The use and usefulness of transgenic systems in the discovery of new medicines

The pharmaceutical industry is faced with the challenge to deliver truly novel medicines to a market under financial pressure when pharmaceutical R&D becomes increasingly difficult and complex. This review will mainly focus on how transgenic systems can help to deliver drug targets that can open up completely new treatment opportunities and also how the technology can help maximise the quality of new chemical compounds. Together this will increase the success rate for compounds during clinical development. It is clear that genetically modified mice have many characteristics making them useful to understand novel disease mechanisms and to find new pharmaceutical treatment paradigms. Mouse and man are very similar on the genetic level, it is possible to measure similar disease parameters in mouse and man and genetic alterations in the mouse often result in functional changes predicting the relevant pharmacological effect in man. Transgenic animals can be used broadly in the discovery of new medicines. Traditionally, the main utility of transgenic animals has been during the target validation phase, ie increasing the possibility that a specific target is a safe and efficient drug target. However, at least as important is the use of knockout animals in analysing the safety, specificity, metabolism and efficacy of future drug candidates.

By Professor Jan Törnell

The pharmaceutical industry is faced with the challenge to deliver novel medicines to a financially constrained market when pharmaceutical R&D at the same time becomes increasingly difficult and complex. The healthcare providers in different countries are forced to cut costs and are therefore increasing their expectations on new medicines. In health-economic studies it is imperative, in my view, to make sure all costs and potential savings in different areas are included when making the cost analysis of new medicines. The organ responsible for covering costs for medicines are often distinct from the authorities paying for sick leave and early retirement. On top of this comes the loss of production and the decreased revenues from taxation. The value for patients in increased quality of life and prolonged life expectancy, which are the most important parameters in this context, is even more difficult to express in monetary terms. In essence,

