




A NEW KIND OF CANCER WARFARE AGENT

NEXT GENERATION MONOCLONAL ANTIBODIES

LARGE PHARMACEUTICAL COMPANIES HAVE CERTAINLY TAKEN NOTICE OF NEXT-GENERATION MAB TECHNOLOGIES. AS THIS TECHNOLOGY GROWS AND CONTINUES TO HELP REVOLUTIONIZE THE WAY CANCER TREATMENTS EVOLVE THERE ARE STILL HURDLES TO OVERCOME. WE TAKE A LOOK AT SOME OF THESE HURDLES TO HELP KEEP THINGS IN PERSPECTIVE AS WELL AS DISCUSS WHERE THE TECHNOLOGY IS HEADING.

BY KARA A. NYBERG, PHD



In view of the fact that traditional chemotherapeutic regimens bombard the body with toxins to kill tumor cells en masse—a process that also claims numerous normal cells as casualties—the therapeutic appeal of monoclonal antibodies (MAbs) has never been greater for researchers. Since their initial clinical development in the late 1970s, MAbs entering clinical study has more than tripled and today they represent one of the hottest areas of oncology drug development.

MAbs are specifically designed to seek and destroy only those cells coated in tumor-associated antigens, thus sparing normal cellular bystanders. Between 1980 and 2005, a total of 206 unique oncology MAbs have been investigated in clinical trials; and out of that total, 9 have gained Food and Drug Administration (FDA) approval.

Rituximab [Rituxan; Genentech, Biogen Idec] was the first oncology MAb to hit the US market in 1997. Since then, roughly one therapeutic MAb has been approved each year for a cancer indication (see Table 1).

According to research compiled by Janice Reichert, PhD, of the Tufts Center for the Study of Drug Development, 109 oncology MAbs are currently under investigation in clinical trials. The concept for MAb technology is rooted in findings originally unearthed in academia.

“When it became appreciated that antibodies could be used for therapeutic intent, biotech was really born,” said Louis Weiner, MD, Vice President of Translational Research and Chairman of the Department of Medical Oncology at Fox Chase Cancer Center. After many initial frustrations and setbacks, several biotech companies were able to hone their MAb technology capturing the attention of pharmaceutical corporation CEOs, who in turn, stepped in to oversee final-stage development.

Targeting the Tumor Cell

The beauty of MAbs lies in their ability to preferentially target tumor cells for destruction—a property that is critically dependent on selecting the appropriate antigen for targeting. Many tumor-associated antigens are expressed on normal cells as well as tumor cells; the key for effective treatment is to select targets that are highly expressed only on tumor cells and not normal cells, especially those comprising vital organs. In addition, ideal tumor targets need to be easily accessible, homogeneous, present on all malignant cells within a tumor, and stationary (i.e., not shed or secreted). Thus far, MAbs have been more successful against hematologic cancers than solid cancers due to improved cell accessibility. [cont. on pg 38 >>](#)



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Table 1: Currently Approved MAb Therapies for Cancer

Drug	Manufacturer	Target	Description	Cancer	FDA Approval	Net U.S. Earnings in 2006 (in millions)
Rituximab (Rituxan, Mabthera)	Genentech, Biogen Idec	CD20	Chimeric	Non-Hodgkin's lymphoma	1997	\$2071
Trastuzumab (Herceptin)	Genentech	ErbB2/HER2	Humanized	Breast cancer	1998	\$1234
Gemtuzumab ozogamicin (Mylotarg)	Wyeth	CD33	Humanized	Acute myeloid leukemia	2000	Unknown
Alemtuzumab (Campath)	Genzyme	CD52	Humanized	Chronic lymphocytic leukemia	2001	Unknown
Ibritumomab tiuxetan (Zevalin)	Cell Therapeutics, Inc.	CD20	Murine, radiolabeled with yttrium-90	Non-Hodgkin's lymphoma	2002	\$17.767
Tositumomab-I131 (Bexxar)	Corixa	CD20	Murine, radiolabeled with iodine-131	Non-Hodgkin's lymphoma	2003	Unknown
Cetuximab (Erbixub)	Bristol-Myers Squibb, ImClone	EGFR	Chimeric	Colorectal cancer, head and neck cancer	2004	\$646
Bevacizumab (Avastin)	Genentech	VEGF	Humanized	Colorectal cancer, non-small-cell lung cancer	2004	\$1746
Panitumumab (Vectibix™)	Amgen	EGFR	Human	Colorectal cancer	2006	\$39*


EGFR = epidermal growth factor receptor; HER2 = human epidermal growth factor receptor-2; VEGF = vascular endothelial growth factor receptor.

