

Antibody Therapeutics – a mini review

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The study of antibodies has been one of the focal points in modern biology and medicine, but antibody in general was mostly a subject for basic research until César Milstein and Georges Köhler developed methods for the isolation of monoclonal antibodies (mAbs) using hybridoma cells in 1975 ⁽¹⁾. Since then, mAbs have not only been used as subjects and tools for break through basic research, but have also been used as clinical diagnostics, reagents for high throughput drug screening, and more importantly, life-saving medicines. In the therapeutic mAb field, the progress was initially slow and intermittent. The first therapeutic mAb, a murine-derived murononab for acute organ rejection, was approved by FDA in 1986, more than a decade after the discovery of the hybridoma technology ⁽²⁾. As a result of technological breakthroughs in the 1980's and 1990's, progress in the therapeutic mAb field has been accelerated. This mini review will provide readers with an overview on the practice of discovery, development, and clinical application of therapeutic mAbs.

Antibody structure

An antibody contains two light chains and two heavy chains, which are linked by multiple disulphide bonds (Figure 1A). The light and heavy chains contain a variable region, also known as the Fab (fragment antigen binding) region, and a constant region, which is also known as the Fc (fragment crystalizable) region. The antigen binding complementarity determining regions (CDRs) are short hypervariable amino acid sequences found in the variable domains of both light and heavy chains. Each of the two chains contains three CDRs (CDR1, CDR2 and CDR3). CDRs are synonymous with hypervariable domains because the majority of the sequence variations associated with antibodies are found in the CDRs. Among the six CDRs in an IgG molecule, CDR3s have the greatest variability. After binding to a target, a mAb can recruit effector cells such as natural killer cells, macrophages or neutrophils or activate complement to destroy the target-associated cells. These actions are referred to as antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Both ADCC and CDC are resulted from the combined functions of Fab and Fc portions of the mAb. The Fc region is the tail region of an antibody. In IgG, IgA and IgD antibody isotypes, the Fc region is composed of two identical protein fragments, derived from the second and third constant domains of the antibody's two heavy chains. The Fc regions in IgM and IgE contain three heavy chain constant domains in each polypeptide chain. The IgG isotype is most commonly used in therapeutic applications.

Sources of therapeutics antibodies

Monoclonal antibodies isolated from immunized animals using hybridoma technology is one of the major sources of therapeutic antibodies. Briefly, B-cells are removed from the spleen of an animal that has been challenged with the relevant antigen. These B-cells are then fused with myeloma tumor cells that can grow indefinitely in culture (myeloma is

a B-cell cancer). This fusion is performed by making the cell membranes more permeable. The fused hybrid cells (called hybridomas), being cancer cells, will multiply rapidly and indefinitely and will produce large amounts of the desired antibodies. Monoclonal antibodies isolated from wildtype animals, such as murine species, induce immunological responses in human. To reduce this response mAbs are commonly modified and produced as murine:human chimeric antibodies or humanized antibodies for therapeutic applications (Figure 1B-D).

