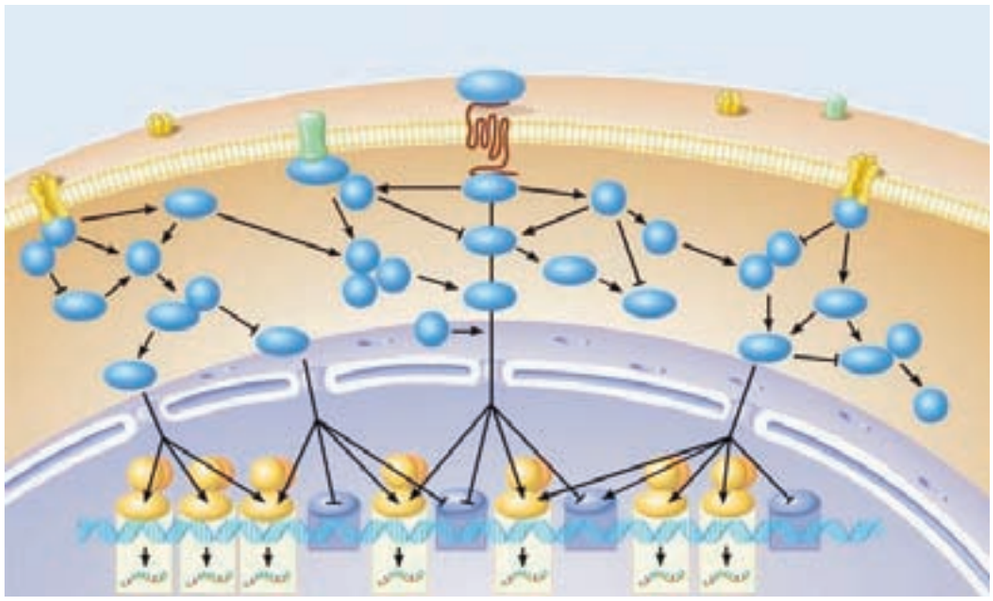

Figure 1
A major challenge to efficient drug discovery and development is the enormous complexity of human cells and tissue. Every cell has hundreds of cellular control pathways and tens of thousands of genes and RNA transcripts which are dynamically regulated

anchored in defining the effect of compounds on isolated targets outside their natural microenvironment have obvious drawbacks.

As an example, kinases are a class of proteins for which target-based drug discovery approaches are particularly well suited. Kinases enzymatically catalyse the transfer of a phosphate group from a donor, such as ADP or ATP, to a protein at serine, threonine or tyrosine residues. Kinase enzymes are involved in many types of cellular signalling and are implicated in several disease processes. This well defined function, combined with the fact that kinases are usually amenable to the development of *in vitro* screens for the identification of compounds that inhibit or enhance kinase activity, has made them the subject of many target-based drug discovery efforts. However, despite numerous discovery programmes that have identified drug candidates that inhibit kinases, few have become approved drugs, in part due to problems with single target-centred drug discovery[2].

Many of the problems with an over-emphasis on single protein target assays as the foundation of drug discovery are akin to the proverb that warns against 'missing the forest while looking at the trees'. An overriding limitation of the *in vitro*, single target approach is that it ignores the enormous complexity of cells and tissues (**Figure 1**). There are many types of cellular activities that are impossible to model effectively outside the context of a functioning cell. These include: dynamic allosteric interactions of a protein target with other cellular components, transport of targets within and in between cellular compartments, and functions that require complex structural interactions between multiple components of the cellular apparatus. It is also the case that other types of molecules including DNA, RNA and lipids play functional and structural roles within cells, but are excluded as targets in the single protein target approach.

Another key problem with the 'single tree' approach is the fact that up to 80% of the hundreds of cellular proteins that are believed to be promising or validated targets for therapeutic intervention are not 'druggable'[3]. For example, many promising targets do not have properties that lend themselves to conventional high-throughput compound screening approaches. For cancer alone, there are numerous examples of well validated potential drug targets that clearly play a key role in disease progression, but for which there is no straightforward approach to pursuing classical compound screening. Examples of this include: myc, stat3, and beta catenin – all of which are well studied oncogenes for which there is strong evidence that their amplification, over-expression, or misregulation plays a key role in cancer progression. However, because of the difficulties in designing *in vitro* screens for their functions, there are few, if any, drug candidates that have been discovered based on inhibiting these well validated oncogenic targets.

