Bombesin/Gastrin-Releasing Peptide Receptor: A Potential Target for Antibody-Mediated Therapy of Small Cell Lung Cancer

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ABSTRACT

Purpose: Bombesin/gastrin releasing peptide (BN/GRP) is a growth factor for small cell lung cancer (SCLC). The receptor (R) for BN/GRP is overexpressed on SCLC cells and other solid tumors. BN/GRP and its receptor form an autocrine loop to promote tumor growth. We developed a novel immunotherapeutic approach targeting surface BN/GRP-R on SCLC cells and immune trigger molecules on host immune effector cells to direct immune effector cells to SCLC cells and mediate targeted cancer cell destruction. Targeted immunotherapy combined with chemotherapy enhanced SCLC cell killing.

Experimental Design: We designed a synthetic BN/GRP antagonist (Antag 2) and constructed a bispecific molecule (BsMol), H22xAntag 2 (humanized monoclonal antibody) for Fc γRI. We tested the binding of the BsMol to several SCLC cell lines, its ability to mediate cytotoxicity of SCLC by IFN-γ-activated human monocytes, and BsMol-mediated immunotherapy in an animal model of SCLC xenograft.

Results: Common chemotherapy (cisplatin, etoposide, and paclitaxel) inhibited thymidine uptake into SCLC cells in a dose-dependent pattern. Antibody-dependent cellular cytotoxicity mediated by the BsMol inhibited thymidine uptake into SCLC cells and was largely dependent on E:T cell ratio. When SCLC cells were treated with antibody-dependent cellular cytotoxicity followed by exposure to chemotherapy agents an additional 25–40% inhibition of thymidine uptake into SCLC cells was observed consistently. With BsMol and IFN-γ-activated human monocytes, tumor burdens were reduced significantly in immunodeficient mice bearing human SCLC xenografts.

Conclusions: Combined chemotherapy and immunotherapy targeting BN/GRP-R with a BsMol significantly enhances targeted SCLC cell killing.

INTRODUCTION

Lung cancer is the second most common malignancy in the United States. As estimated by the American Cancer Society, there will be 171,900 new cases and 157,200 deaths from lung cancer in 2003 (1). SCLC accounts for 15–25% of all lung cancers. Although SCLC is sensitive to both chemotherapy and radiotherapy, the duration of response is usually short-lived. The majority of SCLC patients die from progressive disease.

Human GRP is a mammalian analogue of BN, initially discovered in the frog (2). BN/GRP is produced by majority of SCLC patients and binds to BN/GRP-R on their cell surfaces to form an autocrine loop to promote tumor growth (3). Interruption of this autocrine loop between BN/GRP and BN/GRP-R results in the inhibition of SCLC growth in vitro as well as in vivo (4, 5). There are three subtypes of BN/GRP-R, namely GRP-R, neuromedin B receptor, and BN receptor subtype 3 (6–9). Both BN and GRP bind to three subtypes of receptor with variable affinity. BN/GRP-R is coupled with G protein, which activates multiple signal transduction pathways on the binding of BN/GRP and results in cell proliferation (10–13).

In a cancer-bearing patient, the host immune system has become compromised and cannot mount an effective immune response to the growing tumor. Several approaches have been developed to break such immune incompetence, by activating certain trigger molecules on immune effector cells, by activating costimulatory pathways in T cells, or by eliciting a host immune response with a tumor vaccine. The Fc receptors for IgG (FcγRI), expressed on monocytes, macrophages, and neutrophils, are one of few molecules capable of mediating ADCC. FcγRI is a potent cytotoxic trigger molecule activated by a number of cytokines including IFN-γ, granulocyte/macrophage colony-stimulating factor, and granulocyte-colony-stimulating factor (14). Targeting this receptor has the potential to recruit large numbers of immune effector cells and to redirect their cytotoxic activities toward cancer cells.

Growth factor receptors on tumor cell surfaces are ideal targets for immunotherapy. To our knowledge, there is no mAb directed to human GRP-R. We hypothesized that a BsMol could enhance SCLC cell killing.

