Published in final edited form as:
*Leuk Res*. 2014 October ; 38(10): 1224–1229. doi:10.1016/j.leukres.2014.06.014.

# An improved recombinant Fab-immunotoxin targeting CD22 expressing malignancies

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## Abstract

Moxetumomab pasudotox (HA22) is a recombinant immunotoxin, now in clinical trials, that combines an anti-CD22-Fv with a 38-kDa fragment of *Pseudomonas* exotoxin A. To produce a less immunogenic molecule without reducing the half-life in circulation, we constructed LMB11 combining an anti-CD22 Fab with a less immunogenic version of PE38. We found that LMB11 retains full activity toward CD22-expressing cells. In mice, the half-life of LMB11 is 29 minutes and the antitumor activity of LMB11 is better than that of HA22. Because it can be safely given at much higher doses, LMB11 produced complete tumor remissions in 7/7 mice.

### Keywords

B-cell malignancies; Moxetumomab pasudotox; Less immunogenic PE38

## 1. Introduction

Antibody based therapies have made a major impact on the treatment of malignancies. Twelve unlabeled antibodies are now approved for the treatment of various types of cancer [1,2]. Monoclonal antibodies are also being used for the delivery of low molecular weight cytotoxic agents, radioisotopes and protein toxins [3]. Our laboratory has focused on development of immunotoxins containing a portion of *Pseudomonas* exotoxin A (PE)[1] to kill cancer cells [4,5]. We call these agents recombinant immunotoxins (RITs), because

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Contributions
T.K.B. designed research, carried out experiments, and wrote the paper; M.O. carried out all animal study and antigenicity assays and supplied data; R.J.K. Assayed immunotoxins on cells from patients; and I.P. conceived experiments and supervised their performance and wrote the manuscript. All authors approved the final version of the manuscript.

Conflict of Interest
I. Pastan and R. J. Kreitman are the inventors of many immunotoxin patents, all of which have been assigned to NIH. The authors declare no conflicts of interest.

[1]PE; *Pseudomonas* exotoxin A; RITs; recombinant immunotoxins; Moxe, Moxetumomab pasudotox; SCID, severe combined immunodeficiency

recombinant DNA techniques are used to produce these agents. Moxe (Moxetumomab pasudotox) is a RIT in which the Fv portion of an antibody reacting with CD22 is fused to a 38-kDa fragment of PE (Figure 1A). Moxe has produced complete remissions in nearly 50% of patients with drug resistant HCL [6,7]; on the basis of this high response rate it is now in a pivotal phase III trial for this disease. Moxe also has produced complete remissions in children with refractory ALL [8,9]. We believe that Moxe is effective in these chemo-refractory patients due to its unique mechanism of action, which is to induce apoptosis by inhibiting protein synthesis; thus Moxe is able to kill drug resistant cells.

Although active in patients with HCL and ALL, Moxe and SS1P (anti-mesothelin Fv-PE38), a similar immunotoxin that targets mesothelin expressed on many human solid tumors, can cause capillary leak syndrome, which limits the amount of immunotoxin that can be given. These two agents contain a 38-kDa portion of native PE, which is very immunogenic to humans and as a result neutralizing antibodies to the immunotoxin form and limit the number of cycles that can be given to patients. To overcome these problems, recent efforts in our laboratory have focused on making immunotoxins that have fewer side effects and lower immunogenicity. We have done this by identifying and removing portions of the toxin that are not necessary for activity or are responsible for immunogenicity [10,11]. Moxe contains a 38-kDa fragment of PE, that is composed of domains II and III of PE (Figure 1A). Domain III contains the ADP-ribosylating activity that catalyzes the inactivation of elongation factor 2, arrest of protein synthesis and cell death. Domain II contains the furin cleavage site necessary to separate domain III from the Fv, once the immunotoxin enters the cell. We reasoned that removal of cathepsin cleavage sites within PE38 would diminish the number of peptides available for presentation by MHC class II to T cells and hence lower immunogenicity. We found that the major cleavage sites were located in domain II and removed most of domain II except for the furin cleavage site of HA22 to yield HA22-LR (Figure 1B). HA22-LR has much less non-specific toxicity and is also less immunogenic in mice [10, 11]. Consequently HA22-LR can be given to mice at much higher doses than HA22 and produces better antitumor effects [11]. However the LR immunotoxins with a deletion of domain II are small (molecular weight 48-kDa) and as a consequence are rapidly filtered by the kidney having a very short half-life in the blood [10].

To further decrease the immunogenicity of HA22 we mapped the mouse and human B cell epitopes [12, 13]. Deletion of domain II removes several of these epitopes. B cell epitopes in domain III were silenced by mutating arginine residues on the surface of the protein to alanine, producing an immunotoxin named HA22 dsFv-LR-LO10-458R456A (LMB10); its structure is shown in Figure 1C.

The current study was designed to produce an immunotoxin with a much longer half-life in the circulation using a Fab instead of an Fv (Figure 1D). The resulting immunotoxin (LMB11) should be useful in treating patients with B cell malignancies, because it would have a longer half-life in the blood, it could be given safely at high doses without causing capillary leak syndrome, and it would have low immunogenicity so more treatment cycles could be given.

