Therapeutic antibodies

After years of promise, magic bullets appear to be on the upswing.

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A century ago, Paul Ehrlich referred to antibodies as "magic bullets" and prophesied that they would be multipurpose tools for the treatment of various diseases. Since then, scientists have explored their therapeutic potential. Polyclonal antibodies, mostly in the form of sera from immunized animals, have been used since the 1890s, mainly to treat infectious diseases. Although they are rather effective, the broad clinical application of these antibodies was impeded by their inherent drawbacks. Aside from the molecule of interest, sera contain a multitude of different antibodies that might interfere with the desired activity, other proteins that might be harmful to the recipient, and, possibly, infectious agents. Only with the advent of two major milestones in immunology over the past few decades could antibody-based therapies make a considerable jump forward.

Technical milestones

Historically, researchers believed that the antibody "formed" on the antigen template, but in the 1950s, Niels K. Jerne developed a theory of natural selection for antibody formation, whereby the antibody repertoire was genetically predetermined and the antigen triggered the selection and clonal expansion of selected antibody-producing cells (*1*). The second milestone was the development of the hybridoma technique in 1975 (*2*). This technology allowed the virtually unlimited production of specific monoclonal antibodies (mAbs) in vitro. Since then, a plethora of hybridoma-derived monoclonal antibodies has been developed and used as diagnostic and research reagents and a few as therapeutic drugs.

Monoclonal antibodies, however, have some severe drawbacks that limit their applicability in humans. Most importantly, the human immune system fights the hybridoma antibody (in most cases derived from rodents) as a foreign antigen, inducing human antimouse antibody responses that eliminate the hybridoma antibody and can damage the kidneys. After the first significant results in the early 1980s, researchers' endeavors were overshadowed by sober disappointment about emerging clinical problems and limitations. To partly humanize the molecules and thereby reduce the immunogenicity of the antibodies, researchers developed new techniques such as chimerization and humanization (complementarity-determining region, or CDR, grafting). The first chimeric antibody, introduced into the market in 1994, was ReoPro, which was designed to prevent complications during coronary angioplasty. ReoPro proved to be a solid success in both treatment and business, generating revenues of $384 million in 2002; the accumulated revenue is approaching $2 billion.

More recent developments, in which completely human antibodies are generated by using transgenic mouse systems or phage/phagemid display, are the new driving force behind the fast-paced expansion of antibody product pipelines. The ability to select antibodies in vitro from large repertoire libraries is based on the linkage between phenotype and genotype, the physical connection of the antibody and the gene coding for it. In phage display, the antibody genes are fused to genes that code for bacteriophage coat proteins (*3*). The plasmid is packed within the viral capsid, and the expressed antibody protein is subsequently presented on the bacteriophage surface. The screening process is based on "panning" the library against the immo-

bilized target (molecular or cellular antigen).

Recently, researchers at Affitech A.S. (*www.affitech.com*) developed the AffiScreeN technology, which offers a screening throughput of up to 100,000 clones per round in one week's time (Figure 1). It combines donor-specific antibody libraries with automated filter screening. The exploitation of the already "target-specific enriched" repertoire of suitable donors (e.g., patients or vaccinees) enables researchers to screen the library on a filter with single-chain antibodies instead of phages.

Using AffiScreeN, researchers plate their antibody library on the first day and incubate the plates overnight. The next day, they robotically transfer colonies to multiwell plates for further growth. On the third day, the researchers "print" the colonies onto a membrane coated with nutrient medium and grow colonies in a well-ordered array. The next day, they induce gene expression so that candidate antibodies can react with a capture membrane, which has been treated with the antigen, blocked for nonspecific binding, and placed beneath the colony membrane. Finally, the researchers detect antibodies retained on the capture membrane using a secondary antibody, and then use a robot to "cherry pick" candidate clones.

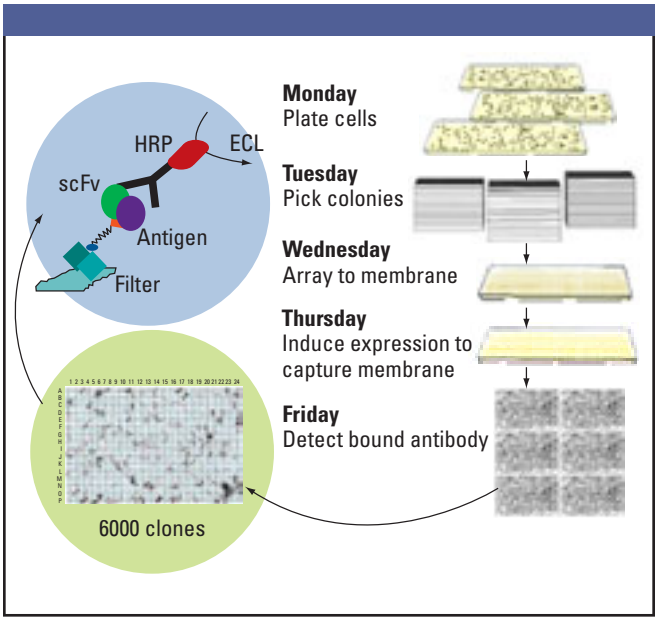

Figure 1. Panning for therapeutic gold. The AffiScreeN system is an automated high-speed, high-throughput technology for screening human antibody libraries. (Image courtesy of Affitech A.S.)

