

Molecular diagnostics in drug development *the changing face of pharmaceutical discovery*

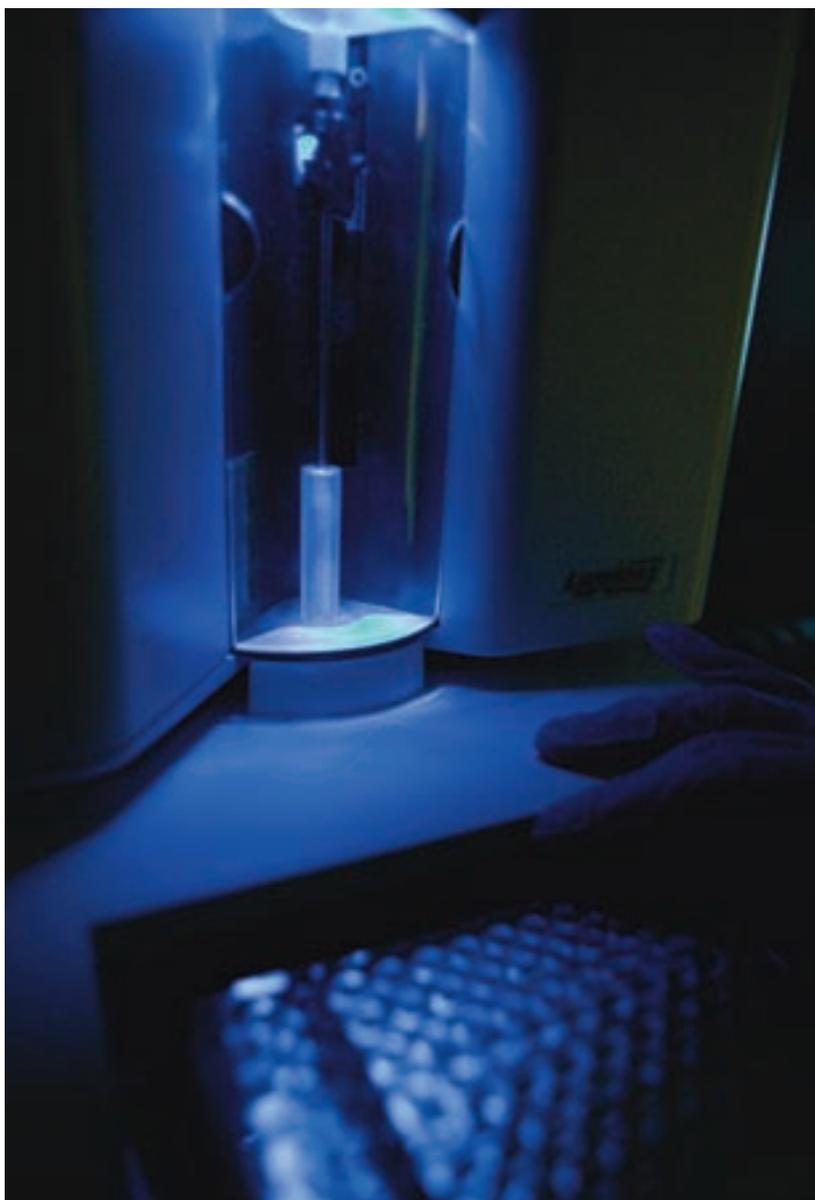
Clinical drug development is moving into an era where high technology new diagnostics are key to the development of the majority of new drugs. Markers are now available that can be used as surrogate endpoints for efficacy while others can be used to pre-screen patient cohorts so that any patient who might be contra-indicated for the study can be removed from the trial. Moreover, the increasing financial and human resource burden accumulating for health service providers because of adverse drug-related events, has alerted the FDA to the fact that pharmacogenetic evidence should be provided for all new drugs wherever possible. This article argues that, with the advent of personalised medicine and the accumulation of new genomic information and technologies, the role of molecular diagnostics is paramount to the future success of the pharmaceutical industry.

The pharmaceutical industry is currently facing up to the fact that it needs to radically alter its drug discovery and development paradigm to face the new challenges that are emerging. The scale of the industry is still immense, from the megalithic multinational giants down to the smallest biotech start-ups and the rewards can be spectacular for those that are successful. Nevertheless, the fact is now becoming increasingly clear that the efficiency and productivity of the industry leaves a lot to be desired. Despite record levels of R&D investment, there were only 20 drugs approved by the FDA in 2005 which conversely means that the level of attrition

of candidate molecules during the drug development pathway is getting higher. Indeed, recent figures indicate that only 8% of all molecules that enter drug development actually make it to successful registration compared to 14% 10 years ago. Even more worrying is the fact that the attrition rates in Phase II and III clinical studies are as high as 70% and 33% respectively. Obviously, the later in the process that a molecule falls down, the greater the cost to the organisation developing it. One of the major challenges, therefore, is to develop systems that can identify problems as early as possible and thus reduce the level of investment in fatally flawed molecules.

