Cancer Therapy with Antibodies and Immunoconjugates

Meeting Report of the Ninth Conference on Cancer Therapy with Antibodies and Immunoconjugates held October 24-26, 2002, in Princeton, New Jersey

Robert M. Sharkey, Edward A. Sausville, and David M. Goldenberg

Garden State Cancer Center, Center for Molecular Medicine and Immunology Belleville, New Jersey 07945 [R.M.S., D.M.G.], and National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 [E.A.S.]

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1 To whom correspondence may be addressed, at Center for Molecular Medicine and Immunology, 520 Belleville Avenue, Belleville, NJ 07945. Fax: 973-844-7020; E-mail: dmg.gscancer@att.net.

This conference, held biennially for the past 12 years, provides a forum where investigators from throughout the world can gather and discuss how monoclonal antibodies (MAb) can be used to improve the treatment of cancer. As in the past, this meeting focused primarily on the use of radiolabeled antibodies in cancer treatment, but this year there were many additional contributions on the use of unconjugated ‘naked’ antibodies for the treatment of cancer, reflecting a growing understanding that antibodies cannot only be used to direct isotopes and drugs to tumors, but can be effective agents in themselves. Other immunoconjugates prepared with toxins, drugs, or other agents were also reported to be highly effective therapeutic agents, some of which are now showing efficacy in clinical trials. In addition, presentations focused on a variety of approaches, including pretargeting, regional delivery, and combinations with other standard treatment regimens designed to optimize antibody-targeted treatment strategies. Although the most efficacious treatments were reported in a variety of hematologic malignancies, there were a number of presentations, primarily in early preclinical development that provided evidence for potential future improvements in the treatment of solid tumors.
**Introduction**

Overall, the central theme of this conference remains one where the translational application of antibodies in the treatment of hematologic and non-hematologic malignancies is highlighted in a series of presentations that focus on the preclinical development and ultimate clinical application of these treatment strategies. Since the inception of this conference in 1978 (1) to highlight the advances made in using radiolabeled antibodies for cancer detection, antibody-directed targeting agents, as well as antibodies that are unlabeled, have steadily been making their way into clinical practice. Indeed, early preclinical and clinical testing of a number of now FDA-approved agents has been discussed over the years at this conference. This year’s meeting was chaired by David M. Goldenberg (Center for Molecular Medicine and Immunology and Gardens State Cancer Center [GSCC], Belleville, NJ), Ralph A. Reisfeld (The Scripps Research Institute, San Diego, CA), and Edward A. Sausville (National Cancer Institute [NCI], Bethesda, MD), and was attended by almost 200 scientists and clinicians from both academia and industry, specializing in immunology, pharmacology, oncology, radiation oncology, nuclear medicine, medical physics, and chemistry, and presenting almost 60 papers. Keynote speakers briefly highlighted several topics, but perhaps one of the key features of a focused forum such as this was the number of succinct presentations that enabled attendees to listen and ask questions of prominent and young investigators alike.

**Immunoconjugates**

Dr. Ira Pastan (NCI) provided an overview of the development of an immunoconjugate for the treatment of CD22-expressing hematologic malignancies, including hairy cell leukemia (HCL), non-Hodgkin’s lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Dr. Pastan highlighted a number of important considerations that made it possible to develop the BL22 anti-CD22 single-chain, *Pseudomonas* exotoxin A, fusion protein for clinical studies, including antibody specificity and ability to internalize appropriately, and the importance of appropriate molecular engineering for both the toxin and the MAb to optimize performance. Clinical studies with this new agent led to new discoveries, such as how to better manage some of the side effects associated with this treatment and the determination that a higher response rate in patients was linked with higher levels of antigen expression. This subsequently led investigators to select a
new affinity-enhanced version of the single-chain antibody that they plan to test clinically in the future. However, promising anti-tumor effects have already been seen in drug resistant HCL with the initial BL22 construct, with 11/12 patients having a complete response to the treatment with durations > 1 year. Dr. M. Jules Mattes (GSCC) also spoke to the importance of MAb internalization. He showed data that even MAbs not noted for internalization are taken into cells, possibly due to membrane turnover by tumor cells. In this regard, Dr. Mattes described the internalization properties of a MAb directed to the CD20 antigen (1F5), which was not considered to be an antibody that internalized after binding to human lymphoma cell lines. His work also highlighted the trafficking of the anti-CD20 MAb into the endocytic recycling compartment rather than a lysosomal compartment. Radioconjugates that residualize inside the cell would be advantageous irrespective of the trafficking mechanism, but different patterns of trafficking will also likely impact on the efficacy of other immunoconjugates. Dr. Raya Mandler (NCI) showed some very promising preclinical data with a geldanamycin conjugate to an anti-HER2 MAb (Herceptin®, trastuzmab). Both *in vitro* and *in vivo* data supported highly specific targeting and improved therapeutic outcome in HER2 over-expressing cell line and xenografts of human breast and gastric cancers when compared to the MAb alone. Dr. Hu (USC Keck School of Medicine, Los Angeles, CA) described studies using a fusion protein between 2 MAbs, one that bind to tumor vascular tissues and the other to Tissue Factor (TF), a cell membrane receptor protein that initiates the extrinsic pathway of blood coagulation. These fusion proteins caused microregional thrombosis in tumor xenografts, suggesting a possible future role for appropriate constructs to inhibit or block the tumor blood supply. Overall, the presentations illustrated how diverse, effective immunoconjugates can be developed.

