Journal of Applied Biology & Biotechnology Vol. 00(0), pp. 1-13, 2024
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2024.172190

Technological advancement in the development of monoclonal antibody therapies: Present, past, and future

Harit Kasana[1,2], Harish Chander[1], Ashwani Mathur[2]*

[1]National Institute of Biologicals (Ministry of Health and Family Welfare, Government of India), Noida, Uttar Pradesh, India.
[2]Department of Biotechnology, Jaypee Institute of Information Technology Noida, Noida, Uttar Pradesh, India.

ARTICLE INFO

Article history:
Received on: October 05, 2023
Accepted on: February 17, 2024
Available online: ***

Key words:
Hybridoma,
Monoclonal antibodies,
Phage display,
Recombinant DNA technology,
Bispecific antibodies,
US FDA.

ABSTRACT

Monoclonal antibodies (mAbs) have been a key player in the field of biopharmaceuticals for an extended period of time, especially in terms of approval and sales, and this dominance is expected to persist. In terms of a single product, mAb-based drugs are the most lucrative class of drugs, making them four of the top ten best-selling medications in terms of both revenue and market shares in 2022. It is estimated that by 2028, mAbs will be worth USD 420–460 billion. The therapeutic potential of mAbs has been recognized through hybridoma technology that was developed in the mid-1970s. Multiple approaches can currently be employed to generate chimeric, humanized, and fully human mAbs. These mAbs represent the cutting edge of biomedical research and offer excellent treatment options for a variety of disorders, such as severe asthma, rheumatoid arthritis, Crohn's disease, multiple sclerosis, infectious diseases, and some types of cancers. Therefore, in this review article, insights regarding one of the fastest-growing biopharmaceutical categories, that is, therapeutic mAb products, and technological advancements in the production of mAbs by different *in vitro* technologies were discussed. In addition, the study provides a comprehensive overview of the authorized mAbs now available in the market, together with their specific targets, forms, and allowed applications.

1. INTRODUCTION

Monoclonal antibodies (mAbs) are immunoglobulins that exhibit a high level of specificity, targeting a single antigen or epitope. mAbs are usually obtained from a clonal proliferation of cancerous human plasma cells that produce antibodies. In 1975, George Köhler and Ceasar Milstein developed hybridoma technology, which spurred new optimism. To create human-derived hybridomas, Kohler and Milstein used human–mouse hybrid cells, which have since become a cornerstone in the large-scale manufacturing of therapeutic antibodies [1,2]. Early therapeutic mAbs, derived from mice, were immunogenic in humans and had limited efficacy in stimulating immune responses in patients, hence restricting their clinical usefulness. In order to overcome these restrictions, methodologies for producing antibodies more human-like were devised throughout the later part of the 1980s [3,4]. However, the advent of novel technologies including recombinant DNA technology, phage display, and transgenic mice has led to the development of numerous kinds of mAbs, primarily chimeric, humanized, and fully human antibodies. These showed lower immunogenicity and greater efficacy as drug products.

mAbs are versatile biomacromolecules that have high specificity for binding to various types of protein and non-protein substrates [5-7]. These mAbs can be produced using numerous approaches to enhance their functioning and utility [Figure 1] [8]. To date, there are currently over 130 therapeutically approved mAbs, with numerous others undergoing preclinical and clinical development [9]. Hybridoma technology is a widely employed technique for the production of mAbs. During this procedure, B lymphocytes that produce antibodies are separated from mice that have been immunized with a specific antigen. These B lymphocytes are then combined with immortal myeloma cell lines to create hybrid cells known as hybridoma cell lines. The hybridoma cells are cultivated in a controlled environment to generate mAbs that target a particular antigen.

mAbs in the market developed by hybridoma technology are given in Table 1. The human mAbs product developed by phage display, transgenic mice, and recombinant technology is given in Table 2. The top best-selling mAb drugs in the year 2022 are given in Table 3.

mAb drugs have developed from clinical research to commercialization over the past few decades. In the past few years, the total number of antibody drugs approved for launch has proliferated, with 130 approved and available on the market [15]. mAb treatments were the

*Corresponding Author:
Ashwani Mathur,
Department of Biotechnology,
Jaypee Institute of Information Technology Noida,
Noida, Uttar Pradesh, India.
E-mail: ashwani.mathur@mail.jiit.ac.in

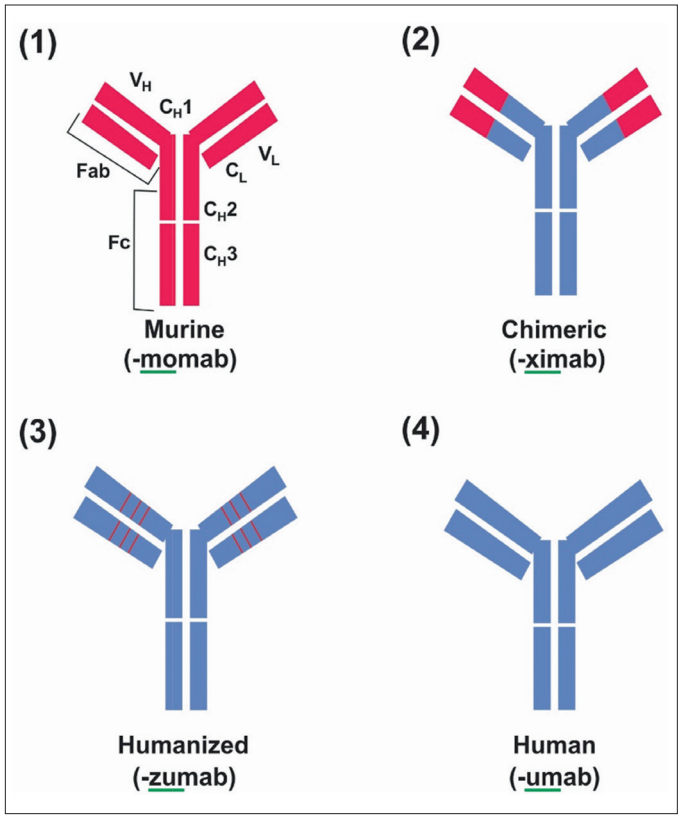

Figure 1: Structure of mAb and modification of murine mAb in therapy. (1) The murine monoclonal antibody. (2) Chimeric monoclonal antibody has variable regions of murine origin and the rest is of human origin. (3) Humanized monoclonal antibody has a hypervariable region of murine origin and the rest is of human origin. (4) Fully human monoclonal antibody.

fastest-growing category in the worldwide biopharmaceutical market in 2022, with four of the top ten best-selling biopharmaceutical products. Keytruda, which is a drug developed by Merck and introduced in 2014, garnered USD 20.9 billion in sales in 2022 [16]. In recent years, the global mAb market has grown, reaching USD 178.5 billion in 2021, which is an increase of 12% year over year. As of April 2022, there were 250 mAb therapies in Phase III clinical trials around the world [17]. Antibody medications are expected to be licensed and marketed in great numbers in the next few years, with a market value of USD 420–460 billion in 2028. The global mAb market has practically been split up by Roche, Johnson & Johnson, Merck, Novartis, AbbVie, and Amgen. The top five antibody drugs in the world by sales in 2022 were Humira, Keytruda, Stelara, Dupixent, and Opdivo, with a combined share of more than 50% of the global market [1]. Therefore, this review article discusses the rapid growth of therapeutic mAb products as well as the technological developments in producing these antibodies using various *in vitro* methods. Furthermore, the study offers a thorough synopsis of the approved mAbs currently accessible on the market, together with their distinct targets, structures, and permissible uses.

2. ANTIBODY STRUCTURE AND FUNCTION

Antibodies are large glycoproteins that belong to the immunoglobulin (Ig) superfamily that help the immune system to recognize foreign antigens, neutralize them, and trigger an immune response. Antibody molecules are made up of light chains (LCs) and heavy chains (HCs). Human immunoglobulins consist of two identical LCs and two identical HCs, forming a Y-shaped protein structure. In biological systems, the combination of one LC and one HC joins with another identical heterodimer creates the complete immunoglobulin. Moreover, antibodies are divided into five classes, namely, IgA, IgD, IgE, IgG, and IgM, are classified based on the type of HC they possess. Furthermore, in immunoglobulin G, disulfide bridges connect two HCs and two LCs to form a 150-kDa protein. One variable domain (VH or VL) and one to four constant domains (CH or CL) make up each chain. Each variable domain splits into three complementarity-determining regions (CDRs) with varying sequences and four framework portions with relatively constant sequences. Three constant domains are located in the HC of immunoglobulin G, while one is located in the LC. Y-shaped fundamental structure characterizes them. The binding location of the antibody, which can bind to the antigen, is determined by the CDRs of the variable chains. These CDRs are complementary to the epitope on the antigen. Variations in the amino acid sequences of the CDRs lend credence to antibodies. The Fc region, located at the base of the Y-shaped structure, enables interactions between antibodies and other components of the immune system [18].

