Immunotoxin Therapy of Small-Cell Lung Cancer
N901-Blocked Ricin for Relapsed Small-Cell Lung Cancer

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Despite its initial chemosensitivity, small-cell lung cancer (SCLC) is rarely cured with chemotherapy alone, and fewer than 5% of patients are alive at 5 years. Immunotoxin therapy appears to offer promise in treating the minimal residual disease that remains after induction chemotherapy. We have studied N901-bR in patients with relapsed SCLC. N901-bR consists of the N901 monoclonal antibody (MoAb) and blocked ricin, an altered ricin molecule in which the galactose binding sites of the ricin B-chain which mediate nonspecific binding of the toxin are blocked through the covalent binding of ligands. N901 is an anti-NCAM (CD56) MoAb which binds to SCLC tumors and cell lines, cardiac muscle, natural killer (NK) cells, and peripheral nerve. N901-bR showed a 2.7 log greater in vitro cytotoxicity to the CD56-positive cell line SE-2 than to the antigen-negative Namalwa cell line. Nineteen patients with relapsed antigen-negative Namalwa cell line. Nineteen patients with relapsed and/or refractory SCLC have been entered into a phase I study at doses ranging from 5 to 40 μg/kg/day given as a 7-day continuous infusion. The dose-limiting toxicity is capillary leak syndrome observed in two thirds of the patients treated at 40 μg/kg/day. One patient at the maximum tolerated dose, 30 μg/kg/day \( \times \) 7 days, has achieved a partial response to N901-bR. No patient has developed clinically significant peripheral or central neuropathy. We plan to begin a phase II study of N901-bR following induction chemotherapy in patients with SCLC.

(Chest 1993; 103:436S-39S)

The treatment of small-cell lung cancer (SCLC) presents a unique challenge in clinical oncology. Tumors are at first quite responsive to chemotherapy, yet this initial sensitivity does not persist. The vast majority of tumors relapse, and cures in patients with even limited stage disease are rare. The early 1980s witnessed a period of optimism; many felt that a cure for SCLC was imminent. Efforts to improve outcome focused on combining chemotherapy with radiation using alternating cycles of non-cross-resistant regimens, and dose escalation of chemotherapeutic agents with autologous bone marrow support. Yet SCLC has shown remarkable resistance to conventional therapy. As we enter the 1990s, we are able to cure less than 5 percent of patients with SCLC, and the vast majority of patients die within 2 years of diagnosis. Clearly if we are to make an impact on the natural history of SCLC, we need to develop therapeutic modalities that are innovative and exploit the biologic differences between SCLC and normal tissue.

Patterns of Failure in SCLC

The emergence of a drug-resistant phenotype is the primary reason for failure of chemotherapy and radiotherapy to cure SCLC. Efforts to circumvent this have included the use of non-cross-resistant alternating combination therapy based on the Goldie-Coldman model. However, alternating combination chemotherapy such as CAV/EP (cyclophosphamide, doxorubicin, and vincristine alternating with etoposide and cisplatin) has not had a dramatic impact on outcome in SCLC. Furthermore, increasing dose intensity, increased duration of therapy, and late intensification have increased response rates, but have not impacted significantly on overall survival. A trial of autologous bone marrow transplantation (ABMT) compared with standard-dose chemotherapy in SCLC yielded a 77% conversion rate from partial response (PR) to complete response (CR) and a disease-free survival benefit, but ultimately, the overall survival benefit was too small to be statistically significant. Clearly, new drugs and approaches are needed.

The patterns of failure following combined modality therapy of limited stage disease are of great importance in designing future therapies. Despite excellent initial control, 40% to 50% of patients with limited stage disease who initially achieved a CR will experience disease relapse in the chest. The brain is also a major site of relapse in SCLC. Autopsy studies have demonstrated that the actuarial probability of developing central nervous system (CNS) metastases during a 2-year period with SCLC is almost 80%. In patients not receiving prophylactic cranial irradiation (PCI), CNS relapse rates range from 25% to 50%. Despite the demonstrated ability of PCI to reduce the CNS-relapse rate, there is no clearly demonstrated impact on survival. As would be expected, leptomeningeal metastasis is a major problem with a 24% incidence of involvement at time of relapse in patients who had previously achieved a CR. The remaining organs involved in relapsed SCLC and the frequency of finding metastatic disease at autopsy include the following: liver, 60%; bone and bone marrow, 44.8%; adrenals, 31.6%; retroperitoneal lymph nodes, 28.2%; pancreas, 13.6%; and kidneys, 8.1%.

