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ORIGINAL ARTICLE

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Immunoconjugates and immunotoxins for therapy of solid tumors

Immunoconjugates and immunotoxins are attracting attention as potential cancer therapeutic agents; however, monoclonal antibodies (MAbs), except in the case of radiolabeled MAbs for B-cell lymphoma [6] and unmodified pan-carcinoma MAb 17.lA for destruction of micrometastases in patients with colon carcinoma [7], have not yet been proven to be clinically effective for this purpose. We believe that the use of appropriate MAbs and linkers will produce immunoconjugates with clinical benefit and that fusion proteins will prove useful for the selective delivery of toxins to tumor cells. However, we also believe that clinical benefits are more likely to be seen in patients with minimal residual disease and that these benefits may be missed if phase I/II trials are confined to patients with bulky tumors. The reasoning behind this conclusion is that mice and rats can be cured of tumors measuring up to 10–15 mm in diameter, which is large for a rodent but not for a human. The absolute tumor size rather than the size in relation to the tumor-bearing host is likely to be crucial because penetration of immunoconjugates and immunotoxins into tumor masses with a high hydrostatic pressure [5] will be easier in tumors of smaller mass.

We have previously reported that immunoconjugates between anti-Le^y MAbs (which bind to most human carcinomas) and the anticancer drug doxorubicin are therapeutically effective against human lung, colon, and breast carcinomas xenotransplanted into nude mice [11–13]. The most dramatic effects, including a high frequency of cures of disseminated tumors including some in orthotopic locations [12], were seen with a thioether immunoconjugate prepared with a chimeric (mouse-human) version of MAb BR96 and doxorubicin (BR96-DOX). This conjugate also cured tumor-bearing nude rats, which, like humans, express the Le^y antigen in the normal gastrointestinal epithelium. We have also reported that a fusion protein between BR96 sFv and *Pseudomonas* exotoxin 40 (PE40) produces cures in several carcinoma models [2, 3, 9]. Furthermore, recent studies carried out in collaboration with Dr. H.O. Sjogren, University of Lund, Sweden, have shown that BR96-DOX produces cures in immunocompetent rats that have a syngeneic, transplanted colon carcinoma growing either subcutaneously or in the liver (unpublished results). These studies are particularly important as they demonstrate for the first time curves of syngeneic tumors, in an immunocompetent model in which the target antigen is expressed both in the tumor and in some normal tissues. The data imply that normal tissues do not act as an antibody "sink" and that cures can be achieved with acceptable side effects by using the appropriate immunoconjugate (such as BR96-DOX for rats). In none of the experiments referred to has doxorubicin alone produced more than occasional partial regressions, and no effect has been detected in animals receiving MAb BR96 alone or a mixture of BR96 and doxorubicin.

It is also encouraging that the immunotoxin BR96 sFv-PE40 has displayed evidence of biological activity, including two partial regressions and one long-term (18-month) stabilization. These results were obtained in a preliminary study in which dogs with Le^y-positive carcinomas were treated by Dr. Carolyn Henry and colleagues, Department of Veterinary Sciences, Washington State University, Pullman, Washington (nine dogs of which two underwent partial remission), and by Dr. Monica Marks, Canyon Park Veterinary Hospital, Bothell, Washington (one dog showing long-term stabilization).

BR96-DOX has just completed phase I clinical trials and will soon enter phase II trials, and an investigational new drug (IND) application for BR96 sFv-PE40 is being filed. The dose-limiting toxicity of BR96-DOX was found to be

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severe nausea and vomiting resulting from damage to the gastrointestinal epithelium. In human patients this toxicity has prevented the delivery of the doxorubicin doses needed to produce cures in large tumors in rodents. Therefore, it is not surprising that the clinical effects seen in the phase I trial were limited to two partial responses and some cases of stabilization. Importantly, the side effects of BR96-DOX in both humans and dogs (in which some of the toxicology studies required for the IND were performed) have been shown to be due to the MAb, i.e., protein, part of the conjugate and not to the targeting of doxorubicin. We do not know whether the clinical effects would have been more impressive had patients with small tumors (or just micrometastases) been treated, although we suspect that this would have been the case.

