Recent technology advances transforming pharmaceutical drug discovery and development

The last decade has seen rapid technological progress but with increasing demands for safe and efficacious drugs, technological solutions that reduce the barriers to clinical success will be of more interest than those that simply shift the bottlenecks and necessitate new compromises in the discovery and development process.

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Developing a commercially successful drug involves a series of compromises, each of which may result in costly bottlenecks, inefficiencies and unacceptably high failure rates. That is the reality of drug discovery today. No more than 1% of drugs in the discovery and development pipeline will reach the marketplace. Failure rates are too high, and all too often drug candidates fail relatively late in development, having already consumed substantial time and resources. The pharmaceutical industry has poured millions of dollars into new technologies over the past two decades, but this has had little impact on the high drop-out rate and cost of drug development. While automation, steps toward miniaturisation, and robotics-based strategies have greatly increased throughputs in compound synthesis and screening, they have not led to truly meaningful gains in the economics of drug discovery and the success rate of new chemical entities. Numerous problem areas remain: better information relative to disease pathophysiology is needed to improve target selection and validation; more predictive, biologically-relevant high throughput screens are essential; and, more emphasis must be placed on acquiring good ADME/Tox data earlier in the discovery process.

Target discovery and validation – impact of nanofluidic real-time qPCR technologies

Central to overcoming these barriers are innovative technologies generating high-value information in a condensed timeframe to minimise the guesswork involved in target, lead and drug candidate selection. Discovery of molecular targets as potential candidates for therapeutic intervention is dictated by whole genome assessment of the differences between normal and disease states for identification of those DNA or RNA transcriptional variants that may play a role in the onset or progression of a disease. The ability to measure large numbers of DNA variants, gene or exon-based transcription in a sample make hybridisation microarrays attractive as the discovery platform of choice although the fast decreasing cost of direct

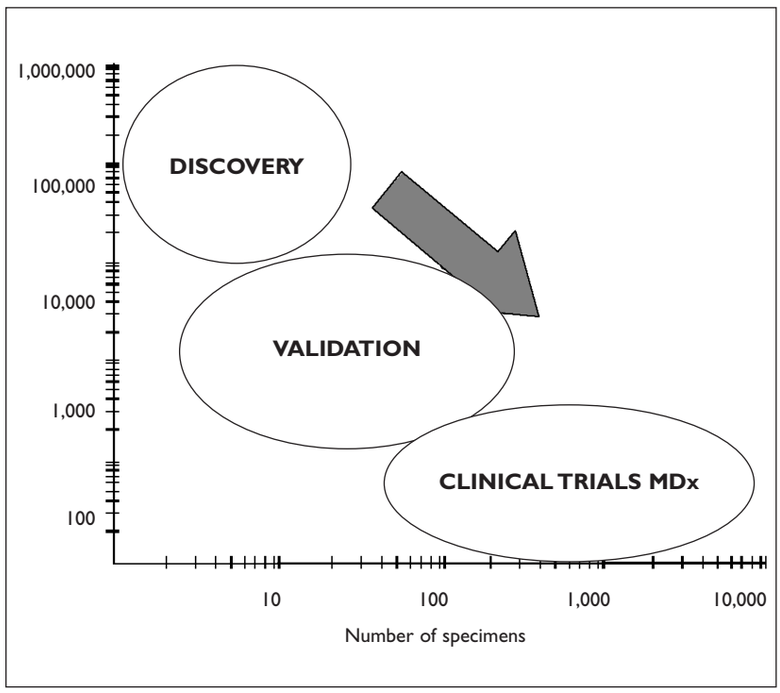

Figure 1
Schematic showing segmentation of numbers of genomic determinants screened versus numbers of specimens in the process leading from early-stage discovery of genetic markers through to their commercialisation as a molecular diagnostic or in biomarker clinical applications

