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Monoclonal antibody discovery mice

Trianni has developed transgenic immunoglobulin mice capable of expressing the full human antibody repertoire, ideal for the discovery of new therapeutic antibody candidates.

Therapeutic monoclonal antibodies (mAbs) have had an enormous impact on medicine, and sales are expected to pass the \$100 billion mark in 2016¹. These mAbs offer several advantages over small molecules: predictable pharmacokinetics, the ability to enhance immune responses, and generally fewer side effects. However, epitopes present on nonhuman immunoglobulin trigger immune responses that can render the antibody ineffective or endanger the patient. To avoid such pitfalls, several approaches have been well validated to 'humanize' antibodies by replacing rodent sequences with human ones. But humanization is both time intensive and labor intensive, and it also increases development risks. Transgenic mice engineered to express human mAb sequences have proven to be the most successful approach to generating fully human antibody therapeutics, as evidenced by the number of such mAbs on the market and in clinical development. Trianni has established the Trianni Mouse, the only mouse carrying immunoglobulin transgenes (transgenic Ig mouse) to express a complete functional human VDJ gene repertoire in a single organism, making it possible to efficiently access the entire range of antibody specificity to discover potent fully-human antibody leads.

Transgenic Ig mice take advantage of B cell maturation processes that essentially 'screen' antibodies for heavy chain and light chain compatibility, robust expression and target selectivity *in vivo*. The Trianni transgenic Ig Mouse produces chimeric antibodies with human variable domains and mouse constant domains. At the gene level, Trianni Mouse variable (V) genes are chimeric: they contain human exons, but all remaining sequences are of mouse origin, including *in silico*-optimized promoters and enhancers.

The Trianni Mouse is healthy, though all mouse VDJ genes have been deleted. B cell counts and circulating antibody levels in naive mice are similar to those found in wild-type parental C57BL/6 mice, and a complete repertoire of 44 human heavy chain, 39 human V_λ light chain and 38 V_κ light chain V genes are present and utilized. Complementarity-determining region (CDR) diversity and length are critically important for broad epitope recognition. In the naive Trianni Mouse, the average heavy chain CDR3 length is 15.6 amino acids (aa), with some as long as 33 aa. Heavy chain CDR3 (CDR-H3) aa utilization frequency is effectively the same in humans and in the Trianni Mouse (Fig. 1). This reproducible utilization pattern confirms that the chimeric human-mouse loci used in the Trianni Mouse are able to recapitulate the fine details of human antibody development *in vivo*.

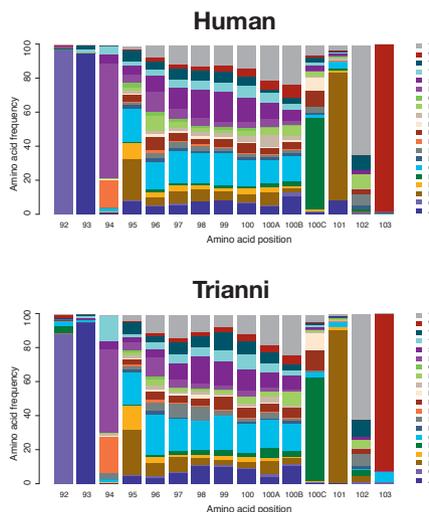


Figure 1: CDR-H3 residue utilization in antibodies derived from human samples and the Trianni transgenic Ig Mouse.

Despite the inherent advantages of transgenic mice, introducing the full complement of human VDJ genes into a mouse is a daunting task. First, the three human loci (H, K and L) together encompass more than 100 genes spread over 2–4 Mb², making genetic manipulation challenging. For this reason, most animals containing immunoglobulin transgenes do not have a complete human variable domain repertoire. Second, the regulation of VDJ rearrangement and gene expression is complex and not perfectly conserved across species. In most transgenic animals, this lack of conservation results in incomplete and irregular transgene expression, reducing antibody diversity and titer. Trianni has leveraged improvements in DNA synthesis technology to overcome both hurdles. Trianni Mouse transgene cassettes encoding each locus were not cloned but instead synthesized, precisely positioning human V gene exons within the context of mouse noncoding DNA. The resulting chimeric cassettes were integrated adjacent to the endogenous mouse constant domain genes. This strategy produced transgenic mice with normal B cell development and immune responses as robust as those observed in wild-type mice.

When Trianni and C57BL/6 or BALB/c wild-type mice are immunized using the same antigens and protocols, the resulting serum IgG antibody titers are the same, within the range of experimental error. Importantly, titers increase in parallel with antigen boosting, demonstrating that the affinity maturation process is normal in the Trianni Mouse. In the hands

of several biopharma partners, the Trianni Mouse has been shown to produce high-affinity antibodies ($K_d < 1$ nM). Trianni Mouse antibodies are also potent: in one benchmarking study, Trianni Mouse candidate antibodies demonstrated both maximal and average functional activity superior to that observed for antibodies derived from wild-type mice. Trianni Mouse leads also show excellent epitope diversity. Once top candidate antibodies have been identified, the mouse constant domains are readily substituted for human domains, with no impairment of function or other properties.

Trianni CEO Matthias Wabl said the company is extending the Trianni Mouse platform by adding "further bells and whistles": the Plasma Ig Mouse maintains fractional cell-surface immunoglobulin expression on plasma cells, enabling cell-based screening of B cells and hybridomas. In the Autoimmune or All Epitope Mouse, self-reactive antibodies that would normally trigger autoimmunity in the mouse are not eliminated, making it possible to discover antibodies to epitopes that are highly conserved between mice and humans. Finally, Trianni is developing a true Bispecific Mouse in which each B cell will express two unique heavy chains and one unique light chain. This platform will enable coevolution of two distinct binding activities for every unique bispecific antibody expressed by each B cell in the mouse.

Trianni's goal is to make its current and next-generation mice widely available to prospective partners on individually tailored terms while working closely with existing licensees to ensure a reliable and consistent supply of animals from Taconic and Jackson Labs. The Trianni Mouse is available directly and through ChemPartner, Evotec and LakePharma as preferred contract research organizations. Please see www.trianni.com for more details.

1. Anonymous. Antibodies market: North America's revenue share to reach 44% in 2016. *Yahoo! Finance* <http://finance.yahoo.com/news/antibodies-market-north-americas-revenue-143000940.html> (2016).
2. Lefranc, M.-P. & Lefranc, G. *The Immunoglobulin FactsBook* (Academic Press, 2001).

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