## 2. Material and Methods

### 2.1. Construction, expression and purification of LMB11

To make a Fab-immunotoxin targeting CD22 expressing cells that contained mutations that silenced B cell epitopes, we constructed two expression plasmids, pTKB Vh and pTKB Vl. The pTKB Vh was constructed to produce the Fd fragment fused to toxin and the pTKB Vl was constructed to produce the light chain [14]. Schematic diagrams of the plasmids used in this study are shown in Figure 2A. The Fd chain containing the Vh sequence of HA22 and the CH1 sequence of human IgG1 was synthesized (Genscript USA Inc) after codon optimization for *E. coli* expression. The light chain containing the Vl sequence of HA22 and the human Cκ constant region was also synthesized after codon optimization for *E. coli* expression. The Fd and the light chain fragment were cloned into an immunotoxin expression vector at NdeI-HindIII and NdeI-EcoRI site, respectively. Plasmids encoding different components of LMB11 were expressed separately in *E. coli* BL21 (λDE3) cultures as described previously [15]. All recombinant proteins accumulated in large amounts as insoluble intracellular inclusion bodies. Soluble recombinant immunotoxins were recovered using a purification scheme that includes ion exchange and size exclusion chromatography previously established for purification of single-chain immunotoxins [15]. The purity of the recombinant immunotoxin collected after passing through the sizing column was assessed by SDS-PAGE analysis by following standard procedure. Briefly, 2 μg of purified LMB11 protein was suspended in SDS-gel loading buffer (Quality Biologicals) with or without 2-mercaptoethanol (Sigma) and analyzed in 4-20% gradient SDS-PAGE. Two μg of pure BSA (bovine serum albumin) was loaded as a reference protein in the gel.

### 2.2. 3D structure of Fv, Fab and PE domains

The predicted 3D structures of various immunotoxin molecules described in Figure 1 were modeled by identifying the closest match of the known antibody structure deposited in the database (http://www.bioinf.org.uk/abs/structures.html). First the sequence of the Vh and Vl of HA22 was aligned with antibody proteins with known structure in the database. The best pdb structure that matched with Vh sequence of HA22 is from 1hil.pdb and for Vl sequence the best match is from 1fbi.pdb. Those pdb files were used to predict the 3D structure of the Fv fragment of HA22 using Discovery Studio software. This modeled Fv fragment was then complexed with Domains II and III of the PE structure (1hkl.pdb), through a loop of 16 amino acid residues, using the Chimera Visualization software.

### 2.3. Cell lines

CD22-positive human Burkitt's lymphoma cell lines (CA46 and Raji) were obtained from ATCC. Acute B-lymphoblastic leukemia (HAL-1, KOPN-8 and SEM) and SuDHL-6 (Diffuse large B-cell lymphoma) cell lines were obtained from Dr. Alan Wayne (National Cancer Institute, Bethesda, MD). REC-1 and JVM-2 (Mantle cell lymphoma) were obtained from Dr. Louis M. Staudt (National Cancer Institute, Bethesda, MD). All cell lines were grown at 37°C with 5% $CO_2$ in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM of sodium pyruvate, 100 U penicillin, and 100 mg streptomycin (Invitrogen).

### 2.4. Cell viability assay

Survival of cells treated with immunotoxins was measured by WST-8 assay using the Cell Counting Kit-8 (Dojindo Molecular Technologies) essentially as described [10]. Briefly, $10^4$ cells/well were incubated with various concentrations of immunotoxin in a 96-well plate for 72 hours, after which 25 μL of the CCK-8 reagent was added to the wells. Plates were incubated until the wells with the maximum absorbance at 450 nm reached values of approximately 1 optical density. Cycloheximide (100 μg/mL final concentration) was used as a control for 100% cell death. Values were normalized between the cycloheximide and PBS controls and fit to a standard 4-parameter sigmoidal equation with a variable slope using the GraphPad Prism (version 2.00; GraphPad Software) program to obtain the concentration of immunotoxin at which there was 50% cell death ($IC_{50}$).

Cells from patients with CLL and HCL were assayed by ATP assay using CellTiter-Glo Reagent (Promega Cat. # G7573) as previously described [10]. Briefly, patient cells were incubated with RITs and control molecules in 3-10 $\times 10^4$/30 μl/well aliquots in U-bottom 96-well plates for 3 days. Each well was pulsed with CellTiter-Glo reagent (10 μl/well) for an additional 30 minutes and the luminescent signal measured. The $IC_{50}$ was defined as the concentration of RIT necessary for 50% inhibition of cell growth. Fifty percent inhibition was defined as the midpoint between maximal inhibitions, as assessed by incubation with cycloheximide 10,000 ng/ml, and no inhibition, as assessed by incubation with media alone.

### 2.5. Thermal stability

To determine their thermal stability, the RITs (20 μg/ml) were heated to various temperatures ranging from 25°C to 80°C for 15 minutes using a thermal cycler. Samples were placed on ice immediately after heat treatment and later evaluated for activity using WST-8 cell viability assay.

### 2.6. Pharmacokinetics

Groups of four female Balb/c mice were injected intravenously with 10 μg of LMB11 or HA22-LR in 0.1 ml containing 0.2% mouse serum albumin in D-PBS. Blood samples were drawn at different times. Sera were analyzed by ELISA as previously described [16].

### 2.7. Antitumor activity

The antitumor activity of RITs was determined in severe combined immunodeficiency (SCID) mice bearing CA46 lymphomas. Cells ($10^7$) with matrigel (0.8 mg) were injected subcutaneously into SCID mice on day 0. Tumors more than 125 $mm^3$ in size developed by day 7. Starting on day 7, animals were treated with intravenous injections of each RIT diluted with 0.1 ml of PBS/0.2% mouse serum albumin in treatment groups of seven mice. RIT was administered once every other day (on days 7, 9, 11, 13 and 15). Tumors were measured with a caliper every 2 or 3 days and the volume of the tumor was calculated by using the formula: tumor volume ($mm^3$) = length × $(\text{width})^2 \times 0.4$. The animal protocol was approved by the National Cancer Institute Animal Care and Use Committee.