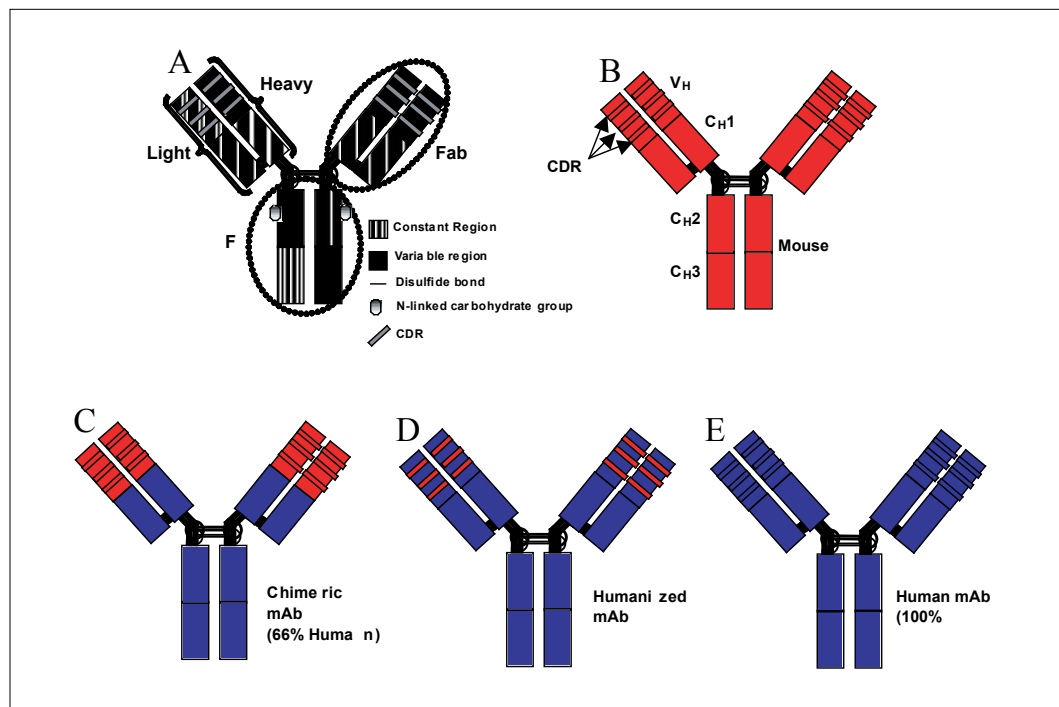


Figure 1. Diagrams of various antibody structures. A, a generic IgG molecule; B, a mouse IgG molecule; C, a murine:human chimeric IgG molecule; D, a humanized IgG molecule; and E, a human IgG molecule.

To circumvent the immunogenicity issue of murine derived antibodies, fully human antibodies can be generated in transgenic mice. This is possible by replacing the innate antibody genes in mice with human antibody genes. As a result, the transgenic mice produce human antibodies in response to immunization with an antigen.

Another source of fully human antibodies is from phage displayed antibody libraries. Either scFv (Single Chain Variable Fragment) or Fab fragment can be displayed on the exterior of the phage virion. This is achieved by directly or indirectly fusing antibody encoding genes, in the form of a library, to a major or minor capsid protein of a bacteriophage. A library can contain over 100 billion distinct antibody scFv or Fab fragments. In addition

to phage, antibody fragment or full IgG molecules can also be displayed on yeast, bacteria, and mammalian cells.

Formats of antibody therapeutics

Most therapeutic antibodies are full length IgG molecules (Table 1). This is because IgG molecules possess several favorable characteristics: 1) they are structurally stable; 2) IgGs have long in vivo half life; and 3) IgGs confer Fc-mediated biological properties. IgG molecules

can be murine, chimeric, humanized, and fully human.

In addition to IgG molecules, antibody fragments can also been developed as therapeutics. Relative to IgG molecules, antibody fragments may have more extensive penetration of tissues (particularly of solid tumors) due to smaller size. The shorter half life of antibody fragments can be extended by modifying the molecules such as PEGylation. Lack of the Fc region in an antibody fragment can eliminate immunogenicity to the Fc region and may reduce side effects caused by the interaction between Fc and

the immune system. Lucentis, a fragment of Avastin, is used for the treatment of wet age-related macular degeneration⁽³⁾. More recently, Certolizumab pegol (Cimzia), a PEGylated antibody fragment was approved for the treatment of rheumatoid arthritis in Europe⁽⁴⁾. Antibody fragment based therapeutics are still considered as a niche opportunity and of the 150 antibody based therapeutics in various stages of clinical development, less than 10 are antibody fragments⁽⁵⁾.

