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Targeting B lymphoma with nanoparticles bearing glycan ligands of CD22

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Abstract

CD22 is a member of the siglec (Sialic acid-binding immunoglobulin-like lectin) family expressed on B cells that recognizes glycans of glycoproteins as ligands. Because siglecs exhibit restricted expression on one or a few leukocyte cell types, they have gained attention as attractive targets for cell-directed therapies. Several antibody-based therapies targeting CD22 (Siglec-2) are currently in clinical trials for treatment of hairy cell leukemia and other B cell lymphomas. As an alternative to antibodies we have developed liposomal nanoparticles decorated with glycan ligands of CD22 that selectively target B cells. Because CD22 is an endocytic receptor, ligand-decorated liposomes are bound by CD22 and rapidly internalized by the cell. When loaded with a toxic cargo, such as doxorubicin, they are efficacious in prolonging life in a Daudi B cell lymphoma model. These B cell targeted nanoparticles have been demonstrated to bind and kill malignant B cells from patients with hairy cell leukemia, marginal zone lymphoma, and chronic lymphocytic leukemia. The results demonstrate the potential of using CD22-ligand-targeted liposomal nanoparticles as an alternative approach for treatment of B cell malignancies.

Standard treatments for patients with B cell lymphoma include systemic chemotherapy and the anti-CD20 antibody rituximab. Although the results of the current therapies are excellent, most patients ultimately relapse and succumb to their disease, creating the need for improved therapies. Among many antibodies under clinical development for B cell depletion therapy, BL22 [1] and CMC-544 [2] are immunotoxins that target CD22, another receptor expressed exclusively on B cells. CD22 is a member of the sialic acid binding Ig-like lectin (siglec) family, which recognize glycan ligands found on glycoproteins and glycolipids, and is well known for its role in regulation of B cell receptor signaling, an activity mediated through ITIM motifs in its cytoplasmic domain [3]. Because CD22 undergoes constitutive endocytosis through a clathrin-dependent mechanism, it is well suited for efficient delivery of the toxin into the cell [4]. In contrast, rituximab relies on complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC) to effect cell killing since CD20 is statically expressed on the cell surface. Thus, CD22 immunotoxins induce B cell killing by a different mechanism than rituximab, and CD22 has become a validated target treatment of non-Hodgkins B cell lymphomas.

It is well documented that CD22 expression varies among the different B cell lymphomas, with chronic lymphocytic leukemia (CLL), marginal zone lymphoma (MZL) and hairy cell leukemia (HCL) expressing low, medium and high levels of surface CD22, respectively. Thus, B lymphoma cells from HCL patients and are anticipated to exhibit high sensitivity to anti-CD22 therapeutics.

CD22 recognizes the glycan sequence NeuAc α 2-6Gal found abundantly on glycans of B cell glycoproteins and other immune cells, and this ligand binding activity modulates its activity as a regulator of B cell signaling. We have exploited the ligand binding activity to target CD22 on B cells as an alternative to anti-CD22 antibodies [5–7]. To accomplish this, we have developed a synthetic high affinity glycan ligand for human CD22, 9-*N*-biphenylcarboxyl-NeuAc α 2-6Gal β 1-4GlcNAc (^{BPC}NeuAc). We had previously found that multivalent polyacrylamide polymers containing ^{BPC}NeuAc are bound and rapidly endocytosed by B lymphocytes [6]. These early results encouraged us to develop a pharmaceutically relevant cell-directed therapeutic for B cell lymphoma that targets CD22 on the surface of the malignant cells using carbohydrate recognition based approach. To accomplish this, we adapted the FDA approved liposomal nanoparticle formulation of the chemotherapeutic DoxilTM to display synthetic high affinity ligands of CD22 to target and deliver chemotherapeutic payloads to B cell lymphoma *in vivo* [5]. To prepare CD22-targeted liposomes, we coupled ^{BPC}NeuAc sialoside to a commercially available pegylated lipid, and the corresponding ^{BPC}NeuAc-pegylated lipid was then incorporated into a liposomal doxorubicin formulation analogous to that in current clinical use (Figure 1A). Formulating the liposomes to contain 5 mol% ^{BPC}NeuAc-pegylated lipids results in a highly multivalent display of the sialic acid ligands on the surface of a 100 nm-sized liposome. The resulting ^{BPC}NeuAc-liposomes bound robustly to and were endocytosed by Daudi human Burkitt's B lymphoma cells and CHO cells that express surface CD22. Upon endocytosis, ^{BPC}NeuAc-liposomes were found co-localized with early endosomes and lysosomes. When doxorubicin was loaded into liposomes, ^{BPC}NeuAc-liposomes efficiently delivered cytotoxic cargo to B cells, resulting in a 33-fold higher potency (IC₅₀ = 1.6 μ M) toward killing Daudi B cells than that of the non-targeted naked-liposomes (IC₅₀ = 53 μ M), a difference predictive of increased efficacy *in vivo* [5].

