Humanizing the Mouse Genome: Generating New Tools for Preclinical Research

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ABSTRACT

Animal models are a key tool for assessing toxicity and efficacy of molecules before they are tested in humans. In many cases, however, potential therapeutic molecules that are active on human targets do not efficiently modulate their counterparts in other species. Expression of human proteins in the mouse is therefore becoming one of the most common approaches to overcome these limitations. The most effective way to generate mouse models expressing human proteins is to insert human genes in the mouse genome, a process defined as genetic humanization. In this poster, we will present examples of genetic humanization projects and discuss the pros and cons of the most common strategies: BAC transgenesis, minigene insertion, and full gene replacement. We will discuss which factors influence the success of these complex projects and which aspects should be taken into consideration while generating a humanized mouse model. Finally, we will present the success rate derived from the analysis and of over 100 independent genetic humanization projects using the two most common approaches, namely gene replacement and minigene insertion, and provide specific guidelines for generation of these preclinical models.

APPLICATIONS OF HUMANIZED MOUSE MODELS

- ***In vivo* drug efficacy testing** by expressing the human target in the mouse
- **ADMET (absorption, distribution, metabolism, excretion, toxicology)** by accurately modeling drug metabolism
- ***In vivo* testing of complex therapeutic approaches** (i.e. *in vivo* genome editing, enzymatic complementation, etc.) by mimicking human diseases
- **Target discovery and validation** by modeling human physiology and pathology
- **Study of infectious disease** by humanizing specific pathogen receptors

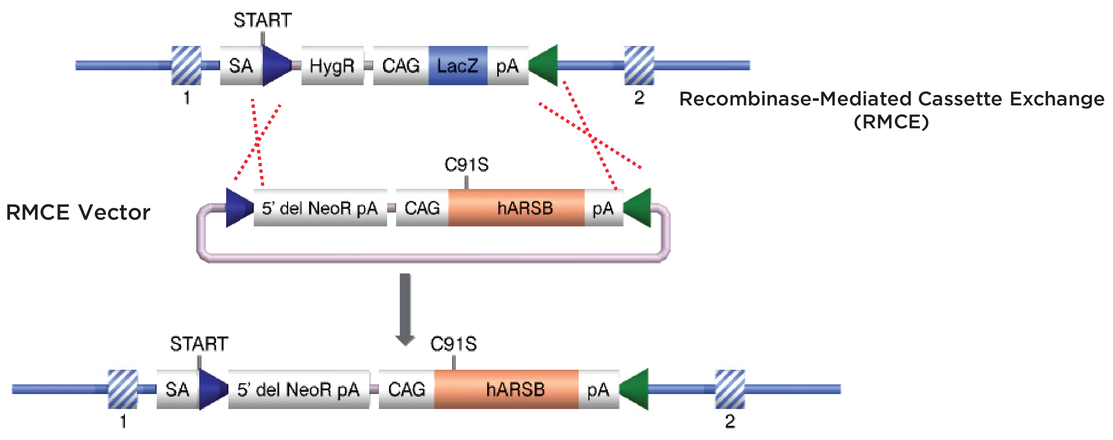
APPROACHES TO HUMANIZING THE MOUSE GENOME

Transgenesis allows the expression of a human gene in the mouse **Pros:** technically straightforward **Cons**: endogenous mouse gene will still be expressed; pattern of expression might not recapitulate expression of the endogenous gene	• Random transgenesis • Targeted transgenesis • Conditional targeted transgenesis
Knock-in allows replacement of the mouse coding sequence with the human counterpart **Pros:** mouse gene is inactivated; expression of the human gene is controlled by the endogenous regulatory elements **Cons:** technically complexity	• Minigene insertion • Gene replacement

HUMANIZATION BY TRANSGENESIS

Experimental Goal	To compare the efficacy of enzyme replacement and gene therapy in treating a monogenic disease (mucopolysaccharidosis V)
Experimental Tool	A mouse model lacking a functional *Arsb* gene and tolerating human ARSB protein
Approach	Generation of a targeted transgenic model expressing a non-functional human ARSB protein and crossed with an *Arsb* knockout mouse model
Results	Using the humanized mouse model, the group of Alberto Auricchio at TIGEM showed that low-dose gene therapy can efficiently substitute enzyme replacement therapy