Antibodies can also be used as carrier agents of small molecule toxins or radiolabeled isotopes, guiding drugs to specific disease sites and limiting undesired effects on healthy cells. This application is commonly deployed in oncology. Two radiolabeled antibodies, Zevalin and

Bexxar, are approved for clinical use (Table 1). These drugs are difficult to administer because a radiologist and an oncologist are needed to oversee the administration. Mylotarg, a humanized anti-CD33 IgG4 antibody conjugated with calicheamicin, is the only antibody used to carry a cytotoxic payload (Table 1). Many challenges still exist in designing antibody:drug conjugates such as choice of linker, stoichiometry, and conjugation chemistry, but increasing evidence suggests that conjugated antibody remains an effective alternative to mAb, small molecular, or radiolabeled isotope monotherapies.

Overcome immunogenicity

The history of therapeutic antibody development parallels the desire of the industry to reduce potential immunogenicity of the drugs. Immunogenicity can reduce efficacy of therapeutic mAbs. In severe cases, immunogenicity can cause anaphylaxis and hypersensitivity reactions. Soon after the approval of the murine-derived monoclonal antibody, muromonab for acute organ rejection in 1986⁽²⁾, it was realized that murine-derived monoclonal antibodies are less than ideal therapeutics

Table 1. Monoclonal antibody therapeutics approved for clinical use.

Generic Name Trade Name Manufacturer	Launch Date	Therapy Area	Major Indication	Target	Protein Form/Isotype	Delivery	Reference
Muromonab Orthoclone/OKT3 Johnson & Johnson	1986	AIID	Transplant rejection	CD3	Murine IgG2a	IV	(16)
Abciximab ReoPro Eli Lilly	1995	CV	Cardiovascular disease	CD41	Chimeric Fab	IV	(8)
Rituximab Rituxan/MabThera Genentech/Roche	1997	Oncology	Non-Hodgkin's Lymphoma	CD20	Chimeric IgG1	IV	(17)
Daclizumab Zenapax Roche	1997	AIID	Transplant rejection	CD25	Humanized IgG1	IV	(9)
Basiliximab Simulect Novartis	1998	AIID	Transplant rejection	CD25	Chimeric IgG1	IV	(18)
Infliximab Remicade Centocor	1998	AIID	Rheumatoid arthritis	TNF alpha	Chimeric IgG1	IV	(19)
Palivizumab Synagis MedImmune	1998	ID	Respiratory syncytial virus	RSV F-protein	Chimeric IgG1	IM	(20)
Trastuzumab Herceptin Genentech	1998	Oncology	Breast cancer	Her2	Humanized IgG1	IV	(21)
Gemtuzumab/ozogamicin Mylotarg Wyeth	2000	Oncology	Acute myelogenous leukemia	CD33	Humanized IgG4 conjugated with ozogamicin	IV	(22)
Alemtuzumab Campath Bayer-Schering	2001	Oncology	Chronic lymphocytic leukemia	CD52	Humanized IgG1	IV	(23)

