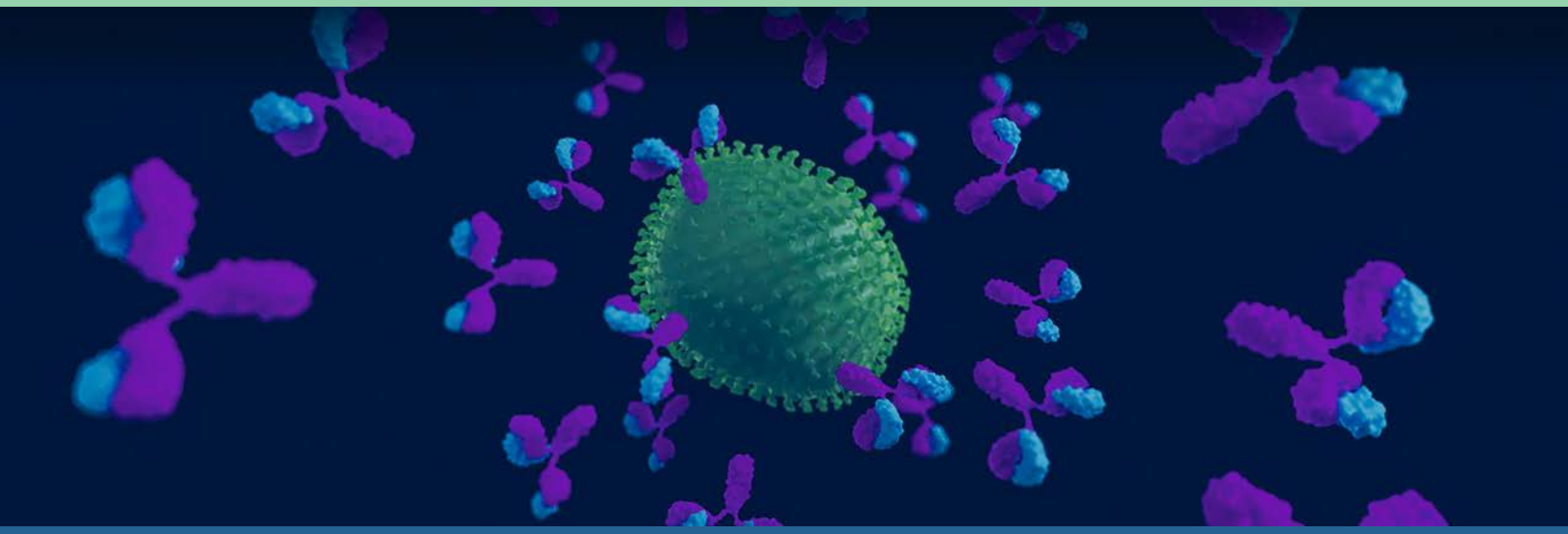
Whitepaper

Transgenic Mice: Transforming Targeted Monoclonal Antibody (mAb) Therapeutics

ABSTRACT: Monoclonal antibodies (mAbs) currently represent the largest class of biopharmaceutical products.[1,2] Market growth is projected to climb at least eight percent each year for the next several years, and the therapeutic antibody market is expected to reach $125 billion by 2020.[2] With the right platform, antibodies are easy to isolate — the immune system generates a library of billions of different antibodies, each antibody with a unique specificity. To isolate attractive leads, scientists expose a host to the antigen of interest and then screen for binding and/or function. Genetically-engineered mice now act as robust engines for the generation of diverse repertoires of affinity-matured, fully-human variable regions with intrinsic drug-like properties necessary for successful development including high potency, specificity, manufacturability, solubility and low risk of immunogenicity. Few existing or contemplated human transgenic mouse platforms express a full human antibody repertoire. The Trianni Mouse is the only one to do so in a single organism. This approach guarantees efficient expression of the full human antibody repertoire and at the same time maintains the natural immune response of the wild-type mouse, allowing researchers to exploit advantages in terms of efficiency in drug discovery and development.