### 2.7. Antigenicity assay

Binding of LMB11 or Moxe to antibodies in human sera was measured in a displacement assay as previously described [12]. Briefly, human sera were obtained under National Institutes of Health Institutional Review Board-approved protocols. Mesothelin-rFc was coated on ELISA plates (50 ng in 50 μl PBS/well) and incubated overnight at 4°. After washing, SS1P (50 ng in 50 μl blocking buffer/well) was added and incubated for 1 hour. In separate tubes, diluted sera were mixed with 2 μg/ml of Moxe or LMB11 and incubated overnight at 4° (immunotoxin-antibody mixture). The SS1P containing plates were washed and followed by the addition of 50 μl of immunotoxin-antibody mixture to each well. Human antibody that did not bind to Moxe or LMB11 was captured by SS1P and quantified using horseradish peroxidase-conjugated rabbit anti-human IgG Fc (Jackson ImmunoResearch Laboratories), followed by a TMB substrate kit (Thermo Scientific). The absorbance at 450 nm was measured with the reference at 650 nm. Binding curves were fit using a four-parameter logistic curve model by SoftMaxPro 4.0 (Molecular Devices). The $IC_{50}$ values indicate the concentration of RIT that inhibit 50% of the antibody reactivity with SS1P.

## 3. Results

### 3.1. Preparation and purification of anti CD22 Fab immunotoxin LMB11

The construction of LMB11 is described in Material and Methods. A diagram showing its structure and location of mutations is shown in Figure 1D. The protein was prepared by ion exchange and size exclusion chromatography from re-natured inclusion bodies as previously described for immunotoxins containing an Fv [15]. Purified protein migrates as a single peak on size exclusion chromatography (data not shown) and the final protein is highly purified when analyzed by SDS PAGE (Figure 2B). A typical yield is 5% of the protein used in the initial refolding step. Most of the material lost is due to aggregation of improperly re-natured protein.

### 3.2. Activity of LMB11 on CD22 expressing cells

We evaluated the activity of immunotoxin LMB11 on eight different CD22-positive human B cell lines of different lineages. Cells were incubated with a series of concentrations of LMB11, Moxe or HA22-LR for 72 hours, and cell viability measured by a WST-8 assay. Figure 3A shows a representative cell viability assay using the Burkitt's lymphoma cell line CA46. The $IC_{50}$ of LMB11 is 0.6 ng/ml and the $IC_{50}$ value for HA22-LR is 0.4 ng/ml in this cell line. In another Burkitt's lymphoma cell line (Raji) the $IC_{50}$ values for LMB11 and HA22LR are 0.21 and 0.13 ng/ml, respectively. The $IC_{50}$ values and the relative activity of LMB11 compared to HA22-LR in other cell lines are summarized in Table 1. Compared with HA22-LR, LMB11 displays excellent cytotoxic activity on all cell lines tested. There is no activity of LMB11 or HA22-LR on CD22 negative cell line A431/H9.

To determine whether LMB11 is also active on cells obtained directly from patients, we tested cells from eight CLL and four HCL patients by ATP assay which is an indicator of cell growth. The data in Table 2 shows that activity of LMB11 is comparable with the

activity of Moxe in every sample tested indicating that LMB11 retains its activity on cells obtained directly from patients.

### 3.3. Thermal stability of LMB11

To be sure that LMB11 was suitable for clinical use we assessed its stability by incubating the immunotoxin protein for 15 minutes at various temperatures ranging from 23° to 80°. As shown in Figure 3B, LMB11 and HA22LR were both very stable and behaved similarly, losing 50% of their activity after incubation at 65°.

### 3.4. Pharmacokinetics of LMB11 in mice

To assess the half-life of LMB11, Balb/c mice were injected with 10 μg of LMB11 or HA22-LR and bled at various intervals between 2 and 120 minutes. We measured the concentration of RIT in mice sera by ELISA as described previously [13]. Data were fit to a single exponential decay function (Figure 4A). LMB11 has a half-life of 28 minutes and HA22-LR a half-life of 10 minutes. We attribute the difference in half-life to a difference in molecular weight because HA22-LR is small and rapidly filtered by the kidney, whereas LMB11 is not. LMB11 has a molecular weight of 73.1 kDa whereas HA22-LR has a molecular weight of 50.5 kDa.

### 3.5. Antitumor activity of LMB11 in mice

The antitumor activity of LMB11 was evaluated in a mouse xenograft model in which CA46 cells grow as subcutaneous tumors [17]. Treatment was begun on day 7, when the tumors reached approximately 150 to 175 $mm^3$ in size. RITs were injected intravenously, every other day × 5. In the first experiment the activity of LMB11 was compared with equimolar amounts of three other immunotoxins in which the toxin is connected to a dsFv instead of a Fab (Figure 1A, 1B and 1C). These are Moxe, HA22-LR (that has a deletion of domain II), and LMB10 (HA22-LR with the same mutations in domain III as LMB11). We used an equimolar concentration of all the immunotoxins to account for the maximum safe dose of Moxe at 6 μg per dose. LMB11 and HA22-LR had similar antitumor activities, whereas Moxe and LMB10 were somewhat less active (Figure 4B).

Because LMB11 contains a modified PE38 toxin which is less toxic to mice because a large portion of domain II is deleted (10) we were able to safely use a high dose (1.5 mg/kg × 5) and found that the tumors in all seven mice treated in this group had completely regressed by day 18 and had not regrown on day 32 (Figure 4C). In the treated mice there was no weight loss and no deaths or other toxicities (Table 3). The body weights of the LMB11 treated group were similar to that of the PBS injected group, indicating 1.5 mg/kg of LMB11 was a non-toxic dose for mice.