Ibritumomab tiuxetan Zevalin Biogen/Iddec	2002	Oncology	Non-Hodgkin's Lymphoma	CD20	Murine IgG1 conjugated with Yttrium 90	IV	(24)
Omalizumab Xolair Genentech/Novartis	2003	Respiratory	Asthma	IgE	Humanized IgG1	SC	(25)
Efalizumab Raptiva Genentech	2003	AIID	Psoriasis	CD11A	Humanized IgG1	SC	(26)
Tositumomab Bexxar GSK	2003	Oncology	Non-Hodgkin's Lymphoma	CD20	Murine IgG2a conjugated with Iodine-131	IV	(27)
Adalimumab Humira Abbott	2003	AIID	Rheumatoid arthritis	TNF alpha	Human IgG1	SC	(11)
Cetuximab Erbix ImClone/BMS	2003	Oncology	Colorectal cancer	EGFR	Chimeric IgG1	IV	(28)
I-131 ch-TNT Shanghai Medipharm Biotech Co.	2003	Oncology	Advanced lung cancer	Intracellular DNA in tumors	Chimeric IgG1 conjugated with I-131	IV	(29)
Bevacizumab Avastin Genentech	2004	Oncology	Colorectal and non-small cell lung cancer	VEGF	Humanized IgG1	IV	(30)
Natalizumab Tysabri Biogen IDEC/Elan	2004	CNS/AIID	Multiple sclerosis	VLA4	humanized IgG1	IV	(31)
Tocilizumab Actemra Roche/Chugai	2005	AIID	Castleman's disease	IL-6R	Humanized IgG1	IV	(32)
Ranibizumab Lucentis Genentech/Novartis	2006	Ophthalmology	Wet age-related macular degen- eration	VEGF	Humanized mab fragment of Avastin	Injection into the eye	(3)
Panitumumab Vectibix Amgen	2006	Oncology	Colorectal cancer	EGFR	Human IgG2	IV	(33)
Certolizumab pegol Cimzia UCB-Schwarz	2007	AIID	Rheumatoid arthritis	TNF alpha	PEGylated Fragment	SC	(4)
Eculizumab Soliris Alexion	2007	Hematology	PNH (chronic hemolysis)	C5a	Humanized IgG2/IgG4 hybrid	IV	(34)

AIID: arthritis, immune and inflammatory disorders; **ID:** infectious disease; **CNS:** central nervous system; **CV:** cardiovascular.

due to their high immunogenicity in human. Several “humanization” strategies, such as chimeric mAb⁽⁶⁾ and CDR grafting⁽⁷⁾, were devised to reduce the human anti-mouse antibody (HAMA) responses. It took a decade for the first chimeric mAb, abciximab for hemostasis, to be approved by FDA in 1994⁽⁸⁾. The first humanized mAb, Zenapax for kidney transplant rejection, was approved for clinical use by FDA in 1997⁽⁹⁾. Humanization alleviated the HAMA response to various degrees, but many other drawbacks became evident. For example, the humanization process is technically demanding and the process may result in reduced antigen binding affinity and decreased efficacy. To avoid the human immune response to murine-derived mAbs and the technical challenge associated with humanizing murine mAbs, two major approaches were developed for generating fully human mAbs. The first approach was to express the human antibody-encoding genes on bacteriophage surfaces. The resulting libraries contain billions of unique human antibodies which can be screened for leads⁽¹⁰⁾. Humira, the first fully human mAb derived from a bacteriophage displayed antibody library, was approved by FDA in 2003 for the treatment of rheumatoid arthritis⁽¹¹⁾. The second approach was to use transgenic mice to produce fully human antibodies^(12,13). This is achieved by replacing the mouse native antibody genes with their human counterparts. Vectibix, an anti-EGFR antibody approved for colorectal cancer therapy in 2006, was the first fully human antibody therapeutic derived from a transgenic mice system⁽¹⁴⁾. The industry trend is to develop more human like antibodies for clinical use. However immunogenicity is a complex biological process and it can not be predicted solely on human content of a mAb. For example, Humira, a fully human antibody, showed relatively high incidence of immunogenicity⁽¹⁵⁾. There are 11 humanized and five chimeric mAbs in clinical use today, but there is little difference in immunogenicity rate between the two classes of antibodies (Table 1).

Manufacturing

Manufacturing of mAbs is expansive. A large scale facility can take about 5 years to build and it costs more than US\$200 million. Mammalian cell culture (Chinese hamster ovary cells, recombinant myelomas or hybridomas) is the dominant production platform for mAb therapeutics. About half of the current marketed mAbs are expressed in CHO. Cimzia is the first mAb (fragment) therapeutic made in a bacterial cell line. Other promising methods of antibody production, such as plants, transgenic animals (milk), eggs, and yeast, are being developed.