genome sequencing and the biological richness intrinsic to sequence data could replace microarrays as the discovery platform of choice. Inherent variability between biological specimens; the relatively low sample throughput of both microarray and sequencing systems; and the need for independent technical validation of the genomic differences measured between specimens in a discovery cohort motivates the need for validation of the microarray or sequencing results with an independent assay system across a larger validation cohort. Real-time quantitative polymerase chain reaction (real-time qPCR) is the reference assay of choice; its high reproducibility and replication fidelity leads to a sensitivity, precision and dynamic range superior to current microarray or sequencing technologies. Statistics and the inherent biological diversity of individuals dictates measurement of smaller numbers of genomic determinants in larger sample populations on transitioning from target discovery to validation (**Figure 1**). Further expansion to accommodate larger sample populations is required for establishing a determinant as an informative biomarker for clinical studies or as a possible diagnostic. Extending the analytical benefits of qPCR to these larger scale, multi-gene studies necessitates development of a technological approach that readily accommodates the expansion of the number of qPCR tests per specimen and the number of specimens analysed in any large scale study.

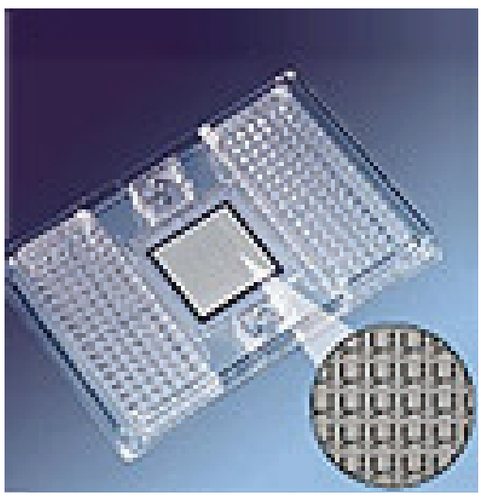

Figure 2
Fluidigm's Biomark dynamic array for qPCR with magnified view of the miniaturised pneumatically-valve configuration

Nanofluidic real-time qPCR

Strategies are varied for economically scaling real-time qPCR to larger numbers of samples and tests per sample. Automating a standard PCR process in multi-well microtiter plates (either 96- or 384-well microplates) is an obvious choice that leverages existing liquid handling and thermal cycler instrumentation, infrastructure and know-how to achieve higher throughput with microlitre-scale reactions. The exponential increase in experimental complexity with number of reactions, the operational expense in reagents and consumables and the capital intensity required to incrementally scale throughput limit this option to those facilities with existing infrastructure and expertise. Multiplexing multiple PCR tests in a single reaction tube and demultiplexing the amplified targets (amplicons) using one of a variety of different methods (eg multiple optical labels, capillary electrophoresis, mass spectrometry or dissociation (melt) temperature) has become a popular route to multi-gene/multi-sample analysis but the inherent complexity of designing primers to ensure independent, optimised PCR amplifications having a common set of thermal parameters without cross-talk ultimately imposes a limit on measurement accuracy relative to the number of assays multiplexed and the composition of the nucleic acid sequences amplified. The requirement to redesign and re-optimise the entire set of PCR assays if only one target is changed imposes a severe penalty on experiment flexibility, particularly as the number of multiplexed assays increases.

PCR in a miniaturised serial or parallel fluidics systems is the final strategic option. Continuous

flow monolithic lab-on-a-chip devices allow rapid sequential PCR amplification and analysis of micro- or nanolitre samples in a micromachined channel passed through multiple, fixed temperature zones followed by detection of the amplified target sequences. Sample throughput is limited by sampling spacing in the channel to prevent cross-contamination and problems endemic to serial microfluidic systems such as microchannel clogging and inefficient interfaces to microplates, the standard fluidics carrier in pharmaceutical drug discovery.