‘Naked’ Antibodies

With the advent of the successful treatment of NHL using a chimeric MAb to the CD20 antigen (rituximab) and an anti-HER2 antibody (trastuzmab) for the treatment of breast cancer with minimal side effects, there has been considerable interest in the use of naked antibodies for the treatment of a variety of malignancies. Dr. Stephen Schuster (Hospital of the University of Pennsylvania, Philadelphia, PA) provided a brief overview of the treatment of NHL with rituximab and, importantly, highlighted the utility for using rituximab as a form of maintenance therapy for this indication. When used in this manner, complete response rates were increased
and duration of responses were extended. Dr. Morton Coleman (Weill Medical College of
Cornell University and New York Presbyterian Hospital, NY, NY) further expanded on the
possibility for using an anti-CD22 MAb (epratuzumab) that alone, in early Phase I/II trials,
showed efficacy in NHL, but he also provided data from early clinical testing that suggest that
the combination of anti-CD22 and anti-CD20 (rituximab) antibodies will improve efficacy in
NHL. Dr. Alexandria Cesano (Amgen, Thousand Oaks, CA) provided an overview of
preclinical studies directed toward understanding the potential mechanism(s) of action and to
explain the anti-tumor responses seen with the anti-CD22 antibody epratuzumab. Dr. Mitchell
Cairo (Columbia University, NY, NY) reported some promising early clinical results using
naked anti-CD20 antibodies rituximab in combination with chemotherapy to improve the
treatment of post-transplant lymphoproliferative disorder. Other studies showed promising new
approaches for naked antibody therapy, including preclinical studies on the use of hMN-14, a
humanized anti-carcinoembryonic antigen (CEA) MAb in medullary thyroid cancer in
combination with chemotherapy, and a bispecific antibody directed to CEA and p-glycoprotein
(PGP) for more selective inhibition of PGP in hopes of reducing multi-drug resistance (Dr.
David Modrak, GSCC). Dr. Ken Foon (Abgenix, Fremont, CA) discussed the development of a
high-affinity, fully human anti-epidermal growth factor (EGF) receptor MAb (ABX-EGF) that
has begun clinical testing. Early results from the trial have indicated some anti-tumor activity
without the development of anti-antibody responses, suggesting that repeated cycles can be
given. Dr. Alan Solomon (University of Tennessee, Knoxville, TN) provided an intriguing
look into the development of a MAb (11-1F4) reactive with amyloid fibers that was shown in
mice to enhance the removal of human light-chain associated amyloidomas by activating an Fc-
mediated immune response. These data suggest that a similar approach might be developed for
other forms of amyloid-associated diseases. Dr. Clair Dobson (Cambridge Antibody
Technology, Cambridgeshire, UK) described their work using phage display to develop single-
chain MAbs to the TRAIL (TNF-related apoptosis-inducing ligands) receptor 1. Early in vitro
studies have identified several candidates that induce apoptosis. Thus, there is a renewed interest
in naked antibody approaches for treating cancer and other diseases as ultimately new targets are
discovered and mechanisms of action are elucidated.
**Radiolabeled Antibodies**

The largest number of presentations involved the use of radiolabeled antibodies for the treatment of cancer. In this year’s meeting, there were a larger number of presentations using antibodies combined with a variety of alpha-emitters, including bismuth-213, astatine-211, and actinium-225. Dr. Senekowitsch-Schmidtke (Universität München, Munich, Germany) presented data for a new MAb that is directed to a mutated, tumor-specific E-cadherin. The MAb specifically reacted with exon-8 or exon-9-deleted E-cadherin and not the wild type, making the reactivity of this MAb highly unique in its specificity for patients with gastric cancers, which express this mutated form of E-cadherin. This antibody (d9MAb) radiolabeled with bismuth-213 and injected intraperitoneally was shown in an animal model mimicking peritoneal carcinomatosis to be highly effective in preventing the growth of the human tumor xenograft. While others discussed the use of alpha-emitter antibody conjugates, liposomally-entrapped, and modified polylysine for targeting alpha-emitters in the treatment of locoregional disease, Dr. Greg Adams (Fox Chase Cancer Center, Philadelphia, PA) showed that an intravenously injected diabody to HER2/neu (C6.5) radiolabeled with astatine-211 was better than the same antibody radiolabeled with yttrium-90 for the treatment of a subcutaneous breast cancer xenograft. This study was particularly interesting, since alpha-emitters have been considered primarily for use in micrometastatic disease, but in this case, it is possible that the pharmacokinetics and other targeting properties of the antibody are well-matched for this use with astatine-211. Other potential applications of alpha-emitter therapy were in myeloid leukemia, where clinical studies presented by Dr. John Burke (Memorial Sloan-Kettering Cancer Center, NY, NY) showed the efficacy of fractionated injections of a bismuth-213-labeled anti-CD33 MAb (HuM195) with minimal side effects.

