Meeting Report

Meeting Report on the Second Targeted Tumor Therapies

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Abstract

This meeting report on the fourth Fabisch Symposium for Cancer Research and Molecular Biology describes the aims of the international meeting, the main topics of the presentations, and the highlights of the conference. The fourth Fabisch Symposium was the second on Targeted Tumor Therapies and held from April 1–3, 2009 in Berlin, Germany. The meeting focused on noncarrier-based targeted tumor therapies and their clinical application. The world’s leading experts in this field presented the state of the art on tumor-specific targeting and tumor growth inhibition, drug design and production, and the description of innovative strategies for improved delivery. The topics concentrated on immunotoxins and other targeted toxins as anticancer drugs, thus providing a specialized meeting platform not existing elsewhere for these therapeutics. Although a number of innovative approaches on the avoidance of immune responses against highly effective toxins were presented, a notable conclusion of the meeting and direction for future research is the acute need to further reduce the immunogenicity of the targeted toxins, which hampers the efficacy of this group of therapeutics in clinical studies. The meeting successfully fostered plans for further research and cooperation between different groups to hopefully achieve advanced translational and clinical studies. Mol Cancer Ther; 9(1); 17–23. ©2010 AACR.

Introduction: The Fourth Fabisch Symposium and Related Presentations

On the basis of the success of the first symposium in 2006 on targeted tumor therapies held in Berlin, with support from the Fabisch Foundation, and numerous requests from the participants, the second Meeting on Targeted Tumor Therapies (fourth Fabisch Symposium for Cancer Research and Molecular Biology) was held from April 1–3, 2009 in Berlin, Germany. The meeting, mainly supported by the Fabisch Foundation, was again organized by Dr. Hendrik Fuchs and Dr. Christopher Bachran.

The main purpose of the symposium was the presentation and discussion of noncarrier-based targeted tumor therapies and their clinical application, with a special focus on target recognition, drug design and production, and innovative strategies for improvement. In addition to our meeting, the field of targeted tumor therapies was one of the many issues at the 100th annual meeting of the American Association for Cancer Research (April 18–22, 2009 in Denver, Colorado). However, immunotoxins, immunotoxins, immunoconjugates, and other targeted conjugate approaches were the topic of only two poster sessions in Denver and thus gathered little attention. The focus of several presentations at the Denver meeting was the development and signaling of non-small cell lung cancer as well as the treatment of the disease with tyrosine kinase inhibitors and nanoparticles (1, 2). Targeted tumor therapy approaches were furthermore the topic of a number of recent articles published in Molecular Cancer Therapeutics. Madhankumar and colleagues described interleukin 13-coated vesicles for the delivery of doxorubicin in an intracranial brain tumor model, and Chen and colleagues increased the delivery of arsenic trioxide nanoparticles by use of folate-coated vesicles (3, 4). Both groups described liposome-dependent delivery of drugs, a secondary topic of the second Targeted Tumor Therapy meeting, which concentrated on the direct delivery of single molecules. Four further recent publications in Molecular Cancer Therapeutics describe such systems. One approach reported targeted delivery of small interfering RNA (siRNA) against the bcl-2 mRNA for apoptosis induction to epithelial cell adhesion molecule-positive cells by fusion proteins containing the designed ankyrin repeat protein for cell binding and internalization and truncated human protamin-1 for siRNA binding (5). Sundaram and colleagues published their work in Molecular Cancer Therapeutics on a conjugation of docetaxel to deslorelin, a luteinizing hormone-releasing hormone superagonist for the treatment of prostate cancer (6). The following two publications were made by groups who also attended and presented their work at the second Targeted Tumor Therapy meeting. Yuan and colleagues designed fusion proteins targeting human ovarian cancer...
cells overexpressing claudin-3 and claudin-4 with the C-terminal fragment of *Clostridium perfringens* enterotoxin fused to tumor necrosis factor (7). Prof. Dr. Michael G. Rosenblum, who presented his results at the meeting, was co-editor of this publication. Mahmud and colleagues recently published their work on ErbB2-targeting fusion proteins containing apoptosis-inducing factor in *Molecular Cancer Therapeutics* (8). Preliminary results of this study were presented at the second Targeted Tumor Therapy meeting.

