Box 1. ONYX-015 gives good Phase II clinical trial results

ONYX-015 is a modified adenovirus that replicates and lyses $p53^-$ but not $p53^+$ human tumour cells. Recently, a Phase II clinical trial reported that ONYX-015 treatment, combined with chemotherapy, was promisingly successful in 30 patients with head and neck cancers. Tumours disappeared completely in eight patients and another 19 experienced a dramatic reduction in tumour size[4]. French Anderson, who pioneered the first clinical trial of gene therapy for the genetic adenosine deaminase (ADA) deficiency in 1990, says that this latest trial of ONYX-015 'is particularly encouraging because the tumours disappeared in significant numbers of patients and have not recurred.'[5]

further.' Moeller also highlights that future studies should also address the problems of gene delivery. 'An adenoviral vector is only useful in solid tumours, and it has to be injected directly, making the treatment of small tumours or metastases very difficult.' Clinical trials might be some way off but he thinks they should investigate whether a combination therapy of p16–p27, chemotherapy and radiotherapy might improve the outcome, 'as it has in the recent and encouraging studies of ONYX-015' (Box 1).

Although unable to give full details at this stage, McArthur says that his team is testing second-generation molecules that are more potent than the p16–p27 fusion product. 'We are currently doing preclinical studies on these molecules and we expect that we can use them to overcome some of the delivery hurdles. We hope that the molecules we put into clinical trials will have broader activity against a wider variety of tumours and will be capable of dispersing throughout the body,' he says.

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Kathryn Senior

Key strategies in functional genomics for drug discovery

Speakers at the IIR conference entitled *Using Functional Genomics in Drug Discovery*, held on 27–28 September 2000 in London (UK) discussed a number of methods of using functional genomics, some of the problems and issues surrounding their use in drug discovery and many future concerns and future directions for the field.

Klaus Lindpaintner (Hoffman-La-Roche, Basel, Switzerland) highlighted that although many diseases are genetically defined, many are the result of a combination of genetics with other factors. For example, in Alzheimer's disease, the presence of the ApoE4 allele is associated with a twofold increase in incidence of the disease. However, although head trauma alone shows no effect on incidence, the presence of this allele together with trauma increases incidence of the disease tenfold. This highlights that diseases are more than just a genetic mutation but are a combination of genes and the environment: diseases such as haemophilia are mostly genetic, while diseases such as lung cancer are significantly induced through the environment (mostly smoking in this example).

John Morrison (AstraZeneca, Macclesfield, UK) presented data on asthma to highlight that combined genetics and genomics approaches are better than the individual approaches alone as it should enable the prioritization of candidate genes to reduce the number from tens of thousands with unknown function to approximately ten. This would be done through the use of data from several differential strategies and then identifying the genetic association with the disease using SNPs followed by searching databases such as Incyte's. There were concerns that the use of so many different technologies would lead to accumulative errors and an increase in the number of false-positives, but Morrison confirmed that these technologies were being run in parallel at AstraZeneca and that the experiments were designed specifically to avoid this problem.

Genomics for target validation

The information gleaned from the sequencing of the human genome should dramatically speed up the process of target validation. There are several methods with which to use this information, four different such approaches being presented at this conference.

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Epidemiological studies

Ralf Tolle (Lion Bioscience, Heidelberg, Germany) discussed the possible impact of genetic epidemiological studies on preclinical target gene validation. One key advantage of this method is the fact that no animal models are required as it uses the human *in vivo* system. However, Tolle highlighted that there are some financial problems associated with this method, such as the data management costs (although IT solutions will decrease the cost of SNP studies substantially) and the assay costs (which should reduce through technological advancements towards producing robust and affordable genotyping assays).

The most significant current concern, however, is the limited availability of well-characterized populations, and this therefore presents the challenge of educating people on the potential benefits this could bring to them. Recent studies conducted by Lion Biosciences where they were recruiting cardiovascular patients produced a surprisingly positive reaction from patients after the process and the benefits of it were clearly explained. In fact, they found that once people found out they had the disease, they were quite pragmatic in their actions. Tolle concluded that he therefore thinks that, in the near future, genetic epidemiology will make the transition into industrial applications to complement existing validation strategies.

Gene knockout technologies

Stephen Harris (GlaxoWellcome, Stevenage, UK) outlined the use of gene knockout technologies in target validation and suggested that investment in this technology would enable comparative, evidence-based, target selection decisions when integrated within a functional genomics pipeline. To make the best use of these technologies, he suggested that there are three key scientific and business-related questions that must be asked when doing target selection using large-scale knockout transgenics.

First, it is important to find out how often a gene knockout gives rise to a phenotype and what types of phenotypes are observed. Second, it is important to know what value is attributed to the phenotype. A recent study examined 50 past G- protein-coupled receptor (GPCR) drug discovery projects at Glaxo-Wellcome for which there were 15 gene knockout phenotypes available. These projects were cat-egorized into nine 'informative', two 'misleading' and four 'positive' projects. The analysis of the results suggested that at least 20% of all knockouts (at least for GPCRs) will have a phenotype that is informative in some way.

The final question is to decide how best to access comparative knockout information at the scale necessary and in a timely manner. This requires a decision between using internal generation of knockouts and phenotyping, and outsourcing. Outsourcing can be done through the traditional fee-for-service providers, although the cost-per-gene can be high and the phenotyping would still have to be done internally. Alternatively, outsourcing can be done through functional genomics alliances, which reduces both the cost-per-knockout and the cost-per-gene, and also enables shared benefits to both companies.