The Fc region of antibodies is accountable for performing effector functions, including antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Antibodies bind to Fc receptors (FcRs) on the surface of effector cells like natural killer (NK) cells and macrophages, causing them to phagocytose or lyse the target cells. The target cells are eliminated when antibodies in the CDC initiate the complement cascade on the cell surface. Human IgG1 exhibits superior efficacy in both CDC and ADCC, making it highly suitable for use in cell treatment to target infections or tumors. IgG1 and IgG3 possess the capacity to elicit ADCC and CDC. However, IgG2 and IgG4 do not possess this capability [19]. Moreover, a novel subset of recombinant antibodies, the single-chain Fv fragment, was first presented by Bird et al. [20]. In order to connect the VH and VL domains, a 15–20 amino acid flexible linker peptide was employed. Compared with intact antibodies, scFv is the smallest form of recombinant immunoglobulin that maintains full activity.

mAbs have significantly transformed biological studies and clinical diagnostics, and their therapeutic potential is currently being strategically positioned for application. The initial application of treatment involved mAbs derived from rodents, which resulted in notable endogenous antibody responses. Additionally, it often fails to consider the initiation of effector activities. These limitations have propelled the search for more compatible and effective alternatives.

In response to these challenges, humanization, which is a sophisticated form of genetic engineering, has emerged as a groundbreaking solution. By integrating only the rodent complementary determining regions with the human variable region framework and constant heavy and LC portions, humanized antibodies achieve 95% homology with genuine human antibodies. This innovative approach not only mitigates the immune response issues associated with rodent-derived antibodies but also enhances the efficacy of mAbs in therapeutic applications [21]. The implementation of antibody phage display enabled the direct selection of human-origin-specific antibodies, thus achieving a targeted antibody production approach. Antibody phage display has emerged as the primary method in antibody engineering due

Table 1: US FDA-approved monoclonal antibody in the market developed by hybridoma technology.

S. No.	Antibody	Brand name	Year of approval	Target	Format	Indication	Company	References
1	Muromonab-CD3	Orthoclone OKT3	1986	CD3	Murine IgG2	Kidney transplant rejection	Centocor Ortho Biotech Products L.P.	[10]
2	Abciximab	Reopro	1994	GPIIb/IIIa	Chimeric IgG1 Fab	Prevention of blood clots in angioplasty	Centocor Inc.	[10]
3	Rituximab	MabThera, Rituxan	1997	CD20	Chimeric IgG1	Non-Hodgkin lymphoma	Biogen Inc.	[10]
4	Palivizumab	Synagis	1998	RSV	Humanized IgG1	Prevention of respiratory syncytial virus infection	MedImmune	[10]
5	Infliximab	Remicade	1998	TNFα	Chimeric IgG1	Crohn's disease	Janssen Biotech Inc.	[10]
6	Trastuzumab	Herceptin	1998	HER2	Humanized IgG1	Breast cancer	Roche, F. Hoffmann-La Roche, Ltd.	[10]
7	Alemtuzumab	Campath, Lemtrada	2001	CD52	Humanized IgG1	Chronic myeloid leukemia	Berlex Inc.	[10]
8	Ibritumomab tiuxetan	Zevalin	2002	CD20	Murine IgG1	Non-Hodgkin lymphoma	Biogen Inc.	[10]
9	Omalizumab	Xolair	2003	IgE	Humanized IgG1	Asthma	Roche, F. Hoffmann-La Roche, Ltd.	[10]
10	Cetuximab	Erbitux	2004	EGFR	Chimeric IgG1	Colorectal cancer	Bristol-Myers Squibb	[10]
11	Bevacizumab	Avastin	2004	VEGF-A	Humanized IgG1	Colorectal cancer	Roche, F. Hoffmann-La Roche, Ltd.	[10]
12	Natalizumab	Tysabri	2004	ITGA4	Humanized IgG4	Multiple sclerosis	Biogen Inc.	[10]
13	Ranibizumab	Lucentis	2006	VEGF-A	Humanized IgG1 Fab	Macular degeneration	Roche, F. Hoffmann-La Roche Ltd.	[10]
14	Eculizumab	Soliris	2007	C5	Humanized IgG2/4	Paroxysmal nocturnal hemoglobinuria	Alexion Pharmaceuticals Inc.	[10]
15	Certolizumab pegol	Cimzia	2008	TNFα	Humanized Fab, pegylated	Crohn's disease	Celltech, UCB.	[10]
16	Tocilizumab	RoActemra, Actemra	2010	IL-6R	Humanized IgG1	Rheumatoid arthritis	Chugai Pharmaceutical Co. Ltd.	[10]
17	Brentuximab vedotin	Adcetris	2011	CD30	Chimeric IgG1	Hodgkin lymphoma	Seattle Genetics Inc.	[10]
18	Pertuzumab	Perjeta	2012	HER2	Humanized IgG1	Breast Cancer	Roche, F. Hoffmann-La Roche, Ltd.	[10]
19	Trastuzumab emtansine	Kadcyla	2012	HER2	Humanized IgG1	Breast cancer	Roche, F. Hoffmann-La Roche, Ltd.	[10]
20	Obinutuzumab	Gazyva, Gazyvaro	2013	CD20	Humanized IgG1	Chronic lymphocytic leukemia	Biogen Inc.	[10]
21	Siltuximab	Sylvant	2014	IL-6	Chimeric IgG1	Castleman disease	Centocor Inc.	[10]
22	Vedolizumab	Entyvio	2014	α4β7 integrin	Humanized IgG1	Ulcerative colitis, Crohn disease	Genentech Inc.	[10]
23	Blinatumomab	Blincyto	2014	CD19, CD3	Murine bispecific tandem scFv	Acute lymphoblastic leukemia	Amgen	[10]
24	Pembrolizumab	Keytruda	2014	PD-1	Humanized IgG4	Melanoma	Merck & Co. Inc.	[10]
25	Idarucizumab	Praxbind	2015	Dabigatran	Humanized Fab	Reversal of dabigatran-induced anticoagulation	BIP	[10]
26	Dinutuximab	Unituxin	2015	GD2	Chimeric IgG1	Neuroblastoma	United Therapeutics Corp.	[10]
27	Mepolizumab	Nucala	2015	IL-5	Humanized IgG1	Severe eosinophilic asthma	Centocor Inc.	[10]
28	Elotuzumab	Empliciti	2015	SLAMF7	Humanized IgG1	Multiple myeloma	Bristol-Myers Squibb	[10]
29	Ixekizumab	Taltz	2016	IL-17α	Humanized IgG4	Psoriasis	Eli Lilly	[10]
30	Reslizumab	Cinqaero, Cinqair	2016	IL-5	Humanized IgG4	Asthma	Celltech, UCB	[10]
31	Atezolizumab	Tecentriq	2016	PD-L1	Humanized IgG1	Bladder cancer	Roche, F. Hoffmann-La Roche, Ltd.	[10]

(Continued)

Table 1: (Continued)