### 3.6. Immunogenic properties of LMB11

LMB11 is engineered as a low immunogenic molecule against humans by silencing human B cell epitopes. For this reason an immunogenicity study using a mouse model is not possible because the immune system of mice is different from humans. Because we are unable to test the immunogenicity of LMB11 in humans, we assessed its antigenicity; that is the binding of LMB11 to antibodies in the blood of patients who were treated with

immunotoxins and developed antibodies to the immunotoxin HA22 (Moxe) as a surrogate measure of the immune response (13). We previously showed that immunogenicity and antigenicity were closely related in mice (12). To do this we performed a competition ELISA assay in which we measured the concentration of RIT that reduced the level of patient serum antibodies reacting with Moxe by 50% [12]. We analyzed sera from nine patients treated with Moxe. Figure 5 shows that relative to Moxe, the binding of LMB11 was reduced in all patients and reduced more than 100-fold in five of nine patients and more than 10-fold in seven of nine patients indicating the antigenicity of LMB11 is significantly less than that of Moxe.

## 4. Discussion

The primary goal of this study was to make an improved version of recombinant Moxe immunotoxin targeting the B-cell differentiation antigen CD22. CD22 is expressed on many B-cell malignancies including HCL, CLL, ALL and non-Hodgkin's lymphoma, making it an attractive therapeutic target. Moxe contains an affinity improved Fv reactive with CD22 fused to PE38. Moxe has been tested in Phase I/II clinical trials to treat drug resistant HCL and CLL patients with very promising responses. However, many patients developed neutralizing antibodies after a few cycles of treatment against the PE portion of the immunotoxin, making it difficult to achieve complete remission in these patients. Recently we have re-engineered the PE38 portion of Moxe by removing the B-cell epitopes to make it less immunogenic in patients. The resulting molecule (LMB10) is 48-kDa in size and cleared from the mouse blood very fast with a half-life of only 10 minutes. We describe in this manuscript the construction and characterization of LMB11 by replacing Fv with a Fab fragment targeting CD22. As for all other therapeutic drugs, the half-life and stability of the immunotoxin is an important factor for its efficacy. Due to the presence of the constant domain of the heavy and light chain, half-life of the LMB11in mouse blood is higher than the Fv containing immunotoxin. As described in Figure 3B, LMB11 is also very stable when incubated at various temperatures.

Non-specific dose-limiting toxicity and the neutralizing antibody formation of PE-based immunotoxins is a major obstacle to successful RIT therapy. We often encounter neutralizing antibodies in patients treated with multiple cycles of Moxe. LMB11 contains a less immunogenic form of PE and in antigenicity assays, its reactivity was reduced in the sera of seven out of nine patient's evaluated.

Due to its larger size LMB11 remains in the mouse blood longer with a half-life of 28 minutes (Fig. 4A). When we inject 2.0 mg/kg of Moxe, all five mice died quickly (10). We did not measure the exact LD50 value, but we were able to give five cycles of a larger dose (1.5 mg/kg) of LMB11 to mice without any sign of weight loss or any other sign of illness (Table 3). It is important to point out that most of the anti-human CD22 antibodies including the one used in this study do not cross-react with murine CD22 and therefore limits the assessment of toxicity and pharmacokinetics properties of the therapeutic agent in a murine model. Because CD22 is only expressed on B cells that are regenerated when immunotoxin therapy is stopped; we believe LMB11 will be well tolerated in humans and that it will have good antitumor activity.

