

ADCC Enhancement Technologies for Next Generation Therapeutic Antibody

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Introduction

Recombinant therapeutic antibodies are a successful new class of drugs developed in the past two decades. Antibody therapy has a long history tracing back to thousands of years ago, as early as 200 BC in China, in the form of vaccination against infectious diseases. In the late 1800s, sera from humans or animals containing antibodies were widely used for prophylaxis and therapy of viral and bacterial diseases^[1,2]. With the advent of molecular biology, it has become possible to produce recombinant antibodies in mammalian cells^[3,4]. Human chimeric and humanization of antibodies reduced immunogenicity of the drugs, paving the way for broad use in patients and disease applications^[5,6]. Fully-human antibodies have become a reality with new technologies such as phage display antibody library and transgenic mouse with human immunoglobulin genes^[7]. Up to date, 23 therapeutic antibodies have been approved by the FDA for the treatment of various diseases (Table 1), including cancer, viral infection, rheumatoid arthritis, and organ graft rejection. It is estimated that about 30% of new drugs in the next decade will be based on antibody products^[8,9].

Monoclonal antibodies have achieved great commercial success, and are considered the most attractive product segment in the prescription pharmaceutical market out to 2012^[10]. Across the 3 main product types (monoclonal antibody, therapeutic proteins and small molecules), monoclonal antibodies are the only product to reach double-digit annual growth, and are predicted to reach \$50 billion by 2013. Five therapeutic antibodies (Avasatin, Herceptin, Humira, Remicade and Rituxan), the “Big Five”, accounted for 77.0% of the total segment sales in 2007, with at least \$3 billion revenue each. Aside from the “Big Five”, there are 8 antibodies (Denosumab, Lucentis, Bapineuzumab, Numax, Golimumab, Actemra, Tysabri, and Cimzia), the “Emerging 8,” which show great promise of significant increases. Among the disease indications, oncology, inflammation and immunity dominate the landscape in both approved drugs and current drug discovery and development.

ADCC (Antibody Dependent Cell-mediated Cytotoxicity), is a major clinical mechanism of action for therapeutic antibodies against cell surface targets (Figure 1) in cancer and chronic inflammation. It takes advantage of patients’ innate immune cells to kill target cells. The functions are primarily triggered through direct interaction of the Fc domain of human immunoglobulin, in most cases, immunoglobulin subclass I (IgG1), with corresponding receptors^[11,12].

Table 1: U.S. FDA approved therapeutic antibodies for clinical use

Product	Brand name	Target	Disease indication	FDA approval date
Muromonab-CD3	Orthoclone OKT3	T-cell CD3 receptor	Transplant rejection	1986
Abciximab	ReoPro	gpIIb-gpIIIa, $\alpha v\beta 3$	Cardiovascular disease	1994
Daclizumab	Zenapax	IL-2 receptor α	Transplant rejection	1997
Rituximab	Rituxan, MabThera	CD20	NHL	1997
Trastuzumab	Herceptin	ErbB2	Breast cancer	1998
Palivizumab	Synagis	Epitope of F protein of RSV	Prevention of RSV infection	1998
Infliximab	Remicade	TNF α	Inflammatory diseases	1998
Basiliximab	Simulect	IL-2 receptor α	Transplant rejection	1998
Gemtuzumab	Mylotarg	CD33	AML	2000
Alextuzumab	Campath	CD52	CLL	2001
Adalimumab	Humira	TNF α	Inflammatory diseases	2002
Efalizumab	Raptiva	CD11a	Psoriasis	2002
Ibritumomab	Zevalin	CD20	NHL	2002
Tositumomab	Bexxar	CD20	NHL	2003
Omalizumab	Xolair	IgE	Asthma	2004
Bevacizumab	Avastin	VEGF	Colorectal cancer	2004
Cetuximab	Erbitux	EGFR	Colorectal cancer	2004
Natalizumab	Tysabri	T-Cell VLA4 receptor	Multiple sclerosis	2006
Panitumumab	Vectibix	EGFR	Colorectal cancer	2006
Ranibizumab	Lucentis	VEGF	Macular degeneration	2006
Eculizumab	Soliris	Complement C5	Inflammatory diseases	2007
Golimumab	Simponi	TNF α	Inflammatory diseases	2009
Canakinumab	Ilaris	IL-1	Muckle-Wells disease	2009