Finally, there is one other important limitation to single target-based drug discovery: very few drugs or drug candidates interact exclusively with a single target. Instead, they often interact with

multiple sites within cells and tissues. There are several examples of well studied drugs, including paracetamol (Tylenol), for which the true mechanism of action is either unknown, or many years after discovery is shown to be quite different than the mechanism that was initially ascribed. This has lead to appreciation, among at least some scientists, that the complexity of cells and tissues is a major issue for drug development that should, perhaps, be embraced[4]. This cross-reactivity of drugs is particularly problematic in highly related protein families such as the kinases. Although it is difficult to quantify the problem, there are many anecdotal stories of kinase inhibitor discovery efforts that have been bogged down by issues of target selectivity.

Despite these drawbacks in target-based drug discovery, there have been several examples of success including several inhibitors of receptor tyrosine kinases that have entered the clinic in the past three years[2]. However, it remains perplexing that the rate of successful drug discovery and development efforts remains dismally low.

Using genomics in drug discovery: an opportunity for a paradigm shift?

In recent years, The Human Genome Project and related efforts have led to an unprecedented proliferation of both public and private databases of genomic data. In parallel, the last decade has seen great advances in the development of molecular biological reagents, robotics, arraying techniques, assay detection technologies and faster computers. This has made it possible to embrace comprehensive monitoring of complex biomolecular events at reasonable costs. In particular, measuring the level of RNA transcripts from tens of thousands of different genes at once, through the use of microarrays and similar technologies, has provided the ability to monitor the expression of essentially the whole genome in the form of individual mRNA levels for a wide variety of situations and settings. This has opened the door to use of molecular profiling, or multi-variant biomarker strategies, for every step in the drug discovery and development process[5,6,7].

Molecular profiling of cellular states is often approached using transcript-based (genomic) arrays, but as other technologies mature such profiling can and will increasingly include proteomics, metabolomics and other 'omics' approaches to multi-variant molecular analysis. Molecular profiling by these approaches has several potential advantages both as a primary anchor to drug discovery and as a complement to more conventional target-based discovery efforts. In both cases, increasing use of molecular profiling is likely to lead to a shift away from the current over-reliance on *in vitro* monitoring of single drug-target interactions in drug discovery – in other words, we are likely to see a shift from a 'single-tree approach' to a 'forest monitoring approach', or, better yet, development of complementary and efficient processes for both (**Figure 2**).

Following are several examples of emerging strategies for the use of molecular profiling in drug discovery and development – mostly in the area of cancer. Cancer is essentially a disease of the genome in that one of the hallmarks of all cancer cells is the presence of profound alterations of their genomic DNA that includes mutations, deletions, amplifications and rearrangements. For this reason both academic and industrial efforts to develop better medicines for cancer have led to many examples of the use of genomic molecular profiling to improve the drug discovery and development process.

Drug discovery – The use of large complex sets of genomic biomarkers, generally in the form of microarrays used to monitor the expression of large sets of genes – has already found its way into standard use in the identification and validation of drug targets[5-8]. Profiling the expression of large gene sets in normal vs disease states can provide critical clues to the activities of cellular control pathways as well as identifying specific genes that play key roles in disease processes. This use of molecular profiling to understand the basic science of disease is gaining widespread acceptance in both academic and industry settings. For example, GeneLogic's GeneExpress® database contains data that have been collected over several years comparing the genomic expression state of many thousands of human tissues. Likewise, there are many published accounts in which molecular profiling has played an important role in defining disease states. One interesting recent example is the use of microRNA profiles to classify cancer types[9].

An exciting use of molecular profiling that has the potential to revolutionise drug screening is its utility in defining cellular states as the primary driver for the identification of drug candidates (rather than compound interactions with a specific target *in vitro*). This is an approach to drug discovery that has been pioneered at Avalon Pharmaceuticals using a proprietary technology it has termed AvalonRx[7]. Using a system called HITS (High-Throughput Integrated Transcriptional Screening), it screens for active