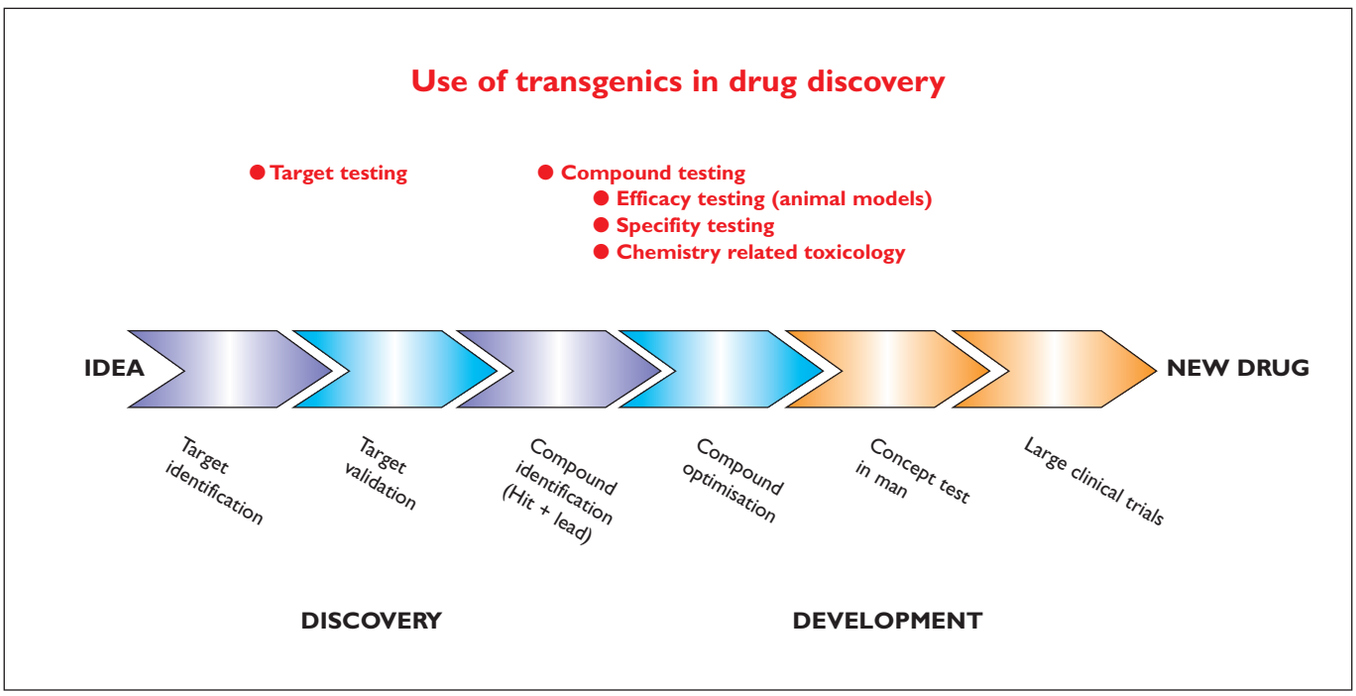

Figure 1
Transgenic technology can be used widely in pharmaceutical R&D. In the early phases transgenic technology can be used for *in vivo* target validation. During the lead optimisation phase the models can be used to understand efficacy, specificity, distribution, kinetics and safety of compounds

the pharmaceutical industry is asked to develop novel breakthrough medicines to a low cost.

This challenge for the pharmaceutical industry is linked to a large opportunity for companies willing to adapt to the more difficult climate. This opportunity comes from the huge investment (more than $3 billion) the worldwide scientific community has made in analysing the structure and function of the human genome. It is a mistake to believe that the human DNA sequence by itself will disclose a large number of new targets. The real value comes from linking the discovered genes to normal function and to disease. By doing this novel drug targets can be found and new medicines, valuable both for society and industry, can be discovered.

This linkage between genes/pathways/networks and normal function/disease will come from a synthesis of data from genome structure, gene expression studies, mouse knockout experiments, *in vitro* studies and clinical studies. The companies having the capacity to refine this information and turn it into knowledge will be the winners for the future.

This review will focus on how experimental models, and in particular mouse models, can be used to increase the value of both targets and compounds. In-depth analysis of targets will help to link specific genes to disease and thereby discover truly novel therapeutic approaches. Using transgenic mice to evaluate efficacy, safety and specificity of compounds will help to focus chemistry and clinical trials on the compounds with the highest likelihood of success and thereby increase the effectiveness of R&D.

I will not go into any detail about the technology used to generate transgenic mice. It is only worth mentioning that although novel technology (eg siRNA) may be important in the future, techniques like gene targeting (gene knockout) is likely to be the workhorse for modification of the genome *in vivo* in the foreseeable future.

What is required if the mouse should be a model for human disease?

At least three conditions need to be fulfilled if the mouse should be an adequate model for human disease: 1) Human and mouse have to be similar on the genetic level to allow for identification of homologues genes and pathways/networks; 2) It must be possible to measure similar functional parameters in mouse and man; and 3) Genetic intervention with transgenic technology of a specific target gene must result in an altered function predicting the effect of pharmaceutical intervention with the corresponding target in man.

Similarity of human and mouse genomes

Both the genomic sequences for man[1,2] and mouse[3] have recently been published and the similarities between the two genomes are striking. Approximately 99% of the genes in mouse have homologues genes in man and vice versa. The opportunity to modify the mouse genome by trans-

genic technology makes it possible to study loss of function or gain of function of particular mouse genes homologues to specific human genes. This is likely to be one of the most powerful tools to understand the functions of human genes in physiology and pathology.

Functional analysis of mouse and man

To be able to evaluate functional consequences of perturbations of the mouse genome that are relevant to human physiology and pathology it must be possible to measure similar functional parameters in mouse and man. This is challenging in many areas but the progress has been tremendously fast in the last five to 10 years.

The blood volume of a mouse is very small and therefore the sample size that can be collected is small. For analysis this requires very sensitive biochemical methods that can handle tiny sample volumes. Such methods are now established. The advantage of a small body size is the minute amount of substance needed when testing potentially novel medicines.

The miniaturisation of methods for analysis of cardiovascular parameters such as blood pressure, ECG and blood flow has advanced enormously in the last five to seven years. By using systems for continuous monitoring of unrestrained conscious animals it is not only feasible to discover small changes in animals not stressed but it is also possible to discover changes only present at a specific time of day.