Therapy for Relapsed SCLC

The treatment of relapsed SCLC is a monumental challenge. Overall, response rates in a recent South West Oncology Group (SWOG) study in patients with relapse ranging from 9% to 27%. In this study, response was influenced by the performance status of patients at the time of relapse as well as prior response to therapy. Median survival from time of relapse was 16 weeks. This study is among the most optimistic as many other authors quote a

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median survival of only 7 to 8 weeks in patients experiencing relapse.  
Small-cell lung cancer presents an interesting opportunity for new drug development. The majority of patients are able to achieve a minimal disease state with conventional chemotherapy. A new agent whose mechanism of action was distinctly different from that of conventional chemotherapy would thus have an excellent opportunity to impact on this disease. Immunotoxins (IT) may represent such a class of drugs. Their mechanism of action differs from that of traditional cytotoxic agents, and they are unlikely to potentiate bone marrow suppression. Thus, ITs may be able to improve the cure rate in SCLC by eradicating drug-resistant cells following effective upfront chemotherapy and radiotherapy.

**Background on Immunotoxins**

In contrast to conventional chemotherapeutic agents, antibodies, with their exquisite specificity, might prove to be agents with which to treat human tumors with minimal nonspecific toxicity. Although the concept of treating tumors with antibodies has been considered for several decades, extensive clinical studies were impossible due to the limited supply of heteroantibodies. The advent of monoclonal antibody technology has provided unlimited quantities of homogeneous antibodies to test as therapeutic agents.

Attention has been focused on the use of monoclonal antibodies as vehicles for the delivery of cytotoxic agents directly to tumor cells. Previous studies examining potential antineoplastic agents have identified several toxins that are 5 to 7 logs more potent than conventional chemotherapeutic agents. Such toxins may be used to develop highly cytotoxic antibody-toxin conjugates. Three major components are necessary for a successful immunotoxin: (1) a specific monoclonal antibody with limited cross-reactivity with normal tissue; (2) a potent toxin which does not bind to normal tissue and only binds to and penetrates cells when bound to the monoclonal antibody; and (3) a stable linkage between the antibody and toxin.

Although the concept of an antibody toxin conjugate appeared to be relatively simple, the development of highly cytotoxic and specific ITs has been more difficult. As a result of early clinical trials, it has become increasingly clear that several variables are important in the development of a therapeutically efficacious IT. First, not only is the specificity of the monoclonal antibody important, but the isotype, avidity, and localization of binding site on the antigen also appear to be very important. Second, the nature of the toxin is equally important. The major classes include naturally occurring polypeptide inhibitors of protein synthesis such as ricin, abrin, saporin, pokeweed-anti-viral protein (PAP), and gelonin. These natural substances kill cells by ribosomal inactivation. Whole ricin and abrin possess 2 distinct chains, 1 chain with lectin-binding properties, and another with cytotoxic activity. These 2-chain toxins are highly toxic to intact cells in their natural state. In contrast, toxins composed of a single polypeptide chain (eg, saporin, PAP and gelonin) demonstrate little intrinsic toxicity to intact cells because they have no mechanism for binding to surface membranes. Moreover, the binding of ricin to normal cells can be inactivated by separating the toxic A-chain from the cell-surface-binding B-chain. For this reason, the first ITs created for clinical trials utilized monoclonal antibodies linked to either single-chain toxins or to purified ricin A-chain.

Over the past 5 years, an increasing number of laboratories have linked single-chain toxins to monoclonal antibodies. These ITs have specifically demonstrated cytotoxicity to those cells expressing the relevant cell-surface antigen. However, the expected cytotoxic efficiency did not manifest for the majority of these single-chain ITs. Additional biologic considerations, including the ability of the antibody toxin conjugate to penetrate the cell surface as well as the number of IT molecules required to kill a cell, appeared to be very important in inducing cell death. Moreover, it became evident that tumor cells could detoxify many single-chain toxin immunoconjugates by enzymatic degradation in the lysosome.

**Background on Blocked Ricin**

Extensive laboratory studies examining the effects of different single-chain toxins as well as modifications of antibody-toxin linker chemistry on the capacity of ITs to internalize and kill target tumor cells have been performed. These studies demonstrated that single-chain toxins were not very effective cytotoxic reagents even with antibodies of the highest avidity and ability to internalize. Therefore a new type of toxin was prepared which retained the toxic properties of the intact ricin molecule but had chemically blocked the ability of the B-chain to bind to normal cells.

