Drug Discovery & Genetically Engineered Models

genOway

Personalized Genome Engineering Service

How Can Drug Discovery Programs Benefit From Genetically Engineered Models?

Success stories have been published and publicized, but costly failures exist!

On average, and for one target, at least 10 different types of Knockout models can be designed (and tens of variations for each type). Numbers are even higher for more sophisticated models, like Knockin, Humanization, etc.

The accuracy and the quality of the *in vitro* and *in vivo* research models are key to the outcome of target validation and compound efficacy studies.

How can success stories become the standard for drug discovery?

Success stories clearly demonstrate that the primary success factor is the physiological relevancy of the model, while failures are often linked to: (1) lack of thorough analysis of the target gene biology; and (2) technical-based model design.

Techniques/technologies should remain a means to achieve a scientific objective and not the other way around. It is physiology that dictates the design.

Well-designed genetically engineered models can only be obtained through a labor-intensive, science-based analysis process, which relies on three main pillars:

- **Biology of the target:** Each target is different and belongs to different pathways; engineering should be done accordingly.
- **Goal of the project:** The model use dictates the design - a model for target validation is different than a model for toxicology studies, even though ultimately the target will be inactivated.
- **Business constraints of the drug discovery program:** The genetically engineered model is a part of the puzzle and it must fit.

Focus on the target

- How does the target work?
- How is it regulated?
- Where is it expressed?
- Which are its partners?
- How does it interact with them?
- And so on.

These are critical questions to address when generating a model for translational medicine, target validation, or efficacy studies.

genOway approach: it's all about the right design

For each new model, (1) a thorough scientific analysis is performed (literature, *in-silico* tools, risk assessment, etc.), and (2) options are ranked and compared, based on the target gene biology, model use, technical feasibility, timelines, and budget.

This well-defined decision-tree process is crucial to the quality of the research model, the quality of data resulting from the use of the model, and its impact on drug discovery programs.

Examples of Applications

1. Target validation

Through tissue-specific expression of the target gene:

- Models for ADC: cell-specific expression of the antigen targeted by the antibody, expression level, and distribution are crucial to the design of a relevant model, enabling one to study the effect of ADC on the tumor microenvironment (ADC targeting TAM)

Through gene ablation, the most commonly used strategy:

- Enables one to predict the consequence of a target-complete inactivation, but should be limited to target, with very little interaction with other pathways (ion channels, secreted proteins, etc.)

Through point mutation:

- Becoming the standard for dissecting between functions of proteins displaying dual activity, such as scaffolding and kinase activities (RIP1, RIP3, JAK, STAT, etc.)

2. Mechanism of action (MOA)

Tissue/time-specific inactivation of a target gene:

- Ideal for MOA studies and investigation of targeted therapies (immune check-point, complexes involved in effector functions, and more)

Tissue/time-specific induction of catalytically inactive enzymes/kinases:

- To inactivate a given function of an enzyme while preserving its expression and interaction with partners; mimics what a small molecule (DNA sensor, PKC, PK, etc.) would do

3. Translational medicine

Understanding of the etiology of the disease in humans is paramount to designing a relevant translational model.

Are the SNPs identified through GWAS causal agent of the diseases? What are the consequences of those mutations at the gene and protein levels?

- Defect in gene-splicing process resulting in no expression of the protein
- Defect in gene-splicing process resulting in expression of a mutated protein
- Defect in the gene expression level and pattern
- Defect both gene expression level and expression of a functional protein

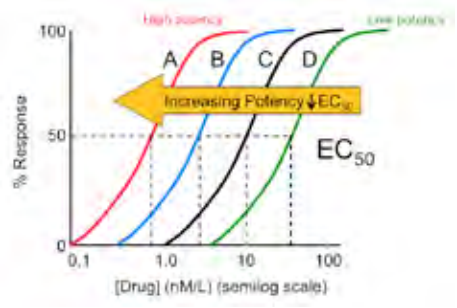
Answers to these questions are often unknown, but a thorough bio-informatics analysis coupled to *in vitro* assessment of some of the hypothesis could clarify the context. This dictates the design of the *in vitro* or *in vivo* model recapitulating the human situation.