S. No.	Antibody	Brand name	Year of approval	Target	Format	Indication	Company	References
32	Obiltoxaximab	Anthim	2016	*Bacillus anthrasis* PA	Chimeric IgG1	Prevention of Inhalational anthrax	Elusys Therapeutics Inc.	[10]
33	Inotuzumab ozogamicin	Besponsa	2017	CD22	Humanized IgG4	Acute lymphoblastic leukemia	Wyeth Pharmaceuticals	[10]
34	Ocrelizumab	Ocrevus	2017	CD20	Humanized IgG1	Multiple sclerosis	Biogen Inc.	[10]
35	Emicizumab	Hemlibra	2017	Factor IXa, X	Humanized IgG4, bispecific	Hemophilia A	Chugai Pharmaceutical Co., Ltd.	[10]
36	Benralizumab	Fasenra	2017	IL-5Rα	Humanized IgG1	Asthma	MedImmune	[10]
37	Gemtuzumab ozogamicin	Mylotarg	2017	CD33	Humanized IgG4; ADC	Acute myeloid leukemia	Pfizer	[10]
38	Mogamulizumab	Poteligeo	2018	CCR4	Humanized IgG1	Mycosis fungoides or Sézary syndrome	Kyowa Hakko Kirin	[10]
39	Galcanezumab	Emgality	2018	CGRP	Humanized IgG4	Migraine prevention	Eli Lilly	[10]
40	Tildrakizumab	Ilumya	2018	IL-23 p19	Humanized IgG1	Plaque psoriasis	Merck & Co. Inc.	[10]
41	Fremanezumab	Ajovy	2018	CGRP	Humanized IgG2	Migraine prevention	Teva Pharmaceutical Industries, Ltd.	[10]
42	Ibalizumab	Trogarzo	2018	CD4	Humanized IgG4	HIV infection	Taimed Biologics Inc.	[10]
43	Ravulizumab	Ultomiris	2018	C5	humanized IgG2/4	Paroxysmal nocturnal hemoglobinuria	Alexion Pharmaceuticals Inc.	[10]
44	Caplacizumab	Cablivi	2019	von Willebrand factor	Humanized Nanobody	Acquired thrombotic thrombocytopenic purpura	Ablynx	[10]
45	Romosozumab	Evenity	2019	Sclerostin	Humanized IgG2	Osteoporosis in	Amgen	[10]
46	Risankizumab	Skyrizi	2019	IL-23 p19	Humanized IgG1	Plaque psoriasis	Boehringer Ingelheim Pharmaceuticals	[10]
47	Polatuzumab vedotin	Polivy	2019	CD79β	Humanized IgG1 ADC	Diffuse large B-cell lymphoma	Roche, F. Hoffmann-La Roche, Ltd.	[10]
48	Brolucizumab	Beovu	2019	VEGF-A	Humanized scFv	Macular degeneration	Novartis Pharmaceuticals Corp.	[10]
49	Crizanlizumab	Adakveo	2019	P-selectin	Humanized IgG2	Sickle cell disease	Novartis Pharmaceuticals Corp.	[10]
50	Eptinezumab-jjmr	Vyepti	2020	CGRP block	Humanized	Migraine	Lundbeck	[11]
51	Isatuximab-irfc	Sarclisa	2020	Anti-CD38	Chimeric	Multiple myeloma	SANOFI AVENTIS	[11]
52	Sacituzumab govitecan-hziy	Trodelvy	2020	Anti-Trop-2; SN-38;	Humanized	mTNBC	IMMUNOMEDICS	[11]
53	Daratumumab and hyaluronidase-fihj	Darzalex Faspro	2020	Anti-CD38,	Human	Multiple myeloma	JANSSEN	[11]
54	Inebilizumab-cdon	Uplizna	2020	Anti-CD19	Humanized	NMOSD (AQP4+)	VIELA	[11]
55	Pertuzumab, trastuzumab, and hyaluronidase-zzxf	Phesgo	2020	Anti-HER2;	Humanized	Breast cancer (HER2+)	GENENTECH	[11]
56	Belantamab mafodotin-blm	Blenrep	2020	Anti-BCMA	Humanized	Multiple Myeloma	GLAXOSMITHKLINE	[11]
57	Satralizumab-mwge	Enspryng	2020	Anti-IL6 receptor	Humanized	NMOSD (AQP4+)	GENENTECH	[11]
58	ATOLTIVIMAB +2	Inmazeb	2020	Zaire ebolavirus glycoprotein	Humanized IgG1κ	Zaire ebolavirus infection.	REGENERON	[11]
59	NAXITAMAB-GQGK	Danyelza	2020	Anti-glycolipid GD2	Humanized IgG1	Neuroblastoma	Y-MABS THERAPEUTICS	[11]
60	MARGETUXIMAB-CMKB	Margenza	2020	Anti-HER2	chimeric IgG	Breast cancer	MACROGENICS	[11]

(Continued)

Table 1: (Continued)

S. No.	Antibody	Brand name	Year of approval	Target	Format	Indication	Company	References
61	RITUXIMAB-ARRX	Riabni	2020	Anti-CD20	chimeric IgG	Non Hodgkin lymphoma	AMGEN	[11]
62	Dostarlimab-gxly	Jemperli	2021	PD-1	Humanized	Endometrial cancer	GSK	[12]
63	Loncastuximab tesirine-lpyl	Zynlonta	2021	CD19	Humanized IgG1	B cell lymphoma	ADC	[12]
64	Dostarlimab-gxly	Jemperli	2021	PD-1	Humanized	Solid tumor	GSK	[12]
65	Ranibizumab-nuna	Byooviz	2021	VEGF	Humanized IgG1	Macular degeneration	SAMSUNG	[12]
66	Ranibizumab	Susvimo	2021	VEGF	Humanized IgG1	Macular degeneration	GENEN TECH	[12]
67	Faricimab-svoa	Vabysmo	2022	VEGF; Ang-2	Humanized IgG1	nAMD; DME	GENENTECH	[13]
68	Sutimlimab-jome	Enjaymo	2022	CP	Humanized	Cold agglutinin disease	BIOVERATIV	[13]
69	Bevacizumab-maly	Alymsys	2022	VEGF	Humanized IgG1	mCRC	AMNEAL	[13]
70	Risankizumabrzaa	Skyrizi	2022	IL-23	Humanized IgG1	Plaque psoriasis	ABBVIE	[13]
71	Ranibizumab-eqr	Cimerli	2022	VEGF	Humanized IgG1	nAMD; RVO; DME;	COHERUS	[13]
72	Spesolimab-sbzo	Spevigo	2022	IL-36R	Humanized IgG1	Pustular psoriasis	BOEHRINGER	[13]
73	Bevacizumab-adc	Vegzelma	2022	VEGF	Humanized IgG1	mCRC; NSCLC;	CELLTRION	[13]
74	Mirvetuximab soravtansine-gynx	Elahere	2022	FRα-DM4	Humanized IgG1	Ovarian cancer	IMMUNOGEN	[13]
75	Teplizumab-mzwv	Tzield	2022	CD3	Humanized IgG1	type 1 diabetes	PROVENTION	[13]
76	Osunetuzumab-axgb	Lunsumio	2022	CD20; CD3	Humanized IgG1	Follicular lymphoma	GENENTECH	[13]
77	Ublituximab-xiiy	Briumv	2022	CD20	Chimeric IgG1	Multiple sclerosis	TG	[13]
78	Teclistamab-cqyv	Tecvayli	2022	BCMA; CD3	Humanized bispecific IgG4	Multiple myeloma	JANSSEN	[13]
79	Elranatamab	Elrexfio	2023	BCMA, CD3	Humanized IgG2	Multiple myeloma	Pfizer	[10]
80	Rozanolixizumab	Rystiggo	2023	FcRn;	Humanized IgG4	Generalized myasthenia gravis	UCB	[10]
81	Talquetamab	Talvey	2023	G protein-coupled receptor 5D, CD3;	Humanized IgG4 bispecific	Multiple myeloma	Janssen	[10]
82	Epcoritamab	Epkinly	2023	CD20, CD3	Bispecific humanized IgG1	Diffuse large B cell lymphoma	Abbvie	[10]
83	Glofitamab	Columvi	2023	CD20, CD3e	Bispecific	Diffuse large B-cell lymphoma	Genentech	[10]
84	Lecanemab	Leqembi	2023	Amyloid beta protofibrils;	Humanized IgG1	Alzheimer's disease	Biogen	[10]
85	Retifanlimab	Zynyz	2023	PD-1	Humanized IgG4	Merkel cell carcinoma	Incyte	[10]

to its capacity to rapidly isolate single-chain Fv fragments derived from human offspring. This technology has been widely employed to choose antibodies targeting diverse compounds of interest. In recent years, new *in vivo* and *in vitro* approaches for the isolation of human antibodies have been discovered, expanding the spectrum of applications for antibody engineering technology. Transgenic mice having human IgG loci instead of natural Ig genes can be used to select human antibodies *in vivo* [22,23]. Bacterial surface display, yeast surface display, and cell-free ribosome display are all *in vitro* possibilities for picking single-chain Fv fragments, and they are all discussed elsewhere.

3. VARIOUS METHODOLOGIES EMPLOYED IN THE DEVELOPMENT OF ANTIBODIES THAT EXHIBIT REACTIVITY TOWARD THE INTENDED TARGET

3.1. Immunize an Animal

The process of creating mAbs is both intricate and fascinating. Initially, an animal (usually a mouse or rat) can be vaccinated with the target antigen. This phase is essential because it primes the animal's system to generate the required antibodies. During the subsequent stage, B cells derived from the animal's immune system are meticulously combined with myeloma cells, leading to the creation of a hybridoma.