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2 The abbreviations used are: SCLC, small cell lung cancer; R, receptor; BN, bombesin; GRP, gastrin releasing peptide; ADCC, antibody-dependent, cell-mediated cytotoxicity; mAb, monoclonal antibody; BsMol, bispecific molecule.
be constructed to target GRP-R on tumor cell surface and a cytotoxic trigger molecule on immune effector cells to activate ADCC (15). We constructed a BsMol consisting of a synthetic BN/GRP peptide and a mAb directed to FcγRI H9253. The BsMol could bind to SCLC cells and mediate specific lysis of SCLC cells in vitro (16, 17). Several clinical trials targeting FcγRI H9253 have been reported with encouraging results. A bispecific antibody composed of anti-FcγRI and anti-HER2 antibody, MDX-210, was evaluated in patients with advanced breast and ovarian cancer. After infusion, cytokine release was documented and tumor regression in patients was observed (18, 19). In a Phase II trial, patients with advanced prostate cancer received multiple doses of MDX-210 in combination with granulocyte/monocyte-colony-stimulating factor. Seven patients (35%) had >50% decrease in PSA levels after treatment (20). Infusion of BsAb consisting of anti-FcyRI and anti-CD33 antibody was evaluated in a Phase I trial in patients with relapsed or refractory acute myeloid leukemia (21). In those trials, activation of host immune responses was documented; most patients tolerated the infusion well.

There is ample laboratory and clinical evidence that combining immunotherapy with conventional chemotherapy results in either additive or synergistic effects on tumor cell killing (22–24). Rituximab (anti-CD20 mAb) in combination with chemotherapy has become a standard treatment for CD20-positive lymphoma (25). Herceptin (anti-HER2 mAb) combined with various chemotherapy agents has been used to treat metastatic breast cancer (26, 27). The mechanism for the enhanced effects of cancer cell killing between chemotherapy agents and mAb is unclear. A simple explanation is that there is additive cell killing mediated by two different agents acting on different targets. Evidence suggests that an antibody targeting a growth factor receptor significantly enhances the effect of chemotherapy by a mechanism called receptor-enhanced chemosensitivity (22, 23). Conversely, chemotherapy may enhance the effect of growth inhibition by an antibody against a growth factor receptor (28–30).

We chose to simplify the steps of chemical conjugation of a BsMol targeting BN/GRP-R, to study the effect of targeted immunotherapy of xenografted human SCLC in a murine model, and to evaluate the effect of combined immunotherapy and chemotherapy on SCLC cells.

MATERIALS AND METHODS

Construction of BsMol. Detailed chemical construction of a BsMol has been published previously (16, 17). To simplify the construction process, we added a cysteine residue to the NH₂-terminal of a BN antagonist, (D-Phe⁶, Leu-NHEt¹³, and des-Met¹⁴) BN(6–14) to create a free sulfhydryl group (31). The peptide (Cys⁵, D-Phe⁶, Leu-NHEt¹³, and des-Met¹⁴) BN(5–14), named Antag 2, was custom synthesized (BACHEM, Torrance, CA). The quantity of free sulfhydryl group on Antag 2 was determined by an Ellman’s test. H₂₂, a humanized mAb F(ab’₂)₂ fragment against FcγRI (Medarex, Inc., Princeton, NJ), was incubated with a cross-linker, N-Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP), to form a 2-pyridyl disulfide-activated antibody. The final conjugation of H₂₂xAntag 2 was achieved by mixing the cysteine-containing Antag 2 with the antibody.

Cell Culture. SCLC cell lines H345 and H69 were purchased from American Type Culture Collection (Rockville, MD). DMS273 was a SCLC cell line established from pleural effusion of a patient (32, 33). DMS273 was provided to us by...
Drs. Olive S. Pettengill and George D. Sorenson of Dartmouth Medical School (Lebanon, NH). All of the cell lines were maintained in serum-free RPMI 1640 medium containing 1 × 10⁻⁸ M hydrocortisone, 5 μg/ml of insulin, 10 μg/ml of transferrin, 1 × 10⁻⁸ M β-estradiol, and 3 × 10⁻⁸ M selenium (HITES medium) (all purchased from Sigma Chemical Co., St. Louis, MO).