TRIANNI

Transgenic Mice: Transforming Targeted Monoclonal Antibody (mAb) Therapeutics

Monoclonal antibodies (mAbs) currently represent the largest class of biopharmaceutical products.[1,2] Late-stage antibody therapeutics increased from 39 candidates in Phase III studies, as of late 2014, to 53 by the end of 2015.[3] Market growth is projected to climb at least eight percent each year for the next several years, and the therapeutic antibody market is expected to reach $125 billion by 2020.[2]

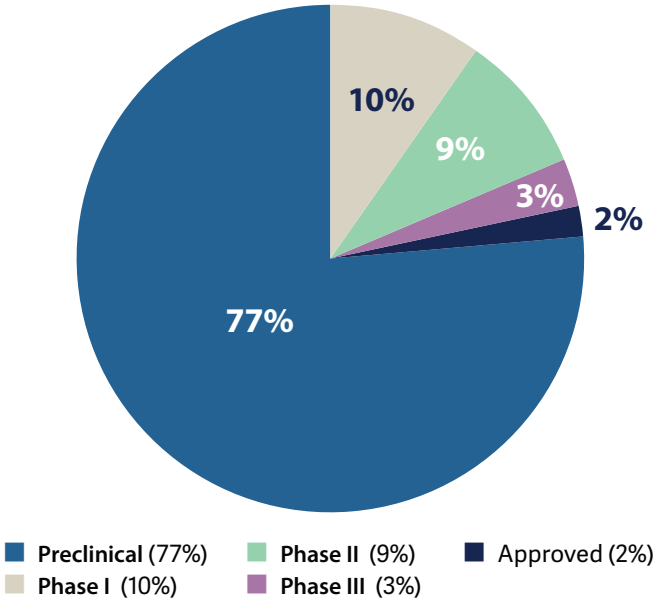
In addition to pioneering applications such as post-organ transplantation immune-regulation, mAb-based drugs have become important in the battle against cancer, inflammatory disease, cardiovascular disease, infection, respiratory disease, and ophthalmologic disease.[3,4,5,6]

Important mAb Advantages

Industry experts say mAbs are preferred in modern drug discovery because they provide:

- Exquisite specificity;
- Superior safety profiles;
- A rapid route to a clinical proof-of-concept for targeted therapeutics.[2,7]

Percentage of Antibodies in Each Clinical Phase



SOURCE: TABS Antibody Database. http://tabs.craic.com 3515 Antibodies – June 2016

The evolution of mAb engineering is resulting in new therapeutic candidates with enhanced effector function, improved half-life and enhanced stability.[7] In addition, recent improvements in mAb production technologies have substantially improved process yields and reduced actual manufacturing costs.

With the right platform, antibodies are easy to isolate – the immune system generates a library of billions of different antibodies, each antibody with a unique specificity. For almost any target, there will be several different antibodies produced. To isolate attractive leads, scientists need to expose a host to the antigen of interest and then screen for binding and/or function. Antibodies offer huge advantages in terms of efficiency in drug discovery and development.

Before, most therapeutic drugs were small molecule chemicals that, while efficacious in their targeted indications, tended to be of poor specificity. Antibodies, on the other hand, are large biological molecules that are highly specific for a particular antigen and that can bind strongly to their target; as therapeutics, mAbs are compelling.

Today, human and humanized mAbs comprise 63% of approved mAb products.[8] Nearly 60 mAbs were approved for human use or in regulatory review by mid-2016. Of those, 29% were derived from transgenic mice engineered to express human antibody repertoires. The percentage of antibodies in Phase III human clinical trials derived from human transgenic

mice was 39%.[9] The trend favoring transgenic mice as the preferred platform for therapeutic antibody discovery increases with mAb drugs in earlier clinical phases.

Transgenic Clarity

Since the original Nobel Prize-winning mAb isolation technology was unveiled in 1975, numerous advances have transformed mAb discovery and development.

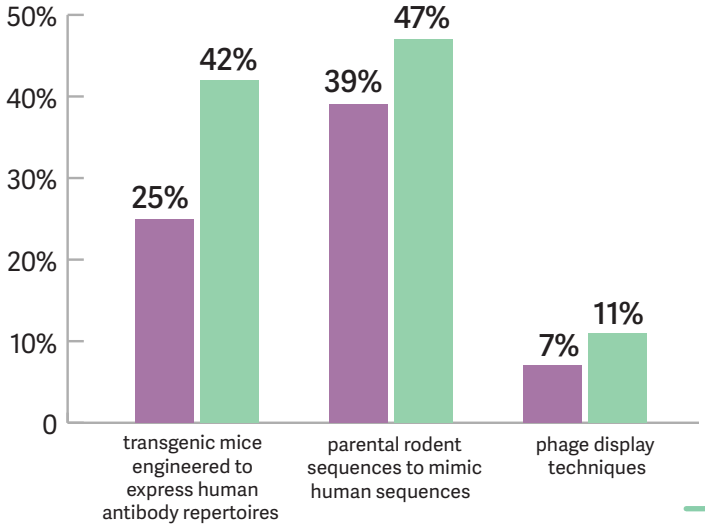
Early attempts to use murine mAbs as human therapeutics met with some success; however, these drugs triggered human anti-mouse immune (HAMA) responses.[10,11] As a result, researchers explored ways to reduce mAb immunogenicity, such as combining murine and human-derived sequences, resulting in chimeric and humanized mAbs.[11]