By Dr Berwyn Clarke

Diagnostics



The Luminex platform, is a multipurpose analyser based on the principles of flow cytometry

The advent of personalised medicine

A second issue for the drug companies is that the regulatory bodies are now becoming aware that recent technological developments mean that the whole concept of clinical trials should be re-addressed. In particular, the availability of specific new diagnostic tools means that for any given drug it is often possible to study the ability of that drug to show clinical benefit in specific patient cohorts with far greater definition than in the past. This has been largely driven by the level of adverse drug-related events which are related to already licensed drugs which creates massive financial burdens for healthcare organisations worldwide. More importantly, the lack of accuracy in assigning

specific drugs or doses to individual patients according to their specific characteristics means that they do not realise maximum benefit from the drug or may indeed experience undesirable toxicities. Consequently, there is a clear indication that the days of generic medicine are indeed numbered and that medicine in the future will be more and more targeted to the individual patient, ie personalised medicine.

The integration of molecular diagnostics into drug development

One of the underlying solutions to these new challenges to the pharmaceutical industry is the increasing value and utility of molecular diagnostics into the drug development pathway. The recent radical advances in molecular diagnostic technology, eg PCR, proteomics, genomics, metabolomics etc have provided key tools for the pharmaceutical industry to not only improve the ways it studies efficacy endpoints but also to introduce ways to reduce attrition rates and to address the concept of personalised medicine. Consequently, the molecular diagnostics industry is now taking a much more significant role in the drug development process than ever before. There are even situations where new diagnostic markers require their own diagnostic clinical trials to be undertaken to fully validate their significance before they are used in a drug registration study. This article is intended to review some of the ways that molecular diagnostics are being utilised to aid the pharmaceutical industry in the drug development process and as core components of the concept of personalised medicine.

The antiviral therapeutic area

In many ways the antiviral therapeutic area has been the pioneer for the development of personalised medicine. The driver for this has been the development of multiple medicines against HIV since the initial discovery of AZT in 1987. Because of the nature of the retrovirus lifecycle, HIV is easily able to mutate its genome in the presence of any selection pressure. Consequently when AZT was first administered in the clinic the appearance of drug-resistant forms of the virus began to emerge within about six months of therapy. Following detailed sequence analysis of the virus genome, the locations of the mutations that caused this resistance were shown to cluster to defined regions within the viral reverse transcriptase gene, the target of AZT. Fortunately, as new HIV agents were developed it was observed that these AZT-resistant viruses were still susceptible to some, but not all, of the new agents. Unfortunately, these agents themselves

induced resistance and mapping of the resistant *loci* often revealed completely different patterns of mutations. As HIV therapy progressed and, to a major extent, in order to overcome drug resistance, anti-retrovirals began to be used in multiple combinations often targeting multiple aspects of the viral lifecycle. Although this did have a great effect on controlling viral replication and inhibiting the incidence of resistance, it did not completely solve the problem and resistance did still appear in the clinic, particularly if the drugs were not administered with adequate compliance. The current state of the HIV field is that there are 19 drugs available representing four distinct classes of attack on the viral lifecycle and many of these are used in specific combinations to maximise clinical benefit. The complication for the clinical management of HIV is that each drug induces its own specific pattern of resistance mutations and, in the presence of other drug combinations, the mutational patterns can become extremely complicated. Furthermore, it is often observed that specific resistance patterns not only affect activity of the particular drug but also confers cross resistance against specific other anti-retroviral agents. Another complication of the appearance of drug resistance is that the forms of HIV that are circulating in the community are now composed of high proportions of drug resistant viruses as well as the classical wild-type forms.

From the perspective of the pharmaceutical industry the evolution of drug resistance and combination therapies has created a new challenge. Firstly, it is clear that any new therapeutic must be able to show efficacy against drug resistance as well as wild-type virus including those virus strains that are circulating predominantly. It is also essential to understand the nature of resistance that is selected during clinical trials both in terms of specific mutational patterns and likely cross-resistance to other agents. Obviously, all these questions would be extremely difficult to answer without the availability of specific molecular diagnostics which are able to rapidly monitor and analyse the appearance of specific drug-resistant viral strains.