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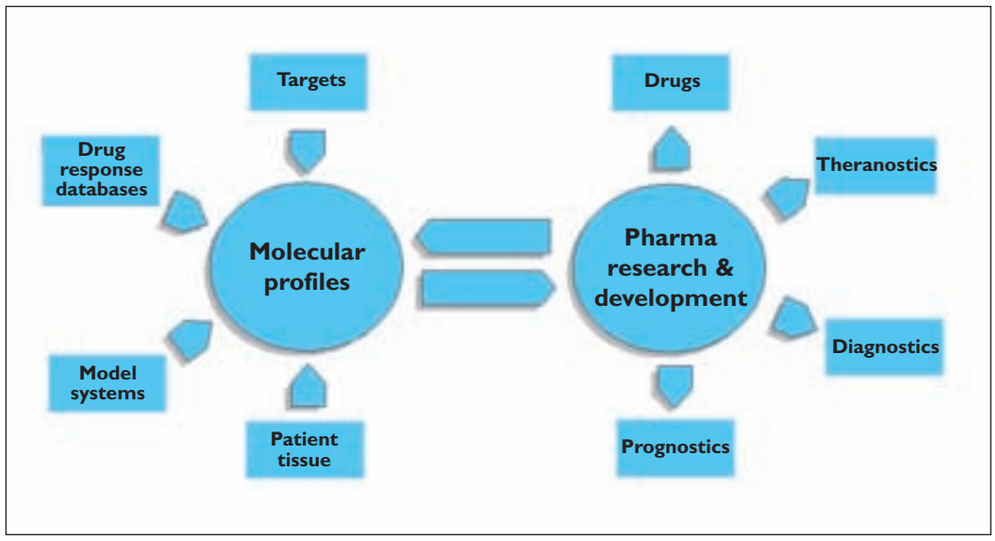

Figure 2
The future of drug discovery will likely see an increasingly central role for molecular profiling

compounds by monitoring genomic response profiles within living cells. One of the most important advantages of this approach is that it allows a straightforward way to design a screen for essentially any target or pathway. This can be done by studying genomic response profiles in cells in which the pathway or target has been disrupted or affected using biological reagents that disrupt specific pathways and that modify a clinically relevant response. For example, RNAi techniques can be used to knockdown the gene expression of an 'undruggable' target and then the resulting whole genome response profile can be mined for a set of genes that are subsequently converted to a screen to identify compounds that cause a similar or identical response. Likewise, drugs can be optimised using a genomic response profile that has been linked to a desired therapeutic response. Another advantage of this approach is that drug discovery and optimisation occurs in the context of the living cell, rendering the precise target(s) of the compound somewhat irrelevant.

After isolating hits from its high-throughput molecular profiling screens, which focus on changes in the expression of only 8-32 genes, Avalon characterises these hits on microarrays where it monitors the 'on target' and 'off target' effects of the compounds on thousands of genes. All of this information is carefully analysed in its METS (Microarray-Based Transcriptional Screening) platform. In this process, it obtains insight on potential mechanism of action, selectivity, specificity and novelty as well as information on toxicity, tumour targets and predictions of *in vivo* efficacy.

As part of the METS platform, Avalon has assembled a database of reproducible transcriptional effects for several hundred reference compounds and novel hits, including well known physiological modulators, drug candidates in the earlier stages of development, marketed drugs as well as failed clinical candidates. Within this database, scientists have clustered compounds that share common known mechanisms and have identified additional secondary mechanism profiles, typically from 8-40 gene sets, that can be used to further distinguish sub-groups of functional analogs within agent families. These sets of distinct descriptor genes can then be used as compound classifiers.

Drug development – Once a drug candidate is in hand, the use of genomic and other forms of molecular profiling data for driving drug development decisions is becoming relatively widespread in the pharmaceutical industry. One of the most important areas for which this approach holds promise is in defining efficacy and toxicity in model systems. For defining efficacy, there have been several published examples of using molecular profiling to correlate a molecular profile elicited by a particular drug or drug candidate to a histopathological readout, disease model, or clinical endpoint[5-8,10,11]. But this approach is still in its infancy. Avalon has built a platform that allows the routine examination of tumours in model animal systems through molecular profiling of the tumour's genomic response to treatment with various drug development candidates. This approach, which is termed RACETraCK (Rapid Assessment of Compound Efficacy, Transcriptional Change,

and Kinetics), has several advantages over conventional monitoring of endpoints such as tumour size. These include: rapid assessment of response (often monitored within 24 hours), information about drug penetration and specific molecular definitions of response. Eventually, it is likely that this type of approach will become a routine part of the assessment of drug candidate efficacy for many models and disease types.

The prediction of toxicity of drug candidates is another area that holds great promise for molecular profiling. Several companies including Iconix, GeneLogic, Curagen and Rosetta/Merck are pioneering methods by which databases of molecular profiles for known toxins are used as a reference for profiling drug candidates. Although there are limited published examples of the success of these methods, they will likely continue to become an increasing robust approach for determining drug candidate toxicity.