Table 2: US FDA-approved human monoclonal antibodies in the market.

S. No.	Antibody	Brand name	Year of approval	Target	Format	Technology	Indication	Company	References
1	Adalimumab	Humira	2002	TNFα	Human IgG1	Phage display	Rheumatoid arthritis	AbbVie Inc.	[10]
2	Panitumumab	Vectibix	2006	EGFR	Human IgG2	Transgenic mice	Colorectal cancer	Amgen	[10]
3	Ustekinumab	Stelara	2009	IL-12/23	Human IgG1	Transgenic mice	Psoriasis	Centocor Ortho Biotech Inc.	[10]
4	Canakinumab	Ilaris	2009	IL-1β	Human IgG1	Transgenic mice	Muckle-Wells syndrome	Novartis Pharmaceuticals Corp.	[10]
5	Golimumab	Simponi	2009	TNFα	Human IgG1	Transgenic mice	Rheumatoid and psoriatic arthritis, ankylosing spondylitis	Centocor Ortho Biotech Inc.	[10]
6	Ofatumumab	Arzerra	2009	CD20	Human IgG1	Transgenic mice	Chronic lymphocytic leukemia	Genmab A/S	[10]
7	Denosumab	Xgeva, Prolia	2010	RANKL	Human IgG2	Transgenic mice	Bone loss	Amgen	[10]
8	Belimumab	Benlysta	2011	BLyS	Human IgG1	Phage display	Systemic lupus erythematosus	GlaxoSmithKline.	[10]
9	Ipilimumab	Yervoy	2011	CTLA-4	Human IgG1	Transgenic	Metastatic melanoma	Bristol-Myers Squibb	[10]
10	Raxibacumab	Abthrax	2012	*B. anthrasis* PA	Human IgG1	Transgenic mice	Anthrax infection	GlaxoSmithKline	[10]
11	Ramucirumab	Cyramza	2014	VEGFR2	Human IgG1	Phage display	Gastric cancer	Eli Lilly	[10]
12	Nivolumab	Opdivo	2014	PD-1	Human IgG4	Transgenic mice	Melanoma, non-small cell lung cancer	Bristol-Myers Squibb	[10]
13	Necitumumab	Portrazza	2015	EGFR	Human IgG1	Phage display	Non-small cell lung cancer	Eli Lilly	[10]
14	Secukinumab	Cosentyx	2015	IL-17α	Human IgG1	Transgenic mice	Psoriasis	Novartis Pharmaceuticals Corp.	[10]
15	Alirocumab	Praluent	2015	PCSK9	Human IgG1	Transgenic mice	High cholesterol	Regeneron Pharmaceuticals Inc.	[10]
16	Evolocumab	Repatha	2015	PCSK9	Human IgG2	Transgenic mice	High cholesterol	Amgen	[10]
17	Daratumumab	Darzalex	2015	CD38	Human IgG1	Transgenic mice	Multiple myeloma	Genmab A/S	[10]
18	Olaratumab	Lartruvo	2016	PDGFRα	Human IgG1	Transgenic mice	Soft tissue sarcoma	Eli Lilly	[10]
19	Bezlotoxumab	Zinplava	2016	*Clostridium difficile* enterotoxin B	Human IgG1	Transgenic mice	Prevention of *Clostridium difficile* infection recurrence	Merck & Co. Inc.	[10]
20	Brodalumab	Siliq, Lumicef	2017	IL-17R	Human IgG2	Transgenic mice	Plaque psoriasis	MedImmune	[10]
21	Guselkumab	Tremfya	2017	IL-23 p19	Human IgG1	Phage display	Plaque psoriasis	MorphoSys	[10]
22	Dupilumab	Dupixent	2017	IL-4Rα	Human IgG4	Transgenic mice	Atopic dermatitis	Regeneron Pharmaceuticals Inc.	[10]
23	Sarilumab	Kevzara	2017	IL-6R	Human IgG1	Transgenic mice	Rheumatoid arthritis	Regeneron Pharmaceuticals Inc.	[10]
24	Avelumab	Bavencio	2017	PD-L1	Human IgG1	Phage display	Merkel cell carcinoma	Merck	[10]
25	Durvalumab	Imfinzi	2017	PD-L1	Human IgG1	Transgenic mice	Bladder cancer	MedImmune	[10]

(Continued)

Table 2: (Continued)

S. No.	Antibody	Brand name	Year of approval	Target	Format	Technology	Indication	Company	References
26	Burosumab	Crysvita	2018	FGF23	Human IgG1	Transgenic mice	X-linked hypophosphatemia	Kyowa Hakko Kirin/ Ultragenyx	[10]
27	Lanadelumab	Takhzyro	2018	Plasma kallikrein	Human IgG1	Phage display	Hereditary angioedema attacks	Dyax Corp.	[10]
28	Erenumab	Aimovig	2018	CGRPR	Human IgG2	Transgenic mice	Migraine prevention	Novartis	[10]
29	Cemiplimab	Libtayo	2018	PD-1	Human mAb	Transgenic mice	Cutaneous squamous cell carcinoma	Regeneron Pharmaceuticals Inc.	[10]
30	Emapalumab	Gamifant	2018	IFNγ	Human IgG1	Phage display	Primary hemophagocytic lymphohistiocytosis	NovImmmune	[10]
31	Moxetumomab pasudodox	Lumoxiti	2018	CD22	Murine IgG1 dsFv	Phage display	Hairy cell leukemia	MedImmune	[10]
32	Teprotumumab-trb	Tepezz	2020	IGF-1R block	Human IgG1	Transgenic mice	Thyroid eye disease	Horizon therapeutics	[11]
33	Evinacu-mab-dgnb	Evkeeza	2021	ANGPTL3	Human	Recombinant	HoFH LDL-C	Regeneron	[12]
34	Amivantamab-vmjw	Rybrevan	2021	EGFR MET Receptor	Human IgG1	Recombinant	NSCLC	Janssen	[12]
35	Aducanumab-avwa	Aduhelm	2021	Amyloid beta	Human IgG1	Recombinant	Alzheimer's disease	Biogen	[12]
36	Anifro-lumab-fnia	Saphnelo	2021	(I)IFNR	Human	Recombinant	SLE	Astrazeneca	[12]
37	Tisotumab vedotin-tftv	Tivdak	2021	Tissue Facto	Human	Recombinant	Cervical	Seagen	[12]
38	Adalimumab-aqvh	Yusimry	2021	TNF	Human IgG1	Recombinant	RA	Coherus	[12]
39	Tezepelumab-ekko	Tezspire	2021	TSLP	Human	Recombinant	Severe asthma	Astra zeneca	[12]
40	Tralokinumab-ldrm	adbry	2021	IL-13	Human IgG4	Recombinant	Eczema	Leo	[12]
41	Nivolumab; relatlimabrmbw	Opdualag	2022	PD-1; LAG-3	Human IgG4	Recombinant	melanoma	Bms	[13]
42	Tremelimumabactl	Imjudo bla 761289	2022	CTLA-4	Human IgG2	Recombinant	Liver cancer	Astra Zeneca	[13]
43	Tremelimumabactl	Imjudo bla 761270	2022	CTLA-4	Human IgG2	Recombinant	Liver cancer	Astra Zeneca	[13]
44	Dalimumab-aacf	Idacio	2022	TNF	Human IgG1	Recombinant	RA JIA PsA	Fresenius	[13]
45	Pozelimab	Veopoz	2023	Complement 5	Human IgG4	Recombinant	CHAPLE disease	Regeneron	[10]
46	Nirsevimab	Beyfortus	2023	RSV	Human IgG1	Recombinant	RSV infection	Sanofi	[10]

Table 3: Top five best-selling monoclonal antibody drugs in 2022.

S. No.	Antibody	Brand name	Company	Target	Technology/ formats	Indication	Revenue (USD)	References
1	Adalimumab	Humira	AbbVie	TNFα	Phage display	Rheumatoid arthritis	21.2 billion	[14]
2	Pembrolizumab	Keytruda	Merck & Co.	PD-1	Humanized IgG4	Melanoma	20.9 billion	[14]
3	Ustekinumab	Stelara	Janssen	IL-12/23	Human IgG1	Plaque psoriasis, psoriatic arthritis	9.7 billion	[14]
4	Dupilumab	Dupixent	Sanofi Genzyme, Regeneron	IL-4Rα	Human IgG4	Atopic dermatitis, asthma, chronic rhinosinusitis with nasal polyps	8.7 billion	[14]
5	Nivolumab	Opdivo	Bristol-Myers Squibb	PD-1	Transgenic mice	Melanoma, non-small cell lung cancer	8.2 billion	[14]

This hybridoma is a biological combination that merges the specificity of the B cell with the lifespan of the myeloma cell, resulting in immortality. Subsequently, by selecting clones that produce mAb with the required specificity for the target antigen, hybridomas can produce an unlimited number of mAb [Figure 2]. Muromonab-CD3 is one of the mAbs created by this method (Orthoclone OKT3).