Treating Infections

Before the introduction of antibiotics and the propagation of population-wide vaccinations, sera were a major treatment for infectious diseases. The increase in microbial antibiotic resistance, the threat of bioterrorism (however likely), and the psychological and economic effects of sudden outbreaks such as the recent SARS epidemics have brought passive serum therapy back into prominence. But the clear drawbacks of this tool, as stated earlier, and the comparatively high costs of bulk production, make monoclonal or recombinant antibodies preferable. The latter technology, in particular, features relatively low costs for mass production.

To date, the most prominent antibody in the infectious disease therapy market is the humanized palivizumab (Synagus) from MedImmune (*www.medimmune.com*), which was developed for preventing respiratory syncytial virus infection in high-risk groups. Even though this product targets a niche market, it has achieved high revenues: $668 million in 2002. Although the infectious disease market seems to be fairly neglected by companies developing antibodies, some promising products are in the development pipeline, such as the human anti-hepatitis virus B and C antibodies from XTL Biopharmaceuticals (Rehovot, Israel).

Immunomodulation

Inflammation and immunosuppression have long been major targets for antibody therapy (Table 1). By blocking a receptor of the inflammation pathway or neutralizing soluble factors of inflammation, it is possible to significantly reduce disease symptoms (*4*). The only monoclonal antibody of murine origin generated during the hype of the early 1980s to reach FDA approval was Orthoclone OKT3. Binding to CD3, it is a rather unspecific immunosuppressive agent that is used to prevent organ transplant rejection.

After the 1994 introduction of the first recombinant antibody (ReoPro), immunomodulation again kicked off the second monoclonal antibody boom with three antibodies approved in 1997 and 1998. Remicade, used to treat rheumatoid arthritis and Crohn's disease, is so far one of the largest blockbusters in the antibody field, generating revenue of $1.2 billion in 2002. It is a chimeric antibody that neutralizes tumor necrosis factor α (TNF α) and inhibits inflammation. In rheumatoid arthritis, this reduces swelling and pain in the joints and slows down joint destruction, improving the quality of life of patients considerably. The two other FDA-approved drugs of the late 1990s, Zenapax and Simulect, block the IL-2 signaling pathway, and both have proven to be efficient in preventing kidney transplant rejection.

Remicade is facing a serious competitor, however, with the recent approval of Humira. Developed by Cambridge Antibody Technology (CAT, *www.cambridgeantibody.com*) and Abbott Laboratories (*www.abbott.com*), Humira is the first fully human antibody, isolated in vitro by phage display, to reach the market. Abbott's CEO, Miles White, described Humira as "the single most important product we ever launched," and annual sales are expected to exceed the billion-dollar barrier. Being the first fully human antibody, it will be interesting to see whether the expected advantages, such as lower immunogenicity and less-frequent dosing, will be observed.

While both Remicade and Humira are directed against TNF α, other targets for treating rheumatoid arthritis with antibodies are under close investigation (*5*). Both CAT and Protein Design Laboratories (*www.pdl.com*) have antibodies against interleukin-12 under development (CAT's J695 is in Phase II trials), and researchers have discussed targeting other interleukins. Another newcomer this year is Xolair, a mAb that has been approved for treating allergic asthma.

Immunomodulation, however, has had recent setbacks; the fact that three mAbs against psoriasis (ABX-IL8, IDEC-114, and Zenapax) have failed in clinical Phase II trials has raised some

eyebrows. But their failure only shows that the choice of target is crucial. Raptiva from Genentech (*www.gene.com*), which blocks a cell adhesion pathway, has been shown to be effective against psoriasis in Phase III trials and is awaiting FDA approval. Although most of the marketed drugs target rheumatoid arthritis and Crohn's disease, the spectrum is starting to broaden, with clinical trials of antibodies against multiple sclerosis, inflammatory bowel disease, ulcerative colitis, and diabetes type I (*6*).