## Acknowledgments

Funding

This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

## References

1. Bast, RC.; Zalutsky, MR.; Kreitman, RJ.; Frankel, AE. Monoclonal serotherapy. Cancer Medicine. Kufe, Dw; Bast, RC.; Hait, WN., et al., editors. Vol. 7. B. C. Decker, Inc; Hamilton, Ontario: 2006. p. 770-85.
2. Scott AM, Allison JP, Wolchok JD. Monoclonal antibodies in cancer therapy. Cancer Immun. 2012; 12:14. [PubMed: 22896759]
3. Modjtahedi H, Ali S, Essapen S. Therapeutic application of monoclonal antibodies in cancer: advances and challenges. Br Med Bull. 2012; 104:41–59. [PubMed: 23118261]
4. Chaudhary VK, Queen C, Junghans RP, Waldmann T, FitzGerald DJ, Pastan I. A recombinant immunotoxin consisting of two antibody variable domains fused to Pseudomonas exotoxin. Nature. 1989; 339:394–7. [PubMed: 2498664]
5. Pastan I, Hassan R, Fitzgerald DJ, Kreitman RJ. Immunotoxin treatment of cancer. Annu Rev Med. 2007; 58:221–37. [PubMed: 17059365]
6. Kreitman RJ, Stetler-Stevenson M, Margulies I, Noel P, Fitzgerald DJ, Wilson WH, et al. Phase II trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with hairy cell leukemia. J Clin Oncol. 2009; 27:2983–90. [PubMed: 19414673]
7. Kreitman RJ, Tallman MS, Robak T, Coutre S, Wilson WH, Stetler-Stevenson M, et al. Phase I trial of anti-CD22 recombinant immunotoxin moxetumomab pasudox (CAT-8015 or HA22) in patients with hairy cell leukemia. J Clin Oncol. 2012; 30:1822–8. [PubMed: 22355053]
8. Wayne AS, Kreitman RJ, Findley HW, Lew G, Delbrok C, Steinberg SM, et al. Anti-CD22 immunotoxin RFB4(dsFv)-PE38 (BL22) for CD22 positive hematologic malignancies of childhood: pre-clinical studies and Phase I clinical trial. Clin Cancer Res. 2010; 16:1894–903. [PubMed: 20215554]
9. FitzGerald DJ, Wayne AS, Kreitman RJ, Pastan I. Treatment of hematologic malignancies with immunotoxins and antibody-drug conjugates. Cancer Res. 2011; 71:6300–9. [PubMed: 21998010]
10. Weldon JE, Xiang L, Chertov O, Margulies I, Kreitman RJ, FitGerald DJ, et al. A protease-resistant immunotoxin against CD22 with greatly increased activity against CLL and diminished animal toxicity. Blood. 2009; 113:3792–800. [PubMed: 18988862]
11. Hansen JK, Weldon JE, Xiang L, Beers R, Onda M, Pastan I. A recombinant immunotoxin targeting CD22 with low immunogenicity, low nonspecific toxicity, and high antitumor activity in mice. J Immunother. 2010; 33:297–304. [PubMed: 20445350]
12. Onda M, Beers R, Xiang L, Lee B, Weldon JE, Kreitman RJ, et al. Recombinant immunotoxin against B-cell malignancies with no immunogenicity in mice by removal of B-cell epitopes. Proc Natl Acad Sci USA. 2011; 108:5742–7. [PubMed: 21436054]
13. Liu W, Onda M, Lee B, Kreitman RJ, Hassan R, Xiang L, et al. Recombinant immunotoxin engineered for low immunogenicity and antigenicity by identifying and silencing human B-cell epitopes. Proc Natl Acad Sci USA. 2012; 109:11782–7. [PubMed: 22753489]
14. Bera TK, Pastan I. Comparison of recombinant immunotoxin against $Le^Y$ antigen expressing tumors: influence of affinity, size and stability. Bioconjug Chem. 1998; 9:736–43. [PubMed: 9815167]
15. Pastan I, Beers R, Bera TK. Recombinant immunotoxins in the treatment of cancer. Methods Mol Biol. 2004; 248:503–18. [PubMed: 14970517]
16. Bang S, Nagata S, Onda M, Kreitman RJ, Pastan I. HA22 (R490A) is a recombinant immunotoxin with increased antitumor activity without an increase in animal toxicity. Clin. Cancer Res. 2005; 11:1545–50. [PubMed: 15746059]

17. Kreitman RJ, Wang QC, FitGerald DJ, Pastan I. Complete regression of human B-cell lymphoma xenografts in mice treated with recombinant anti-CD22 immunotoxin RFB4(dsFv)-PE38 at doses tolerated by cynomolgus monkeys. Int J Cancer. 1999; 81:148–55. [PubMed: 10077166]

## HIGHLIGHTS

- LMB11 has a Fab toxin targeting CD22 expressing cancer
- LMB11 has a human Fab to increase half-life in mice
- LMB11 has mutations to decrease immunogenicity
- LMB11 can be safely given at high doses and produces total tumor regression