First-generation transgenic mice, meanwhile, remain immunocompromised compared to wild-type mice. Such mice are unable to mount robust immune responses to target antigens and fail to generate complete therapeutic candidate antibody repertoires. First-generation mice also contain varying subsets of the total human antibody heavy and light chain complement and are limited in the theoretical diversity of antibody sequences they can produce.

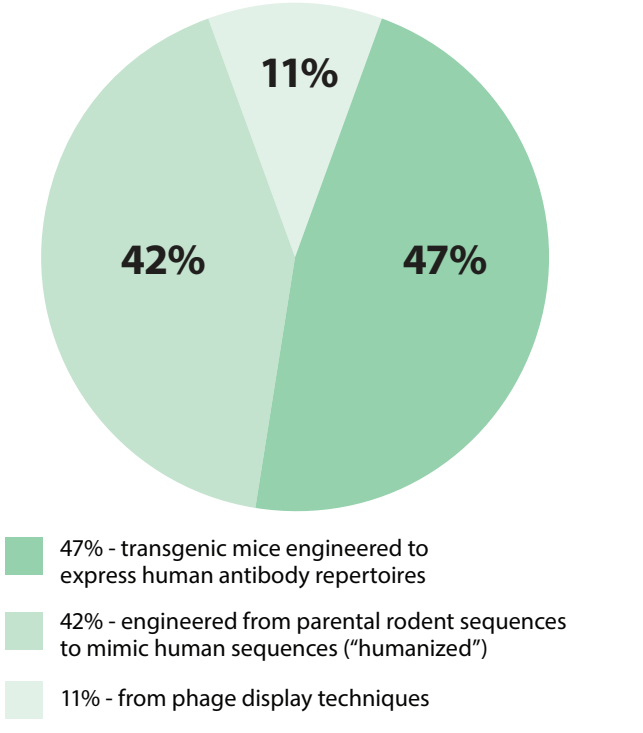
Second-generation transgenic solutions either remain limited by the extent of genetically encoded human immunoglobulin (Ig) repertoire or fail to recapitulate wild-type murine antibody responses.

Human mAb therapeutics, derived from transgenic mouse or phage display technologies, first entered clinical

Of ~60 mAbs Approved for Human Use (as of 2016)



The Percentage of Antibodies in Phase III Human Clinical Trials



SOURCE: TABS Therapeutic Antibody Database.

MONOCLONAL ANTIBODIES DEVELOPMENT

1974 Rudolf Jaenisch (with Bernice Mintz) inserts retroviral DNA into an early-stage mouse embryo and demonstrates that the inserted sequences are present in the genome.

1975 Niels Jerne, César Milstein, and Georges Köhler use the hybridoma technique to immunize against a specific epitope on an antigen. They receive the Nobel Prize in Medicine or Physiology in 1984.

1985 George Smith introduces novel phage expression vectors in a seminal ***Science*** article.

Perceived Phage Advantages

- Can create large libraries, allows researchers to clone human antibodies once library is created
- Provides a technique for obtaining large number of leads in short amount of time
- No immunizations are required; in vitro process
- Ability to generate human-rodent cross-reactive leads

Perceived Phage Challenges

- Heavy-light pairing is random
- Extensive affinity maturation is typically required
- Limited library sequence diversity, excessive redundancy
- Labor intensive procedure for candidate identification
- Prone to generation of leads exhibiting promiscuous binding and poor CMC properties in general
- More steps – both in paperwork and in laboratory processes

1986 Orthoclone OKT3 (muromonab-CD3) approved for use in preventing kidney transplant rejection (murine IgG2a isotype/effector function-mediated T-cell depletion in patients suffering from acute rejection of renal allotransplant).

Perceived Phage Advantages

- Production efficiencies

Perceived Phage Challenges

- Patient develops anti-mouse antibodies

1988 With his testing of Campath-1H, Greg Winter (with Lutz Riechmann, Mike Clark, Marianne Brüggeman, Carol Bindon) pioneers techniques to humanize mAbs.

