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# Efficacy of a CD22-targeted antibody-saporin conjugate in a xenograft model of precursor-B cell acute lymphoblastic leukemia

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ABSTRACT

Targeted therapies, such as those using imatinib and rituximab, have revolutionized the treatment of Philadelphia chromosome-positive and CD20-positive acute lymphoblastic leukemia (ALL) respectively, yet these therapies are effective in only a subset of patients and remission is generally not durable. The next generation of targeted therapies includes the use of antibodies conjugated to potent cytotoxic agents and are classified as antibody drug conjugates (ADC). For B-lineage ALL, CD22 is an ideal target for ADC therapy because it is expressed on the majority of B-lineage ALL cells and because antibody binding mediates receptor internalization. HB22.7-SAP is a conjugate of our anti-CD22 monoclonal antibody (mAb), HB22.7, and the ribosome inhibiting protein, saporin (SAP). *In vitro*, HB22.7-SAP effectively bound to CD22 on the surface of pre-B ALL cell lines and exhibited potent and specific cytotoxicity. In a NOD/SCID xenograft mouse model of pre-B ALL, when compared to the vehicle-treated control, HB22.7-SAP increased the median survival time from 20 days to over 50 days without significant toxicity.

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## 1. Introduction

Of the nearly 6000 new cases of ALL diagnosed in the Unites States in 2011, approximately 80% were of B cell lineage and pre-B ALL was the most prevalent phenotype. In children, ALL is highly curable with a 5-year survival rate of 89%, however, a subset of children have a poor prognosis with a 5-year survival rate of only 30% [1]. Furthermore, current treatment for children with standard ALL consists of 2½ to 3 years of highly toxic combination chemotherapy. Children treated with chemotherapy suffer not only from acute side effects but also "late effects", serious lifelong effects which involve multi-organ systems such as the endocrine, skeletal, cardiovascular, and neural systems [2]. Secondary malignancies are a potential long term complication as well. Although mostly thought of as a childhood disease, the incidence of ALL is approximately 1/100,000 for people over the age of 15 in the United States [3]. The long-term survival rate for adults with ALL remains poor (40–50%) but recent advances in treatment are improving outcomes [4]. Precise definition of prognostic factors and optimized risk stratification have been important developments. Superior chemotherapy, adaptation of pediatric-based chemotherapy protocols and improvements in stem cell transplantation are also increasing survival [5]. Despite these advances, treatment-related mortality and the inability to tolerate and complete therapy is a limiting feature in patients over 50 years of age. To avoid the limitations and significant side effects of current ALL treatments, recent approaches have focused on the development of novel targeted therapies to improve efficacy and reduce toxicity.

One approach has been to use mAbs targeted to antigens (*e.g.*, CD19, CD20, CD22, CD33, and CD52) that are expressed on the surface of leukemic blasts [6]. The rationale for antibody therapy is that the cytotoxicity is specific; it affects only cells which express the target antigen. Recent studies have demonstrated promising results yet antibody therapy is primarily used as an adjuvant to chemotherapy and, therefore, does not preclude systemic chemotherapy-mediated toxicity. The next generation of antibody-based therapy involves the use of mAbs directly conjugated to potent cytotoxic agents for specific intracellular delivery. In ALL, ADCs have demonstrated remarkable pre-clinical and clinical efficacy and may at some point, obviate the need for systemic chemotherapy or reduce the necessary dose [7–9].

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Lymphoid-associated surface antigens are ideal targets for ADC therapy because of their lineage-specific expression. In particular, CD22 has gained significant interest as a target partly because the lack of CD22 expression on hematopoietic stem cells allows for the regeneration of normal B cells after ADC-mediated elimination. CD22 is a 140-kDa sialo-adhesion protein specifically expressed by normal and malignant B cells and appears to be involved in the regulation of B cell function and survival [10]. Additional motives for targeting CD22 include its expression by the majority of B-lineage lymphoma and ALL (>90% of pre-B ALL cases express CD22) and rapid internalization upon antibody binding [11–13].