For the meeting in Berlin we gathered the leading scientists working in this ever-evolving field of tumor medicine to support the development of new ideas and collaboration between the different groups, and to provide a summary of the current status of these creative therapies. The importance of targeted tumor therapy approaches, not only for scientific goals but also for pharmaceutical purposes, was emphasized by the great number of scientists from academic institutions in the United States and Europe who attended the symposium, as well as several representatives of pharmaceutical companies. This participation clearly shows the continuously increasing high interest in targeted drug delivery for tumor therapy for both scientific and pharmaceutical reasons (9). A total of 28 key speakers presented their latest research results for the improvement of targeted tumor therapies. In addition, eight short communications were chosen from submitted abstracts for oral presentation. The remaining abstracts were presented in two poster sessions. A total of 31 posters documented further results on targeted tumor therapy approaches. The subject of the oral presentations and posters concentrated on specifically delivered protein toxins for the treatment of certain tumor entities. These protein-based drugs are named immunotoxins or targeted toxins and are fusion proteins consisting of antibodies (or antibody fragments), growth factors or cytokines as tumor-specific ligand, and bacterial or plant protein toxins as toxic moiety. Variants contain human enzymes to mediate cell death or enzymes that activate prodrugs into cytotoxic drugs in tumor tissue. To date, only one targeted toxin is approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with cutaneous T-cell lymphoma whose malignant cells express the interleukin 2 receptor. However, many other targeted toxins under development, and in clinical trials, were presented during the meeting. The key speakers presented their work in relation to five main topics: (i) generation and design of chimeric toxins, (ii) directed enzyme prodrug therapy, (iii) targeted RNases, (iv) innovative tools to improve targeted tumor therapies, and (v) targeted tumor therapies in clinical applications. All abstracts are available at the symposium Web site.¹

¹ http://www.charite.de/fabisch/

Clinical Data Presented

The scientific presentations started with the Luzie Fabisch Memorial Lecture given by Prof. Dr. A.E. Frankel (Cancer Research Institute of Scott & White, Temple, Texas), who presented the results of an ongoing clinical phase I trial on a new immunotoxin (Fig. 1A). This immunotoxin, composed of the catalytic and translocation domain of diphtheria toxin and a single chain Fv antibody fragment (scFv) of an antibody against CD3ε, revealed promising results in seven patients with cutaneous T-cell lymphoma. A potentially limiting factor for this therapy is the development of anti-immunotoxin antibodies observed in all patients. The evolving immune response against the drugs is one of the main obstacles for tumor therapy with immunotoxins and other targeted toxins. Prof. Dr. H. Dürkop (Charité—Universitätsmedizin Berlin, Germany) presented clinical data on the biodistribution of a fusion protein consisting of a scFv directed against the extra-domain B of fibronectin. Radiolabeled fusion protein was successfully traced in three lymphoma patients toward the lymph nodes. In addition, two Hodgkin lymphoma patients showed a partial response to the treatment, thus indicating a potential therapeutic success of the fusion protein. The results of clinical studies with targeted toxins for the treatment of brain tumors were presented by Dr. W.A. Hall (Upstate Medical University, Syracuse, New York). He presented his work on convection-enhanced delivery for local application of drugs into the brain (Fig. 1B). Further clinical data presented at the symposium in context with drug improvement are introduced below.

New Targeting Options

One of the current problems of targeted toxins is insufficient specificity and efficacy. Many groups are working on new targeted toxins or other means to circumvent these problems. The following results were presented in the sessions for the topics (i) and (iv). A number of speakers worked with antibodies against tumor-specific cell surface antigens to achieve highly specific delivery of the toxic moiety to the tumor cells. Prof. Dr. D.A. Valleria (University of Minnesota Cancer Center, Minneapolis, Minnesota) presented his work on bispecific fusion proteins (directed against epidermal growth factor receptor and either interleukin 4 or interleukin 13 receptor) with increased specificity to tumor cells (Fig. 1C). Prof. Dr. U. Elsässer-Beile (Universitätsklinikum Freiburg, Germany) and Prof. Dr. M. Colombatti (Policlinico G.B. Rossi, Verona, Italy) worked in recent years on targeted toxins for the treatment of prostate cancer (Fig. 1D). These therapeutics use antibodies against tumor specific antigens such as prostate-specific membrane antigen or the epithelial cell adhesion molecule. In animal models the immunotoxins proved to be highly specific and seem to be favorable for future therapy of prostate carcinoma.
Dr. E. Pogge von Strandmann (Universitätsklinikum Köln, Germany) showed the feasibility to activate the patient's immune system to eliminate tumor cells by using bispecific antibodies to direct natural killer cells to the tumor. The highly interesting findings of Prof. A. Thorburn (University of Colorado Denver, Colorado) on apoptosis- and autophagy-induction by targeted toxin showed that inhibitory effects may vary in different tumor cells and that it may be advantageous to manipulate treatment regimen in respect to autophagy induction. However, more research on autophagy and apoptosis in relation to tumor therapy is necessary to achieve a better understanding of the induced effects.