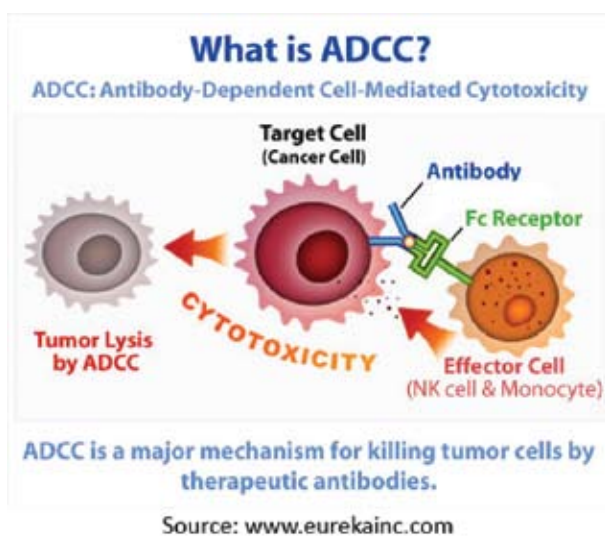


Figure 1: Diagram of ADCC: Antibody-Dependent Cell-Mediated Cytotoxicity

Clinical importance of ADCC activity has been highlighted by recent studies on genetic polymorphism in cancer patients. Strong correlation has been established between ADCC response and long-term survival rates in metastatic cancers. Pioneering work by Cartron G. et al.^[13], demonstrated that a better clinical response to Rituximab (anti-CD20) was associated with the 158 valine (V) allotype of Fc γ RIIIa, a key receptor for mediating ADCC activity. Similar results have been obtained in at least 4 more cohorts of patients with non-Hodgkin's lymphoma treated with Rituximab. Fc γ RIIIa polymorphism at position 158 results in different levels of ADCC mediated by the same antibody. PBMCs from homozygous valine (V/V) allotype show much higher ADCC activity than that from phenylalanine-carrier (F/V; F/F) allotype patients. However, data for antibodies against solid tumors remained elusive. In fact, the initial study with trastuzumab (anti-ErbB2, Herceptin) was negative. Recent study by Musolino et al, for the first time, showed convincingly that a better response to trastuzumab is associated with 158 V/V genotype^[14]. This correlation provides strong evidence linking clinical outcome to ADCC response to antibody treatment. This result is consistent with data acquired with Rituximab. Similar findings have been published that associates the 158 V/V genotype of Fc γ RIIIa with a better response to cetuximab^[15].

Therapeutic antibodies with enhanced ADCC are anticipated to have a clinical advantage owing to increased specific lysis of target cells, such as cancer cells, mediated by Fc receptors present on natural killer cells, mac-

rophages, and other immune cell types. Figure 2 shows an example of tumor cell killing by ADCC-enhanced anti-Her2 antibody with freshly isolated human PBMC. A higher percentage of tumor cells are killed at maximal dosage, while significant cell lysis is still observed at a much lower dose with the enhanced antibody as compared with the wild type. More importantly, dramatically improved tumor cell killing is observed with human PBMC with F/F genotype, which showed minimal activity with the wild type antibody (Figure 3). Antibodies with optimized binding to Fc receptors show enhanced ADCC activity against target-expressing cancer cell lines with human PBMC. Furthermore, markedly improved anti-tumor activity has been observed in xenograft models in FcRIII-knockout mice that express the low-binding allele of human CD16A. Recently, a primate study of anti-CD19 recombinant antibody with enhanced

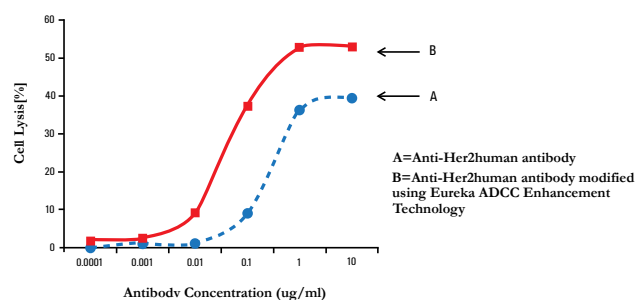


Figure 2: ADCC Activity Assay of Anti-Her2 human antibody against SKBR3 (high Her-2 expression ovarian cancer cell line) by human PBMC with V/V genotype

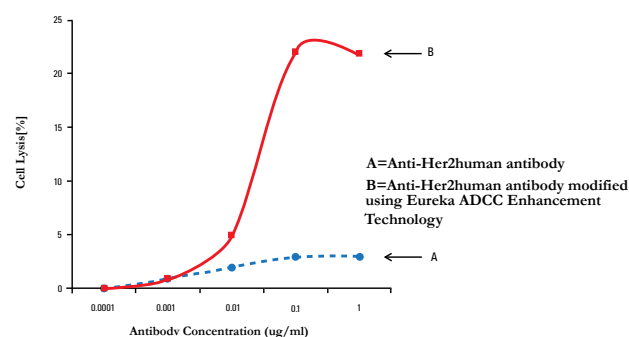


Figure 3: ADCC Activity Assay of anti-Her2 human antibody against MDA-MB-231 (low Her-2 expression breast cancer cell line) by human PBMC with F/F genotype

ADCC by Xencor technology showed positive efficacy results in eliminating B cell population. Table II provides a summary of ADCC enhancement technologies.