The human brain with all its complex cognitive and emotional functions is probably the most challenging organ system in man to try to model in lower species like mice. A challenge in analysing emotional and cognitive functions in the mouse is that only behavioural signs can be studied in contrast to human patients where the diagnosis is largely based on a dialogue. Rather than trying to find a 'depressed' or 'schizophrenic' mouse, these diseases are probably best studied by observing behaviours in the mouse that will represent a component of the disease[4].

The mass of a mouse is several orders of a magnitude smaller than a human being. This will make imaging more challenging in some aspects but, for

References

1 Lander, ES et al. Initial sequencing and analysis of the human genome.[see comment][erratum appears in Nature 2001 Aug 2;412(6846):565 Note: Szustakowki J [corrected to Szustakowski J]]. Nature, 2001. 409(6822): p. 860-921.

2 Venter, JC et al. The sequence of the human genome.[see comment][erratum appears in Science 2001 Jun 5;292(5523):1838]. Science, 2001. 291(5507): p. 1304-51.

3 Waterston, RH et al. Initial sequencing and comparative analysis of the mouse genome.[see comment]. Nature, 2002. 420(6915): p. 520-62.

4 Bucan, M and Abel, T. The mouse: genetics meets behaviour. Nature Reviews Genetics, 2002. 3(2): p. 114-23.

5 Zambrowicz, BP and Sands, AT. Knockouts model the 100 best-selling drugs – will they model the next 100? Nature Reviews Drug Discovery, 2003. 2(1): p. 38-51.

6 Jankowsky, JL et al. Transgenic mouse models of neurodegenerative disease: opportunities for therapeutic development. Current Neurology & Neuroscience Reports, 2002. 2(5): p. 457-64.

7 Inui, A. Transgenic approach to the study of body weight regulation. Pharmacological Reviews, 2000. 52(1): p. 35-61.

8 Powell-Braxton, L et al. A mouse model of human familial hypercholesterolemia: markedly elevated low density lipoprotein cholesterol levels and severe atherosclerosis on a low-fat chow diet. Nature Medicine, 1998. 4(8): p. 934-8.

9 Carmeliet, P, Moons, L and Collen, D. Mouse models of angiogenesis, arterial stenosis, atherosclerosis and hemostasis. Cardiovascular Research, 1998. 39(1): p. 8-33.

10 Mangiarini, L et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell, 1996. 87(3): p. 493-506.

Continued on page 89

technical reasons, it is possible to perform image analysis in smaller animals with higher resolution than in man.

Correlation between the phenotype of a mouse knockout and the effect of a drug acting on the corresponding target in human

A fundamental question is if it is possible to validate a human drug target by deletion of the homologues mouse gene with knockout technology. In other words, will the functional consequences of the mouse knockout predict what pharmaceutical intervention will yield in man?

To answer this question I performed a retrospective analysis of more than 100 targets for approved drugs. For almost 60% of the targets the corresponding mouse knockout had a relevant functional deviation (unpublished data). This high degree of predictability of mouse knockouts for human pharmaceutical intervention is very promising. In approximately one third of the cases I could not identify any published knockout data supporting the clinical indication and in a few per cent some supporting data could be found. Possible explanations for these targets where no supporting mouse knockout data could be found in the public domain includes developmental consequences of the gene inactivation, it may be challenging to measure relevant parameters in certain disease areas or genuine biological differences between mouse and Man. Understanding the limitations and the opportunities of mouse models for human disease is very important.

In a similar analysis the targets of the 100 best-selling drugs were analysed[5]. The authors found that the phenotypes of the specific knockouts correspond well to the efficacy of drugs acting on respective target.

The conclusion would be that i) the mouse is very similar to man on a genetic level enabling homologues genes to be identified, ii) similar functional parameters can be measured in mouse and man making it possible to compare disease/marker deviations, and iii) functional consequences of deleting a gene with knockout technology correspond well to the comparable pharmacological effect in man.