Targets

Antibodies do not readily cross cell membranes or the brain blood barrier (BBB), but they can engage a wide range of extracellular drug targets such as membrane bound proteins or circulating ligands and cytokines (Table 1). Unlike enzymes, GPCRs, ion channels, and nuclear receptors, extracellular signaling (ECS) targets generally do not interact with small molecules because the natural biological roles of ECS targets are typically achieved through protein-protein interactions. ECS proteins have been successfully targeted by antibodies. The therapy areas in which therapeutic antibodies have the strongest presence in terms of marketed products and mAbs in clinical development are oncology and AIID (Arthritis, Immune and Inflammatory Disorders).

Market outlook

Of the 24 monoclonal antibody products in clinical use, five (Avastin, Herceptin, Humira, Remicade, and Rituxan) of them accounted for 80% of sales in 2006. Small molecule drugs and monoclonal antibodies accounted for 83.3% and 4.6% of total pharmaceutical revenue in 2006, respectively. Datamonitor predicts that monoclonal antibodies together with therapeutic proteins will account for 70% of the growth in prescription pharmaceutical annual sales between 2006 and 2012. Revenues from antibody therapeutics are predicted to increase from US\$19,573 million in 2006 to US\$43,381 million in 2012. This is because mAbs have the advantage of primarily addressing high unmet need therapy areas such as oncology and AIID. Of the approximately 180 monoclonal antibodies in clinical use and in clinical development (72 PhI, 65 PhII, 21 PhIII, 23/marketed/preregistration), 100 of them are being developed for oncology. The second concentrated therapeutic category is in the AIID area with 31 antibodies in clinical development. Infectious disease is becoming a major disease area for antibody therapeutics with about 27 antibody molecules in various stages of clinical development. It is encouraging to see that more than 27 antibody drugs that are in clinical use or in clinical development are in the CNS, CV, women’s health, diabetes/endocrinology, hematology, ophthalmology, and respiratory disease areas⁽⁵⁾. It is also worth noting that about 20 of the 180 antibody therapeutics in various stages of clinical use and development are antibody:drug and antibody:radioisotope conjugates.

Biogenerics, biosimilars, and follow-on biologics

Unlike small molecule drugs, monoclonal antibodies are much larger and more complex molecules that are

not easily characterized by analytical tools. Additionally antibody therapeutics are produced as heterogeneous mixtures of molecules that vary slightly in molecular structure, such as different glycoforms. The complex nature of mAbs has contributed to the lack of a consensus definition for generic biopharmaceuticals. Multiple terms are being used in describing generic biopharmaceuticals, such as biogenerics, biosimilars, and follow-on biologics. Development of generic antibody therapeutics will be expensive because clinical trials are needed. Some predict that it will take at least ten years before the technology is advanced to a stage whereby the safety and bioequivalence of a biosimilar can be verified without clinical testing (Janet Woodcock, FDA). Despite the regulatory and technological barriers to the development of generic biopharmaceuticals, it is certain that successful biopharmaceuticals will eventually face generic competition.

Summary

At the end of 2007, at least 24 mAb therapeutics have been approved for clinical use by regulatory agencies around the world and more than 150 therapeutic mAbs are in various stages of clinical trials⁽⁵⁾. There is no doubt that antibody therapeutics will play an ever increasing role in combating life threatening and debilitating diseases.