Ricin consists of 2 subunits, the A-chain and the B-chain, which are linked by a single disulfide bond. The A-chain is an enzyme that inactivates the 60S subunit of eucaryotic ribosomes, and the B-chain binds to galactose-terminated oligosaccharides, ubiquitous on eucaryotic cell surfaces. Intact ricin immunoconjugates are potent cytotoxic agents; however, such immunotoxins demonstrate nonspecific cytotoxicity because they bind to all cells via the B-chain. An approach to limit the toxicity of monoclonal antibody-whole ricin conjugates by chemically blocking the 2 galactose binding sites of the ricin-B chain with linking natural ligands into the binding sites in a chemically stable way has been developed. Monoclonal-antibody-blocked ricin conjugates retain the specificity of the monoclonal antibody with only slightly less cytotoxicity than was observed for the whole ricin molecule and significantly more than is seen with ricin A-chain IT. These biochemical modifications do not appear to affect the ability of the B-chain to enhance toxin transport across the cell membrane.

N901-bf is an immunoconjugate linking the N901 anti-NKH1 antibody to a "blocked" whole ricin molecule. The process as described in the above section produces a highly purified IT combining the specificity of N901 with a toxicity significantly greater than ricin A-chain alone. The nonspecific toxicity of free blocked ricin is reduced by more than 100-fold relative to whole ricin, even though the activity of the blocked ricin A-chain is identical to that of native ricin A-chain, as measured by their capacity to inhibit protein synthesis in a cell-free system. By linking the blocked ricin to N901, a conjugate is formed that has the same general toxicity, but has a 500-fold increased cytotoxicity for the
targeted cell population specified solely by the antibody.

**Characteristics of the N901 Monoclonal Antibody**

Monoclonal antibodies (MAB) have been utilized as a means of investigating the cell lineage of SCLC and of clarifying the relationship between SCLC and other tumors. For example, SCLC cells express neural-related antigens on their surface. In this respect, SCLC resembles the gastrointestinal tumors derived from cells engaged in amine precursor uptake and decarboxylation (APUD), such as pheochromocytoma, neuroblastoma, and carcinoid. This has led to the generation of MAB reactive with SCLC and the investigation of immunophenotyping as a means of distinguishing SCLC from other cancers.58 A second goal in generating these antibodies is to provide a means of "purging" bone marrow of patients prior to high-dose chemotherapy with autologous marrow reinfusion.61 Finally, these antibodies, which are selective for SCLC, provide candidate molecules for development as immunonconjugates. The N901 monoclonal antibody (anti-CD56) recognizing the NKH1 antigen has been chosen to develop an IT with activity against SCLC.

The NKH1 antigen is a 180 to 200 kDa surface structure that is expressed by normal human natural killer cells. The NKH1 antigen is selectively expressed on 10% to 15% of peripheral blood lymphocytes but not on granulocytes, monocytes, erythrocytes, platelets, or earlier hematopoietic precursors. Previous studies have demonstrated that expression of NKH1 is restricted to those cells with non-MHC-restricted cytolytic function, an operational definition of NK cells.62 These include resting NK as well as activated lymphokine-activated killer (LAK) effectors. In malignant hematopoietic cells, NKH1 is commonly expressed on large granular lymphocytic leukemias and about 20% of acute myelogenous leukemia cases, but rarely on other lymphoid malignancies, with the exception of myeloma. Although the majority of NKH1+ cells also express CD2 (T11/E rosette receptor) antigen, only 20% to 25% coexpress a CD3/T-cell receptor complex. Interestingly, both CD3+ and CD3- fractions of NKH1+ cells are able to mediate non-MHC restricted killing that is characteristic of NK cells.63

Extensive screening of NKH1 antigen expression in non-hematopoietic tissue has also been carried out by immunoperoxidase staining of frozen tissue samples. The strongest staining of N901 occurs with peripheral nerves of all types. It appears that the N901 antibody binds to the intraneural fibroblasts and not to the neuron itself or to Schwann cells. In addition, staining of astrocytes and neuropol in the CNS was observed by immunoperoxidase methods. Staining of cardiac and skeletal muscle fibers was also observed. Additional staining was noted in thyroid follicular epithelium and in the cytoplasm of cortical stromal cells of the ovary.

In one recent study, the expression of NKH1 in SCLC was compared to a panel of 21 other MAB against NK cells, other leukocyte antigens, cytokeratins, or non-lineage-specific antigens.64 All SCLC specimens were stained diffusely and strongly with NKH1 and OKT9 which is specific for the transferrin receptor. Most non-small-cell lung cancer also reacted with OKT9, but NKH1 antigen was only found in SCLC samples.