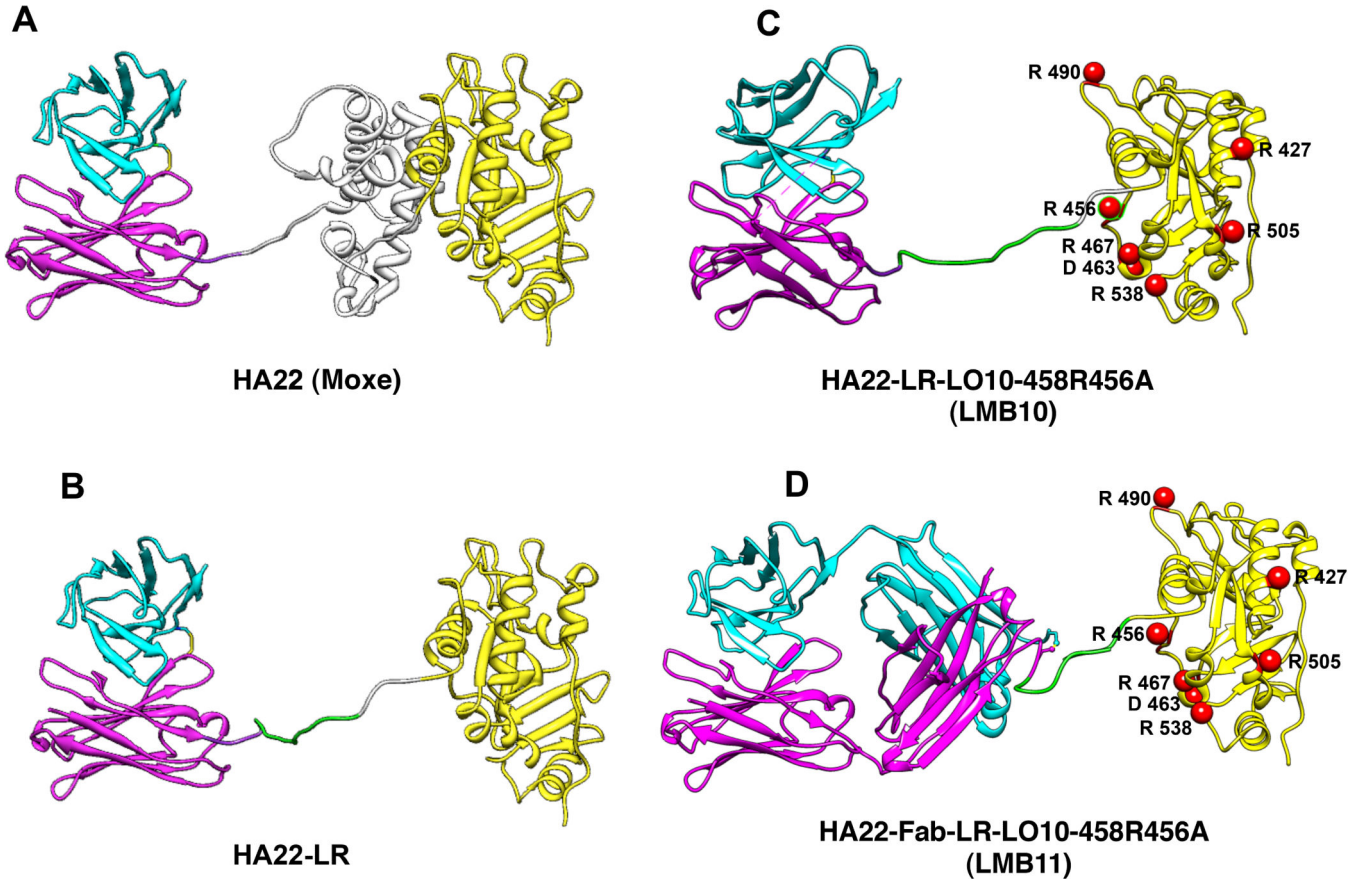


**Fig. 1.**

Ribbon drawing of HA22 immunotoxin variants. The light chain (cyan) and the heavy chain (magenta) were modeled using the X-ray structure data from 1fbi.pdb and 1hil.pdb, respectively. Domains II and III of PE data were taken from 1hkl.pdb and are represented in grey and yellow, respectively. The numbers in domain III represent the mutated residues. The linker between Fv and the toxin containing the furin cleavage site is green. Length and conformation of the linker were chosen arbitrarily.

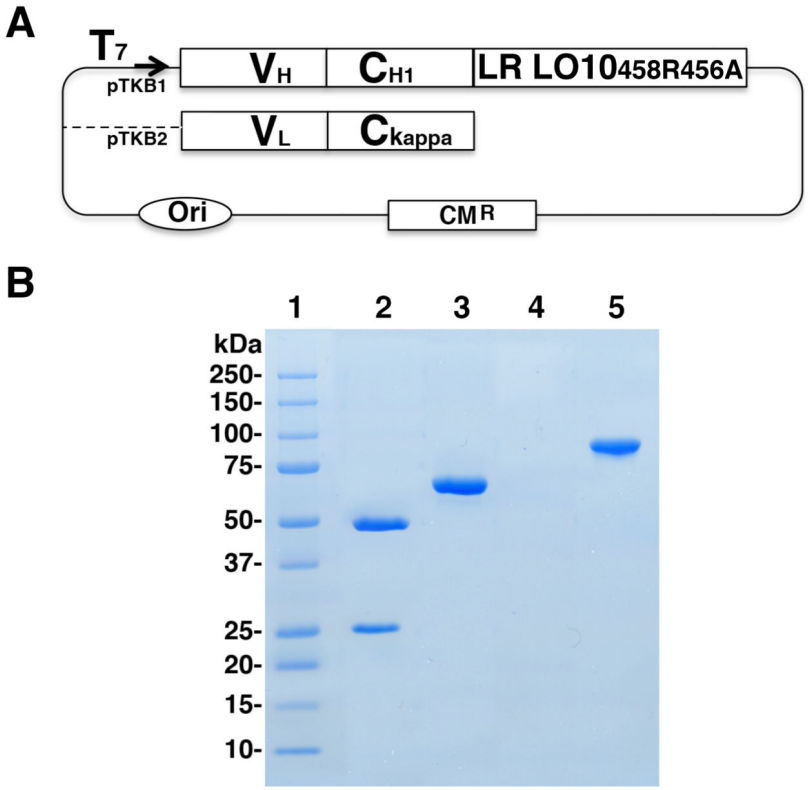


**Fig. 2.**

Plasmids for expression and SDS-PAGE of the LMB11 immunotoxin. A. The plasmid pTKB1 encodes fusion protein of the Vh portion of Moxe and the CH1 sequence of human IgG1 with a modified PE toxin LR-LO10-458R456A. The plasmid pTKB2 encodes fusion protein of the Vl fragment of Moxe and human Cκ sequences. B. SDS PAGE analysis of purified immunotoxin. The purified proteins (2 μg) were analyzed in 4-20% gradient SDS polyacrylamide gel under reducing (lanes 2 and 3) and non-reducing (lane 5) conditions. Lane 1, standards; Lane 2, LMB11; Lane 3, BSA; Lane 5, LMB11.