Humanization Strategy by Transgenesis



Targeted Allele (After Recombination-Mediated Cassette Exchange)

Alliegro, M.; Ferla, R.; Nusco, E.; Leonibus, C. D.; Settembre, C.; Auricchio, A. Low-Dose Gene Therapy Reduces the Frequency of Enzyme Replacement Therapy in a Mouse Model of Lysosomal Storage Disease. *Molecular Therapy* **2016**, *24* (12), 2054–2063.

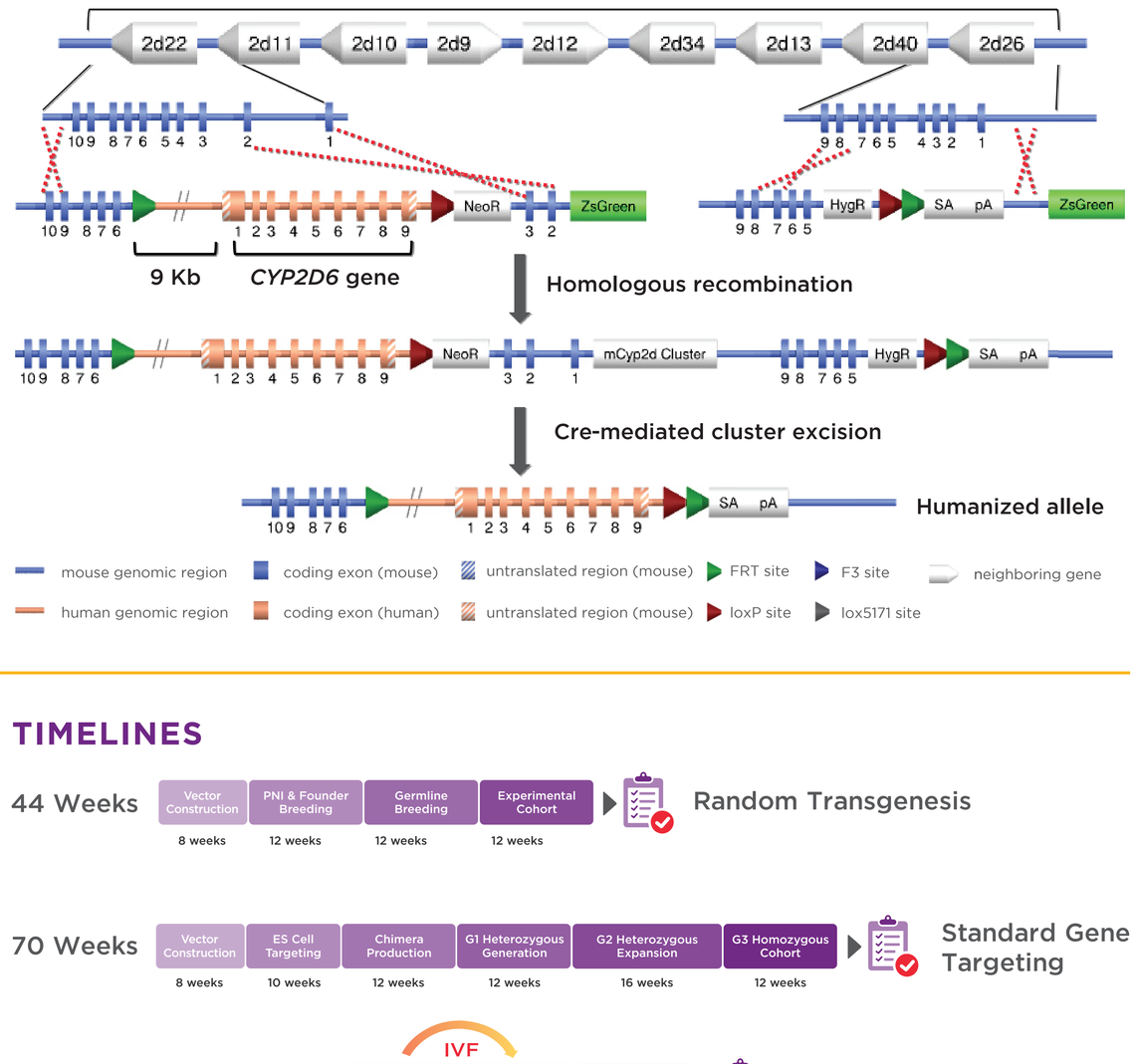
Ferla, R.; Claudiani, P.; Cotugno, G.; Saccone, P.; Leonibus, E. D.; Auricchio, A. Similar Therapeutic Efficacy Between a Single Administration of Gene Therapy and Multiple Administrations of Recombinant Enzyme in a Mouse Model of Lysosomal Storage Disease. *Human Gene Therapy* **2014**, *25* (7), 609–618.