The efficacy of doxorubicin-loaded CD22 targeted liposomes was evaluated in a systemic Daudi B lymphoma model in NODSCID mice [5]. The disseminated Daudi lymphoma was established by tail vein injection, followed by dosing mice on days 1 and 3 with doxorubicin-loaded ^{BPC}NeuAc-liposomes or non-targeted liposomes at 3 mg doxorubicin/Kg per dose. We observed a significant improvement in extending mean time of survival (MTS) in tumor bearing mice that were treated with CD22 targeted liposomes compared to those left untreated or received non-targeted liposomal doxorubicin ($p = 0.0001$). Mice treated with liposomes displaying 5 mol% ^{BPC}NeuAc-ligands exhibited a MTS greater than 100 days with 5 of 8 long-term survivors being healthy at the end of the study (day 100). Follow up analysis revealed a high engraftment level of Daudi lymphoma cells in the bone marrow of both untreated or naked-liposome treated animals. In contrast, residual tumor cells were not detectable in the bone marrows of the long-term survivors that were treated with ^{BPC}NeuAc-liposomes, further demonstrating the efficacy of the CD22-targeted liposomal regimen.

CD22 targeted ^{BPC}NeuAc-liposomes are also avidly bound by neoplastic B cells from patients with hairy cell leukemia (HCL), chronic lymphocytic leukemia (CLL), or splenic marginal zone lymphoma (MZL). Binding of ^{BPC}NeuAc-liposomes to patient malignant B cells strongly correlated with the expression level of CD22, regardless of their prior treatment history. Classic HCL (Figure 1B) and MZL express brighter surface CD22 than CLL, hence B cells from the HCL and MZL patients bound strongly to the ^{BPC}NeuAc-liposomes while B cells from CLL patients bound at lower levels [5]. Despite variation of CD22 expression, malignant B cells from all patients were sensitive to cell killing by the doxorubicin-loaded ^{BPC}NeuAc-liposomes in an *in vitro* vital dye based cell viability assay while spared by the non-targeted regimen. Indeed, as illustrated in Figure 1C, more than 85% hairy cells were killed after 1-hour exposure to the targeted liposomal doxorubicin at as low as 10 μ M. Since the CD22-targeted ^{BPC}NeuAc-liposomes breach the cells by an

endocytic mechanism, it is anticipated that the therapeutic benefit will be synergistic with anti-CD20 rituximab and other immune-mediated therapies for treatment of hairy cell leukemia and other B cell lymphomas.

Although the glycan ligand used in the current targeted liposomes is highly effective for targeting B cells, there is still ample room to identify sialic acid substituents with increased affinity and selectivity for CD22. We found that ^{BPC}NeuAc ligand was also recognized by both human and murine sialoadhesin (Sn), another siglec (Siglec-1) that is expressed on a subset of macrophages. In mice, this cross-reactivity causes the liposomes to be cleared by macrophages, which effectively compete with the B lymphoma cells for binding and endocytosis. In mice, sialoadhesin is the only other siglec responsible for binding the ^{BPC}NeuAc-liposomes, since there is no difference in clearance of ‘naked’ and ^{BPC}NeuAc-liposomes in Sn knockout mice. Using systematic methods of identifying substituents that increase the selectivity of siglec ligands [8], we expect that 2nd generation ligands that do not cross-react with sialoadhesin will effect even greater efficiency for targeting CD22 on human B cells.

In summary, liposomal doxorubicin displaying high affinity glycan ligands of CD22 target and deliver chemotherapeutics cargo to CD22 expressing cells. The ^{BPC}NeuAc-liposomes induce complete remissions in mice with a human B lymphoma and kill malignant cells from patients with B cell lymphomas, including hairy leukemia. These results demonstrate the potential of a platform that uses a glycan ligand-based approach for B cell depletion therapy as an alternative to antibody-based targeting strategies. We anticipate that ligand based targeting of siglecs will provide improved treatment options for patient with hairy cell leukemia or other B cell lymphomas.

Acknowledgments

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Figure 1.

Liposomal nanoparticles displaying glycan ligands of CD22 for targeting and killing hairy cells leukemia. (A) Schematic illustration of a doxorubicin-loaded liposomal formulation comprising ^{BPC}NeuAc-pegylated lipids for active targeting to CD22. (B) ^{BPC}NeuAc-liposomes bind to hairy cells in the patient peripheral blood. Data shown are FACS analysis of HCL (red) and normal B cells (green), using fluorescently labeled ^{BPC}NeuAc-liposomes or naked liposomes. (C) Cytotoxicity of ^{BPC}NeuAc-liposomes toward HCL. Patient blood cells were subjected to liposomal doxorubicin at 10 or 40 μM for 1 hr. Cells were thoroughly washed and incubated in fresh medium for an additional 5 days prior to viability assay. Data shown are percent viability (means of triplicate $\pm$ s.d.) of patient blood lymphocytes

evaluated by the standard MTT assay. Cells left untreated were defined as the maximal cell viability. Complete cell killing was determined from the Triton X-100 lysed cells. $*P < 0.05$ as compared to control treatments of naked-liposomes. Representative data from one of four samples are shown.