The use of transgenic mice in pharmaceutical research

Transgenic animals can be used widely in the process of discovering new medicines, eg in validation of potential new drug targets and in understanding of specificity, efficacy and safety of compounds.

Target validation

The sequencing of the human genome has meant that the challenge of identifying novel genes, that are potential drug targets, has been minimised. The great challenge today is to validate targets, ie to link some of the 30,000+ genes to specific diseases and prioritise the best targets. Interaction with a 'perfect' target should result in prevention or regression of disease, not in serious target-related side-effects and the target should belong to a class of molecules that could be affected by small orally available molecules. Ideally, this analysis should be done before the company invests too large resources to generate an optimised small chemical entity. Over the years several technologies have been used for this purpose eg antibodies, antisens, ribozymes, etc. These technologies all have their challenges for *in vivo* use including delivery, ability to penetrate cell membranes and specificity. The most widely used *in vivo* technology is the transgenic technology. There are several advantages with transgenic technology (eg specificity, useful to target most [all?] genes) but the technique also has several limitations (eg time and effort required to generate the models, vital developmental functions of certain genes and possibly developmental compensation in some cases). Some of these challenges can be overcome by using conditional transgenic technology but this certainly adds time and effort.

Compound validation

One area for using transgenic mice in drug discovery that has been largely neglected is the possibility to test compounds and potential future medicines.

Efficacy evaluation

One of the most expensive problems for the pharmaceutical industry is that the single largest reason for failure of compounds in clinical trials is lack of efficacy. This is related both to that the target is not properly linked to disease (not properly validated, see above) and/or that the compound has not been tested in relevant efficacy models. In some diseases it is possible to measure parameters in healthy animals (eg measure blood pressure when testing compounds for treatment of hypertension or stomach acid secretion when searching for drugs against stomach ulcers). In other diseases it is crucial to have disease alterations in the animals that could be prevented or reversed. This is the case in diseases like Alzheimer's disease, different forms of cancer, atherosclerosis, obesity, osteoarthritis and many more. In some cases transplantations can be used (eg xenografts in studies of cancer), others can

be studied in animal models where a disease phenotype has been induced surgically, chemically, by a spontaneous mutation or by transgenic technology. In diseases like Alzheimer's disease, obesity and atherosclerosis, transgenic technology is very important in helping to establish efficacy models[6-9] and these models are widely used in the pharmaceutical industry.

Specificity evaluation

It is a challenging task to understand if a specific compound is acting only through the target it is aimed at. A number of tests can be done *in vitro* in cell-free and cell-based systems. For example, the proposed specific compound can be tested on closely related targets and some information may come from dose-response curves or time-courses. The information is, however, limited by the fact that the chemical compound may act through a completely different system or that all members of a target class may not be known.

It is an opportunity to use knockout animals in this analysis. If an effect can be recorded in animals lacking the proposed targets, the drug is most likely acting through some other mechanism(s). This understanding may help in achieving a better efficacy/safety ratio by making compounds more specific.

Safety evaluation

As described above, safety of new medicines is a priority both for the pharmaceutical industry and regulatory authorities. This is even more important when novel drugs will be used for prevention of disease or when chronic diseases will be treated. Knockout animals can be used both to understand target (mechanism) related toxicity and off-target toxicity.

Off-target toxicity can be investigated in a similar way as described for specificity analysis above. If adverse reactions are observed in animals lacking the target for the compound, these side-effects are likely to be mediated by a mechanism not related to the target (and thereby to the efficacy). With this result there is a fair chance to get around the side-effects by making a more specific compound. If, on the other hand, adverse events seen in wild-type animals disappear when the target is deleted in the knockout animals the side-effect is likely to be mediated through the target. It will then be difficult to get around the adverse events by making more specific compounds. Instead, dosing may have to be adjusted or the bioavailability to certain toxicology related compartments in the body might need to be restricted.