**Variants of the Toxic Load**

Several speakers introduced their results on targeted toxin variants with toxic loads other than the often-used *Pseudomonas* Exotoxin A (PE) or diphtheria toxin. Dr. S.H. Leppla (NIH, Bethesda, Maryland) reported the results of his studies on genetically engineered anthrax toxin (Fig. 2A). This antitumor agent comprises two proteins; protective antigen binds cells and delivers lethal factor into a target cell after proteolytic activation. The genetically modified protective antigen contains a tumor-specific protease-cleavage site, thereby leading to high specificity and toxicity. Another mechanism of tumor growth inhibition was achieved by a number of proapoptotic proteins, which are used to induce cell death by apoptosis after specific delivery to tumor cells. These fusion proteins were presented by Prof. Dr. M.G. Rosenblum (The University of Texas, Houston, Texas) and Prof. Dr. W.S. Wels (Georg-Speyer-Haus, Frankfurt a. M., Germany; Fig. 2B), both presenting immunotoxins containing the serine protease granzyme B, and Dr. W. Helfrich (University Medical Center Groningen, The Netherlands).
who discussed fusion proteins containing soluble tumor necrosis factor, Fas ligand, and tumor necrosis factor-related apoptosis-inducing ligand. In addition to the desirable induction of apoptosis for elimination of tumor tissue, these pro-apoptotic proteins are of human origin and thus potentially not immunogenic for future applications in patients. This finding is of great relevance because earlier clinical studies with highly immunogenic bacterial or plant toxins indicated low efficacy owing to strong immune responses. Dr. M.K. Tur (Helmholtz-Institute for Biomedical Engineering/Universitätsklinikum RWTH Aachen, Germany) showed the efficacy of targeted proteins consisting of scFvs and human kinases (Fig. 2C). The fusion of the death-associated protein kinase 2 (a pro-apoptotic kinase) with human CD30 ligand resulted in a fully human therapeutic protein with tumor growth inhibitory properties in vivo. A further strategy to evade the immune response and simultaneously deliver inhibitory molecules to tumor cells was addressed by using siRNA coupled to aptamers targeting the prostate-specific membrane antigen (Prof. Dr. S. Barth, Fraunhofer-Institut für Molekularbiologie & Angewandte Ökologie, Aachen, Germany). The elongation factor 2-specific siRNA delivered to antigen-positive tumor cells resulted in specific cytotoxicity. This aptamer-siRNA-based strategy is very promising because these molecules are expected to be nonimmunogenic and are easier to produce in vitro compared with protein-based therapeutic molecules (Fig. 2D). The concept of RNAi-mediated tumor therapy using liposomal delivery to downregulate essential proteins in tumor cells was further discussed by Dr. A. Santel (Silence Therapeutics, Berlin, Germany). One of the nonimmunogenic loads, the RNases, have received considerable attention over recent years, thus one session was dedicated to this topic and to immunoconjugates; and included presentations by Dr. K. Shogen (Alfacell Corporation, Somerset, New Jersey; Fig. 3A), Dr. S.M. Rybak (Bionanomics, Green Cove Springs, Florida; Fig. 3B), Dr. J. Krauss (Universität Heidelberg, Germany), Dr. T. Schirrmann (Technische Universität Braunschweig, Germany), and Dr. C. De Lorenzo (University of Napoli Federico II, Naples, Italy; Fig. 3C). The most advanced work in this area has been achieved with the amphibian onconase, which has successfully been tested in clinical trials. Targeting of onconase and other RNases is
thus a potential therapeutic option to overcome the problem of high immunogenicity of immunotoxins. During the session on RNases, further immunoconjugates containing human RNases were presented. Linked to human antibodies, these fusion proteins should cause no drug-neutralizing immune response.