The cancer market

Compared with their use in immunomodulation, the demands for antibodies to fight cancer are fairly different and more challenging. It is not simply a matter of binding antibodies to the tumor cell and waiting placidly for its destruction, as one could more easily do with viruses and bacteria. In contrast to in vitro models, and partly animal–human xenograft systems, tissue cells in vivo seem to express molecules for defense against cellular immune systems as well as against complement (*7*). Although these defense mechanisms are still poorly understood, they provide some hints as to why many potential therapeutics perform marvelously in vitro and in animals, but a fairly high portion of them still fail in early clinical development. These examples demand the consideration of additional strategies besides the simple recruitment of immune functions by the antibody's Fc part. Promising strategies that have already been investigated include immunotoxins and antibody-targeted radiotherapy (*8*), the disruption of signaling pathways, and the use of bispecific antibodies (*9*).

However, the early attempts—and still the most successful ones—have simply used full-length mAbs targeting hematologic neoplasms, and the newer chimerized and humanized antibodies are already becoming a main treatment tool for leukemia and lymphoma. Since its approval in 1997, rituximab (Rituxan) has developed into one of the most commercially successful cancer drugs ever, with total U.S. net sales of $779 million in 2001 and $1.08 billion in 2002. Being a chimeric mAb specific for the CD20 surface receptor that is overexpressed on B-cell non-Hodgkin's lymphoma, rituximab elicits antibody-dependent cell apoptosis in the target tissue, and its use has expanded during recent years from aggressive refractory or recurrent non-Hodgkin's lymphoma to other CD20-positive leukemias and lymphomas. Many clinical trials have explored possible combinations of rituximab with other chemotherapies than the original regimens, other antibodies, new small-molecule drugs, biologic response modifiers, and even gene therapy (*10*). The radiolabeled version of rituximab, ibritumomab (Tiuxan), has been used for imaging (^{111}In) and therapy (^{90}Y).

Approved in 2001, alemtuzumab (Campath) is a humanized mAb that targets CDw52, a protein highly expressed on various malignant lymphoid tumors but which is unfortunately also expressed on normal T and B cells. However, clinical results with the therapeutic are convincing and are reflected by product profit and royalty revenues of $4.5 million in the first quarter of 2003.

The FDA approval of gemtuzumab–ozogamicin (Mylotarg) in mid-2000 started a new era in antibody therapy because it is the first (plant) toxin-conjugated antibody on the market. Its target, CD33, is a protein expressed by most myeloid leukemic blast cells but not by stem cells. The toxin ozogamicin (calicheamicin) inhibits DNA synthesis and induces apoptosis in the target cell.

Table 1

Antibodies for the treatment of inflammatory diseases and immune regulation

Drug	Source	Type	Target and indication
FDA-approved antibodies *(approval year)*			
Omalizumab (Xolair) *(2003)*	Genentech, Novartis, Tanox	Humanized mAb	IgE, allergic asthma
Infliximab (Remicade) *(1999)*	Centocor	Chimeric mAb	TNF α, rheumatoid arthritis
Infliximab (Remicade) *(1998)*	Centocor	Chimeric mAb	TNF α, Crohn's disease
Adalimumab, D2E7 (Humira) *(2003)*	Cambridge Antibody Technology, Abbott	Fully human mAb	TNF α, rheumatoid arthritis
Muromonab-CD3 (Orthoclone OKT3) *(1986)*	Ortho Biotech, Johnson & Johnson	Murine mAb	CD3, organ transplant rejection
Daclizumab (Zenapax) *(1997)*	Protein Design Labs	Humanized mAb	CD25, kidney transplant rejection
Basiliximab (Simulect) *(1998)*	Novartis	Chimeric mAb	CD25, kidney transplant rejection
Drugs in late-stage development			**Phase, target, and indication**
Raptiva (formerly Xanelim)	Genentech, Serono, Xoma	Humanized mAb	BLA[a] submitted, CD11a, psoriasis
Natalizumab (Antegren)	Elan, Biogen	Humanized mAb	Phase III, VLA-4β1[b], Crohn's disease, multiple sclerosis
CDP-870	Celltech, Pfizer/ Pharmacia	Humanized mAb	Phase III, TNF α, rheumatoid arthritis, Crohn's disease

[a]Biologics License Application. [b]Very late antigen 4β1.

Although the side effects are comparable to those of conventional chemotherapy, the clinical acceptance of Mylotarg was quick, as reflected by the $7.6 million in net revenues already in the fourth quarter of 2000.

Since the obstacles in antibody therapy are greater for solid tumors than for leukemia and lymphoma, it is not surprising that few anti-solid-tumor antibodies have reached the market. Edrecolomab (Panorex), which was approved in Germany in 1995, targets a specific cell adhesion molecule (EpCAM/17-1A) and is suited for the treatment of colorectal cancer. Unfortunately, although the clinical data are positive, the FDA declined approval, in all likelihood because of the completely murine design of the antibody. However, trastuzumab (Herceptin), a humanized antibody directed against HER-2/neu, was approved by the FDA mainly for mammary carcinoma but also for some lung and pancreatic tumors. Herceptin achieved net sales of $745 million in 2002, a 33% increase over 2001 sales.