Driven by the clinical need therefore, the HIV molecular diagnostic area has developed into an mini-industry in its own right. Firstly, sophisticated sequence analysis assays were developed which had complex interpretational software which could rapidly analyse sequence patterns and relate them into predicted phenotypic information. Partly because of the complexity of this information alternative methods were then developed whereby the relevant viral target was amplified by PCR and using elegant molecular techniques was reintroduced specifically

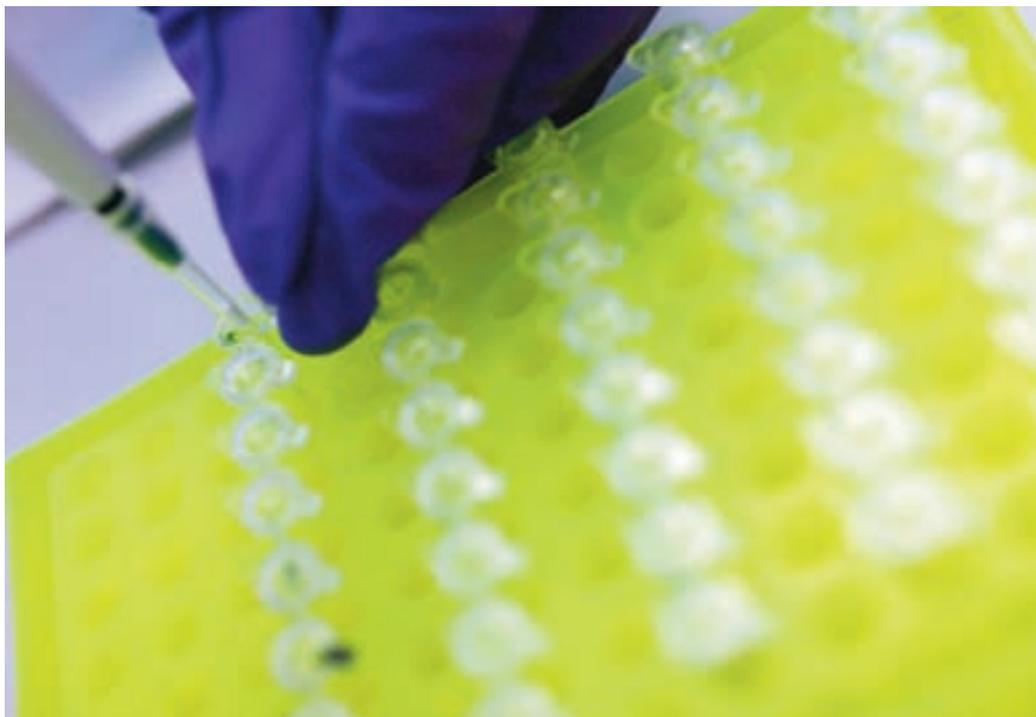
into laboratory strains. In this way the actual consequence of the mutations could be analysed in terms of the altered sensitivity to new or existing drugs. These phenotypic assays are typically longer and more complex to undertake than the straight genotypic analysis but the data they provide is often critical to the drug development process. Initially the viral phenotyping assays were focused on the viral reverse transcriptase and protease targets but now that other viral functions such as the integrase, the fusion proteins and the gag protein are all being exploited so the requirement for new molecular diagnostic assays to support antiviral drug development are likely to increase further.

While resistance analysis is a key component of antiviral drug development, the requirement for sensitive robust molecular diagnostics extends to other areas such as the measurement of viral load and clinical markers such as CD4/CD8 ratios. From the perspective of the diagnostics industry the additional bonus of developing these molecular diagnostics for clinical trials is that their utility does not cease when the drug is registered. Because of the emergence of drug resistance, HIV patients need to be regularly monitored to detect early signs of resistance so that their therapy can be modified. In this way the patient derives maximum benefit and the healthcare provider does not waste money on the use of expensive drugs on a patient whose virus will no longer respond. This concept of regular monitoring of patients is really the classic example of the pioneering concept of personalised medicine which is now beginning to emerge as a major development in modern medicine in many therapeutic areas.

Personalised medicine and pharmacogenetics

Although the antiviral area paved the way for the real implementation of personalised medicine, it has been known for many years that genetic variation in the host can have significant implications for drug response. One of the first indicators was in the use of drugs metabolised by the enzyme N-acetyl transferase (NAT) which is used in surgical paralysis. Variations in the NAT gene divides people into slow or fast acetylators and has significant impact on the rate they recover from surgical paralysis.

A more recent finding was the determination of variation in the enzyme thiopurine methyltransferase (TPMT) which is responsible for the metabolism of several anti-tumour agents. Genetic polymorphism in this gene means that it is difficult to achieve an effective dose of these drugs in patients



Sample preparation in PCR tubes awaiting multiplex PCR

with childhood leukaemia. Children with TPMT deficiency exhibit severe haematopoietic toxicity when exposed to these drugs whereas those with high activity polymorphism require high doses for any clinical benefit.