HB22.7 is an anti-CD22 mAb originally developed (and humanized) by our group for the treatment of non-Hodgkin's lymphoma (NHL). Among other anti-CD22 mAbs, HB22.7 is unique because of its independent cytotoxic effect [14]. In addition to being efficacious as a single agent in xenograft models of NHL, we have shown that when conjugated to the surface of liposomal payloads, HB22.7 can act as a targeting ligand and enhance cellular uptake [15,16]. In the present study, we examined the potential for HB22.7 to serve as a vehicle for the targeted delivery of a directly conjugated cytotoxic agent. The protein toxin, SAP, was chosen for conjugation to HB22.7 due to its high enzymatic activity, resistance to proteolytic degradation and ease of chemical conjugation [17]. Several groups have developed mAb-SAP conjugates targeted to a variety of tumor cells and demonstrated significant preclinical anti-tumor activity [18–23]. To this end, we examined the *in vitro* cytotoxicity and *in vivo* activity of HB22.7-SAP using CD22-positive pre-B ALL cell lines.

## 2. Experimental

### 2.1. HB22.7 and saporin

The anti-CD22 mAb, HB22.7, was prepared and characterized as described previously [14]. SAP, mouse-IgG-SAP and the custom conjugate, HB22.7-SAP, were purchased from Advanced Targeting Systems (San Diego, CA).

### 2.2. Cell lines

REH, JM1 and Jurkat cells were purchased from the American Type Culture Collection (Rockville, MD) and maintained at 37 °C, 5% $CO_2$ and 90% humidity. REH and Jurkat cells were maintained with RPMI and JM1 cells were maintained with IMDM media supplemented with 10% fetal bovine serum, 50 units/mL penicillin G and 50 μg/mL streptomycin sulfate.

### 2.3. Flow cytometry

To assess CD22 expression, $0.5 \times 10^6$ cells per sample were resuspended in 100 μL FACS buffer (PBS + 4% FBS) and chilled on ice. HB22.7 (10 μg/mL) was incubated with cells for 30 min on ice, followed by 3 washes with ice cold FACS buffer. Cells were then incubated with a 1/50 dilution of goat anti-mouse IgG-FITC (Invitrogen, Camarillo, CA) for 30 min on ice and in the dark. Cells were washed 3 times and 10,000 events were analyzed on a FACScan (BD, San Jose, CA).

To confirm human cell engraftment in the xenograft model, mice that developed hind limb paralysis (a frequent finding due to advanced disease in this model) were euthanized and cells were harvested from bone marrow for flow cytometry analysis. After washing with PBS, $1 \times 10^6$ cells per sample were resuspended in 90 μL FACS buffer and 10 μL APC anti-human HLA-A, B, C antibody (BioLegend, San Diego, CA). The cells were kept on ice and in the dark for 20 min then washed with FACS buffer and analyzed on a FC500 (Beckman Coulter, Brea, California). Bone marrow from healthy NOD/SCID mice and REH cells were used as negative and positive controls, respectively.

### 2.4. MTS assay

An MTS assay was used to evaluate the *in vitro* cytotoxicity of HB22.7-SAP [24]. REH, JM1 and Jurkat cells were seeded in 96-well plates at a density of $1 \times 10^4$ cells/well in 90 μL media. HB22.7-SAP was serially diluted with media and 10 μL of each dilution was added to the appropriate well. As a control, cells were also treated with a combination of free HB22.7 and free SAP at concentrations equivalent to each component of HB22.7-SAP. After a 72 h incubation, cell viability was assessed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) according to the manufacturer's instructions. MTS solution (20 μL) was added to each well and cell viability assessed after a 1 h incubation at 37 °C. Cell viability as a percent of the untreated control was calculated as follows: $[(OD_{490}\ \text{treated} - OD_{490}\ \text{background})/(OD_{490}\ \text{control} - OD_{490}\ \text{background}) \times 100]$. The mean ± standard deviation (SD) of 3 separate experiments performed in triplicate is shown.