*Panitumumab was approved in the US in the 4th quarter of 2006; this figure represents only 4th-quarter sales.

Although certainly a step in the right direction, selecting a good target is not a surefire path to success. The first generation of MAb consisted of monoclonal anti-human antibodies created in mice. When used in humans, these murine MAb could find their cancer targets, but were rapidly recognized as non-human, prompting the immune system to mount an anti-mouse antibody response. Attack

of the murine MAb resulted in side effects ranging from mild allergic reactions to, more rarely, anaphylactic shock. Moreover, even if the MAb did successfully reach their targets before being destroyed, the majority proved to poorly activate the human immune system to trigger an anti-tumor response, and many had fleeting serum half-lives.

To reduce intolerance to murine MAb and bolster their effectiveness, researchers replaced the offending immunogenic portions with chunks of human antibodies. The next



generation—chimeric MAbs—consisted of a human constant region, typically the kappa light chain and IgG1 heavy chain, also known as the fragment crystalline part of the molecule—fused to a murine fragment antigen binding part—the region that contains the variable domains that recognize the target.

Roughly 65% human, these MAbs are generally human enough to slip through the host's immunologic radar, are more adept at recruiting immune proteins to facilitate tumor cell killing, and they have increased serum half-lives. Subsequent MAb iterations have led to the generation of humanized MAbs, which are created by grafting small pieces of murine variable domains onto human antibodies to produce MAbs that are about 95% human. And, finally fully-human MAbs were created using transgenic mice or phage display libraries.

The Big Three

Among the approved oncology MAbs, Weiner considers rituximab, trastuzumab [Herceptin; Genentech], and bevacizumab [Avastin; Genentech] as the “big three”. Rituximab, the first oncology MAb approved by the FDA, has had a good showing in the cancer clinic and for good reason: it works, and it works well. Rituximab attaches itself to the CD20 protein, which is present on more than 95% of B-cell lymphomas, and then recruits immune effector proteins to kill targeted cells. In the pivotal Phase 3 clinical trial that helped rituximab garner FDA approval, the anti-CD20 antibody cut the cancer cell burden in half (48%; 80/166) for patients with relapsed or refractory, low-grade or follicular non-Hodgkin's lymphoma. Patients experienced limited side effects.

Clinical outcomes were even more striking when rituximab was combined with chemotherapy. Data showed that by adding the MAb to standard chemotherapy regimens can produce a response in up to 95% of patients and can extend progression-free survival on the order of years, depending on the type of lymphoma and the disease setting in which it is administered (i.e., adjuvant, salvage). Moreover, the agent does not cause excess toxicity when added to chemotherapy—an important consideration for use in elderly individuals.

In addition to revolutionizing the treatment of lymphoma, rituximab has taught researchers that in some cases, it is acceptable for the antibody to target an antigen that is present on both tumor cells and, to a lesser extent, normal cells without wreaking bodily havoc.

“This freed us from the obligation of searching for purely tumor-specific targets,” said Weiner. Trastuzumab, he noted, first “taught us that modest effects in the laboratory can translate to major effects in people, which has given us all pause as we think about how to interpret the results of animal model studies. Secondly, it taught us that modest effects in the advanced disease setting can translate to much more striking effects when the antibody is used in an earlier stage of disease.” Bevacizumab “has led the way in showing that targeting the tumor microenvironment, as opposed to targeting the tumor cells themselves, can be very productive.”

These agents may also be considered the big three based on the blockbuster sales they rake in for Genentech. The three led US product sales for the company in 2006, bringing in more than \$5 billion. Total [cont. on pg 40 >>](#)

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US product sales for the company amounted to \$7.2 billion for the year. Rituximab led the three with sales totaling \$2.1 billion, followed by bevacizumab at \$1.7 billion, and trastuzumab at \$1.2 billion.

Looking Ahead: Smaller, Heartier, and Cheaper

As effective as MAb technology has become, these agents still suffer from some major drawbacks—namely, size, delivery, and cost. The large size of MAbs often precludes them from penetrating deep into tumor tissues and sometimes inhibits their ability to bind in tight antigen cavities or receptor clefts. These agents cannot be administered orally, and they are expensive due to production costs. Trastuzumab costs a little over \$3000 a month for the treatment of breast cancer, while bevacizumab approaches \$9000 a month for the treatment of lung cancer. “It will be a major advantage when less expensive strategies are developed, because these are extraordinarily expensive treatments,” said Weiner.