Devices for parallel PCR processing involve thermal cycling PCR reagents and sample template in a high density array (>1 well/mm^2) of micro- or submicrolitre wells. Although a number of experimental systems have been reported[1], the technology has matured to the point where there are several commercial high throughput products for quantitative real-time PCR. Fluidigm (www.fluidigm.com) sells the Biomark dynamic array system for quantitative measurement of gene expression[2]. A dynamic array is an integrated fluidic circuit (IFC) comprised of an array of miniature pneumatically-actuated valves that form a high density array of nanolitre reaction containers when actuated. Available in either 48 x 48 or 96 x 96 array formats, sample and qPCR reagents are dispensed into access ports compatible with fluidic transfer from a SBS-standard microplate. Actuation of the valves allows 48 samples to be tested for 48 gene transcripts (2304 qPCR tests) or 96 samples against 96 transcripts (9216 qPCR tests) in 6.7 nanolitre reactions in a single thermal cycle run. Roche Applied Sciences (www.applied-science-system.com) offers an alternative approach based on high density 1536 well microplates designed to run on its 480 LightCycler with a modified thermal block. Samples and reagents in standard microplates are robotically combined and thermally cycled in 0.5-2 microlitre reactions in the higher density microplate. Reagent savings and assay-sample customisation are important strengths of these systems.

BioTrove Inc (www.biotrove.com) offers an alternative solution through its OpenArray® system where up to 3,072 individual qPCR tests can be performed in a single OpenArray plate against multiple specimens simultaneously[3,4] (**Figure 3**). The plates are pre-printed and quality checked prior to shipment with either customer-supplied SYBR Green I or TaqMan® qPCR assays or those pre-validated from BioTrove's extensive qPCR library. Customers can purchase, off-the-shelf, BioTrove's Pathways™ OpenArray plates for analysing four samples per plate with comprehensive panels of more than 600 pre-validated qPCR assays. The gene assay panels are organised to allow quantitative, system-level investigations of expression profiles across different diseases (eg cancer, cardiovascular, diabetes), mechanisms of biological action (eg apoptosis, signal transduction, toxicity) or gene families (kinome) for human, rat and mouse specimens. The spatial independence of each qPCR assay allows a high

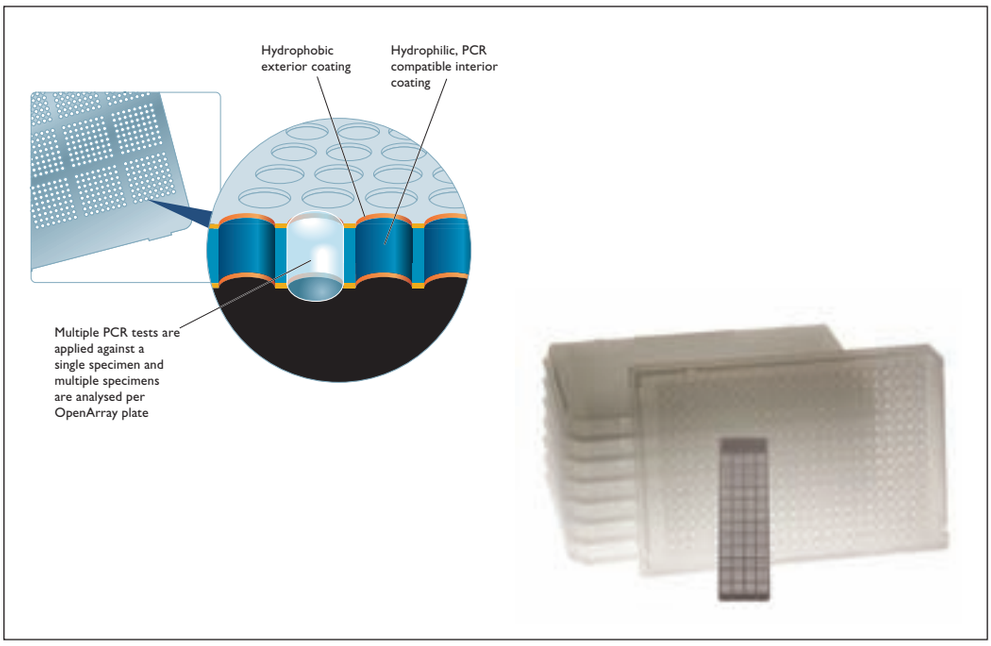

Figure 3
Each OpenArray® plate is a micromachined array of 3,072 through-holes in a platen the size of a standard microscope slide (upper left). The polymer coating on each plate ensures isolated and independent PCR assays against one or more specimens. One OpenArray plate equals the analytical capacity of eight 384-well microplates (lower right)