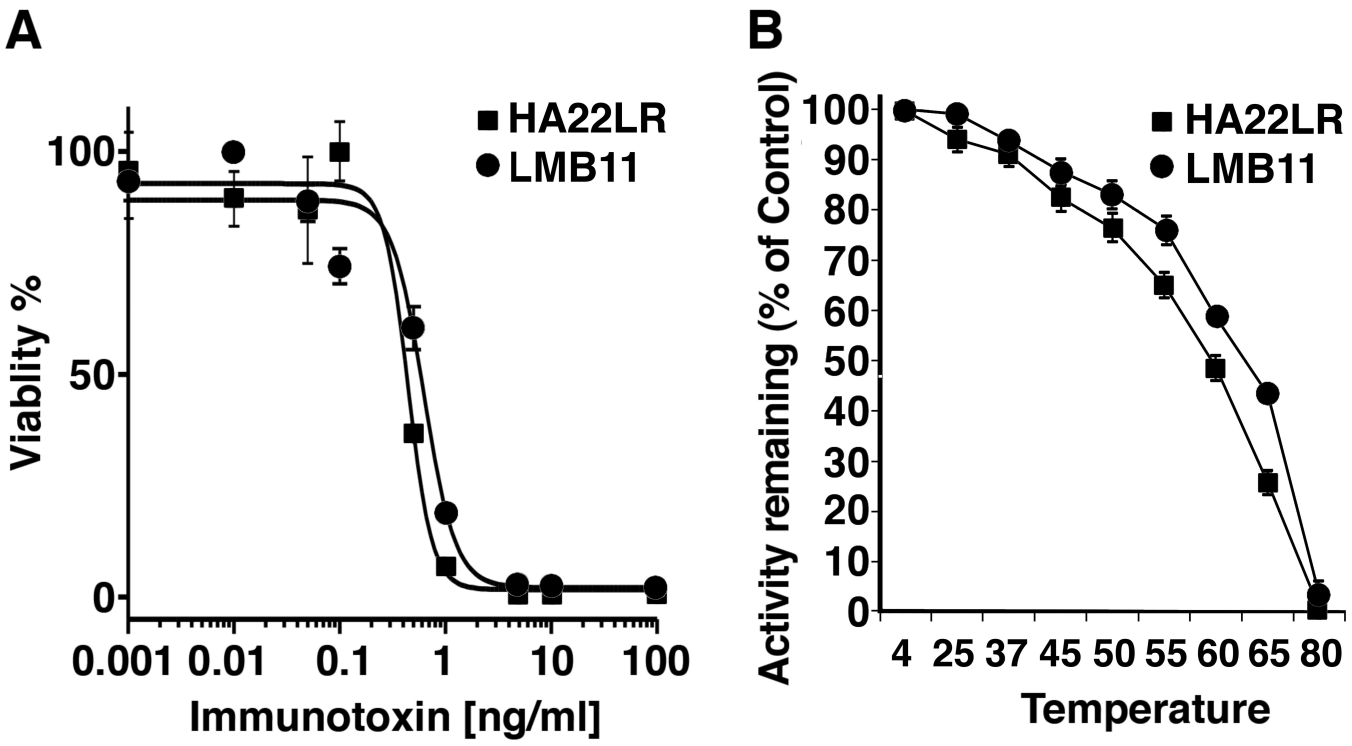


**Fig. 3.**

Properties of LMB11 immunotoxin on cultured cell lines. A. Cytotoxic activity of LMB11 on CD22 positive cell line. Representative cytotoxicity assays on CD22 positive CA46 cells using WST8 after 3 days of incubation with immunotoxin. B. Thermal stability of LMB11 immunotoxin. RITs were heated for 15 minutes at various temperatures as described in Material and Methods and the activity remaining was evaluated after each incubation. Data presented as percent activity remaining as compared with untreated control (sample incubated in ice).

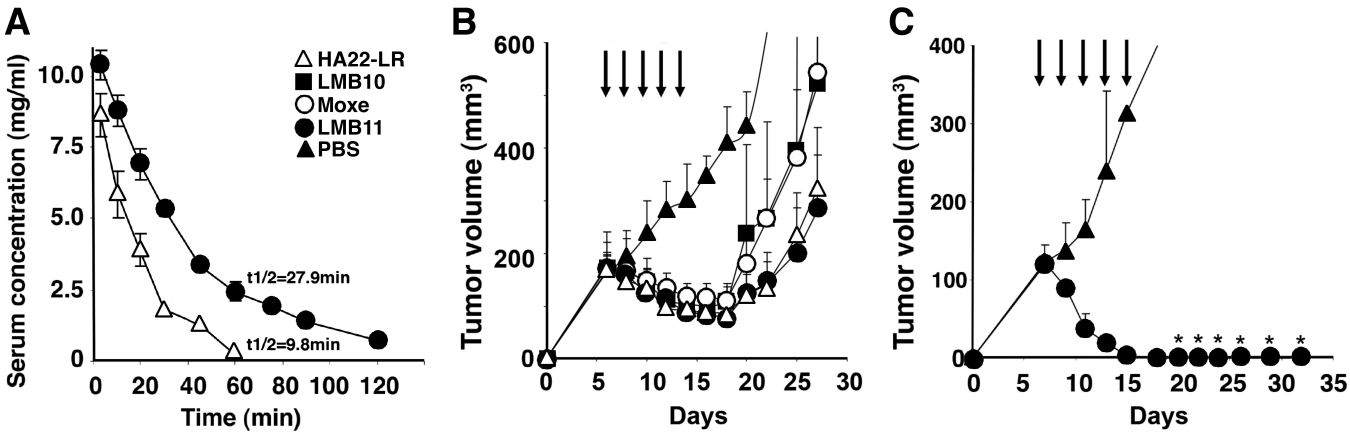


**Fig. 4.**

LMB11 mouse studies. A. Pharmacokinetics of LMB11 in mice. Balb/c mice were injected intravenously with 5 μg of LMB11 (●) or HA22-LR (△). Blood samples were drawn at different times. The levels of immunotoxin in mouse blood were measured by ELISA. Results are the average of four animals for each time point + SE. B. Antitumor activity. Group of five SCID mice bearing CA46 tumors were treated every other day × 5 (↓) with 0.2 % mouse serum albumin in PBS (▲), 5 mmol/kg doses of Moxe (O), HA22-LR (△), LMB10 (■), or LMB11 (●). C. Group of seven SCID mice bearing CA46 tumors were treated every other day × 5 (↓) with 0.2 % mouse serum albumin in PBS ( ▲) or LMB11 at 1.5 mg/kg (●). *No measurable tumors in 7/7 mice.