Other presentations focused on improving chelation of radioisotopes and appropriate selection of radioconjugates for matching to the internalization or pharmacokinetic properties of the MAb. Dr. Gerald DeNardo (UC Davis, Sacramento, CA) showed how designing chelates that can be selectively degraded by liver enzymes could reduce the hepatic uptake of chelated radiometals, and Dr. Sally DeNardo (UC Davis) presented results comparing traditionally conjugated chelates to cathepsin-sensitive chelates, indicating that a similar reduction in hepatic uptake could be achieved clinically. Another topic of interest was the potential for combining
radiolabeled antibodies with chemotherapy. Dr. Rosalyn D. Blumenthal (GSCC) gave a detailed account of in vitro studies directed to developing an approach to rationally select agents and how they might be used in combination with a radiolabeled MAb. One unexpected finding was that certain combinations could potentially be antagonistic, while using it in a different manner could provide additive anti-proliferative effects. Dr. David V. Gold (GSCC) showed in a human pancreatic cancer animal xenograft model that a yttrium-labeled MAb to pancreatic cancer (PAM4) could significantly improve the therapeutic response seen with gemcitabine, even when it was given at very low doses along with a standard dose regimen of gemcitabine. Clinically, therapeutic studies with radiolabeled antibodies focused primarily on the use of a humanized anti-CD22 MAb, epratuzumab, in the treatment of NHL. These studies included the use of yttrium-90- and rhenium-186-labeled antibodies, both of which have shown anti-tumor activity in Phase I trials.

Pretargeting approaches were also discussed at the meeting, focusing primarily on the use of bispecific antibodies. An overview presentation showing the importance of timing of the steps and the blood kinetics of the bispecific antibody was given by Dr. Robert M. Sharkey (GSCC). Dr. Edmund Rossi (IBC Pharmaceuticals, Inc., Morris Plains, NJ) presented data with a series of molecularly-engineered, recombinant, bispecific antibodies, showing how such constructs could significantly improve targeting by having highly favorable binding and pharmacokinetic properties. Pretargeting studies in animal models illustrated the potential advantage of pretargeting radiolabeled peptides using a novel anti-hapten antibody system. Early clinical studies designed to optimize a bispecific pretargeting approach also were presented (Dr. Jacques Barbet, INSERM, Nantes, France). The data indicated excellent targeting ratios, but full optimization of the procedure was not yet achieved.

Summary

The conference in some sense represented a real milestone for this field. The past year had marked the regulatory approval for marketing of a radiolabeled anti-CD20 MAb (Zevalin®), with good prospects at the time of the meeting for similar approval for a distinct but related agent (Bexaar®). These reflected the documentation of clinical activity with perceived benefit in a variety of indications, including indolent and transformed, chemorefractory, lymphomas, as well
as post-transplant lymphoproliferative disorder. Manageable toxicities were associated with these treatments. The diversity of approaches illustrated that the field supports several innovative technologies, particularly the engineering of novel forms of targeting agents, including “diabodies”, “minibodies”, and the like. New targets are clearly being addressed with these strategies.

However, a number of issues remain and challenges to the radioimmunotherapeutic field in general were clearly apparent in the discussions of the meeting. First, the “challenge” of solid tumors must be acknowledged. Indeed, the sense emerged that the ongoing dosimetric evaluations reinforces the likely continued reliable delivery of radiation doses concordant with sterilization of hematopoietic tumors. But real concern about the doses possible with various delivery strategies in relation to the known sensitivity of epithelial tumors must be considered further by investigators in this field. Second, delivery of MAb-based approaches to solid tumor masses remains a challenge, and reinforces the need for focus in solid tumors in adjuvant or minimal-disease settings, with manipulation of pharmacology, antibody affinity, or “loading” of labeled constructs to optimize delivery of radiation. A better understanding of the determinants of the tumor micro-circulation would go hand-in-hand with the generation of novel approaches.

In the case of non-labeled MAbs, one must further be concerned that without a better understanding of the biology underlying the basis of responding tumors, or eliciting novel target antigens that engage complementary pathways, a potential limit to the value of the current agents may be expected. With regard to current versions of immunotoxins, although strategies to manage vascular leak syndrome have been zealously pursued, full optimization of the potential value of these approaches would attempt to develop intoxicating mechanisms distinct from the protein synthesis poisons represented by *Pseudomonas* constructs. These would specifically be chosen and optimized to minimize the now almost stereotypical toxicities expected from these constructs that could be potentially problematic in projecting to more widespread clinical use, except perhaps in patients with circulating tumor cells or bulky disease.

The recognition of these prospects and issues should actually be regarded as quite a healthy set of actual opportunities. While one cheers when a “Gleevec®” bursts upon the clinical
scene, there is frequently very little that can easily be done to re-engineer such a molecule to address limitations of its use. In contrast, the confluence of advances and ingenuity in protein engineering, biology, and the accumulating evidence of clinical benefit will assure continued vigorous growth and innovation in antibody-based approaches in the coming years.

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