1990 Human mAb from phage display / recombinant libraries.

1994 New technologies for generating human mAbs from transgenic mice are first described.

2002 Adalimumab becomes the first fully-human mAb to be approved by the US Food and Drug Administration (FDA).

2006 Panitumamab, the first fully-human therapeutic antibody derived from a transgenic mouse, is approved for epidermal growth factor-receptor (EGFR) positive colorectal cancer.

Perceived Phage Advantages

- Efficient production of large, diverse lead panels
- Repeated immunization/boosting generates high affinities
- Screening cascade is not “black box” (can select for function early on)
- Cell lines provide stable supply of purified antibody
- All derived antibodies extensively vetted by murine immune system/in vivo B-cell selection process and so tend to exhibit favorable CMC/drug-like properties

Perceived Phage Challenges

- Quantity of mice
- More intensive immunization approaches
- Robust screening methods required for isolation of desirable leads

2010 Trianni Mouse chimeric genes efficiently generate human variable region repertoires through improved V(D)J recombination, optimized antibody expression and robust class switching and affinity maturation.

development during the 1990s. Creation of transgenic mice carrying human antibody genes is a particularly noteworthy event as this technology promises a rapid means for the isolation of therapeutic antibodies. Transgenic mice have been successfully leveraged to generate new drugs with decreased immunogenicity and improved potency, specificity and stability.[2,4,7] In particular, candidates derived from transgenic mouse platforms have considerably higher Phase II to III and Phase III to approval transition rates than those of the entire cohort of human therapeutic mAbs.[12] The use of transgenic mice expressing human immunoglobulins avoids HAMA responses and maintains technical advantages of mouse hybridomas. In the transgenic approach, natural diversification and selection are exploited as integrated loci are under the control of the animal's immune system where they undergo normal processes of VDJ gene rearrangement and somatic hypermutation.[4]

Improving Outcomes

Recent research has focused on improving the efficacy of existing mAbs by optimizing affinity and adding other beneficial modifications.[13]

Remarkable progress has been made in the development of display technologies (and Next Generation Sequencing), improving affinity and specificity. Moreover, antibody engineering technologies are being employed to continuously improve effector function and serum half-life. Still, optimal delivery to the target tissue is required to avoid unwanted side effects.[14] Antibodies discovered using phage libraries continue to suffer from limited diversity and non-native pairing of immunoglobulin heavy and light

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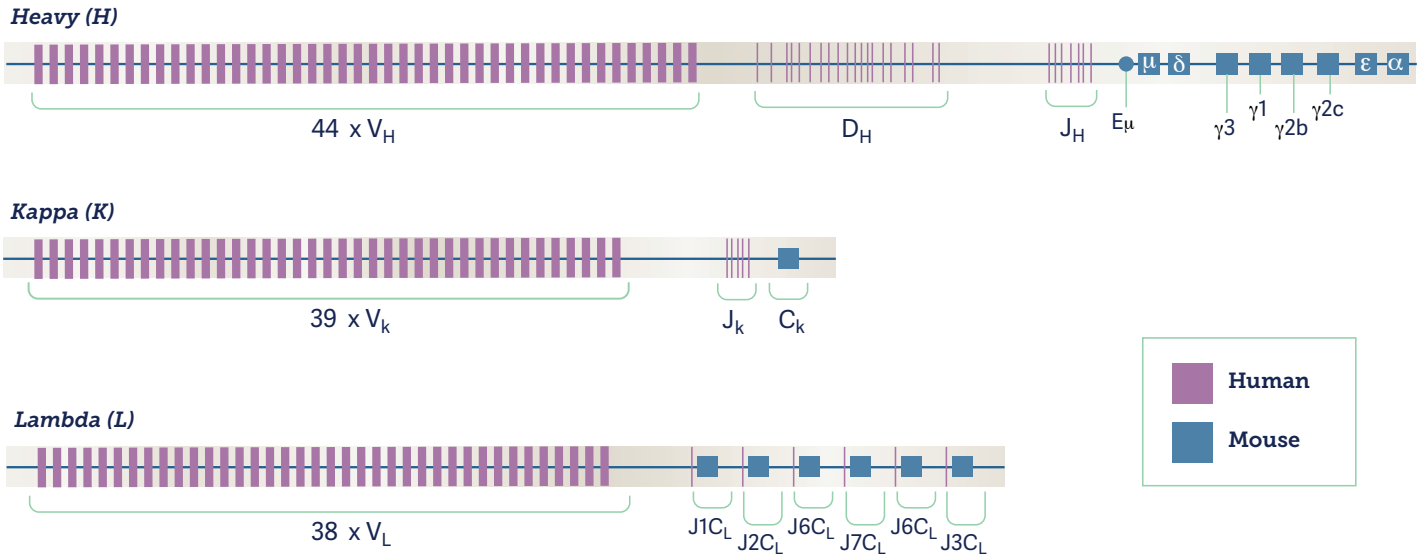
chains. Additionally, affinity maturation requires iterative, time-consuming, in vitro methods, which don't benefit from endogenous mammalian cell filters known to bias selection toward well-behaved leads. Phage antibodies often exhibit suboptimal biophysical attributes, leading to difficulties in manufacture and in poor pharmacokinetics and other CMC properties. They also can be immunogenic in patients, leading to attenuation of their efficacy over time.[15]