**Colony Assay.** SCLC cells (5 × 10⁴) were suspended in 1 ml of HITES medium containing 0.3% agarose. Cells were plated over a base layer of 1 ml of HITES medium of 0.5% agarose. BN, Antag 2, and the BsMol were added at different concentrations. Cells were incubated for 14 days; colonies were counted under a reversed-phase light microscope. A colony was defined as a distinct aggregate of >50 cells.

**Binding Study by Flow Cytometry Analysis.** Cells were washed with PBS containing 0.1% BSA and 0.1% sodium azide (PBA). Cells (3 × 10⁶) in 100 μl of PBA were incubated with different concentrations of BsMol for 1 h at 4°C. After incubation, the cells were washed twice and incubated with goat F(ab)2 antihuman IgG FITC (Jackson ImmunoResearch Inc., West Grove, PA) for 30 min at 4°C, then fixed in 1% paraformaldehyde. To test the specificity of the binding, the cells were incubated with Antag 2 or BN at different concentrations for 30 min before adding the BsMol. Unconjugated H22 and angiotensin (BACHEM), a structurally unrelated peptide, were used as negative controls. Samples were analyzed by FACSscan using Cellquest software (Becton-Dickinson Immunossytems, San Jose, CA).

**Western Blot Analysis.** Freshly cultured SCLC cells and peripheral blood lymphocytes were washed with PBS, lysed in the triple-detergent lysis buffer [50 mM Tris, 150 mM NaCl, 0.1% SDS, 1% NP40, 0.5% sodium deoxycholate, 100 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin (pH 8.0)]. The cell lysate at 20 μg of protein per lane was loaded onto a 10% SDS-polyacrylamide gel. After electrophoresis, the gel was transferred to a nitrocellulose membrane. The membrane was incubated first with 5% nonfat milk for 2 h and then with BsMol for 2 h at 4°C. Unconjugated H22 was used as negative control. After incubation, the membrane was washed and a peroxidase-conjugated goat F(ab)2 antihuman IgG (Jackson ImmunoResearch) was added to a final dilution of 1:1000. After 1 h of incubation, the membrane was washed extensively and incubated with a chemiluminescent reagent according to the manufacturer’s instructions (Pierce Chemical Co., Rockford, IL). The signal was detected by exposing the membrane to a Kodak film.

**Thymidine Incorporation Assay.** Cisplatin and paclitaxel were obtained from Bristol Myers-Squibb Company (Princeton, NJ). Etoposide was obtained from Bedford Laboratories (Bedford, OH). Growth inhibition of tumor cells was measured by a [%³H]thymidine incorporation assay. Cells in complete medium were seeded (2.5–5 × 10⁴/well) onto a 96-well plate. After 48 h of culture, individual drugs were added at various concentrations. Cells were incubated with each agent continuously for 72 h. [%³H]Thymidine was added during the last 8 h of incubation.

**Preparation of Immune Effector Cells.** Mononuclear cells were separated from peripheral blood of healthy donors using Ficoll-Hypaque density gradient centrifugation. After 2 h of incubation, nonadherent cells were removed. Adherent cells were cultured in RPMI 1640 containing 10% fetal bovine serum and 150 units/ml of IFN-γ overnight. Cells were detached from the culture dish by incubating with HBSS medium and 2 mM EDTA for 5 min. The phenotype of the cells and the expression of FcyRI were determined by flow cytometry. The cell preparation contained 80–90% monocytes.