Although using knockouts early in the discovery process is very expensive and is associated with high-risk multipliers, many companies such as Glaxo-Wellcome and Bristol-Myers Squibb have already looked at the value they expect to gain from the data it produces and have decided to take this route. Harris did however express concerns over the use of conditional knockouts, as although he thinks they have value, there are still uncertainties over solely using conditional knockouts for everything.

Proteomics

Jon Terrett (Oxford Glycosciences, Oxford, UK) described the use of proteomics for the discovery and validation of targets, markers and antigens. Intracellular targets for cancer are being discovered by large-scale comparisons of tumour and normal material at the protein level. A similar strategy has been used to find proteins in serum that correlate with the progression of cancer from benign to highly metastatic states. Candidate antigens for immunotherapy are being defined from membrane preparations of cell line and clinical tumour material. These proteomics-based discoveries are being combined with genomic and bioinformatic analyses. Three of these antigens show massive upregulation in primary breast cancers and a prevalence of up to 40%, and some have already been committed to progression to therapeutic antibodies by Medarex.

Bioinformatics

Bob Belevieu outlined the Human Genome Sciences (HGS, Rockville, MD, USA) strategy for using bioinformatic searching of their extensive EST database to find targets in classes that are recognized as tractable for small-molecule intervention and also as protein therapeutics in their own right. The examples described related to proteins containing a secretion signal sequence at their N-termini that were recognized partially by a hydropathy plot and then more fully characterized by cloning and sequencing the full-length genes. HGS has a repertoire of patents on such proteins and some are already committed to clinical trials as therapeutic proteins and drug targets.

Predictions for the future

Although progress in functional genomics has been significant, many, such as Stephen Harris, think that there is still much work to do in defining the criteria to make the correlations between the genotype and the mechanisms of disease pathophysiology. Many other areas were mentioned that need much more careful consideration and are discussed briefly here.

Effects on preclinical and clinical studies

With the advent of pharmacogenomics, we should be able to move from empirical prescription using 'mass markers' to rational 'individualized' prescriptions, avoiding trial-and-error prescribing as well as reducing the impact of the side effects from inappropriate drugs. In the future, we should therefore be able to profile molecular targets and apply this knowledge to patients.

Lindpaintner predicted that although pharmacogenomics will enable more testing to be done prior to animal studies, there will always be a need to go into animals at some stage before going into humans. However, there should be an overall reduction in animal testing as animals should only need to be exposed to pharmacogenomically tested drugs. Meanwhile, in clinical trials, Jon Morrison suggested that as there will be a need for stratification of patients into homogenous groups for clinical trials, diseases will need to be reclassified at the molecular level and patients will be classified on the variability of expression of drug targets.

Diagnostic use

Lindpaintner predicted that in the future there will be a need for an increased use of *in vitro* diagnostics, for more differential molecular diagnosis and for an increase in the integration of diagnostics and therapeutics. This increased use of diagnostics should shorten discovery and development cycles, maybe not so much on individualized projects but to a more significant degree overall, due to fewer failures because of targeting *bona fide* disease mechanisms. In the short-term, Lindpaintner suggested that pharmacogenetics would include adverse effect profile pharmacogenetics and efficacy profile pharmacodynamics; in the mid-term, it would cover genotyping for target identification and expression profiling; in the long-term, there would be a move towards causative and predictive pharmacogenetics as well as new target discovery using, for example, expression profiling.

Public perceptions

Lindpaintner also expressed concern over the way that the bioethical problems are being handled. He suggested that, at the moment, there is a widespread fear by the public of abuse of genetic information and that a focus on confidentiality will limit the utility and use of information for the patients' benefit. He therefore felt that there is a need for a societal consensus that protects the individual while enabling the beneficial use of the information. A prerequisite to this, therefore, is that genetic scientists discuss these issues openly with the public. He also felt that as the process of pharmacogenomic testing proves to be effective, people will be more willing to have predisposition testing.

Antibody drugs

Carl Webster (Cambridge Antibody Technology, CAT, Royston, UK) discussed the use of phage antibody libraries and proposed that they are a key part of future target validation and drug discovery strategies. He suggested that the advantages of using these libraries are clonal diversity and that the antibodies have a high level of affinity. These antibodies can be used for high-throughput validation of genomics targets, as research reagents and for proof-of-principle studies. Webster also emphasized that monoclonal anti-bodies against these genomics targets are expected to become a substantial drug class of the future. There are several human monoclonal antibodies already in clinical trials, with S2E7 (BASF, Ludwigshafen, Germany), the potential rheumatoid arthritis agent, being the first (and only one so far) to enter Phase III trials.

The future

Lindpaintner concluded by predicting that genetics should change medicine in evolutionary and incremental terms because of more sophisticated patient-specific information. However, he said that conceptually, genetics would make no difference as it is just another step along in the history of medicine, as really genetic information is just biological information but on a different level.

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Rebecca N. Lawrence

Pseudocomplementary strategy strengthens PNA therapeutic potential

Peptide nucleic acids (PNAs), synthetic oligomers that mimic naturally occurring DNA and RNA, have the potential to act as antisense and antigene drugs, but several limitations have so far hindered their development as therapeutic agents. Recently, Vadim Demidov, Maxim Frank-Kamenetskii (Center for Advanced Biotechnology, Boston University, MA, USA) and colleagues published a new study on the properties of pseudocomplementary PNAs

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