A significant limitation of this technology is the immune response triggered by mouse antibodies in certain individuals, leading to the development of human-anti-mouse antibodies (HAMA). The primary drawback of this method is the activation of the immune system by mouse antibodies in specific individuals, resulting in the formation of HAMA. This immune response not only reduces the effectiveness of the original mouse mAbs but also prevents the use of any mAbs that have similar sequences to mice because of the potential for allergic reactions and decreased availability in the body. As a result, the use of mouse mAbs in medical conditions is significantly limited.

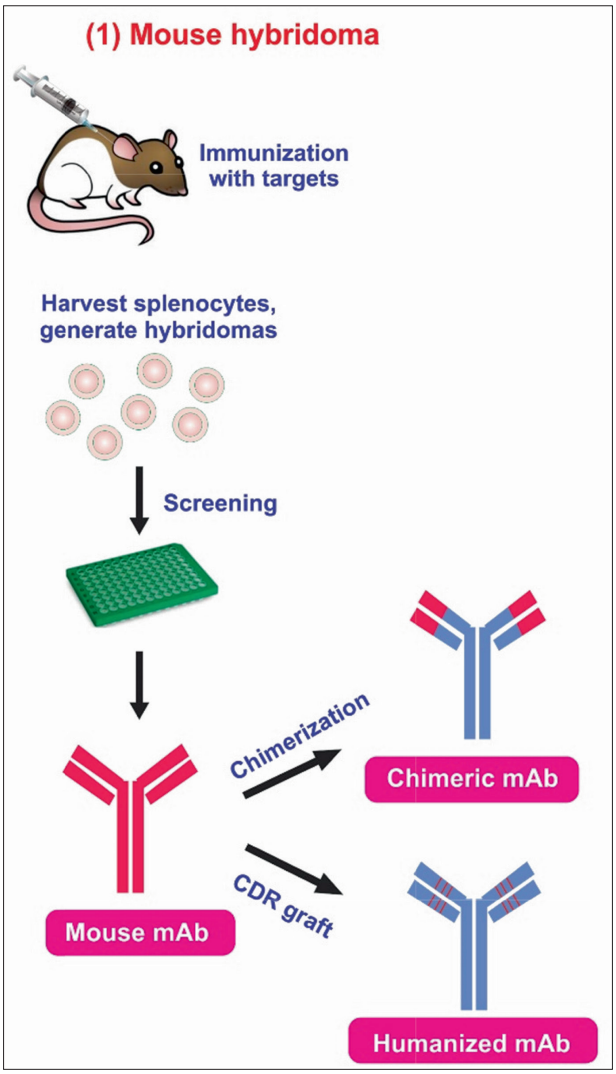

Figure 2: Development of mouse monoclonal antibody by hybridoma technology. Chimeric antibodies are developed by joining a sequence of murine variable domains with the human constant domain region. The humanized antibodies are developed by transplanting the murine CDR sequence to the human framework sequence. This technique is termed CDR grafting.

Furthermore, the process of genetic engineering was employed to produce chimeric antibodies by merging the variable regions of mice with the constant segments of humans. This measure was implemented in order to reduce the probability of mouse antibodies eliciting an immunological response in humans [Figure 2] [24]. Despite the fact that chimeric antibodies are less immunogenic than murine MAbs, human anti-chimeric antibody reactions have been found [25]. Approaches to engineer alterations to the immunoglobulin molecule, such as humanizing the antibody or producing a chimeric antibody, have been established and used in the majority of mAbs selected in animals. In order to minimize the presence of mouse elements, the process of humanizing non-human antibodies involves grafting the CDRs from non-human antibodies onto human frames. For the development of these humanized antibodies, human frameworks that have the closest similarity to the framework regions of non-human antibodies as recipients for CDR grafting were selected [26,27]. However, CDR grafting by this method results in a loss of affinity for their specific targets [28]. Therefore, key framework residues that support the conformations of CDR loops in the murine antibody are also grafted onto the human template in order to restore the affinity of the parental murine antibody. Based on these progressions in antibody engineering, the development of recombinant mAbs signifies another significant achievement in the pursuit of more effective and ethically manufactured therapeutic agents.

3.2. Recombinant mAbs Development

Recombinant antibodies refer to mAbs that are artificially created in laboratory conditions through the use of synthetic genes. In contrast to mAbs, recombinant antibodies are produced using advanced technology that does not employ hybridomas or animals. Recombinant mAbs offer effective therapies for cancer, autoimmune disorders, and various other disorders. Additionally, these antibodies can be employed in biomedical and toxicological studies. mAbs are extensively used in biomedical science and medicine because they have the capacity to attach to, neutralize, or eliminate antigens that are particular to certain cells [29]. However, the ascites method of manufacturing causes considerable agony and misery to the animals involved. Basically, recombinant antibodies are mAbs generated *in vitro* using synthetic genes [Figure 3]. The technology involves recovering antibody genes from source cells, amplifying and cloning the genes into an appropriate vector, introducing the vector into a host (bacteria, yeast, or mammalian cell lines), and achieving the expression of adequate amounts of functional antibody [30,31].

3.3. Phage Display

3.3.1. Phage-display vectors

Phages are viruses that infect bacterial cells, and many of the vectors used in recombinant DNA studies are phages that infect *Escherichia coli*, which is the most common recombinant DNA host. Recombinant DNA vectors, including phages, have the capacity to include segments of "foreign" DNA, which can be derived from human DNA or chemically synthesized. The foreign "insert" is copied alongside the vector DNA in its *E. coli* host as a guest. The distribution of phages seems to be entirely stochastic. Phage capsid fusions are used to provide extensive collections of encoded peptides or proteins in combinatorial libraries. The library can be used to identify phages that carry the appropriate peptides or proteins, which can then be decoded via phage DNA sequencing [32,33]. By employing panning and selection techniques, it is possible to isolate specific phage clones that produce desired recombinant antibodies from vast populations of phages, which may

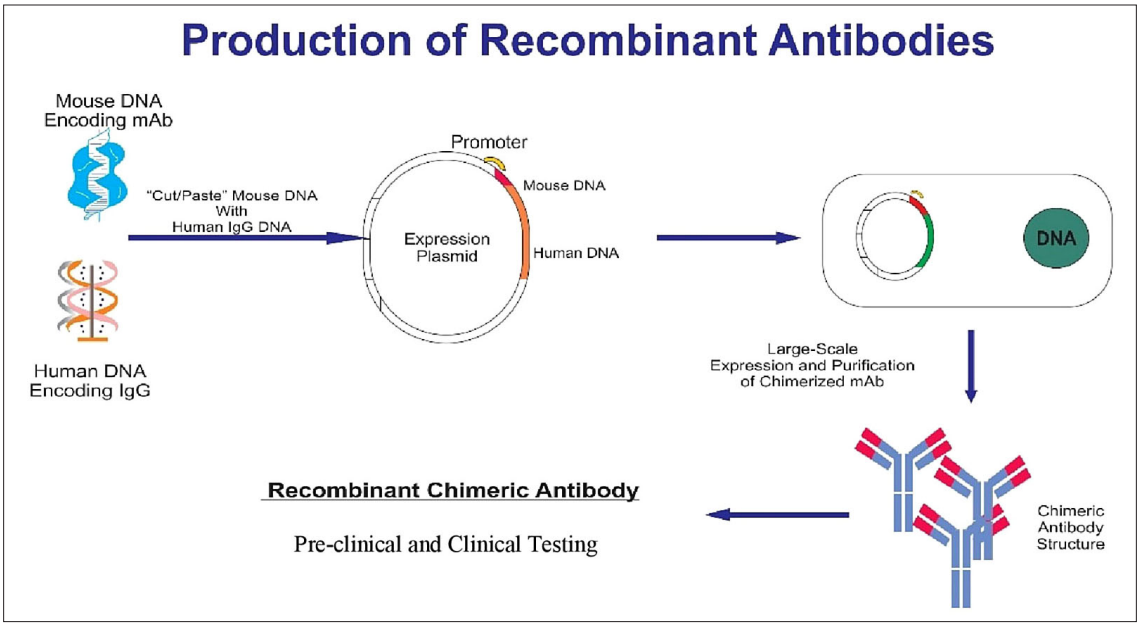

Figure 3: Schematic overview of production and development OF Recombinant antibodies. Recombinant antibodies are a type of monoclonal antibodies that are generated *in vitro* from a synthetic gene without immunizing any animals or cultivating any hybridomas.

consist of tens of millions of different clones [Figure 4]. The utilization of genetically modified antibody fragments in combination with the phage display technique highlights the adaptability and effectiveness of this procedure in the selection of targeted antibodies.