It is not uncommon that compounds have strict species specificity and in some occasions it may be difficult to monitor target related toxicity in the standard species used for safety assessment. Knockout animals can be used to understand target related toxicity by analysing the phenotype of the gene-targeted animal. There are some limitations with this approach. The knockout resembles a complete inhibition of the target and this is not always seen with pharmacological inhibition. Another caveat may be that by deleting the gene (and thereby the target) very early during embryonic development there may be both false positive and false negative results. False positive results may come from that the target has important developmental functions that may not be a problem when treating adult patients. False negative data may be generated because deletion of the target already during embryonic development may lead to activation/establishment of mechanisms compensating for the loss of the target.

Another possibility to investigate human-specific substances in experimental species is to 'humanise' the mouse in respect of the particular target. A human variant of the target is introduced by transgenic technology either by gene-addition technology if the mouse gene can be left intact or by 'knock-in' technology if the mouse gene has to be deleted (as is the case when an antagonist is to be tested). Several studies shows the feasibility of 'humanisation' with many of the expected down-stream effects of the human target[10,11].

Analysis of pharmacokinetics and drug metabolism

Understanding of how a compound is absorbed, distributed in the body and excreted is essential in the generation of a new medicine. Transgenic technology may offer help to understand the molecular basis for these phenomena. One model that has been widely used for understanding drug transport is the knockout of the mdr1a gene resulting in a mouse lacing the P-glycoprotein (Pgp)[12].

Metabolism via the P450 system of enzymes is an important mechanism for inactivation of drug substances. Several strains of mice have been generated lacking specific P450 enzymes[13-15]. The involvement of specific P450s in toxic responses can be studied. For example, CYP2E1 -/- mice have reduced metabolism of benzene[16]. These mice also show less toxic response to benzene, well in line with the concept that most of the toxicity is caused by metabolic activation.

Continued from page 88

11 Bonaventure, P et al. Humanization of mouse 5-hydroxytryptamine1B receptor gene by homologous recombination: in vitro and in vivo characterization. Molecular Pharmacology, 1999. 56(1): p. 54-67.
12 Schinkel, AH et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell, 1994. 77(4): p. 491-502.
13 Heidel, SM et al. Cytochrome P4501B1 mediates induction of bone marrow cytotoxicity and preleukemia cells in mice treated with 7,12-dimethylbenz[a]anthracene. Cancer Research, 2000. 60(13): p. 3454-60.
14 Buters, JT et al. Cytochrome P450 CYP1B1 determines susceptibility to 7, 12-dimethylbenz[a]anthracene-induced lymphomas. Proceedings of the National Academy of Sciences of the United States of America, 1999. 96(5): p. 1977-82.
15 Lee, SS et al. Role of CYP2E1 in the hepatotoxicity of acetaminophen. Journal of Biological Chemistry, 1996. 271(20): p. 12063-7.
16 Valentine, JL et al. Reduction of benzene metabolism and toxicity in mice that lack CYP2E1 expression. Toxicology & Applied Pharmacology, 1996. 141(1): p. 205-13.

Conclusions

It is clear that genetically modified mice fulfil many of the criteria for making them useful models to understand disease mechanisms and to find new pharmaceutical paradigms. Mouse and man are very similar on the genetic level allowing for identification of homologous genes, pathways and networks. It is also possible to measure similar parameters in mouse and man allowing for proper comparison of disease mechanisms/markers in the two species. Moreover, genetic alterations in the mouse often result in functional changes predicting the relevant pharmacological effect in man.

Transgenic animals can be used broadly in the discovery of new medicines. Historically, the main utility has been during the target validation phase, ie increasing the possibility that a specific target is a safe and efficient drug target. However, at least as important is the use of knockout animals in analysing the safety, specificity, metabolism and efficacy of future drug candidates. DDW

Professor Jan Tornell *(MD, PhD) is Global Director of AstraZeneca Transgenics and Comparative Genomics. In 1996 he was recruited to Astra to build and lead a core unit (Astra Transgenic Centre) to generate and analyse transgenic animals for the company worldwide. After the merger with Zeneca he was appointed to the role of leading a unit for Transgenics and Comparative Genomics for AstraZeneca globally. Prior to joining AstraZeneca he ran a research group at Gothenburg University and has had a major role in building transgenic technology in Scandanavia. He was appointed Professor of Physiology in 1998.*