SL-401, a targeted therapy directed to the interleukin-3 receptor (CD123), and SL-801, a reversible inhibitor of Exportin-1 (XPO1), display synergistic anti-tumor activity against hematologic malignancies in vitro

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ASH 2016 #4724

Abstract

Background: Novel combination therapies have shown success in combating tumor heterogeneity and drug resistance. SL-401 is a targeted therapy directed to the interleukin-3 receptor (IL-3R/CD123), which is overexpressed on a number of hematologic malignancies. SL-401 has demonstrated high single-agent activity in patients with advanced hematological malignancies, including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), as well as multiple myeloma (MM) in combination with other agents. SL-801 is a novel oral small molecule that reversibly inhibits exportin-1 (XPO1), which is critical for the nuclear export of key anti-cancer drugs and RNA transcripts. 

Methods: The human K562 CML, MV-4-11 AML, RPMI-8226 MM, and L-428 HL cell lines were treated with varying concentrations of SL-401 and SL-801 alone or in combination for 48 hours. Cell viability was assessed using the CellTiter-Blue® assay. Combination index (CI) values were calculated using Compusyn software. 

Results: As single agents, SL-401 and SL-801 demonstrated anti-tumor activity in all four cell lines tested. MV-4-11 cells were the most sensitive to both drugs, with I_{50} values of 34 pM for SL-401 and 21 nM for SL-801. In the other cell lines, the IC_{50} for SL-401 were 17.5 nM in K562, 25 nM in RPMI-8226 cells, and 100 nM in L-428 cells, and the IC_{50} for SL-801 were 99 nM in K562 cells, 51 nM in RPMI-8226 cells, and 440 nM in L-428 cells. When combined with each other, SL-401 and SL-801 potently inhibited cell growth in all cell lines, and CI calculations indicated that the interaction between the two drugs was synergistic at most dose combinations. Notably, CI values < 0.35 were observed in L-428, RPMI-8226, and MV-4-11 cells, indicative of strong synergy. Consistent with these observations, the combination of SL-401 and SL-801 also induced higher levels of caspase activation and LDH release in K562, MV-4-11 and L-428 cells than either drug alone.

Conclusion: These findings demonstrate that SL-401 and SL-801, when combined, act synergistically in their in vitro anti-tumor activity against CML, AML, MM, and HL cells. Investigations into the molecular mechanisms underlying the observed synergy are in progress. These promising results provide rationale for further development of SL-401 and SL-801 combination therapy in the treatment of a broad range of hematologic malignancies.

Materials and methods

Cell culture: K-562 and MV-4-11 cells were cultured in IMDM supplemented with 10% FBS. L-428 and RPMI-8226 cells were cultured in RPMI-1640 supplemented with 10% FBS. Cell lines were treated with varying concentrations of SL-401 and SL-801.

In vitro cytotoxicity assay: The CellTiter-Blue® assay was used to assess cell viability after exposure to SL-401, SL-801, or both drugs simultaneously. Cells were grown in 96-well plates and incubated with drugs for 48 hours. The CellTiter-Glo reagent was then added to wells according to the manufacturer's instructions, and luminescence was measured on a Thermolab Systems Luminesco Ascent Reader at room temperature.

Combination index calculations: Combination index (CI) values were calculated using Compusyn software by the method of Chou and Talalay, and treatment was considered to be synergistic when CI < 1.

Caspase activity assay: The Caspase-Glo® 3/7 Assay was used to measure caspase activity in cells treated with SL-401, SL-801, or the combination. Briefly, cells were incubated with drugs for 24 hours and then analysed for caspase 3/7 activity.

Lactate dehydrogenase (LDH) release assay: The LDH release assay was used to measure LDH release from cells treated with SL-401, SL-801, or the combination. Briefly, cells were incubated with drugs for 24 hours and then analysed for LDH activity.

Cell western blot: Whole cell extracts were prepared from MV-4-11 cells treated with 0.1 nM SL-401, 60 nM SL-801, or the combination for 3, 6, 12, and 24 h. Western blots were performed using RIPA buffer with antibodies to caspase 3 and caspase 7 from Santa Cruz Biotechnology and developed by enhanced chemiluminescence.

Conclusions

- Several combinations of SL-401 and SL-801 exhibit strong synergistic anti-tumor activity in L-428 HL, RPMI-8226 MM, and MV-4-11 AML cells, and are demonstrated by CI values < 0.35.
- The combination of SL-401 and SL-801 enhances caspase 3/7 activation and LDH release in L-428 and MV-4-11 cell lines.
- Cellular expression of EF2, the molecular target of SL-401’s payload, remains unchanged with SL-801 treatment.
- Investigations into the molecular mechanisms underlying the observed synergy are ongoing.
- These promising results warrant further development of SL-401 and SL-801 combination therapy in the treatment of a broad range of hematologic malignancies.

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