- *Endogenous V, D and J gene segments deleted and positionally substituted with optimized cassette*
- *Chimeric gene segments (human open reading frames paired with mouse regulatory regions)*
- *Chimeric antibodies (human Vs with mouse Cs)*
- *Precise genetic integration enables rapid production of next-gen variants*

Steady improvement has also been achieved in creation of mouse platforms that express a full human antibody repertoire. Researchers have developed the technology needed to insert large segments of DNA at the right locations in the mouse genome to facilitate complete human antibody repertoire availability.[16]

As a result, genetically-engineered mice now act as robust engines for the generation of diverse repertoires of affinity-matured, fully-human variable regions with intrinsic drug-like properties necessary for successful development including high potency, specificity, manufacturability, solubility and low risk of immunogenicity.[5]

Transgenic mice represent a revolutionary tool to leverage the B cell in discovering novel therapeutic mAbs.

Few existing or contemplated human transgenic mouse platforms express a full human antibody repertoire, though the TRIANNI Mouse is the only one to do so in a single organism. The TRIANNI Mouse responds to an antigen as efficiently as a wild-type mouse. In developing the TRIANNI Mouse, scientists designed immunoglobulin loci containing human variable domain exons within the context of remaining mouse sequence including computationally optimized promoters and enhancers. The resulting DNA sequences were then chemically synthesized, the associated cassettes inserted in place of the corresponding loci in the mouse genome. The result is chimeric V genes proximal to endogenous constant domain genes that combine for optimal expression of human therapeutic antibody candidates. This approach guarantees efficient expression of the full human antibody repertoire and at the same time maintains the natural immune response of the wild-type mouse.[16] In the end, TRIANNI mice make antibodies just like humans – a high-end solution for anyone interested in discovering therapeutic monoclonal antibodies.[9]

The Trianni Mouse Characteristics

- Novel chimeric antibody gene segments comprised of **human** coding sequences combined with **mouse regulatory** genomic sequences;
- Expression of a full repertoire of human heavy and light chain variable domains;
- Multiple enhancements to antibody gene segments improve V(D)J recombination and expression;
- Retention of all mouse constant domain;
- Designs allow for facile future modifications to loci;
- Normal genomic [V(D)J] rearrangement in developing B cells;
- Normal B cell development;
- Normal immune responses; and
- Robust class switching and somatic hypermutation.

Emerging antibody agents provide valuable new treatment options.[12] As scientists hypothesized 25 years ago, mammalian Ig locus function is highly conserved; this was confirmed in early experiments using a germ-line-configured chimeric construct and refined during recent genetic engineering advances.[4,17] Transgenic mice represent a revolutionary tool to leverage the B cell in discovering novel therapeutic mAbs.

