Several immunotoxins are currently in clinical trials or preclinical hybrid proteins that consist of antibody variable fragments. Moxetumomab paseudotox targeting CD22 produces complete and is highly expressed in all MM cells from patients. Because of the restricted expression of BCMA to plasma cells and its role in growth as well as cell survival of MM, the BCMA antigen has been investigated as the targets in various immunotherapeutic strategies. These include antibody-based therapy,\(^9\) chimeric antigen receptor therapy\(^8\) and therapy with BiTEs.\(^10\)

To develop immunotoxins that target BCMA we have generated a panel of monoclonal antibodies (mAbs) by immunizing mice with recombinant BCMA protein using hybridoma technology. We produced hybridomas producing anti-BCMA mAbs as described (Supplementary Materials and Methods). Because BCMA, TACI and BAFFR and BCMA share the same natural ligands, we tested the reactivity of each anti-BCMA mAb with two structurally closely-related TNFRs (TACI or BAFFR) expressed on transfected 293 T cells by flow cytometry (Supplementary Figure S1A) and TNFR:Fc fusion proteins by enzyme-linked immunosorbent assay (Supplementary Figure S1B). Based on this analysis, we selected BM24 and BM306 because they bind to BCMA antigen on the cell surface with high affinity and specificity. The binding affinity (KD) of both mAbs are \(<1 \times 10^{-10}\) M. We cloned the VH and the VL \(-\) oligo primers\(^11\) and used the LR version of the PE indicates.

Multiple myeloma (MM) is a B-cell malignancy that originates in the bone marrow (BM). Although there are FDA-approved antibody-based therapies available for the treatment of some B-cell malignancies, no very effective antibody-based therapy is yet available for MM.\(^6\) The B-cell maturation antigen (BCMA) belongs to the tumor necrosis factor receptor (TNFR) superfamily and is highly expressed in all MM cells from patients.\(^7\) Because of the restricted expression of BCMA to plasma cells and its role in growth as well as cell survival of MM, the BCMA antigen has been investigated as the targets in various immunotherapeutic strategies. These include antibody-based therapy,\(^9\) chimeric antigen receptor therapy\(^8\) and therapy with BiTEs.\(^10\)

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### Supplementary Information

Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

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**Recombinant immunotoxins targeting B-cell maturation antigen are cytotoxic to myeloma cell lines and myeloma cells from patients**

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Novel antibody-based therapies for cancer are predictably effective if they can target cancer cells without damaging normal organs.\(^1\) We have developed various recombinant immunotoxins (RITs) against different targets on cancer cell surfaces.\(^2\) RITs are hybrid proteins that consist of antibody variable fragments attached to a truncated portion of *Pseudomonas* Exotoxin A (PE). Several immunotoxins are currently in clinical trials or preclinical development.\(^2\)-\(^4\) We have reported previously that immunotoxin moxetumomab paseudotox targeting CD22 produces complete remissions in many patients with refractory hairy cell leukemia.\(^5\) This agent has recently completed a phase 3 trial. In addition, a RIT that targets mesothelin showed promising clinical responses in patients with chemotherapy-resistant malignant mesothelioma.\(^6\)

Multiple myeloma (MM) is a B-cell malignancy that originates in the bone marrow (BM). Although there are FDA-approved antibody-based therapies available for the treatment of some B-cell malignancies, no very effective antibody-based therapy is yet available for MM.\(^6\) The B-cell maturation antigen (BCMA) belongs to the tumor necrosis factor receptor (TNFR) superfamily and is highly expressed in all MM cells from patients.\(^7\) Because of

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encoding the immunotoxins is shown (Supplementary Figure S1C and D). After expression and purification, we obtained highly purified RITs for both BM24 and BM306. The corresponding immunotoxins are named LMB38 and LMB70, respectively. A sodium dodecyl sulfate gel showing that the immunotoxins are highly purified is shown (Supplementary Figure S1E).

We tested the cytotoxic activity of LMB38 and LMB70 on BCMA expressing cell lines using a cell proliferation assay (WST-1).
Representative cell-killing curves are shown for LMB70 (Figure 1a) and LMB38 (Supplementary Figure S1F). The IC\textsubscript{50} values are summarized in Table 1. The IC\textsubscript{50} of LMB38 on H929, U266B, JJN3, RPMI-8226, LP-1 and KMS-18 are 1.2, 1.9, 2.5, 6.9, 25 and 55 ng/ml, respectively. Similarly, the IC\textsubscript{50} values for LMB70 on those cell lines are 1.1, 1.9, 2.5, 6.9, 25 and 55 ng/ml, respectively. LMB38 and LMB70 have no activity on Jeko-1 and the HUT-102 cell line that is BCMA negative.

Because WST-1 assays measure both cell growth inhibition and cell death, we measured the cell killing by 3 days incubation with LMB70 for indicated time, washed three times and incubated further with complete media. Three days after seeding, cells were stained with Annexin V PE and 7-ADD and cell viability was analyzed using flow cytometry. Annexin V- and 7AAD-negative cells were considered viable.

To determine whether the apoptosis pathway is induced after exposure of H929 cells to LMB70, we performed western analysis of proteins involved in apoptosis. As shown in Supplementary Figure S2D, the level of Mcl-1 and Bcl-XL was markedly diminished after 6-h exposr of immunotoxin. Also, Caspase 3, 8 and 9 underwent cleavage during the 6-h period. These changes are consistent with rapid induction of apoptosis.

Conflicts of Interest

This study was supported by grants from the National Cancer Institute, National Institutes of Health, Cancer Biology Research, Bethesda, MD, USA; American Cancer Society, and the American Association for Cancer Research, and the T32 Training grants RODS from the National Institute of Health. The remaining authors declare no conflicts of interest.
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