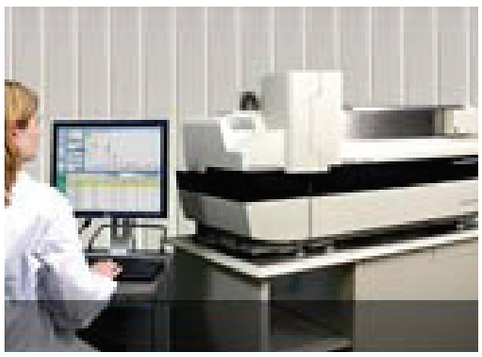

Figure 4
The Life Technologies FlashQuant™ system couples a MALDI ion source with a triple quadrupole mass spectrometer

degree of assay multiplexing without compromising data quality or assay performance. Furthermore, assay independence means optimised semi-custom or custom assay panels are generated by replacing specific assays or adding to the panel without re-design of the existing assays. Fluidic management via passive capillary action makes the system reliable, robust and greatly simplifies workflow integration with SBS-standard microplates.

Advances in early stage compound screening: impact of high throughput, microfluidic mass spectrometry

More predictive, biologically-relevant high throughput screening techniques are necessary to improve early stage compound screening. Rejecting compounds early in development based on actionable, high quality safety and efficacy data from assays relevant to the target disease and human physiology will bring substantial benefits by avoiding the much higher expense of failure down the line in human clinical trials.

Many high-throughput screens involve solution-phase biochemical assays in which compounds are tested for biological activity against a selected enzymatic target. An example of a biochemical assay is a kinase assay in which the ability of a test compound to attenuate the incorporation of one or more phosphate groups into a protein or peptide substrate is monitored. There are several methods by which the phosphopeptide product can be quantified, including the use of radiolabelled phosphate, ELISA-based methods, or a derivatised substrate incorporating a fluorescent label[5].

In many cases enzymatic targets are not amenable to conventional assay methods due to the characteristics of the substrates and products involved. In other cases, an economically viable HTS assay cannot be developed. In either event, potentially valuable targets are often not pursued. As a consequence compromises are made in assay design that lead to suboptimal screens in which biologically active compounds are not detected. Advancing suboptimal leads through the drug discovery process can lead to a significant waste of resources and productivity.

Ideally, an HTS assay facilitates the use of the native biological substrate of a particular target, does not require the use of radioisotopes, and provides accurate and precise quantification without compromising assay design. Label-free technologies offer a number of distinct advantages over label-dependent or artificial reporter assay formats[6]. First, they are, by nature, non-invasive and require minimal manipulation of reaction components, such as proteins or cells. This augments the potential for measuring biologically meaningful data. Second, label-free methods do not suffer from possible assay artifacts such as compound autofluorescence or quenching as no fluorescent dye or label is involved. Third, because there is no requirement for target modification, they can be used to study primary cells as opposed to genetically altered cell lines.

Mass spectrometry may be used to detect given analytes in a complex mixture based on their mass-to-charge ratio (m/z) with excellent selectivity and sensitivity, making mass spectrometry particularly relevant for trace-level quantitation of unmodified biochemical entities. A wide variety of chemistries, including those targets previously deemed intractable with conventional HTS technologies, may be detected using MS. Many of these challenging analytes are from target classes with substrates including lipids, fatty acids, phospholipids, steroids, prostaglandins and others. Rapid assay development can be accomplished with MS, for neither surrogate substrates nor indirect detection approaches (optical, isotopic or antibody-based) are needed. Mass spectrometry may be used to quantify substrate and product directly, providing a percent conversion. Mass spectrometry is also insensitive to many of the test-compound-specific interferences or artifacts that influence other assays (eg, auto-fluorescence or amended reactivity in the case of optical detection of fluorescent reporter groups, or crossreactivity in a coupled or antibody-based assay format). Nonetheless, mass spectrometry also presents several shortcomings. Mass spectrometers have a high capital and operational cost limiting the scalability of the platform. Additionally, MS requires extensive upstream sample preparation and purification, usually through some form of chromatography,

to isolate the analytes of interest from the confounding components in the assay, ultimately limiting the throughput of this platform.

