SL-101, a Novel Monoclonal Antibody-Conjugate That Targets Interleukin-3 Receptor Alpha (CD123), Possesses Preclinical Anti-Tumor Activity Against Hodgkin's Lymphoma.

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Background: The interleukin-3 receptor alpha chain (CD123) is over-expressed on the tumor bulk and cancer stem cells of multiple hematologic malignancies relative to normal stem cells. CD123 has also been shown to be up-regulated by the tumor bulk of a variety of lymphomas, and is also expressed by Reed-Sternberg cells in Hodgkin's lymphoma (HL). The prognosis for patients with HL who fail to achieve a durable remission with approved therapeutics or transplantation is poor. Therefore, novel treatment strategies for such patients are needed. SL-101, a novel monoclonal antibody-conjugate that targets CD123 with high affinity, was constructed. The potency and activity of SL-101 was assessed in HL.

Methods: A panel of anti-CD123 monoclonal antibodies was previously screened for binding affinity to CD123 and for receptor internalization. Three antibodies were selected and the V_H and V_L domains of each were sequenced and used to generate scFv constructs. The scFv regions were genetically fused, via recombinant DNA technology, to a truncated Pseudomonas exotoxin (PE38) containing its translocation and ADP-ribosylation domains. Candidates were then screened based on several functional parameters, including affinity of the parental antibodies, expression of stable antibody conjugates, and CD123 internalization. The coding sequences of the lead antibody-conjugate candidate, SL-101, was codon optimized for expression in E. coli, cloned into an expression vector, and the resulting protein was refolded and purified from inclusion bodies. CD123 receptor internalization was measured using the Mab-ZAP® assay. The sensitivity of CD123-expressing lymphoma cell lines to SL-101 was assessed using a CellTiter Glo® in vitro cytotoxicity assay. Cell lines were incubated with SL-101 (2.54 to 450 pM), or buffer control, for 48 h and then assessed for viability.

Results: After 24 h incubation with an anti-CD123 antibody and the Mab-ZAP® reagent, approximately 75% of CD123-expressing THP-1 cells were killed at the highest concentration tested. Importantly, the degree of cell death was concentration-dependent, indicating that CD123 efficiently internalized upon binding the antibody-Mab-ZAP complex. Based on these results, the sensitivity of CD123-expressing HL cell lines to SL-101 was evaluated. The cell lines
studied were sensitive to SL-101 in a concentration-dependent manner. Furthermore, a wide range of drug sensitivity was observed, with IC\textsubscript{50} values as low as < 0.2 nM.

**Conclusions:** These results indicate that SL-101 is internalized following binding to CD123 and has demonstrated cytotoxicity against HL cells. The expression of CD123 on multiple hematologic cancers coupled with these results provides a rationale for further development of SL-101 in patients with HL and potentially other CD123-expressing hematologic malignancies.