Multiple strategies have been used to achieve ADCC enhancement, including glycoengineering and mutagenesis, all of which seek to improve Fc binding to low-affinity activating FcγRIIIa, and/or reducing binding to the low-affinity inhibitory FcγRIIb. The mutagenesis approach identified critical residues in the Fc region involved in the interaction with FcγR through alanine scanning of surface-exposed residues^[16], mutagenesis guided by a protein structure design algorithm^[17], or using yeast surface display system^[18].

ADCC enhancement through glycoengineering is becoming the preferred technology platform, because of low probability of immunogenicity and less impact on overall antibody protein structural stability introduced by

altered glycosylation. Kyowa Hakko Kirin (better known as Biowa, which is a US subsidiary of Kyowa Hakko Kirin) developed Fut8 knockout CHO cell lines that produce antibodies with an altered glycosylation with the removal of fucose^[19]. Another approach by GlycArt (acquired by Roche in 2005) uses the overexpression of recombinant beta 1,4-N-acetylglucosaminyltransferase III (GnT-III) in production cell lines to produce antibodies enriched in bisected oligosaccharide^[20]. A novel approach developed recently by Eureka Therapeutics produces antibodies with glucosylated oligosaccharide through engineering CHO production cell line. All 3 approaches achieved dramatically enhanced ADCC activity in modified antibodies, and are able to overcome the F/F and F/V genotypes which are only able to support low ADCC with wild type antibodies. The mechanism of all 3 approaches improves the binding affinity to low-affinity activating FcγRIIIa, while defers binding to the low-affinity inhibitory FcγRIIb (Table 2). The clinical

Table 2: ADCC enhancement technologies

Company	Technology	FcγRIIIa	FcγRIIb	Validation
Xencor	Fc mutagenesis	Increased affinity	No change	Preclinical
Macrogenics	Fc mutagenesis	Increased affinity	Reduced affinity	Preclinical
Kyowa Hakko/Biowa	Glycoengineering	Increased affinity	No change	Phase I
GlycArt (Roche)	Glycoengineering	Increased affinity	n/a	Phase I
Eureka Therapeutics	Glycoengineering	Increased affinity	Reduced affinity	Preclinical

Table 3: Clinical trials of antibodies with ADCC enhancement through glycoengineering

Product/Company	Target	Disease indication	Status
KW 0761/Kyowa Hakko	CCR4	allergic rhinitis	Phase I completed
BIW-8962/Biowa	Ganglioside GM2	Multiple myeloma	Phase I in progress
BIW-8405 /Biowa, Medimmune	IL-5 receptor	Asthma	Phase I completed
MDX1401 /Biowa, Medarex	CD30	Lymphomas	IND enabling
Roche, GlycArt	EGFR	Colorectal cancer	IND enabling
Afutuzumab/Roche, glycArt	CD20	NHL	Phase I completed
Neuceptin/Eureka Therapeutics	ErbB2	Breast cancer	IND enabling

relevance of differences in Fc γ RIIb binding remains to be seen in patients. However, a combined increased binding to activating Fc γ RIIIa and reduced binding to inhibitory Fc γ RIIb are believed to be advantageous, especially in recruiting PMN (polymorphonuclear leukocyte) for ADCC.

Multiple candidates with ADCC enhancement have entered clinical trials (Table 3). These candidates are mainly for cancer and autoimmune diseases, although the technology is expected to have major application in antibody drugs against infectious diseases as well. It has been shown that ADCC-enhanced antibodies, with much enhanced potency, are well tolerated. The dramatic reduction of effective dosage in the case of anti-CCR4 program for allergic rhinitis is impressive, which supports the possibility of lowering antibody drug cost by the technology. The effect of the engineered antibodies on F/F and F/V genotype patients has not been fully addressed yet, which is expected to be addressed in Phase III studies.

In summary, ADCC enhancement is a key strategy for improving therapeutic antibody drug efficacy. Recent clinical studies provided further evidence in support of the technology. It has the potential of lowering effective drug dosage for benefits of lower drug cost. Antibodies with ADCC enhancement are expected to eliminate variations of patients' response to antibody treatments caused by genetic polymorphism and improving survival of cancer patients. The commercial value of the technology has been demonstrated by the recent licensing agreement between Amgen and Kyowa Hakko Kirin on anti-CCR4 antibody, which includes \$100 million upfront fee plus \$420 million milestone payment and double digits royalties. We enhancement will become a core technology for developing next generation therapeutic antibody drugs with favorable clinical outcomes.

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