In a nude mouse model, Koros et al58 noted some SCLC tumor reductions using the N901 antibody. In this model a SCLC cell line was introduced into nude mice; the resultant tumors were treated with N901 as serotherapy. Some modest tumor reductions were noted.

**Preclinical Studies with Anti-N901-bR**

**In-Vitro Toxicity:** In vitro cytotoxicity of N901-blocked ricin (bR) was determined on the N901-antigen-positive SCLC cell line SW-2. Cytotoxicity was measured following incubation with varying concentrations of the conjugate using the clonogenic assay described by Scott et al.66 Non-specific toxicity was determined on the N901 antigen-negative Burkitt's lymphoma cell line Namalwa by the same procedure. Toxicity values are presented as an IC50, which is the concentration of conjugate required to kill 63% of cells following a 24-h exposure of the cells to conjugate. The IC50 values for N901-blocked ricin towards SW-2 cells and Namalwa cells are 0.022 nmol and 7.8 nmol, respectively. Four to five logs of SW-2 cells can be killed at an N901-bR concentration of 3.5 nmol.

**Myocardial Toxicity:** N901-bR has demonstrated reactivity with myocardial cells. This may result from binding to a neural cell adhesion molecule isoform expressed on the myocellular cell surface, or it may reflect non-specific toxicity. To further investigate the effects of N901-bR on cynomolgus monkeys, we examined cardiac pathologic condition in monkeys who had received N901-bR by either bolus infusion for 6 days or by continuous infusion over the same time period. Of 12 monkeys who received a total dose of N901-bR of 900 mg/kg over 6 days, 3 had microscopic evidence of significant myocardial inflammation and damage. Of the remaining 8, 6 had evidence of minimal inflammatory changes. No monkeys had any clinical evidence of myocardial insufficiency or arrhythmia during the study. It should be further noted that the dose of drug that produced these changes is well above the likely maximum tolerated dose in humans due to hepatic toxicity.

The mechanism of this tissue response is not clearly explained by the light microscopic findings but is consistent with an immunologically mediated phenomena. In many ways, the pathologic findings are similar to those seen in acute cardiac graft rejection. This requires that attention be given to possible cardiac toxicity in human patients.

**CNS Toxicity:** To clarify the effect of N901-bR on nervous tissue, further studies were performed using high doses of N901-bR administered by continuous infusion for 6 days in cynomolgus monkeys. At total doses of 750 μg/kg and 1,000 μg/kg, mild abnormalities of motor conduction were detected by nerve conduction studies. The monkeys had no apparent clinically significant abnormalities in either motor or sensory function. These electromyographic abnormalities did not develop until 21 days after therapy.

**NK Toxicity:** Since N901 is reactive with NK cells in this species, NK activity was monitored at various times after infusion of N901-bR. In one animal in which this was examined, no functional NK activity could be identified 2 days after infusion. However, by 30 days postinfusion, NK activity had returned to normal.

**Phase I Study in Patients with Relapsed SCLC**

After the completion of extensive preclinical studies which
showed that N901-bR could specifically kill SCLC cells and could safely be given to primates, we began a phase I study of N901-bR in patients with relapsed or refractory SCLC. Patients were given N901-bR in a phase I dose escalation design at doses ranging from 5 μg/kg/d × 7 days to 40 μg/kg/d × 7 days by continuous infusion. Patients were assessed for cardiac toxicity with serial Holter monitors, serial radionuclide ventriculograms, and serial cardiac enzyme measurements. Neurologic toxicity was assessed with serial neurologic exams, serial nerve conduction studies, and electromyelograms.

Results to Date

To date, 19 patients have been treated with N901-bR. We have found that this drug can be given safely in terms of cardiac and neurologic parameters. Capillary leak syndrome is the dose-limiting toxicity at 40 μg/kg/d × 7 days. Serum drug levels have been achieved that are of a level that is cytotoxic in vitro. All patients have produced human anti-mouse antibody and human antiricin antibody after one course of treatment.

We have observed a clinical response to N901-bR in a patient with refractory SCLC. In addition, we have documented drug binding to tumor in the lung, bone marrow, and liver.

Ideally, a drug such as N901-bR would be used when patients are in a state of minimal residual disease, following aggressive induction chemotherapy and radiotherapy. We are initiating a phase II study in patients with SCLC following induction chemotherapy.

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