To overcome these limitations, several biotech companies are striving to develop the next generation of MAb technologies. Many of the therapeutics in development are stripped down versions of MAbs consisting of the smallest functional fragments. With names like Nanobodies [Ablynx], Domain Antibodies [Domantis], and UniBodies [Genmab], it’s not hard to guess the appeal of these agents: They are very small.

Typical MAbs are on the order of 150 kDa in size, while these smaller versions range from

6-15 kDa. Being only one-tenth as big as traditional MAbs provides several advantages:

- the ability to slip into receptor cavities to bind
- the ability to recognize hidden epitopes
- better tissue penetration
- low immunogenic potential
- improved stability that allows for oral or pulmonary administration as well as a long shelf-life
- the ability to cheaply manufacture large quantities in bacteria or yeast

Moreover, the half-life of these agents can be tailored via pegylation or by attaching an anti-serum albumin molecule.

To appreciate the potential power of small antibody fragments, consider the Nanobody engineered by Ablynx, a Belgian biopharmaceutical company. Nanobodies are the smallest functional fragment of naturally occurring antibodies. Derived from animals in the camelidae family (i.e., camels and llamas), these antibodies have only heavy chains, meaning that the antigen-binding portion is composed of only one immunoglobulin variable region, which provides for more stability. All the information for each Nanobody is contained within a single gene that can be quickly produced in bacteria and yeast. This yields a large amount of product at a tenth of the cost it takes to produce a traditional MAb. “The beauty of the Nanobody platform is that we can very rapidly advance early-stage candidates into preclinical development—much faster than conventional antibodies and certainly much faster than small-molecule

In vitro analysis of the trivalent, bispecific anti-EGFR Nanobodies revealed that the agents could completely block EGF-mediated signaling

research,” said Eva-Lotta Allan, Chief Business Officer of Ablynx.

Ablynx recently put their technology platform to the test by developing a panel of potential Nanobodies for cancer therapy that were specifically directed against the epidermal growth factor receptor (EGFR). After inducing a humoral anti-EGFR response in llamas and creating Nanobodies based on the resulting anti-EGFR antibodies, three Nanobodies were selected from the vast pool of candidates for further characterization based on their ability to efficiently block epidermal growth factor (EGF) binding to EGFR.

Creating combinations of these candidates in which two anti-EGFR Nanobodies were fused together in the same molecule dramatically increased receptor blockage, while tacking on an anti-mouse serum albumin domain boosted the half-life from 1.5 hours to 44 hours. In vitro analysis of the trivalent, bispecific anti-EGFR Nanobodies revealed that the agents could completely block EGF-mediated signaling and could almost completely staunch EGF-dependent cellular proliferation. In vivo studies showed Nanobody administration significantly delayed tumor outgrowth in mice carrying solid tumor cells. Based on these findings, these antibody fragments may serve as building blocks for further rational design of anti-EGFR oncology therapeutics.

Weiner and Reichert both agree that the next step for MAb evolution involves the development of designed antibodies, including both antibody fragments as well as full-sized MAbs. “There are an incredible number of companies with technologies to produce full-size MAbs, fragments, or ‘antibody-like’ molecules with altered characteristics (e.g.,

glycosylation pattern) that might optimize the performance of the molecule,” said Reichert. To illustrate the appeal of designer full-sized MAbs, Biolex Therapeutics has developed the technology to produce MAbs with homogeneous glycosylation structures devoid of fucose and xylose sugars. These cleaner and more uniform crystalline fragment portions are more adept at binding to immune proteins that carry out cell-mediated cytotoxicity, thereby enhancing the potency and efficacy of the MAbs.

Weiner predicts that structurally optimized, full-sized antibodies will comprise the next wave of MAbs to hit the clinic, followed then by MAb fragments. He said, “There will be indications for using smaller, customized antibody pieces, especially for imaging purposes and possibly to provide a targeting capacity to other kinds of therapeutic principles,” such as by attaching radioactive isotopes or toxins to create a “targeting warhead.” Reichert’s research indicates that there are 9 designed antibody candidates in clinical study as of July 2006 (see Table 2). “Based solely on the averages for oncology MAbs, we might expect one of the fragments currently in oncology clinical studies to be approved [by the FDA] in 3-5 years,” she said.