The expanding number of antibody products for treating cancer that are approved or in late-stage clinical development (Table 2) restores confidence that by careful choice of the target and prudential design of the drug, antibodies will soon make a deep impact on cancer treatment and the cancer drug market.

The final analysis

Generally, antibodies have seen a remarkable renaissance during the last years. A recent survey conducted by Pharmaceutical Research and Manufacturers of America (*www.phrma.org*) stated that 25% of the 371 biotechnology medicines in clinical development are mAbs. Since, over the past few years, companies working in the antibody field have been able to fine-tune technologies and fill the product pipeline, therapeutic antibodies doubtlessly will become more and more prominent in the market, claiming a growing share of the total drug revenues.

References

(1) Pizzi, R. A. *Mod. Drug Discovery* **2003,** *6* (6), 21–22.
(2) Lesney, M. S. *Mod. Drug Discovery* **2003,** *6* (7), 53.
(3) McGregor, D.; Fitzgerald, K. *Mod. Drug Discovery* **2003,** *6* (3), 23–24.
(4) Andreakos, E.; Taylor, P. C.; Feldmann, M. *Curr. Opin. Biotechnol.* **2002,** *13,* 615–620.
(5) Taylor, P. C. *Intern. Med.* **2003,** *42,* 15–20.
(6) Hibi, T.; et al. *J. Gastroenterol.* **2003,** *38* (Suppl. 15), 36–42.
(7) Kirschfink, M. *Immunol. Rev.* **2001,** *180,* 177–189.
(8) Pastan, I.; Kreitman, R. J. *Curr. Opin. Invest. Drugs* **2002,** *3,* 1089–1091.
(9) Withoff, S.; et al. *Curr. Opin. Mol. Ther.* **2001,** *3,* 53–62.
(10) Ross, J. S.; et al. *Amer. J. Clin. Pathol.* **2003,** *119,* 472–485.

Table 2

Antibodies for the treatment of cancer

Drug	Source	Type	Target and indication
FDA-approved antibodies *(approval year)*			
Edrecolomab (Panorex) (*1995*[a])	GlaxoSmithKline	Murine mAb	EpCAM[b], colorectal cancer
Rituximab (Rituxan) *(1997)*	IDEC	Chimeric mAb	CD20, NHL[c], CLL[d], rheumatoid arthritis
Trastuzumab (Herceptin) *(1998)*	Genentech	Humanized mAb	HER-2/neu ($p183^{neu}$), mammary cancer, non-small-cell lung cancer, pancreatic cancer
Gemtuzumab (Mylotarg) *(2000)*	Wyeth/AHP	Humanized mAb/ calicheamicin	CD33, AML[e]
Alemtuzumab (Campath) *(2001)*	BTG; Millennium Pharmaceuticals	Humanized mAb	CDw52, CLL[d], CML[f], MS[g]
Ibritumomab (Zevalin) *(2002)*	IDEC	Murine mAb/^{90}Y	CD20, NHL[c], low-grade and follicular lymphoma
Daclizumab (Zenapax) *(2002)*	Protein Design Labs	Chimeric mAb	IL-2R; leukemia
Tositumomab (Bexxar) *(2003)*	Corixa, GSK	Murine mAb/^{131}I and murine mAb	CD20, NHL[c], CLL[d]
Selected antibodies in clinical Phase III			
CeaVac	Titan Pharmaceuticals	Murine mAb	CEA[h], mainly colorectal cancer
IGN-101	Igeneon	Murine mAb	EpCAM, colorectal cancer
Mitumomab (BEC2)	ImClone Systems	Murine mAb	Mimicking GD3 ganglioside, mainly melanoma, glioma
Epratuzumab (LymphoCyde)	Immunomedics, Amgen	Chimeric mAb	CD22, NHL[c], autoimmune diseases
MDX-210 (IDM-1, Osidem)	Medarex, Immuno-Designed Molecules	Chimeric biAb[i]	HER-2/neu + FcγRI, ovarian cancer
Endrecolomab (Panorex)	Johnson & Johnson	Humanized mAb	17-A1, colorectal cancer
Pentumomab	Antisoma, Roche	^{90}Y-murine mAb	Muc-1, ovarian cancer

[a]Approved for use in Germany only; no FDA approval. [b]Epithelial cell adhesion molecule. [c]Non-Hodgkin's lymphoma. [d]Chronic lymphocytic leukemia. [e]Acute myelogenous lymphoma. [f]Chronic myelogenous lymphoma. [g]Multiple sclerosis. [h]Carcinoembryonic antigen. [i]Bispecific antibody.

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KEY TERMS: automation, cell biology, clinical, drug delivery, genomics, high throughput, imaging, informatics, proteomics, regulations, screening