Anti-leukemia efficacy and mechanisms of action of SL-101, a novel anti-CD123 antibody-conjugate in acute myeloid leukemia

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Abstract

Outcomes of acute myeloid leukemia (AML) remain poor and warrant the development of novel therapeutic agents. CD123 (interleukin-3 receptor alpha subunit), is overexpressed on AML blasts and leukemia progenitor cell lines (LSCs) compared to normal hematopoietic cells (Jordon et al. Leukemia 2000). SL-101 is a novel anti-CD123 antibody-conjugate comprised of anti-CD123 scFv fused to a truncated and optimized pseudomustoxin (PE) lacking its native targeting domain. We have previously demonstrated SL-101’s cell killing efficacy in AML cell lines (Han et al. ASH 2013). Here we report the anti-tumor efficacy of SL-101 against primary AML cells and the underlying mechanisms of its cytotoxicity.

Fourteen genetically diverse primary AML samples were treated with various doses of SL-101 for 48 h. Six samples reached high levels of CD123 (median 89.6%, range 20.4–99.5%) and intermediate levels of CD13 (median 54.6%, range 1–94.4%), the IL-3 beta subunit. SL-101 was highly active against AML samples, with an IC50 of 0.19 μg/ml (range 0.003 – 0.56 μg/ml). No significant correlation was found between SL-101 activity and levels of CD123 or CD13 (p=12), SL-101 also selectively and significantly suppressed the colony formation (69.5% ± 15.0% inhibition of total colonies, n=7) while sparing normal bone marrow (5.6% ±3.3% inhibition, n=4.00001).

We next investigated the mechanisms of the cytotoxic activity of SL-101. Using AnnexinV/DAPI flow cytometry, we first evaluated the induction of apoptosis in AML blasts and phenotypically defined CD34+CD123-CD34+CD123+ LSCs. After 48 h treatment, SL-101 at 1.0 μg/ml induced higher specific apoptosis in the AML LSCs (51.2 ± 2.45% than in blasts (39.4 ± 1.9%, p=0.006, n=10). Quantification of the AnnexinV+/DAPI- viable cells using counting beads demonstrated further reduction of cell numbers by SL-101 (52.6% blasts, 64.4%), indicating additional mechanisms of cell growth inhibition.

It was recently demonstrated that upon internalization, PE traffics through the endoplasmic reticulum to the cytosol, where it inactivates protein synthesis by catalyzing ADP-ribosylation of elongation factor 2 and causes non-apoptotic cell death (Wyane et al. Blood 2014). To examine the contribution of the direct inhibition of protein synthesis by PE, we first studied SL-101 internalization utilizing DyLight 890-labelled SL-101 by flow cytometry and fluorescence imaging. In CD123-expressing AML cell lines MV4-11 and MOLM13, the intracellular median intensity of DyLight 890 signal increased by 5.4-fold, respectively, within 4 h of treatment and 22.5- and 16.3-fold after 24 h. Fluorescence imaging confirmed cytosolic localization of SL-101 in both cell lines, demonstrating efficient cellular uptake of SL-101. We also examined the efficacy of SL-101 in inhibiting nascent protein synthesis in MV4-11 cells using an AHA Alexa Fluor 488 protein synthesis assay. SL-101 significantly reduced protein synthesis (40.3%, p<0.0005) within 4 , even at low concentrations (0.01 μg/ml), which was comparable to the positive control cycloheximide (44.8% ± 7.9%, p<0.0001). These findings confirmed the potential of SL-101 to efficiently internalize and persist within the cell's protein synthesis machinery.

We further investigated the ability of SL-101 to inhibit intracellular signaling in response to L-3. To this end, cytokine-dependent MoM5e leukemia cells were serum starved and pre-treated with SL-101 at 1.0 μg/ml overnight, followed by stimulation with L-3. SL-101 significantly suppressed L-3-induced activation of p-SERK (71.7% ± 2.6% inhibition, p<0.003) and modestly inhibited p-AKT (17.4% ± 5.4% inhibition, p<0.04), but not p-ERK signaling.

In summary, our data demonstrate that the novel anti-CD123 antibody-conjugate, SL-101, is highly active in AML and induces growth arrest and apoptosis in AML blasts and LSCs by inhibiting protein synthesis and interfering with L-3 signal transduction pathways. Ongoing studies that will be reported at this meeting investigate the in vivo anti-leukemia efficacy of SL-101 in NGSJ mice engrafted with primary AML cells. In conclusion, SL-101 is a novel, potent antibody-conjugate directed against AML blasts and LSCs, and our studies warrant further development of this agent.

In vitro cytotoxicity of SL-101 against AML cell lines

Figure 1. Cell surface binding and intracellular processing of SL-101. (A) Immunoreactions were developed to label CD123 only, which bypasses the dependence on other substrates to form intact L-3 receptor. The light and heavy chains were linked and fused as solid fragments in a 10:1 fragment of the pseudomustoxin. (B) After binding the CD123 antigen, SL-101 immunoreaction is internalized and processed with endosomal contributions. It traffic through the endoplasmic reticulum to the cytosol where it enzymatically inactivates protein synthesis and induces cell death.

Figure 2. In vitro cytotoxicity of SL-101 against AML cell lines. (A) SL-101 was tested in 12 AML cell lines. IC50 values were calculated based on the number of viable cells determined by Annexin V-DAPI flow cytometry. (B) Pearson correlation analysis between IC50 values and L-3 receptor subunit expression.

Methods of anti-leukemia activities of SL-101

Figure 4. Mechanisms of anti-leukemia activities of SL-101. (A) Hematopoiesis of AML cell line was incubated at 1 millimolar with SL-101-DyLight 890 at 100 ng/ml for 30 mins on ice to facilitate binding. Followed by incubation at 37°C for 0, 3, and 24 hrs. The membrane-bound SL-101 was stained with gelase bound flow cytometry analysis. MI indicates SL-101-DyLight 890. (B) For positive control, 1 μg/ml SL-101 treated without wash. (C) Dose- and time-dependent cell killing were determined using Annexin-V/DAPI flow cytometry. (D) Apoptosis was determined in CD123+ AML blasts and CD44+CD123+ LSCs. (E) Protein synthesis inhibition was measured in MV4-11 and MOLM13 AML lines using the Click-iT-Alexa Fluor 488 protein synthesis HCS assay. (F) The cytokine-dependent MoM5e leukemia cells were serum starved and pre-treated with SL-101 at 1.0 μg/ml overnight. SL-101 was treated with L-3. After fixation and permeabilization, cells were stained with antibodies against pERK, pAKT, and β-actin and analyzed by FACS. (G) MOLM13 leukemia cells were ethanol-fixed and stained with FITC for the cell cycle analysis using flow cytometry.

Conclusions

The novel anti-CD123 antibody-conjugate SL-101 is highly active in AML and induces cell growth arrest and apoptosis in AML blasts and LSCs. The cell killing is mediated by inhibition of protein synthesis and interference with L-3 signal transduction pathways. Ongoing studies investigate the in vivo anti-leukemia efficacy of SL-101 in NSG mice engrafted with primary AML cells.

Conflict of Interests

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