**ADCC and Combined Treatment.** Target cells (T) at 2.5–5 × 10⁴/well were seeded onto a 96-well plate. IFN-γ-activated monocytes (effector cells) were added at E:T ratios of 30:1, 15:1, and 7.5:1. The BsMol or unconjugated H22 was added in a final concentration of 1 μg/ml. To evaluate the specific killing mediated by BsMol, free Antag2 was incubated with target cells at 5 μg/ml, and unconjugated H22 was incubated with effector cells at 10 μg/ml for 15 min before adding BsMol. The cell mixture and control cells (tumor cells alone) were cultured for 48 h. Individual chemotherapy agent, cisplatin, etoposide, or paclitaxel, was added and cultured continuously for another 72 h. [%³H]Thymidine was added during the last 8 h of culture. Cells were harvested by a Tomtec cell harvester (Perkin-Elmer, Downers Grove, IL) and counted in a liquid scintillation counter. All of the assays were performed in triplicate. Thymidine incorporation was calculated as: (experimental cpm – monocytes cpm)/tumor cells alone cpm × 100%. The inhibition of thymidine incorporation was calculated as: [1-(experimental cpm – monocytes cpm)/tumor cells alone cpm] × 100%.

**In Vivo Study.** Six to 8-week-old NOD.CB17-Prkd SCID mice were (Jackson Laboratory, Bar Harbor, ME) maintained in a pathogen-free facility. In each experiment, mice were divided into three groups (tumor cells alone, immunotherapy with unconjugated H22, and immunotherapy with BsMol) with 3–4 mice in each group. They were irradiated with 300cGy immediately before the injection of 1 × 10⁶ DMS273 cells i.p. on day 1. IFN-γ-activated human monocytes (1 × 10⁷) were mixed with H22 or BsMol (50 μg/mouse) and injected i.p. on days 3 and 6. All of the mice were sacrificed on day 28. The peritoneal cavity was washed with 5 ml of normal saline twice, and the peritoneal exudate was drawn into the syringe and collected for flow cytometry analysis. The abdominal cavity was then dissected and carefully inspected. All of the visible tumor nodules were resected, weighed, and fixed in 10% buffered formalin solution. They were embedded in paraffin and sectioned at 5 μm for light microscopic examination. Tumor burden was determined by: (a) total weight of all resectable tumor nodules in the peritoneal cavity; and (b) percentage of human CD15/CD56 dual-positive cells in the peritoneal exudate. DMS273 expresses both CD15 and CD56, allowing the detection of remaining SCLC cells.

**Statistics.** The Student t test was used to compare two groups of samples. The significance level was determined when P was <0.05 by two-sided analysis. The results are presented as mean ± SD.

**RESULTS**

**Specific Binding of the BsMol to SCLC Cell Lines.** The BsMol bound to 60–80% of cells from three SCLC cell lines in a dose-dependent pattern (Fig. 2). This binding profile
was consistent with previous data (17), demonstrating that the addition of a cysteine residue at the NH$_2$-terminal of the known antagonist (\(\alpha\)-Phe$^6$, Leu-NHEt$^{13}$, and des-Met$^{14}$) BN(6–14), had no adverse effect on the binding capacity. The mean fluorescence intensity, as an indirect estimation of binding sites, increased in a dose-responsive pattern. The unconjugated H22 alone did not bind to these cell lines and was used as a control.

The BsMol did not bind to human peripheral blood lymphocytes. To determine the specificity of the binding to BN/GRP-R, cells were preincubated with Antag 2 (50 \(\mu\)M), BN (50 \(\mu\)M), angiotensin (50 \(\mu\)M), or unconjugated H22 (10 \(\mu\)g/ml). The binding of BsMol to SCLC cells was partially blocked by Antag 2 (45–60%) and BN (30–40%), but was not blocked by angiotensin and H22 (Fig. 3).
On Western blot analysis, the BsMol stained a single band from three SCLC cell lines with a molecular weight of M, 75,000–80,000 (Fig. 4). Swiss 3T3 cells and human PBL were used as positive and negative control, respectively. The molecular weight of the detected protein was consistent with BN/GRP-R (6–8).