Additional methods for presenting information, including displaying it on the surface of *E. coli* or *Saccharomyces cerevisiae*, as well as employing ribosome display, were created based on the phage display principle. The efficacy of *in vitro* display methods in finding binders specific to antigens is undisputed, and in the future, these methods may be combined to mutually enhance each other. Despite the advantages of antibody phage display, such as the capacity to avoid animal vaccination, isolate antibodies against hazardous or non-immunogenic antigens, and manufacture conformation-specific antibodies, vaccinated mice approaches provide the vast majority of licensed therapeutic antibodies. This is due to the immune system's filtration mechanism, which allows human antibodies to possess superior biophysical characteristics compared with phage display antibodies [33].

3.4. Transgenic Mouse

Transgenic mouse strains with human immunoglobulin repertoires provide an alternative approach to select therapeutic mAbs that have a lower likelihood of causing an immune response. Transgenic technology is used to genetically modify mice strains in order to produce human sequence antibodies, unlike antibody engineering techniques that primarily involve modifying and optimizing individual protein components. These antibodies can be directly used in drug discovery and can be moved into clinical use without the need for additional optimization. The immunoglobulin transgenic mice have been validated as drug discovery platforms with the regulatory approval of their first product, panitumumab, 12 years after its initial publication in the scientific literature [34-36].

In 1994, the HuMabMouse [37] and the Xeno-Mouse [22] were the first transgenic mouse lines released using this technology. Genetic modifications of these lines were done in a manner, where human immunoglobulin (Ig) genes were inserted into the genome, replacing the indigenous Ig genes and allowing the animals to manufacture fully human antibodies [Figure 5] [35,37]. Following a comparable approach, the generation of neutralizing human antibodies from human B cells has also exhibited encouraging results for the treatment of infectious diseases. If feasible, it is preferable to obtain antibodies directly from humans rather than humanizing them from other animals for the treatment of infectious diseases. Human B cells are currently widely employed as a starting material for isolating human mAbs using techniques such as *in vitro* display, B cell immortalization, and single B cell expression cloning [38]. Human antibodies that may neutralize infectious pathogens may exhibit enhanced efficacy when derived directly from human B cells. This can be caused by the fact that the coupling of heavy and LCs has already been selected *in vivo* through rearrangement and has been well-tolerated in individuals, either following infection or immunization.

The progress of bispecific antibodies offers new possibilities for the advancement of novel protein treatments. Bispecific antibodies, through virtue of their ability to bind to two separate targets, have long held the potential of broadening the possibilities of mAb treatments. Bispecific act through a range of different mechanisms, including forming immunological synapses—or interfaces—between immune effector cells (macrophages, or T or NK cells) and tumor cells, staging a double blockade of disease-related pathways, cross-linking receptors, or bridging a gap in the coagulation cascade. The future use of transgenic animals that produce antibodies in their milk remains uncertain, although they have already been successfully engineered.

4. RECOMBINANT EXPRESSION AND PURIFICATION STRATEGIES OF ANTIBODIES

Currently, extensive bioreactors are employed to sustain mammalian cell culture for the production of clinically applicable antibodies. Consequently, the cost of a refined antibody for therapeutic use

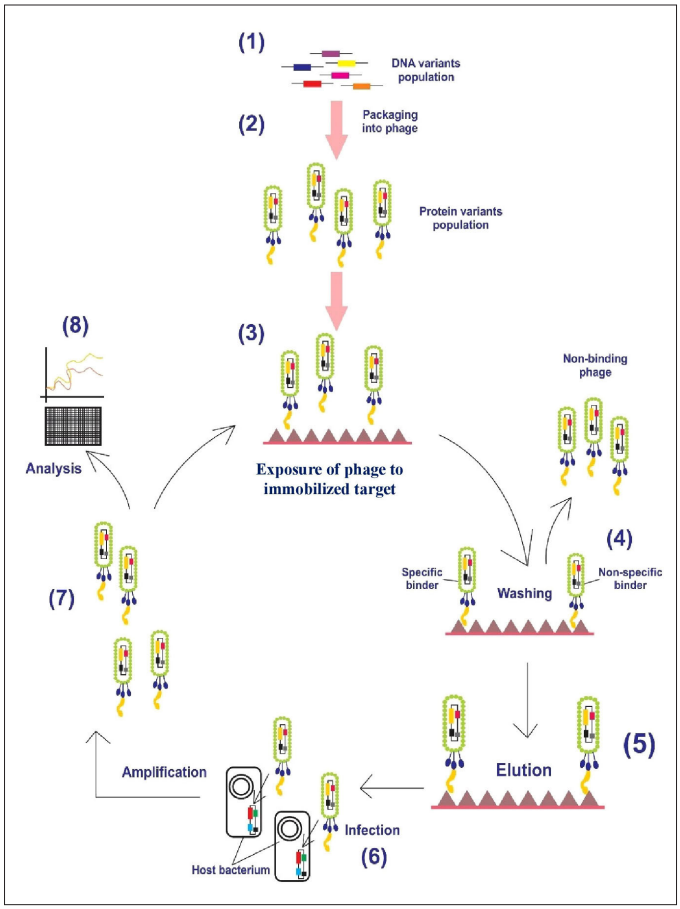

Figure 4: The phage display cycle. (1) A library of DNA variant sequences encoding peptides or proteins is created and (2) thus cloned into phage genomes as fusions to a coat protein gene. (3) The phage library exhibiting variant peptides or proteins is revealed to target molecules and phages with appropriate specificity are captured. (4) Non-binding phages are washed off. (5) Bound phage is eluted by disrupting the interaction between the displayed peptide or protein and the target. (6) Eluted phage is infected into host bacterial cells and thereby amplified. (7) This amplified phage population results in a secondary library that is greatly enriched in phage-displaying peptides or proteins that bind to the target. On repeating the bio-panning steps (3–6), the phage population becomes less and less diverse as the population becomes more and more enriched in the limited number of variants with binding capacity. (8) After several (usually three to five) rounds of bio-panning, monoclonal phage populations may be selected and analyzed individually.

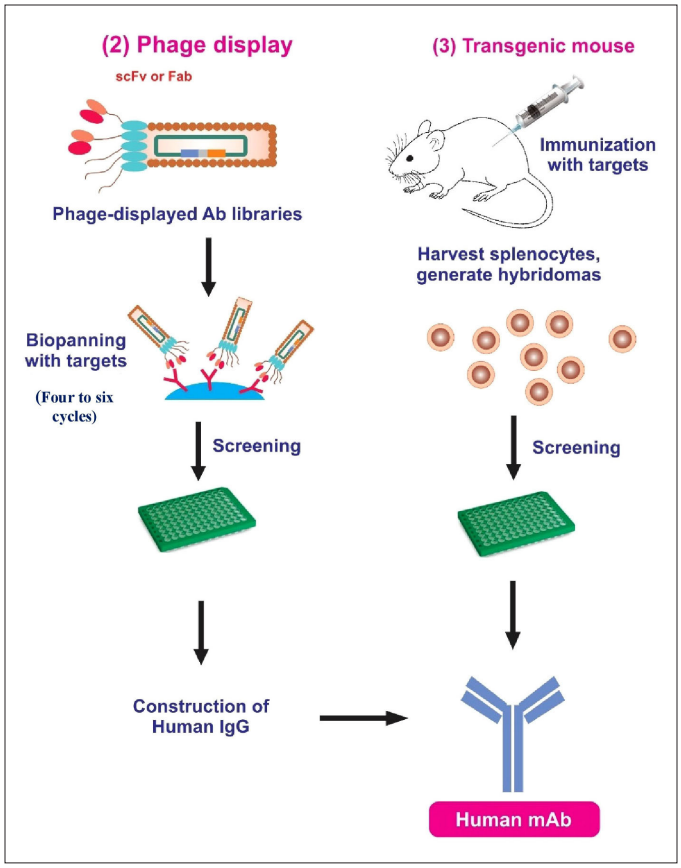

Figure 5: Development of Human monoclonal antibody by phage display and transgenic mouse technology. The characteristic of phages is that they house human DNA, this insert replicates along with vector DNA in *E. coli* host, and then DNA sequences are analyzed to construct and express human IgGs. The transgenic mouse is genetically modified by replacing endogenous Ig with human Ig genes to produce human IgGs.

may exceed up to USD 1000 per gram, in contrast to only USD 5 per gram for standard small molecules produced through chemical syntheses [5].