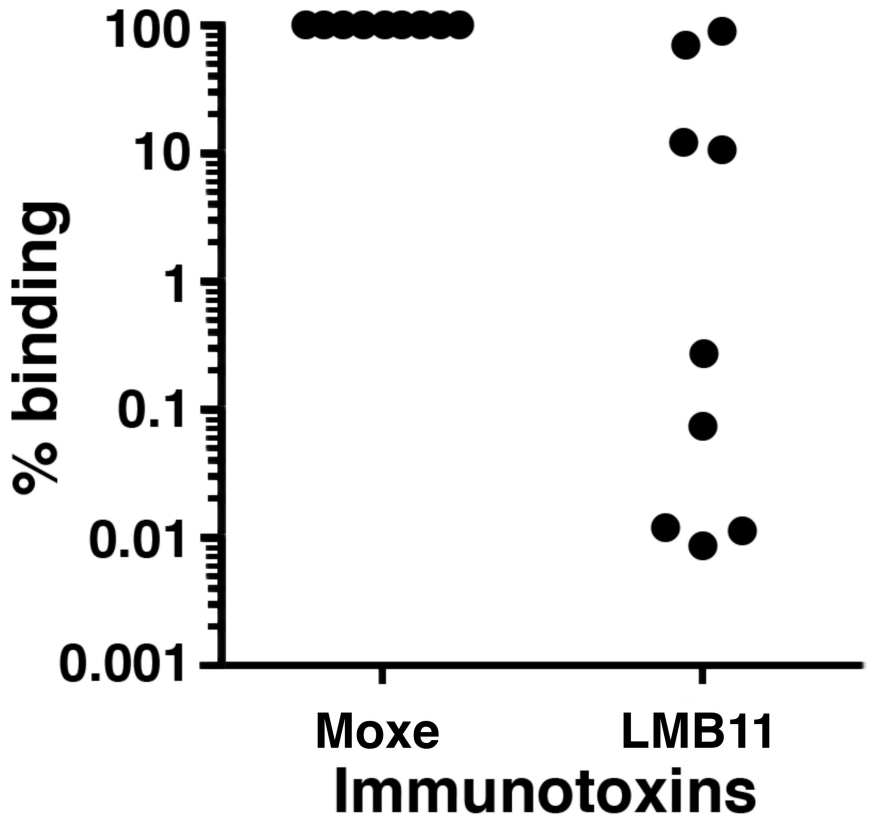


**Fig. 5.**

The reactivity of LMB11 and Moxe with human sera. Ratio of binding of LMB11 to binding of Moxe from nine patients treated with Moxe.

## Table 1

Activity of HA22-LR and LMB11 on CD22-positive Cell Lines

| | | $IC_{50}$ (ng/ml)) | | $IC_{50}$ (pM) | | |
|---|---|---|---|---|---|---|
| **Cell Line** | **Cell Types** | **HA22-LR** | **LMB11** | **HA22-LR** | **LMB11** | **HA22-LR/LMB11 Relative Activity** |
| CA-46 | BL | 0.4 | 0.6 | 8.7 | 8.6 | 1.01 |
| SEM | B-ALL | 7 | 13 | 145 | 175 | 0.83 |
| Raji | BL | 0.13 | 0.21 | 2.7 | 2.8 | 0.96 |
| HAL-1 | B-ALL | 2.3 | 2.33 | 47 | 31 | 1.52 |
| KOPN-8 | B-ALL | 0.9 | 2.0 | 19 | 27 | 0.70 |
| REC1 | MCL | 0.76 | 0.74 | 16 | 10 | 1.60 |
| JVM2 | MCL | 0.8 | 1.4 | 17 | 19 | 0.89 |
| SuDHL | DLBCL | 0.6 | 0.9 | 12 | 12 | 1.00 |
| A431/H9 | EC | >1000 | >1000 | >1000 | >1000 | -- |

BL, Burkitt's lymphoma; B-ALL, Acute B-lymphoblastic leukemia; MCL, Mantle cell lymphoma; DLBCL, Diffuse large B-cell lymphoma; EC, Epidermoid Carcinoma

## Table 2

Growth Inhibition Assay of LMB11 Immunotoxin in HCL and CLL Patients Cells

| | | $IC_{50}$ (ng/ml) | | |
|---|---|---|---|---|
| **Patient** | **Cell Type** | **Moxe** | **LMB11** | **Moxe/LMB11 Molar Relative Activity** |
| 1 | CLL | 64 | 10.4 | 7.0 |
| 2 | CLL | 1.2 | 5.9 | 0.2 |
| 3 | CLL | 5.4 | 28.7 | 0.2 |
| 4 | CLL | 3.1 | 4.6 | 0.8 |
| 5 | CLL | 5.8 | 6.7 | 1.0 |
| 6 | CLL | 73.4 | 68 | 1.3 |
| 7 | CLL | 5.1 | 8.5 | 0.7 |
| 1 | HCL | 3.98 | 5.45 | 0.9 |
| 2 | HCL | 1.56 | 5.15 | 0.4 |
| 3 | HCL | 0.61 | 2.63 | 0.3 |
| 4 | HCL | 0.45 | 0.90 | 0.6 |
| 5 | HCL | 0.17 | 0.44 | 0.5 |

CLL, Chronic lymphocytic leukemia; HCL, Hairy cell leukemia

## Table 3

Body weight of mice during the treatment of immunotoxin

| Treatment Groups | Average weight of mice in grams after treatment on day indicated | | | |
|---|---|---|---|---|
| | 11 | 15 | 18 | 22 |
| LMB11 (1.5mg/kg ×5) | 18.8±1.1 | 17.8±1.5 | 17.5±1.6 | 18.2±1.4 |
| PBS (×5) | 18.3±1.3 | 17.7±1.3 | 18.3±1.3 | 18.9±1.3 |

No death of mice (n=7) in this dose.

Data expressed as average ± SD.