The inherent advantages provided by MS-based analysis have led to a search for solutions to the problem of limited throughput. Ultra performance liquid chromatography (UPLC) systems such as the Waters Acquity® system (www.waters.com) use small micron-size beads to attain better separation with faster run times and lower system volumes. Turbulent flow® chromatography from ThermoFisher Scientific (www.thermofisher.com) permits higher flow velocities. Another approach is to use multiple liquid chromatography systems interfaced to a single MS. This amortises the cost of the latter while compensating for the relative slowness of the former. The commercially available Gilson-Waters multiplexed parallel sample acquisition interface (MUX) further epitomises these efforts. In this approach, a single MS is equipped with up to eight indexed electrospray ionisation sprayers. The FlashQuant™ system from Life Technologies (www.appliedbiosystems.com) couples a MALDI ionisation source with a triple quadrupole mass spectrometer for the MS analysis of large numbers of samples that have been prepared offline by parallel solid phase extraction techniques.

The RapidFire® system available from BioTrove, Inc is an approach that directly couples SPE to MS analysis, and that facilitates automated sample purification and MS analysis. Through the use of miniaturisation and automation, the RapidFire system is capable of throughputs as fast as 5sec per sample, which translates to more than 5,000 individual analyses in eight hours. The RapidFire system is fully automated and compatible with 96 and 384-well microtiter plates. The system has a capacity of up to 18 microtiter plates for the unattended operation of ~7,000 samples. The features of BioTrove's RapidFire platform address integration into the workflow of a true HTS environment.

Incorporating ADME/Tox data earlier in drug discovery

Elimination of unsuccessful candidates earlier in the drug discovery process would help to reduce the resources consumed and high cost of bringing a drug to market. Due to the inherent slow throughput of MS-based techniques, ADME/Tox assays are typically pursued late in drug development. The use of high throughput MS systems have been extended to reduce bottlenecks in *in vitro* ADME assays such as CYP inhibition, metabolic stability and permeability, increasing throughput and capacity to allow for the processing of these samples much earlier in the drug discovery process. In particular, the RapidFire technology has been incorporated into a new product offering, the RapidFire 300, specifically designed

Figure 5
BioTrove's RapidFire System directly couples SPE purification to a mass spectrometer for high throughput analysis

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to implement these standard ADME assays at throughputs that far exceed current HPLC-MS capabilities as well as other competing high throughput MS methods. The RapidFire 300 software is designed to give users the flexibility for running these different assays in a standardised format which efficiently integrates with their existing processes and workflow.

Conclusion – the future

As demands for safe and efficacious pharmaceutical drugs grow, technological solutions will be of increasing interest that reduce barriers to clinical success rather than simply shifting bottlenecks and necessitating new compromises in the discovery and development process. Critical are those solutions providing better target selection and validation from improved information on disease pathophysiology; more predictive and biologically-relevant high throughput assays; and acquisition of ADME/Tox earlier in the discovery process with the goal of identifying and rejecting earlier in the process those chemical structures having poor safety profiles. Miniaturised, quantitative assays implemented in high throughput hold the promise to impact these important areas of need. Nanofluidic quantitative PCR moves the superior analytical properties of the PCR assay into high throughput screening by enabling the capability of quantitatively measuring gene expression in multiple genes in multiple specimens simultaneously with the potential of providing better quality information by biologically validating a target's role in disease pathophysiology. The dual concepts of miniaturisation and quantification is further extended to screening functions in the discovery and development process where the goal is to generate safe and efficacious compounds for subsequent testing in humans. Introduction of high throughput mass spectrometry methods in the initial screening phase and following *in vitro* ADME/Tox testing phase allows testing of putative drug compounds with assays of greater biological relevance to the disease pathology combined with information on compound safety earlier in development. Better quality data from identifying biologically effective compounds based on application of these new technologies will ultimately impact the odds of clinical success; jump-starting a new era in drug discovery that will allow the industry to realise the gains promised by more than a decade of rapid technological progress. DDW

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