**Effect of Antag 2 and the BsMol on Cell Proliferation.** In the presence of BN, the number of SCLC colonies was increased. The growth stimulatory effect of BN was effectively blocked by the addition of Antag 2. The presence of the BsMol, H22xAntag 2 at 1–10 μg/ml, had no significant effect on the number of SCLC colonies (Fig. 5). This observation also confirms our hypothesis that the addition of a cysteine residual at the NH2 terminus had no adverse effect on the biological function of the Antag 2.

**Effect of Chemotherapy Agents on Thymidine Uptake into SCLC Cells.** Inhibition of thymidine uptake into SCLC cells was dependent on the concentration of the chemotherapy agent. After continuous exposure of 72 h, 80–100% inhibition of thymidine uptake was achieved in all three of the SCLC cell lines. The sensitivity to chemotherapy agents varied. The IC50, defined as the concentration resulting in 50% inhibition of thymidine uptake, was 5 nM, 4 nM, and 2.5 nM of paclitaxel for H345, DMS273, and H69 cells, respectively. The IC50 was 0.1 μM, 0.25 μM, and 0.5 μM of etoposide for H345, DMS273, and H69 cells, respectively. The IC50 was 0.5 μM of cisplatin for all three of the cell lines. Dose-response curves are shown in Fig. 6. To demonstrate additional inhibition of thymidine uptake from the addition of ADCC, we chose concentrations of each agent approximating IC20 to IC30 for subsequent experiments described later.

**Effect of ADCC and Combined Treatment on SCLC Cells.** Thymidine uptake into control cells incubated with medium alone for 5 days was defined as 100%. Inhibition of thymidine uptake by BsMol-mediated ADCC was dependent on E:T ratio. At E:T ratio of 30:1, 80–90% inhibition of thymidine uptake was achieved. At E:T ratio of 7:5:1, 20–40% inhibition was observed. When tumor cells were preincubated with free Antag 2 and monocytes preincubated with unconjugated H22 before adding BsMol, the inhibition of thymidine uptake was blocked by 50–70% in both DMS273 and H69 cells. When we combined ADCC with each chemotherapy agent at IC50, an additional 25–40% inhibition was consistently observed compared with chemotherapy alone. A typical experiment with an E:T ratio of 7:5:1 is presented in Fig. 7. The activity of effector cells varied from individual donors, and the sensitivity of SCLC cells to individual donor cells also varied. The summary of four experiments using effector cells from four different donors is presented in Tables 1 and 2. For DMS273 cells, chemotherapy alone resulted in 10–30% inhibition, ADCC alone in 30–40% inhibition, and combined treatment in 60–80% inhibition of thymidine uptake. Compared with chemotherapy alone, a relative increase of 50–80% was observed. Compared with ADCC alone, a relative increase of 35–60% was observed. For H69 cells, chemotherapy, ADCC, and combined treatment resulted in 20–30%, 30–40%, and 50–70% inhibition of thymidine uptake, respectively. Compared with chemotherapy and ADCC alone, combined treatment resulted in a relative increase of 35–60% and 25–50% increase of inhibition. The observed increase of inhibition was highly statistically significant.

**In Vivo Experiments.** All of the control mice injected i.p. with 1 × 10⁶ DMS273 cells had tumor growth in the peritoneal cavity by day 28. The tumor weight ranged from 0.4 to 1.4 gram/mouse. Tumor nodules were usually well capsulated. Most mice developed obstructive jaundice, without distant metastasis in the liver, spleen, gastrointestinal tract, kidney, or reproductive tissues. The total number of peritoneal exudate cells were 6.8 ± 6.4 × 10⁷, and 8.4 ± 2.3% of those cells were positive for human CD56/CD15. The timing and frequency of treatment were determined from preliminary experiments. One injection of BsMol and effector cells was not adequate for control tumor growth. The number of human monocytes injected was determined based on in vitro experiments; an E:T ratio of 10:1 was used. The results from three experiments are summarized in the Table 3. Eleven of 12 mice treated with human monocytes and unconjugated H22 had visible tumor growth. The tumor volume was not significantly different in the control mice. Mice treated with human monocytes and BsMol had significantly less tumor volume compared with the mice treated with human monocytes and H22 (P = 0.001, two-sided). Two of the 11 mice treated with human monocytes and BsMol had no macroscopic tumor in their peritoneal cavities.