The efficacy of a therapeutic antibody is not solely determined by its specificity and affinity. Regarding mAb lead candidates, additional quality attributes, including solubility, viscosity, expression yield, heat, and long-term stability, are crucial [39,40]. The amino acid sequence has a significant impact on the physicochemical properties of antibodies [41]. Some mAbs may exhibit unfavorable developability traits such as increased immunogenicity, physical instability, self-aggregation, higher viscosity, non-specific binding, short half-life, and low expression levels [42,43]. mAb potency, bioavailability, and immunogenicity can be affected by low solubility during biomanufacturing [44-48]. Structural and functional integrity as well as intrinsic qualities depend on thermal stability [48,49]. In addition, immunogenicity problems have made aggregation a major obstacle in the progress of therapeutic mAbs [51-54].

Antibodies and antibody fragments can be produced by other expression systems, including bacteria, yeasts, and plants. *E. coli* bacteria are best at producing small, nonglycosylated, affinity-purifiable fragments of Fab and scFv [55,56]. If there is a need to avoid using affinity tags, alternative purification methods that involve size exclusion chromatography, ion-exchange chromatography, and ammonium sulfate precipitation can be employed to further refine the purification of full-length antibodies and scFv fragments. These methods can be used after the initial purification using the Protein A- and Protein L-binding matrices [57]. The lack of post-translational modifications in bacteria could pose a problem for antibody fragments or fusion proteins that require glycosylation.

5. CONCLUSION

mAbs are treasured for their specificity, selectivity, and binding affinity. All these properties, combined with their ease of production in recombinant mammalian systems, make them successful in a wide

array of applications. Antibodies are the preferred choice in therapy due to their minimal toxicity and high specificity, making them advantageous for treating a diverse array of human diseases including asthma, rheumatoid arthritis, Crohn's disease, multiple sclerosis, infectious diseases, and some types of cancers. In addition to this, mAbs, which are a type of drug, can be used to treat a number of other diseases. Considering the higher production costs compared to small molecules synthesized chemically, antibody-based therapeutics provide various advantages, such as their specificity and the ease of selecting antigen-specific binders. Therapeutic mAbs have gained significant recognition as a type of biological substance that is extensively used in research, development, and commercialization. The significance of mAbs in both the biopharmaceutical sector and the market is undeniable. As mAbs can be tailored to specific targets and pathways, they have transformed the approach to treating human diseases. New methods and techniques are being created to develop novel therapeutic mAbs. mAbs in advanced stages of development for new therapeutic applications are expected to significantly alter previously unexplored targets [58,59]. Besides new mAbs, biosimilars have become increasingly popular due to the impending expiration of patents for numerous highly successful mAbs. For instance, the patent on rituximab, which is an extensively used drug in the fields of cancer, hematology, rheumatology, nephrology, and other related areas, expired in Europe in 2013 and in the United States in 2018 [60,61]. Following the expiration of patents on original products, the creation of biosimilars is intended to make biotherapeutics cheaper. The production of a biosimilar entails a series of progressive comparability exercises, beginning with a comparison of the quality features of biosimilars and reference. Regulatory agencies approve biosimilars based on their demonstrated similarity to a relevant reference in terms of quality, nonclinical, and clinical criteria.

6. AUTHORS' CONTRIBUTIONS

HK wrote the manuscript. AM conceived the idea and methodology. HC and AM edited the manuscript. All authors read and approved the manuscript.

7. FINANCIAL SUPPORT AND SPONSORSHIP

There is no funding to report.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

12. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Schwaber J, Cohen EP. Human mouse somatic cell hybrid clone secreting immunoglobulins of both parental types. Nature 1973;244:444–7.
2. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495–7.
3. Shin SU, Morrison SL. Production and properties of chimeric antibody molecules. Methods Enzymol 1989;178:459–76.
4. Weiner GJ. Building better monoclonal antibody-based therapeutics. Nat Rev Cancer 2015;15:361–70. https://doi.org/10.1038/nrc3930
5. Schoonbroodt S, Steukers M, Viswanathan M, Frans N, Timmermans M, Wehnert A, et al. Engineering antibody heavy chain CDR3 to create a phage display Fab library rich in antibodies that bind charged carbohydrates. J Immunol 2008;181:6213–21. https://doi.org/0.4049/jimmunol.181.9.6213
6. Zhang P, Sun F, Liu S, Jiang S. Anti-PEG antibodies in the clinic: Current issues and beyond PEGylation. J Control Release 2016;244:184–93. https://doi.org/10.1016/j.jconrel.2016.06.040
7. Watanabe H, Nakanishi T, Umetsu M, Kumagai I. Human anti-gold antibodies: Biofunctionalization of gold nanoparticles and surfaces with anti-gold antibodies. J Biol Chem 2008;283:36031–8. https://doi.org/10.1074/jbc.M805547200
8. Saeed AF, Wang R, Ling S, Wang S. Antibody engineering for pursuing a healthier future. Front Microbiol 2017;8:495. https://doi.org/10.3389/fmicb.2017.00495
9. Kaplon H, Muralidharan M, Schneider Z, Reichert JM. Antibodies to watch in 2020. MAbs 2020;12:1703531. https://doi.org/10.1080/19420862.2019.1703531
10. The Antibody Society. Therapeutic monoclonal antibodies approved or in review in the EU or US. (date accessed); Available from: www.antibodysociety.org/resources/approved-antibodies.
11. Cai HH, Pandit A. Therapeutic monoclonal antibodies approved by FDA in 2020. Clin Res Immunol 2021;4:1–2.
12. Cai HH, Pandit A. Therapeutic monoclonal antibodies approved by FDA in 2021. Acta Sci Microbiol 2022;5(11): 81–3.
13. Cai HH, Pandit A. Therapeutic monoclonal antibodies approved by FDA in 2022. J Clin Exp Immunol 2022;8(1);533–5.
14. Buntz B. Drug Discovery and Development Report: Best selling pharmaceuticals of 2022. Available from: https://www.drugdiscoverytrends.com/.
15. Urquhart L. Top companies and drugs by sales in 2019. Nat Rev Drug Discov 2020;19:228. https://doi.org/10.1038/d41573-019-00049-0
16. Walsh G. Biopharmaceutical benchmarks 2018. Nat Biotechnol 2018;36:1136–45. https://doi.org/10.1038/nbt.4305
17. Grilo AL, Mantalaris A. The increasingly human and profitable monoclonal antibody market. Trends Biotechnol 2019;37:9–16. https://doi.org/10.1016/j.tibtech.2018.05.014
18. Murphy, K. Immunobiology, 9th ed. New York, NY: Garland Science; 2017.
19. Weiner LM, Surana R, Wang S. Monoclonal antibodies: Versatile platforms for cancer immunotherapy. Nat Rev Immunol 2010;10:317–27.
20. Bird RE, Hardman KD, Jacobson JW, Johnson S, Kaufman BM, Lee SM, et al. Single-chain antigen-binding proteins. Science 1988;242(4877):423–6. https://doi.org/10.1126/science.3140379