Clinical biomarkers – Perhaps the area where molecular profiling is likely to have the most widely recognised benefit in the near term will be in the development of multi-variant clinical biomarkers. It would be tremendously beneficial to have sets of biomarkers that could guide dosing decisions or identify patients who are likely to respond to a particular drug. Both molecular profiling-based data sets and individual biomarkers for potential use in the clinic are beginning to be discovered by many groups. However, the challenges of validation are such that few of these have yet to find routine use. Despite this, it is likely to be only a matter of time before molecular profiling approaches lead to novel prognostic, diagnostic, and theranostic products[11,12].

The Oncotype DX test, marketed by Genomic Health, is one early example of the clinical promise of tests based on molecular profiling. This prognostic test predicts the likelihood of breast cancer recurrence in women with newly diagnosed, early stage invasive breast cancer and can be used to assess the potential benefit of certain types of therapy. Molecular profiling of mRNA levels was used extensively in the development of the test as well as being the technical foundation for the test itself.

One particularly promising area for the application of molecular profiling is in the development of theranostics (predictive tests that link a drug to use in patients with a particular molecular profile or other characteristic). There has been a limited number of theranostic developments based on individual biomarkers, one example being the pairing of Herceptin with patients who have overexpression or amplification of the *her2/neu* gene, but

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the emergence of molecular profiling approaches should provide many more opportunities for identifying effective theranostics[12]. In the case of cancer, this rise in molecular profiling tests will be accompanied with single marker or genomic mutation tests[13,14]. Having embraced this future, Avalon is developing its lead drug candidate, AVN944, in conjunction with a comprehensive molecular profiling strategy during clinical trials that will lay the foundation for the development of companion theranostics tests[15,16].

In a move that may help speed a paradigm shift in the use of biomarkers in drug discovery, in 2003 the FDA placed a focus on the development of approaches that provide more specific markers of drug response and disease by announcing its Critical Path Initiative. While this initiative is not specifically focused on molecular profiling, such approaches are certain to play a major role in efforts along this path. One interesting development in response to the FDA Critical Path Initiative has been the formation the C-Path Institute, whose purpose is to co-ordinate efforts for clinical studies to fulfill the spirit of the FDA's critical path initiative. Currently, C-Path is co-ordinating an effort involving more than 20 companies and institutions with the goal of designing diagnostic and theranostic approaches for the use of Epidermal Growth Factor (EGF)-targeted drugs for non-small-cell lung cancer[17].

Finally, a major driver that will shape how future molecular profiling-based diagnostics are developed is oversight and guidance from the FDA and other regulatory agencies. Traditionally, many diagnostic tests have been relatively loosely regulated as 'home brew' kits or services that are not subject to full regulatory scrutiny. However, recently the FDA has suggested that multivariate tests, such as those based on molecular profiling using genomics, should be developed and approved through already established processes for other non-home brew diagnostic tests[18].

The future

The use of molecular profiling throughout the drug discovery and development process is likely to increase dramatically over the next few years. This will be based on the clear advantages to multivariate biomarker approaches including: the ability to provide a broad view of the biological state of a cell or tissue; the increased predictive power of monitoring multiple parameters simultaneously; and the power of correlating specific molecular phenotypes to clinical, histopathological or disease model endpoints. In some cases, particularly in cancer, developing molecular profiling of genomic expression will likely also dovetail with basic discoveries about the mechanisms and characteristics of the disease.

It is possible that molecular profiling will eventually replace the current single target based dogma as the central paradigm of drug discovery (**Figure 2**). However, the question as to the speed with which this will occur is difficult to answer. There are many challenges to fulfilling the promise of molecular profiling including: limitations and costs associated with current technologies, the fact that validation of profiles can be time consuming and expensive, and resistance to change in organisations – particularly large pharmaceutical companies – that are entrenched in the dogma of single target based drug discovery. Thus, if a paradigm shift occurs, it will likely involve significant upheaval[13].

Until then, it is clear that the increased use of molecular profiling will continue to make an important contribution to drug discovery and development efforts worldwide and will hopefully lead to lower failure rates, faster progression through the development process, and increasingly precise tests to match the right medicine with the right patient.

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As a co-founder and CEO of Avalon, ***Dr Kenneth C. Carter*** *has overseen the company's rapid growth as a chemical genomics-based drug discovery and development company with a pipeline of cancer drug programmes. Avalon's progress has won numerous accolades for the company and Dr Carter, including Red Herring's Top 100 Innovative Companies and Fiercebiotech's 'Top 15 Companies' awards. Prior to co-founding Avalon, Dr Carter was at Human Genome Sciences, Inc, where he directed the company's gene mapping initiative. In this capacity, he played a role in the discovery, cloning and chromosomal mapping of dozens of novel human genes. Dr Carter has more than 50 published scientific articles and multiple patents.*