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WHITEPAPER REFERENCES

1. Walker N, *Innovation at the Heart of Biopharmaceutical Industry Growth*, Pharma's Almanac, Global Pharmaceutical Supply Chain Trends. Q2, 2016; pp. 19.
2. Ecker D, Jones S, Levine H., *The Therapeutic Antibody Market*, BioProcess Technology Consultants, mAbs January/February 2015; 7:1: 9-14. doi: 10.4161/19420862.2015.989042. Reichert J, *Antibodies to watch in 2016*, mAbs, 2016; 8:2, 197-204. doi: 10.1080/19420862.2015.1125583.
3. Reichert J. Antibodies to watch in 2016. mAbs. 2016;8:2, 197-204. doi: 10.1080/19420862.2015.1125583
4. Brüggemann M, Osborn M, Ma B, et al., *Human Antibody Production in Transgenic Animals*. Arch. Immunol. Ther. Exp. 2015; 63:101-108. doi 10.1007/s00005-014-0322-x.
5. Green L, *Transgenic Mouse Strains as Platforms for the Successful Discovery and Development of Human Therapeutic Monoclonal Antibodies*. Current Drug Discovery Technologies. Mar. 2014; 11(1):74-84.
6. Doig A, Ecker D, Ransohoff T. *Monoclonal Antibody Targets and indicators*. American Pharm. Review (2015). http://www.americanpharmaceuticalreview.com/Featured-Articles/177490-Monoclonal-Antibody-Targets-and-Indications. Accessed August 9, 2016.
7. Scolnik P, *A business perspective*. mAbs. March/April 2009; 1(2): 179–184.
8. Meininger D. *Trends in Therapeutic Monoclonal Antibody Discovery Technology*. www.proteins-congress.com.
9. http://tabs.craic.com
10. Schroff R, Foon K, Beatty S, et al. *Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy*. Cancer Res. 1985; 45: 879–885.
11. Chernajovsky Y, Nissim A (ed). *Therapeutic Antibodies. Handbook of Experimental Pharmacology*. 2008; vol. 181.
12. Nelson A, Dhimolea E, Reichert J. *Development trends for human monoclonal antibody therapeutics*. Nature Reviews | Drug Discovery. October 2010; vol. 9, pp. 772. doi:10.1038/nrd3229.
13. Liu J. *The history of monoclonal antibody development – Progress, remaining challenges and future innovations*. Ann Med Surg (Lond). December 2014; 3(4):113-116. http://dx.doi.org/10.1016/j.amsu.2014.09.001.
14. Nissim A, Chernajovsky Y. *Historical development of monoclonal antibody therapeutics*. Handbook Exp. Pharmacol. 2008;(181):3-18.
15. Lee E, Liang Q, Hanif A, et al. *Complete humanization of the mouse immunoglobulin loci enables efficient therapeutic antibody discovery*. Nature Biotechnology. April 2014; 32(4):356-367. doi:10.1038/nbt.2825.
16. Wabl M. *Inside View: Trianni*. Nature Biotechnolgy. 2016.
17. Brüggemann M, Caskey H, Teale C. *A repertoire of monoclonal antibodies with human heavy chains from transgenic mice*. Proc. Natl. Acad. Sci. (USA) 1989; vol. 86, pp. 6709-6713.

TIMELINE REFERENCES

Jaenisch R, Mintz B. *Simian virus 50 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA*. Proc. Natl. Acad. Sci. U.S.A. 1974; 71 (4): 1250-1254. Doi: 10.1073/pnas.71.4.1250. PMID 4364530.

Milstein C. *Monoclonal Antibodies*. Scientific American. 1980; vol. 243, pp. 56-65.

Wade N. *Hybridomas: The Making of a Revolution*. Science. 1982; vol. 215, pp. 1073-1075.

Nobel Prizes and Laureates. The Nobel Prize in Physiology or Medicine 1984; http://www.nobelprize.org/nobel_prizes/medicine/laureates/1984/press.html

Smith GP. *Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface*. Science. 1985; 228:1315–1317.

Bazan J,Całkosiński I, Gamian A. *Human Vaccines & Immunotherapeutics*. 2012; 8:12, 1817-1828.

Green L. *Transgenic Mouse Platforms for Human Therapeutic Antibody Discovery*. Current Drug Discovery Technologies. 2014; vol. 11, No. 1, pp 79.

Zwick M, Shen J, Scott J. *Phage-displayed peptide libraries*. Curr Opin Biotechnol. August 1998; 9(4): 427-436. PMCID: PMC3987777.

Lonberg N. *Fully human antibodies from transgenic mouse and phage display platforms*. Curr Opin Immunol. 2008; Aug: 20(4): 450-9. Doi:10.1016/j.coi.2008.06.004.

Liu J. *The history of monoclonal antibody development - Progress, remaining challenges and future innovations*. Annals of Medicine and Surgery 3. 2014; 113e116.

Hu D, Hu S, Wan W, et al. *Effective Optimization of Antibody Affinity by Phage Display Integrated with High-Throughput DNA Synthesis and Sequencing Technologies*. PLoS one. 2015; 10(6): e0129125. doi: 10.1371/journal.pone.0129125. PMCID: PMC4457833.