Macleod, A. K.; Mclaughlin, L. A.; Henderson, C. J.; Wolf, C. R. Application of Mice Humanized for CYP2D6 to the Study of Tamoxifen Metabolism and Drug-Drug Interaction with Antidepressants. *Drug Metabolism and Disposition* **2016**, *45* (1), 17–22.

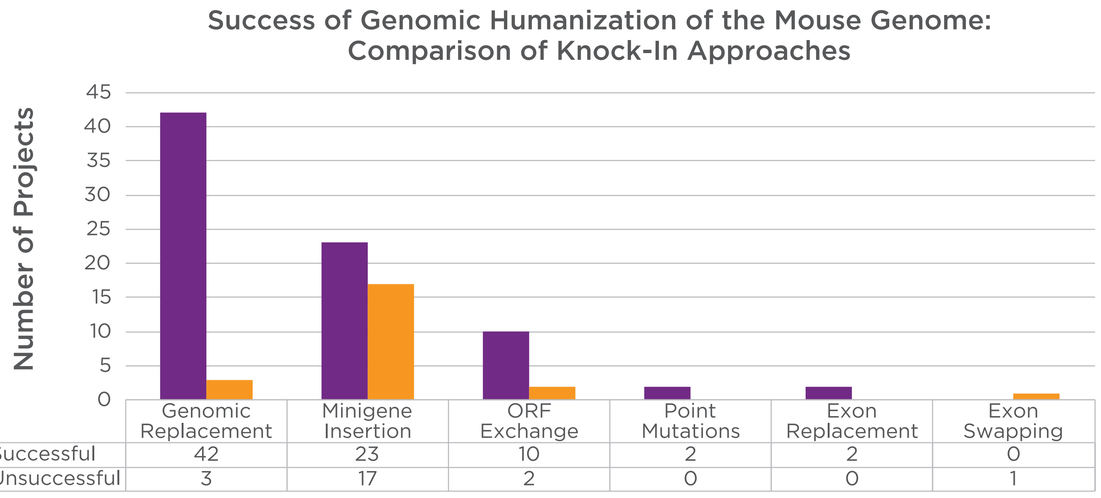
HUMANIZATION BY GENE REPLACEMENT

Experimental Goal	To test the activity of drug metabolism by cytochrome P450 2D6 (CYP2D6) *in vivo*
Experimental Tool	A mouse model where the *Cyp2d* cluster has been replaced by the human *CYP2D6* gene
Approach	Knock-in of the human *CYP2D6* gene and deletion of the entire mouse *Cyp2d* cluster
Results	Using the humanized mouse model, McLeod et al. proved drug-drug interactions *in vivo* between antidepressant drugs and Tamoxifen

Humanization Strategy by Gene Replacement



TACONIC PROJECT SUMMARY



	Genomic Replacement	Minigene Insertion	ORF Exchange	Point Mutations	Exon Replacement	Exon Swapping
Successful	42	23	10	2	2	0
Unsuccessful	3	17	2	0	0	1

Successful means that the humanized allele has an expression of a similar level (60% or more) of the endogenous mouse gene and the same pattern of expression as measured by RT-qPCR. Number of projects analyzed: 102.

TIMELINES

Timelines for gene replacement projects can be shortened using the ExpressMODEL® approach

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