Market Trends for the Next Generation of MAbs

Large pharmaceutical companies have certainly taken notice of next-generation MAb technologies. GlaxoSmithKline (GSK) has recently paid top dollar for two biotech companies cultivating designed MAb technologies. GSK closed a deal in January to acquire Domantis, the developer of Domain Antibodies, for \$454 million. Just a month earlier in December 2006, GSK **cont. on pg 42 >>**



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Table 2: MAb Fragments Currently in Clinical Development

Drug	Manufacturer/ Collaborator	Target	Description	Potential Indication	Phase of Development
MDX-1401	Medarex/BioWa	CD30	MAb with reduced carbohydrate content	Hodgkin's lymphoma	Phase 1
MT103/MEDI-538	Micromet/MedImmune	CD19/CD3	Bispecific, single-chain molecule with 4 variable domains	Non-Hodgkin's lymphoma	Phase 1
IMC-11F8	ImClone Systems	EGFR	IgG1 Fab fragment	Solid tumors	Phase 2
MDX-214	Medarex	CD89	MAB fragment linked to EGF	EGFR-expressing cancers	Phase 2
L19-1311	Philogen/Schering AG	Fibronectin	Single-chain fragment linked to iodine 131	Cancer	Phase 2
L19-IL2	Philogen/Schering AG	Fibronectin	Single-chain fragment fused with IL2	Solid tumors	Phase 2
CDP-791	UCB/ImClone Systems	VEGFR-2	Pegylated MAB fragment	Non-small-cell lung cancer	Phase 2
Medi-522 (Abegrin,)	MedImmune	Alpha-v beta-3 integrin	IgG1 MAB with deamination site removed	Melanoma, colorectal cancer, prostate cancer	Phase 2
VB4-845 (Proxinium,/ Vicinium,)	Viventia Biotech	EpCAM	Single-chain MAB fragment linked to Pseudomonas exotoxin	Head and neck cancer, bladder cancer	Phase 2

EGF = epidermal growth factor; EGFR = epidermal growth factor receptor; EpCAM = epithelial cell adhesion molecule; Fab = fragment antigen binding; IL2 = interleukin 2; VEGFR = vascular endothelial growth factor receptor. Source: Janice M. Reichert, PhD, Senior Research Fellow with the Tufts Center for the Study of Drug Development

signed a record \$2.1 billion deal for Genmab's ofatumumab agent [HuMax-CD20].

As part of this deal, Genmab granted GSK the exclusive option to buy its CD20 UniBody, which consists of an IgG4 fragment that has been modified for enhanced stability. "This shows that Glaxo is very interested in exploring the UniBody platform, perhaps for targeting CD20 or perhaps for other targets," said Jan van de Winkel, PhD, the Executive Vice President and Chief

Scientific Officer of Genmab. Given GSK's two major purchases of fragment-antibody technologies of late, van de Winkel said that "Glaxo has a pretty thorough knowledge of what the next generation of antibody technologies should look like."

Ofatumumab is a fully-human anti-CD20 MAB that, like its mouse-based cousin, rituximab, targets the CD20 antigen on lymphoma and leukemia cells. The agent is currently in a Phase 3 analysis in refractory B-cell chronic lymphocytic leukemia (CLL). In the Phase 1/2 studies leading up to the Phase 3 trial, 13 of 26 evaluable patients (50%) who received

GSK signed a record \$2.1 billion deal for Genmab's ofatumumab agent [HuMax-CD20].

the highest ofatumumab dose had an objective response, with responders holding out a median of 23 weeks (range: 20-31 weeks) before experiencing disease progression. Ofatumumab is also being tested in a Phase 2 trial in combination with fludarabine and cyclophosphamide for first-line treatment of CLL as well as in a Phase 3 study of rituximab-refractory non-Hodgkin's lymphoma.

The efficacy of ofatumumab has not yet been directly compared with that of rituximab in clinical trials, but in vitro and preclinical data suggest that ofatumumab is more effective at killing CD20-expressing cells, even when CD20 expression levels are very low—a situation in which rituximab usually falters. Moreover, ofatumumab is effective at killing cells resistant to rituximab.

Genmab has demonstrated proof-of-concept of numerous UniBodies in in vitro systems and is currently evaluating UniBody efficacy in animal models. “We have had involved discussions with major pharmaceutical and major biotechnology companies to give them access to the UniBody platform. Right now we are generating fully-human UniBodies as therapeutic candidates, but we haven't yet announced when they will move to the clinic,” said van de Winkel. He surmises, however, that these preclinical compounds may enter into clinical studies in late 2008 or early 2009. If all goes well, oncology UniBodies could gain FDA approval 7 to 8 years after this. In terms of profitability, van de Winkel notes that UniBody-based therapeutic approaches may be more economically favorable than traditional MAb approaches due to lower manufacturing costs. At the very least, he suspects that each

UniBody therapeutic will have the potential to generate more than \$1 billion in revenue, comparable with traditional MAbs.



Dr. Jan van de Winkel, PhD, EVP and Chief Scientific Officer, Genmab.

For more information:

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