4. Efficacy studies

The design of the humanized model must take into consideration the family of the target gene and type of therapeutics developed.

Beside its primary function, does the target gene interact with partner molecules? If yes, would a humanization of the target abolish these interactions?

- IL-4Rα interacts with the γc chain to create functional IL-4 receptors, but also interacts with IL-13Rα1 to create the IL-13 receptor
- Recognition of TLR2 by its ligands requires an heterodimerization with TLR1 and TLR6



Is it required to humanize the whole target gene or shall we humanize only the epitope recognized by the antibody and preserve the interaction with other subunits of a complex?

- CD3ε partners with CD3γ, ζ and δ to create a functional CD3 complex
- Lack of interaction with partners would results in immune-compromised models

Shall a humanized model express only the canonical form of the target (appropriate for efficacy studies) **or the canonical and secreted forms that could act as decoys?** (More relevant for PK and PD studies)

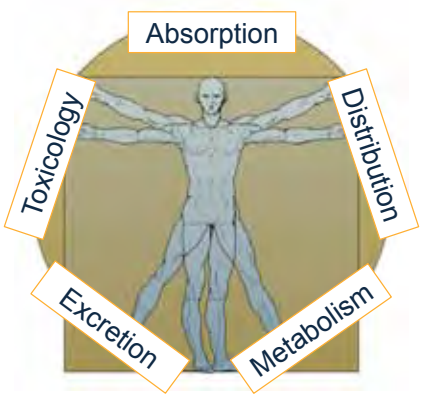
Does the type of therapeutics developed influences the model design?

- Use a chimeric molecule to enhance the signaling of the human protein into mouse cells if often suggested but not adapted to GPCR targeted by small molecules since it could modify the binding pocket

5. Pharmacokinetics and ADMET

What type of models should be used for ADMET studies?

- Selection of the species to be used for the study and its genetic background are essential parameters to be taken into account
- Easy readouts, early response detection, and enhanced throughput could be obtained using in vivo monitoring of selected biomarkers



How to reproduce the human pharmacokinetics?

- Humanization of pathways known to be crucial for PK and PD assessment of biologics and small molecules increases the predictability toward human data: humanization of HSA and FcRN (compound recycling binds HSA complex) closely mimic human pharmacokinetics

How to detect off-target activities in genetically engineered models?

- Physiological relevancy of the model is indispensable to avoid false positives; i.e., most standard KO models exhibit genetic compensation and pathway deregulation
- Most studies are done without the appropriate control models, yielding false results and misleading conclusions

In vivo studies: cost & duration

How can the time to first P.O.C be shortened?

- Use of new embryology technologies combined with *in vitro* phenotyping
- State of the art time requested to get first POC data is less than one year (which includes model design and creation, animal production, and POC experiment)

Could the cost of *in vivo* studies be reduced?

- Over the several years of use, well-designed genetically engineered models showed reduced expenses in both human resources and expenses, i.e., less animals per experiments, while meeting ethical considerations
- Delivery of cohorts of ready to use, fully validated animals combined with optimized screening methodologies

genOway

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«Thorough project assessment, in-depth scientific analysis of your current and future needs, exhaustive technology portfolio, excellence in production, and dedicated customer care are the core pillars that distinguish our personalized genome engineering service. We provide you with the most adapted and physiologically relevant research model.»

At a glance

With 100 employees, genOway has created more than 2000 genetically modified models, while serving clients in:

- 28 countries worldwide
- 380 academic institutions and 13 EU framework projects
- 170 Life Sciences companies, including 17 of the Top 20 pharma

YOUR SUCCESS IS OUR SUCCESS

Gulbins E et al.
Acid sphingomyelinase-ceramide system mediates effects of antidepressant drugs.

Nature Medicine

Adolph TE et al.
Paneth cells as a site of origin for intestinal inflammation.

Nature

Clement JP et al.
Pathogenic SYNGAP1 mutations impair cognitive development by disrupting maturation of dendritic spine synapses.

Cell