### 2.5. ALL xenograft mouse model

Female, 6–8 week old, NOD/SCID mice were purchased from Taconic Farms (Hudson, NY) and maintained in micro-isolation cages under pathogen free conditions. All animal experiments were performed in compliance with institutional guidelines and according to protocol No. 16322 approved by the Animal Use and Care Administrative Advisory Committee at the University of California, Davis. Mice were allowed to acclimate for at least 4 days prior to the start of any experiment. The ALL xenograft model was induced by injecting $5 \times 10^6$ REH cells into the tail vein. Mice were then separated into 3 treatment groups ($n = 6$–8) consisting of PBS, free HB22.7 (0.12 mg/kg) plus free SAP (0.072 mg/kg) and HB22.7-SAP (0.2 mg/kg). The doses of free HB22.7 and free SAP were equivalent to each component in the HB22.7-SAP conjugate. Treatment was initiated 24 h after REH cell injection (designated as day 0) and continued twice weekly for four weeks.

Body weight and other symptoms of toxicity (unkempt fur, ataxia) were observed daily. Mice were euthanized at the onset of hind limb paralysis or when they lost >20% of their starting body weight. On days 6, 13 and 20, blood was drawn from the lateral saphenous vein of animals in each group ($n = 3$) and collected into EDTA-lined tubes for complete blood counts (CBC).

### 2.6. Statistical analysis

Statistical analysis was performed by Student's *t*-test for two groups, and one way ANOVA for multiple groups. All results were expressed as the mean ± SD unless otherwise noted. A value of $p < 0.05$ was considered statistically significant. Survival analysis was carried out using GraphPad Prism 4 software.

## 3. Results

### 3.1. HB22.7-SAP in vitro cytotoxicity

The expression of CD22 on the pre-B ALL cell lines, REH and JM1, was validated using flow cytometry with HB22.7 as a probe (Fig. 1). Nearly 100% of REH and JM1 cells were CD22-positive and between the two cell lines, REH exhibited higher levels of CD22. Next, we compared the binding of HB22.7 to that of HB22.7-SAP and determined that SAP conjugation to HB22.7 had no effect on its binding to CD22. Additionally, the CD22-negative cell line, Jurkat, showed no binding of HB22.7 or HB22.7-SAP (data not shown).

To evaluate *in vitro* cytotoxicity, REH and JM1 cells were incubated with HB22.7-SAP continuously for 72 h followed by measurement of cell viability using an MTS assay. The $IC_{50}$ values of HB22.7-SAP were 0.67 and 1.2 ng/mL in REH and JM1 cells, respectively (Fig. 2). As a control, cells were also treated with free HB22.7 plus free SAP at molar concentrations equivalent to each component in the HB22.7-SAP conjugate. In both cell lines, free HB22.7 plus free SAP had no cytotoxic effect. Additionally, an isotype-matched control, mouse-IgG-SAP, was not cytotoxic to either cell line (data not shown). To further demonstrate the specificity of HB22.7-SAP, CD22-negative Jurkat cells were incubated with HB22.7-SAP and at all tested concentrations, no cytotoxicity was observed.

### 3.2. In vivo efficacy and toxicity of HB22.7-SAP in a xenograft model of pre-B ALL

A xenograft model of pre-B ALL was established by injecting $5 \times 10^6$ REH cells into the tail vein of NOD/SCID mice. It has been previously established that REH cells disseminate throughout the body after intravenous injection and cause hind limb paralysis; therefore, this was used as the endpoint [25,26]. Between days 18 and 21, all of the mice from the PBS and the free HB22.7 plus free SAP groups developed hind limb paralysis (Fig. 3). The median survival time for both treatment groups was 20 days and the onset of hind limb paralysis was preceded by a significant reduction in body weight (data not shown). On the other hand, the treatment