Pharmacogenetics is clearly now becoming a major issue in clinical development. This has largely been driven by the prevalence of adverse drug related events (ADRs). Each year in the UK, adverse drug reactions (ADRs) account for an estimated 6% of hospital admissions, £500 million in healthcare costs and more than 10,000 deaths¹. They are now acknowledged to be one of the leading causes of death in hospital in-patients. As a consequence of the accumulation of similar data in the US, the FDA is now beginning to recognise this as a genuine requirement for clinical trial data submission. Furthermore, the FDA is now considering inclusion of pharmacogenetic information on selected drugs including TPMT. In the past the information carried the warning that inherited deficiency of the enzyme could increase the risk of severe bone marrow suppression. In the future it will recommend that people who develop bone marrow suppression while receiving these drugs should be tested for genotypic TPMT deficiency. Another example is the recommendation that Camptosar includes information on UGT1A1 genetic variants that may affect the metabolism and clinical utility of the drug.

Collectively there is a large body of information on many drugs, particularly those metabolised by the CYP450 family of enzymes that indicates that many drugs should not be administered generically and that specific diagnostic tests should be applied to the patient prior to prescription of these drugs. From the patients' perspective this will have enormous, perhaps even life-saving, implications because they will be given the right drug at the right dose at the right time, every time. In this way patients will avoid many instances of ADRs due to dose-related toxicity and only be given drugs that are clinically proven to work on their genotype. For the healthcare industry this will also be advantageous because drugs will not be used on patients who are likely to be non-responders and the knock-on costs from ADRs such as hospitalisations, etc will be avoided. From the perspective of the patient and the provider there are, therefore, clearly compelling reasons for introducing pharmacogenetic analysis into mainstream clinical management. For the pharmaceutical industry the reality is more complex. Firstly, it might be argued that pharmacogenetics will effectively stratify the target population into patients that may not respond to the drug whereas, in the past, these patients would have been prescribed generically. On the other hand, the frequency and potential of ADRs arising during clinical trials and the fact that patient populations can be selected during

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studies who can be predicted to respond better to therapy, will inevitably improve the apparent clinical efficacy of the drug. Therefore the attrition rate during clinical trial will certainly improve because patients who are likely to respond badly to the drug will have been removed due to their specific genotype. This, together with the recommendations from the FDA, means that the pharmaceutical industry is generally agreed about the importance of personalised medicine and that they can develop drugs that have proven efficacy for specific patients then that must be good for the industry. The implications of this for the molecular diagnostic industry are obvious. Firstly, the industry will need to provide the appropriate reference laboratories which are able to undertake the sophisticated technologies required. Secondly, there will be a demand to introduce robust, reliable, high throughput assays for new biomarkers to support specific drugs if the relevant assays are not commercially available. Thirdly, this is likely to drive closer partnerships between the drug companies and the diagnostic companies right through the drug development process.

Companion diagnostics

As an extension to the personalised medicine concept there are other areas where molecular diagnostics are of huge value to the pharmaceutical industry in addition to the pharmacogenetic aspect. It is now well recognised from the Herceptin story that there are clear genetic reasons why particular drugs only work in particular patients. If the target of the drug is not present or is altered in some way then the drug clearly cannot be expected to work. Similarly there are now accumulating data to show that genetic markers can be used to segment patients in different ways. Some tests can predict patients that are predisposed to particular diseases, others that are likely to progress faster to serious disease or are genetically more likely to show long-term benefit. It is even possible to identify patients who are predisposed to cardiac disease, for example, and it might be prudent for the drug company to restrict their participation in clinical trials. So, again, there are now compelling reasons why the pharmaceutical industry would need to utilise a series of specific molecular diagnostic assays during the drug development process.

Molecular diagnostics in drug discovery – the future

It is clear that the advent of personalised medicine and the accumulation of new information and

technologies from sources such as the Human Genome Project will have significant implications for the pharmaceutical industry. Future studies will be required to address key biomarkers relating to either the target or the host. As more relevant parameters are identified, the likely outcome is that patients who are recruited into clinical trials, as well as the population at large, will ultimately possess their own personal pharmacogenetic and genetic profile. This information will significantly aid in the development of novel molecular medicines and will substantially drive closer interaction between the molecular diagnostic industry and the pharmaceutical industry. **DDW**

Dr Berwyn Clarke is Chief Scientific Officer of Lab21 Limited. He spent most of his career in the pharmaceutical industry before moving to clinical diagnostics in 2000. After initially joining Virco Diagnostics he became VP for Research at Visible Genetics before its acquisition by Bayer. Following the acquisition he formed a new company, Oneida Theradiagnostics, prior to the launch of Lab21 in 2005.