As already observed in clinical trials, one way to evade the immune response against highly immunogenic protein toxins is the concealing of the drug in other structures. Dr. H.H. Bäumler (Charité—Universitätsmedizin Berlin, Germany) presented his work on loading red blood cells with cytostatics to achieve uptake of the loaded red blood cells into tumor cells while the drug is not accessible to the immune system.

We observed that the combination of targeted toxins with saponins (glycosylated plant triterpenoids or ster-oids) drastically increases the impact of the drug on tumor cells; results were presented by Dr. H. Fuchs (Charité—Universitätsmedizin Berlin, Germany). This combination therapy resulted in a number of benefits: (i) the amount of toxin necessary to inhibit tumor growth in mice is much lower in comparison to the treatment with the targeted toxin alone resulting in absent or largely reduced side effects (hair loss, transaminase induction, lethargy); (ii) the immune response was significantly lowered in comparison to the single treatment; and (iii) the tumor growth inhibitory effect of the targeted toxin is stronger upon the combination with saponin (Fig. 3D). A further strategy to evade immune responses against bacterial toxins was described by Dr. M. Onda (NIH, Bethesda, Maryland) who identified and subsequently mutated a large number of B-cell epitopes on the bacterial protein toxin PE and thus achieved the design of new immunotoxins containing mutated PE with less immunogenic properties while retaining full enzymatic activity.

Dr. M. Sutherland (University of Bradford, Bradford, United Kingdom) developed a number of nontoxic cytostatics that become activated within tumor cells by enzymes of the cytochrome P450 superfamily showing elevated expression in a number of different human cancers. Thus targeting of these enzymes by prodrugs mainly converted in tumor cells is an elegant way to mediate tumor specific cell killing. Moreover, the designed prodrugs are well tolerated in vivo and induced significant tumor growth retardation.

Figure 3. Example slides of the presentations given by A, Dr. K. Shogen, B, Dr. S.M. Rybak, C, Dr. C. De Lorenzo, and D, Dr. H. Fuchs.
The Antibody-Dependent Enzyme Prodrug Therapy System

In addition to the clinical and preclinical data on targeted toxins, a session of the meeting focused on antibody-dependent enzyme prodrug therapy (ADEPT). The principle of ADEPT is that an enzyme coupled to a tumor-specific antibody converts a subsequently administered prodrug into a cytotoxic cell-permeable drug resulting in accumulation of the drug at the site of the tumor and cell death. Prof. Dr. K.D. Bagshawe (Imperial College London, United Kingdom) gave a comprehensive overview on the history of ADEPT, a strategy that he initiated. Dr. P.M. Deckert (Klinikum Brandenburg, Germany) and Dr. K. Chester (University College London, United Kingdom) presented their data on current ADEPT projects. Dr. Deckert showed tumor growth inhibition in a mouse model for fusion proteins containing a scFv directed against the glycoprotein A33, which is overexpressed in colon carcinomas, and cytosine deaminase for prodrug activation. Dr. Chester presented her work on clinical studies with an ADEPT-fusion protein directed against carcinoembryonic antigen and a bacterial carboxypeptidase G2 as prodrug-converting enzyme. However, although the ADEPT showed evidence of efficacy in phase I and II trials, the induced immune response was too strong for prolonged treatment. Thus, as for targeted toxins, the development of nonimmunogenic or even human moieties for targeted therapeutic fusion proteins is necessary for clinical success.

Future Research

Reduction of the immune response against immunotoxins or other targeted therapeutics emerged as the most important issue during the meeting. The majority of presentations at the fourth Fabisch Symposium were thus focused on strategies to use human toxins, fewer immunogenic variants of bacterial or plant toxins, fewer immunogenic proteins or even RNAi as toxic load to overcome this therapeutic limiting effect. It is clear that in the next few years research in this area will focus on nonimmunogenic agents. The number of research groups developing protein-based targeted therapies is relatively small compared with other fields of anticancer agents; as a result the take-home message from this symposium was improved collaboration between these groups and sharing of results, so that hopefully the problem of immunogenicity can be identified and overcome at an earlier stage. The result of this early identification will be a large reduction in development costs, as currently this therapeutic limiting side-effect is only identified after years of laboratory research and early clinical trials. However, this symposium has clearly highlighted the efficacy and tremendous potential of these innovative therapeutics and has featured promising new treatments encouraging further research into these innovative targeted therapies. We wish to thank all participants for contributing to a very interesting symposium and look forward to seeing you all, and hopefully a number of new research groups, at the next symposium.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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