**DISCUSSION**

The BN/GRP-R is expressed on malignant cells from patients with SCLC, as well as other cancers such as breast and prostate. Our novel targeting molecule is composed of a peptide...
ligand for the receptor and a mAb directed to the high-affinity Fc receptor expressed on mononuclear phagocytes and granulocyte-colony-stimulating factor-activated neutrophils. This BsMol is capable of mediating ADCC of SCLC cells. Combining the commonly used anticancer agents cisplatin, etoposide, and paclitaxel, and the BsMol targeting BN/GRP-R significantly inhibits SCLC cell growth in vitro. Targeted immunotherapy is dependent on E:T cell ratio in vitro. At a low E:T ratio, chemotherapy predominates. Monocyte activity varied among donors, as did the susceptibility of different SCLC cell lines to monocytes from different donors. Multiple variations made it difficult to determine whether combining chemotherapy with ADCC had an additive or a synergistic effect. However, a significant increase in tumor inhibition over ADCC alone was consistently observed when the E:T ratio was above 7.5:1.

We also tested the efficacy of BsMol-mediated ADCC alone in human SCLC xenografts in NOD/scid mice. In these “proof-of-principle” studies, the tumor volume of xenografted human SCLC was significantly reduced by administrating BsMol and human monocytes on days 3 and 6. Determining optimal conditions for this immunotherapy regarding timing, dosing of cells/antibody, and frequency requires additional study.

The simplified method reported here of BsMol construction is more suitable for large-scale production. We previously used a conjugation linker to create a sulfhydryl group on a

Table 1  Cytotoxicity of DMS273 SCLC cells

<table>
<thead>
<tr>
<th></th>
<th>Chemotherapy</th>
<th>ADCC</th>
<th>Combination</th>
<th>$P^b$</th>
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<tr>
<td>Etoposide</td>
<td>24 ± 14</td>
<td>36 ± 18</td>
<td>68 ± 9</td>
<td>0.005/0.03</td>
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<td>Cisplatin</td>
<td>31 ± 11</td>
<td>36 ± 18</td>
<td>70 ± 4</td>
<td>0.003/0.03</td>
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<tr>
<td>Paclitaxel</td>
<td>10 ± 8</td>
<td>36 ± 18</td>
<td>59 ± 9</td>
<td>0.01/0.02</td>
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</tbody>
</table>

$^a$ Average percentage of cytotoxicity from four experiments.  
$^b$ Chemotherapy versus combined therapy/ADCC versus combined therapy.

Table 2  Cytotoxicity of H69 SCLC cells

<table>
<thead>
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<th>Chemotherapy</th>
<th>ADCC</th>
<th>Combination</th>
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<tr>
<td>Etoposide</td>
<td>22 ± 1</td>
<td>34 ± 15</td>
<td>52 ± 9</td>
<td>0.004/0.05</td>
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<tr>
<td>Cisplatin</td>
<td>28 ± 8</td>
<td>34 ± 15</td>
<td>54 ± 5</td>
<td>0.002/0.03</td>
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<tr>
<td>Paclitaxel</td>
<td>24 ± 4</td>
<td>34 ± 15</td>
<td>63 ± 12</td>
<td>0.01/0.03</td>
</tr>
</tbody>
</table>