21. Queen C, Schneider WP, Selick HE, Payne PW, Landolfi NF, Duncan JF, et al. A humanized antibody that binds to the interleukin 2 receptor. Proc Natl Acad Sci U S A 1989;86:10029–33.
22. Mendez MJ, Green LL, Corvalan JR, Jia XC, Maynard-Currie CE, Yang XD, et al. Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice [published erratum appears in Nat Genet 1997 Aug;16(4):410]. Nat Genet 1997;15:146–156.
23. Ishida I, Tomizuka K, Yoshida H, Tahara T, Takahashi N, Ohguma A, et al. Production of human monoclonal and polyclonal antibodies in TransChromo animals. Cloning Stem Cells 2002;4:91–102; http://dx.doi.org/ 10.1089/153623002753632084; PMID:12006160.
24. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci U S A Nov 1984;81(21):6851–5. https://doi.org/10.1073/pnas.81.21.6851
25. Bell SJ, Kamm MA. Review article: The clinical role of anti-TNFalpha antibody treatment in Crohn's disease. Aliment Pharmacol Ther 2000;14(5):501–14. https://doi.org/10.1046/j.1365-2036.2000.00777.x; PMID: 10792111.
26. Pelat T, Bedouelle H, Rees AR, Crennell SJ, Lefranc MP, Thullier, P. Germline humanization of a non-human primate antibody that neutralizes the anthrax toxin, by *in vitro* and *in silico* engineering. J Mol Biol 2008;384:1400–07.
27. Robert R, Streltsov VA, Newman J, Pearce LA, Wark KL, Dolezal O. Germline humanization of a murine Abeta antibody and crystal structure of the humanized recombinant Fab fragment. Protein Sci 2010;19:299–308.
28. Pavlinkova G, Colcher D, Booth BJ, Goel A, Wittel UA, Batra SK. Effects of humanization and gene shuffling on immunogenicity and antigen binding of anti-TAG-72 single-chain Fvs. Int J Cancer 2001;94:717–26.
29. Kettleborough CA, Saldanha J, Heath VJ, Morrison CJ, Bendig MM. Humanization of a mouse monoclonal antibody by CDR-grafting: The importance of framework residues on loop conformation. *Protein Eng* 1991;4(7):773–83. https://doi.org/10.1093/protein/4.7.773
30. Dhankar R, Rathee P, Jain AK, Arora S, Kumar MS, Rath G, et al. HER-2-targeted immunonanoparticles for breast cancer chemotherapy. J Appl Pharm Sci 2011;132–9.
31. Nabiel A, Yosua Y, Sriwidodo S, Maksum IP. Overview of refolding methods on misfolded recombinant proteins from *Escherichia coli* inclusion bodies. J Appl Biol Biotech 2023;11(3):47–52. https://doi.org/10.7324/JABB.2023.112204
32. Smith GP. Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virion surface. Science 1985;228(4705):1315–7. https://doi.org/10.1126/science.4001944
33. Hoess RH. Protein design and phage display. Chem Rev 2001;101(10):3205–18.
34. Lonberg N, Taylor LD, Harding FA, Trounstine M, Higgins KM, Schramm SR, et al. Antigen-specific human antibodies from mice comprising four distinct genetic modifications. Nature 1994;368(6474):856–9. https://doi.org/10.1038/368856a0; PMID: 8159246.
35. Green LL, Hardy MC, Maynard-Currie CE, Tsuda H, Louie DM, Mendez MJ, et al. Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. Nat Genet 1994;7(1):13–21.
36. Gibson TB, Ranganathana A, Grothey A. Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer. Clin Colorectal Cancer 2006;6(1):29–31. https://doi.org/10.3816/CCC.2006.n.01
37. Lonberg N, Taylor LD, Harding FA, Trounstine M, Higgins KM, Schramm SR, et al. Antigen-specific human antibodies from mice comprising four distinct genetic modifications. Nature 1994;368:856–9.
38. Wilson PC, Andrews SF. Tools to therapeutically harness the human antibody response. Nat Rev Immunol 2012;12(10):709–19.
39. Chennamsetty N, Voynov V, Kayser V, Helk B, Trout BL. Prediction of aggregation prone regions of therapeutic proteins. J Phys Chem B 2010;114:6614–24. https://doi.org/10.1021/jp911706q
40. Jain T, Sun T, Durand S, Hall A, Houston NR, Nett JH, et al. Biophysical properties of the clinical-stage antibody landscape. Proc Natl Acad Sci U S A 2017;114:944–9. https://doi.org/10.1073/pnas.1616408114
41. Lecerf M, Kanyavuz A, Lacroix-Desmazes S, Dimitrov JD. Sequence features of variable region determining physicochemical properties and polyreactivity of therapeutic antibodies. Mol Immunol 2019;112:338–46. https://doi.org/10.1016/j.molimm.2019.06.012
42. Lavoisier A, Schlaeppi J-M. Early developability screen of therapeutic antibody candidates using Taylor dispersion analysis and UV area imaging detection. MAbs 2015;7:77–83. https://doi.org/10.4161/19420862.2014.985544
43. Dobson CL, Devine PWA, Phillips JJ, Higazi DR, Lloyd C, Popovic B, et al. Engineering the surface properties of a human monoclonal antibody prevents self-association and rapid clearance *in vivo*. Sci Rep 2016;6:38644. https://doi.org/10.1038/srep38644
44. Bye JW, Platts L, Falconer RJ. Biopharmaceutical liquid formulation: A review of the science of protein stability and solubility in aqueous environments. Biotechnol Lett. 2014;36:869–75. https://doi.org/10.1007/s10529-013-1445-6
45. Garidel P, Kuhn AB, Schäfer LV, Karow-Zwick AR, Blech M. High-concentration protein formulations: How high is high? Eur J Pharm Biopharm 2017;119:353–60. https://doi.org/10.1016/j.ejpb.2017.06.029
46. Trevino SR, Scholtz JM, Pace CN. Measuring and increasing protein solubility. J Pharm Sci 2008;97:4155–66. https://doi.org/10.1002/jps.21327
47. Gibson TJ, Mccarty K, Mcfadyen IJ, Cash E, Dalmonte P, Hinds KD, et al. Application of a high-throughput screening procedure with PEG-induced precipitation to compare relative protein solubility during formulation development with IgG1 monoclonal antibodies. J Pharm Sci 2011;100:1009–21. https://doi.org/10.1002/jps.22350
48. Sormanni P, Amery L, Ekizoglou S, Vendruscolo M, Popovic B. Rapid and accurate *in silico* solubility screening of a monoclonal antibody library. Sci Rep 2017;7:8200. https://doi.org/10.1038/s41598-017-07800-w
49. Seeliger D, Schulz P, Litzenburger T, Spitz J, Hoerer S, Blech M, et al. Boosting antibody developability through rational sequence optimization. MAbs 2015;7:505–15. https://doi.org/10.1080/19420862.2015.1017695
50. Chennamsetty N, Voynov V, Kayser V, Helk B, Trout BL. Design of therapeutic proteins with enhanced stability. Proc Natl Acad Sci U S A 2009;106:11937–42. https://doi.org/10.1073/pnas.0904191106
51. Lowe D, Dudgeon K, Rouet R, Schofield P, Jermutus L, Christ D. Aggregation, stability, and formulation of human antibody therapeutics. Adv Protein Chem Struct Biol 2011;84:41–61. https://doi.org/10.1016/B978-0-12-386483-3.00004-5
52. Perchiacca JM, Tessier PM. Engineering aggregation-resistant antibodies. Annu Rev Chem Biomol Eng 2012; 3:263–86. https://doi.org/10.1146/annurev-chembioeng-062011-081052
53. Rosenberg AS. Effects of protein aggregates: An immunologic perspective. AAPS J 2006;8:E501–7. https://doi.org/10.1208/aapsj080359
54. Li W, Prabakaran P, Chen W, Zhu Z, Feng Y, Dimitrov DS. Antibody aggregation: Insights from sequence and structure. Antibodies (Basel) 2016;5:19. https://doi.org/10.3390/antib5030019

55. Skerra A, Pfitzinger I, Plückthun A. The functional expression of antibody fv fragments in *Ischhuchia coli*: Improved vectors and a generally applicable purification technique. Biotechnology (N Y) 1991;9(3):273–8.
56. Terpe K. Overview of tag protein fusions: From molecular and biochemical fundamentals to commercial systems. Appl Microbiol Biotechnol 2003;60:523–33.
57. Bouvet JP. Immunoglobulin Fab fragment-binding proteins. Int J Immunopharmacol 1994;16(5-6):419–24.
58. Verma V. Leveraging monoclonal antibodies as therapeutics to address antimicrobial resistance in bacteria. J App Biol Biotech 2023;11(3):53–60. https://doi.org/10.7324/JABB.2023.90087
59. Adnyana IMO, Tedyanto EH, Widyadharma IPE. Efficacy and safety of eptinezumab as migraine preventive therapy: A systematic review and meta-analysis. J Appl Pharm Sci 2023;13(02):011–20. https://doi.org/10.7324/JAPS.2023.130202
60. DUBLIN--(BUSINESS WIRE)--May 2, 2019--The "Global and China Monoclonal Antibody Industry Report, 2019–2025". Available from: https://www.businesswire.com.
61. First World Pharma, Truxima, The First Biosimilar mAb in Oncology, Granted EU Marketing Authorisation, 2017.

How to cite this article:
Kasana H, Chander H, Mathur A. Technological advancement in the development of monoclonal antibody therapies: Present, past, and future. J App Biol Biotech. 2024. http://doi.org/10.7324/JABB.2024.172190
DOI: 10.7324/JABB.2024.172190