References

- Kohler G & Milstein C (1975) *Nature* **256**, 495-497.
- Thistlethwaite JR, Jr., Haag BW, Gaber AO, Stuart JK, Aronson AJ, Mayes JT, Lloyd DM, & Stuart FP (1987) *Transplant Proc* **19**, 1901-1904.
- Kenneth TE & Kertes PJ (2006) *Clin Interv Aging* **1**, 451-466.
- Rutgeerts P, Schreiber S, Feagan B, Keininger DL, O'Neil L, & Fedorak RN (2007) *Int J Colorectal Dis*.
- Datamonitor (2007) 245 Fifth Avenue, 4th floor, New York, NY 10016.
- Morrison SL, Johnson MJ, Herzenberg LA, & Oi VT (1984) *Proc Natl Acad Sci U S A* **81**, 6851-6855.
- Kettleborough CA, Saldanha J, Heath VJ, Morrison CJ, & Bendig MM (1991) *Protein Eng* **4**, 773-783.
- Faulds D & Sorkin EM (1994) *Drugs* **48**, 583-598.
- Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, Neylan J, Wilkinson A, Ekberg H, Gaston R, et al. (1998) *N Engl J Med* **338**, 161-165.
- Vaughan TJ, Williams AJ, Pritchard K, Osbourn JK, Pope AR, Earnshaw JC, McCafferty J, Hodits RA, Wilton J, & Johnson KS (1996) *Nat Biotechnol* **14**, 309-314.
- Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, Teoh LA, Fischkoff SA, & Chartash EK (2003) *Arthritis Rheum* **48**, 35-45.
- Russell ND, Corvalan JR, Gallo ML, Davis CG, & Pirofski L (2000) *Infect Immun* **68**, 1820-1826.
- Lonberg N (2005) *Nat Biotechnol* **23**, 1117-1125.
- Chua YJ & Cunningham D (2006) *Drugs Today (Barc)* **42**, 711-719.
- Bender NK, Heilig CE, Droll B, Wohlgemuth J, Armbruster FP, & Heilig B (2007) *Rheumatol Int* **27**, 269-274.
- Cohen DJ, Benvenisty AI, Cianci J, & Hardy MA (1989) *Am J Kidney Dis* **14**, 19-27.
- Maloney DG, Grillo-Lopez AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, Janakiraman N, Foon KA, Liles TM, Dallaire BK, et al. (1997) *Blood* **90**, 2188-2195.
- Nashan B, Moore R, Amlot P, Schmidt AG, Abeywickrama K, & Soullillou JP (1997) *Lancet* **350**, 1193-1198.
- Onrust SV & Lamb HM (1998) *BioDrugs* **10**, 397-422.
- Storch GA (1998) *Pediatrics* **102**, 648-651.
- Albanell J & Baselga J (1999) *Drugs Today (Barc)* **35**, 931-946.
- Sorokin P (2000) *Clin J Oncol Nurs* **4**, 279-280.
- Ferrajoli A, O'Brien S, & Keating MJ (2001) *Expert Opin Biol Ther* **1**, 1059-1065.
- Krasner C & Joyce RM (2001) *Curr Pharm Biotechnol* **2**, 341-349.
- Davis LA (2004) *Ann Pharmacother* **38**, 1236-1242.
- Gauvreau GM, Becker AB, Boulet LP, Chakir J, Fick RB, Greene WL, Killian KJ, O'Byrne P M, Reid JK, & Cockcroft DW (2003) *J Allergy Clin Immunol* **112**, 331-338.
- Davies AJ (2004) *Q J Nucl Med Mol Imaging* **48**, 305-316.
- Kies MS & Harari PM (2002) *Curr Opin Investig Drugs* **3**, 1092-1100.
- Chen S, Yu L, Jiang C, Zhao Y, Sun D, Li S, Liao G, Chen Y, Fu Q, Tao Q, et al. (2005) *J Clin Oncol* **23**, 1538-1547.
- Kerr DJ (2004) *Nat Clin Pract Oncol* **1**, 39-43.
- Rudick RA & Sandrock A (2004) *Expert Rev Neurother* **4**, 571-580.
- Paul-Pletzer K (2006) *Drugs Today (Barc)* **42**, 559-576.
- ohenuram M & Saif MW (2007) *Anticancer Drugs* **18**, 7-15.
- Rother RP, Rollins SA, Mojcik CF, Brodsky RA, & Bell L (2007) *Nat Biotechnol* **25**, 1256-1264.