$^a$ Average percentage of cytotoxicity from four experiments.  
$^b$ Chemotherapy versus combined therapy/ADCC versus combined therapy.
BN/GRP peptide, a method requiring labor-intensive and time-limiting purification of intermediate products twice by high-performance liquid chromatography. Because the COOH terminal of BN/GRP is the active binding site, we hypothesize that manipulating the NH2 terminus may not interfere with binding and functioning of BN/GRP. We designed a BN/GRP antagonist, adding a cysteine residue to the NH2 terminus of the peptide (o-Phe1, Leu-NHEt13, and des-Met14) BN(6–14). The cysteine provides a sulfhydryl group for chemical conjugation. This BN/GRP antagonist with cysteine residue on the NH2 terminus maintains its functions by inhibiting the growth stimulatory effect of BN on SCLC cells. The new construction is a simple two-step procedure without high-performance liquid chromatography purification. The synthetic BsMol, H22xAntag2, binds specifically to BN/GRP-R on SCLC cells. On Western blot analysis, the BsMol stains a single band of protein consistent with the molecular weight of BN/GRP-R. These data support our hypothesis that the addition of a cysteine residue at the NH2 terminus of the BN/GRP antagonist does not alter its biological functions. The new method simplifies the chemical conjugation process.

Cisplatin and etoposide both are the most effective and commonly used chemotherapeutic drugs against SCLC. Paclitaxel is also an active chemotherapy agent to treat SCLC. As a single agent, the overall response to paclitaxel was 53–68% (34). Phase II studies combining paclitaxel with the platinum and etoposide regimen reported response rates of 71–100% in both limited-stage and extensive-stage SCLC (34–36). In vitro data suggest that binding of BN/GRP-R increased expression of epidermal growth factor receptor in SCLC cells; the BN/GRP-R antagonist inhibited tumor growth by down-regulating epidermal growth factor receptor (37–38). Because chemotherapy and immunotherapy act through different mechanisms, such a combination is likely to increase tumor cell killing and overcome resistance to chemotherapy.

Targeting BN/GRP-R is an attractive treatment option for SCLC. Although BN/GRP-R is expressed on normal tissues and is involved in a number of physiological functions, the administration of a BN/GRP-R antagonist had only minimal adverse effects in animals (5). Our new approach of immunotherapy targeting FcγRI and recruiting immune effector cells, including monocytes, macrophages, and activated neutrophils, has potential clinical application. Additional studies of this BsMol in the treatment of SCLC are warranted.

**References**


### Table 3

<table>
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<tr>
<th>Exp.</th>
<th>Groups</th>
<th>Mice</th>
<th>Body weight (g)</th>
<th>Tumor weight (g)</th>
<th>PECw</th>
<th>Pw</th>
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<tr>
<td>1</td>
<td>Control</td>
<td>4</td>
<td>31.0 ± 1.2</td>
<td>0.71 ± 0.37</td>
<td>7.4 ± 4.3</td>
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<td>Monocytes + H22</td>
<td>4</td>
<td>28.5 ± 1.4</td>
<td>0.63 ± 0.22</td>
<td>5.1 ± 1.9</td>
<td>0.22</td>
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<td>29.9 ± 1.2</td>
<td>0.09 ± 0.05</td>
<td>2.2 ± 1.6</td>
<td>0.03</td>
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<tr>
<td>2</td>
<td>Control</td>
<td>4</td>
<td>19.5 ± 2.5</td>
<td>1.07 ± 0.29</td>
<td>17.5 ± 12.7</td>
<td>0.14</td>
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<td>22.5 ± 2.3</td>
<td>0.59 ± 0.55</td>
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<td>23.6 ± 0.9</td>
<td>0.05 ± 0.05</td>
<td>2.7 ± 1.8</td>
<td>0.03</td>
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<tr>
<td>3</td>
<td>Control</td>
<td>3</td>
<td>29.0 ± 1.0</td>
<td>0.49 ± 0.09</td>
<td>12.9 ± 0.5</td>
<td>0.03</td>
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<tr>
<td></td>
<td>Monocytes + BsMol</td>
<td>3</td>
<td>26.8 ± 3.0</td>
<td>0.10 ± 0.09</td>
<td>0.4 ± 0.1</td>
<td>0.03</td>
</tr>
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</table>

w PEC, peritoneal exudate cells represented as percentage of human CD15/CD56 positive cells.
w P comparing tumor weights from mice treated with monocytes + BsMol to those with monocytes + H22.

One mouse died before day 28.
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