Based on the information available, experiments in which attempts are being made to remove those parts of the BR96 protein suspected to be involved in causing destruction of the stomach epithelium are being performed. Other approaches being used include replacement of the chimeric version of the BR96 MAb with a higher avidity, humanized version that binds to tumor cells and has a lower off-rate without showing increased toxicity in dogs; more stable MAb-doxorubicin linkers are being identified; and the effects of different treatment schedules are also being investigated in preclinical models. It appears that frequent delivery of smaller conjugate doses (likely to be more tolerable in patients) is more efficient than injection of larger doses at greater intervals. We have recently reviewed this area [1, 4, 8, 10], and refer the reader to these reviews and the publications cited above.

References

1. Casazza AM, Trail PA, Hellstrom KE (1994) Drug immunotargeting for carcinomas: a reality at last? Ann Oncol 5: 703
2. Friedman PN, McAndrew SJ, Gawlak SL, Chace D, Trail PA, Brown JP, Siegall CB (1993) BR96 sFv-PE40, a potent single-chain immunotoxin that selectively kills carcinoma cells. Cancer Res 53: 334
3. Friedman PN, Chace DF, Trail PA, Siegall CB (1993) Antitumor activity of the single-chain immunotoxin BR96 sFv-PE40 against established breast and lung tumor xenografts. J Immunol 50: 3054
4. Hellström I, Hellström KE, Siegall CB, Trail PA (1995) Immunoconjugates and immunotoxins for therapy of carcinomas. In: Garattini S (ed) Advances in pharmacology. Academic Press, San Diego, p 349
5. Jain R (1994) Barriers to drug delivery in solid tumors. Sci Am 271: 58
6. Press OW, Eary JF, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, Glenn S, Butchko G, Fisher D, Porter B, Matthews DC, Fisher LD, Bernstein ID (1993) Radiolabeled antibody therapy of B cell lyphoma with autologous bone marrow support. N Engl J Med 329: l219
7. Riethmuller G, Schneider-Gadicke E, Schlimok G, Schmiegel W, Raab R, Hoffken K, Gruber R, Pichlmaier H, Hirche H, Pichlmayr R, Buggisch P, Witte J, Eigler FW, Facklerschwalbe I, Funke I, Schmidt CG, Schreiber H, Schweiberer L, Eibleibesfeldt B (1994) Randomized trial of monoclonal antibodies for adjuvant therapy of resected Dukes′ C colorectal carcinoma. Lancet 343: 1177
8. Siegall CB (1994) Targeted toxins as anticancer agents. Cancer 74 [Suppl 3]: 1006
9. Siegall CB, Chace D, Mixan B, Garrigues U, Wan H, Paul L, Wolff E, Hellström I, Hellström KE (1994) In vitro and in vivo characterization of BR96 sFv-PE40. A single-chain immunotoxin fusion protein that cures human breast carcinoma xenografts in athymic mice and rats. J Immunol 152: 2377
10. Siegall C, Wolff E, Gawlak S, Paul L, Chace D, Mixan B (1995) Immunotoxins as cancer chemotherapeutic agents. Drug Dev Res 34: 210
11. Trail PA, Willner D, Lasch SJ, Henderson AJ, Greenfield RS, King D, Zoeckler ME, Bralawsky GR (1992) Antigen specific activity of carcinoma reactive BR64-Adriamycin conjugates evaluated in vitro and in human tumor xenograft models. Cancer Res 52: 5693
12. Trail PA, Willner D, Lasch SJ, Henderson AJ, Hofstead S, Cassazza AM, Firestone RA, Hellström I, Hellström KE (1993) Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science 261: 212
13. Willner D, Trail PA, Hofstead SJ, King HD, Lasch SJ, Braslawsky GR, Greenfield RS, Kaneko T, Firestone RA (1993) (6-Maleimidocaproyl)hydrazone of doxorubicin – a new derivative for the preparation of immunoconjugates of doxorubicin. Bioconjugate Chem 4: 521