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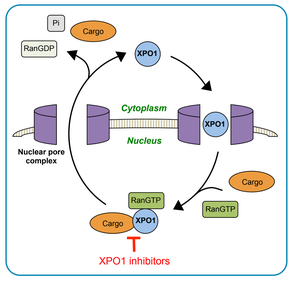
SL-801, a novel, reversible inhibitor of Exportin-1 (XPO1) / Chromosome Region Maintenance-1 (CRM1) with broad and potent anti-cancer activity

Janice Chen[1], Peter McDonald[1], Jonathan D. Schwartz[1], Rebecca S. Schneider[1], Keiichi Sakakibara[2], Naoya Saito[2], Takuji Sato[2], Takumi Kawabe[2], Christopher L. Brooks[1]

[1]Stemline Therapeutics, Inc., New York, NY, USA,[2]CanBas Co., LTD., Numazu, Shizuoka, Japan

Abstract

XPO1/CRM1, the principal nuclear export protein in eukaryotic cells, is required for the nuclear-cytoplasmic transport of both proteins and RNAs. Overexpression of XPO1 is reported in many cancers, causing dysregulated protein localization, aberrant cell proliferation, and resistance to apoptosis, and is associated with aggressive characteristics and poor patient outcome. Recent work has revealed XPO1 to be a clinically relevant target, and nuclear export inhibitors have emerged as a new class of anti-cancer agents with clinical activity in multiple hematologic and solid malignancies. SL-801 is a novel small molecule that binds covalently to Cys528 of XPO1, blocking the ability of XPO1 to interact with substrate cargos (e.g., p53, FOXO, p21, p27, and others). In contrast to the prototypical XPO1 inhibitor leptomycin B, which binds irreversibly to XPO1 and caused significant toxicities in Phase 1 trials, SL-801 binding to XPO1 is reversible, a characteristic that may be exploited to maximize its therapeutic index. Exposure to SL-801 results in potent inhibition of XPO1-dependent nuclear export, cell cycle arrest, and induction of apoptosis in a time- and dose-dependent manner. Here, the anti-tumor activity of SL-801 was investigated against a panel of 240 cell lines representing a broad range of solid and hematologic malignancies and confirmed in several SCID xenograft models.



The OncoPanel™ high content screening platform was used to evaluate the cytotoxicity of SL-801 against 205 solid tumor and 35 liquid tumor cell lines. SL-801 demonstrated potent activity, with 50% growth inhibitory (GI_{50}) values ≤ 10 nM in 51/240 (21.3%) cell lines and GI_{50} values ≤ 100 nM in 230/240 (95.8%) cell lines. SL-801 sensitivity was independent of cell proliferation rate or XPO1 expression levels. While SL-801 was broadly cytotoxic, cell lines of hematopoietic origin exhibited greater sensitivity. GI_{50}s in hematologic cancers ranged from 3-93 nM in leukemias, 1-103 nM in lymphomas, and 3-11 nM in multiple myelomas. SL-801 also inhibited solid tumor growth, with GI_{50}s ≤ 10 nM in several breast, brain, cervical, ovarian, gastric, kidney, liver, lung, melanoma, prostate, and sarcoma lines. In addition, a 5-fold increase in active caspase-3 staining was observed at SL-801 concentrations ≤ 100 nM in 117/240 (48.8%) cell lines, consistent with induction of apoptosis. To understand tumor sensitivity to SL-801, results of the cell line cytotoxicity screen were analyzed against publicly available genomic datasets. This analysis revealed that SL-801 was cytotoxic towards cell lines regardless of mutation status of key oncogenes (e.g., KRAS) and tumor suppressor genes (e.g., TP53).

The *in vitro* cytotoxicity of SL-801 against tumor cell lines was further validated in several xenograft models in SCID mice. In the RPMI-8226 multiple myeloma xenograft model, tumor growth was significantly inhibited at oral SL-801 doses of 31.25 mg/kg administered daily for five days for two weeks. In the ARH-77 human multiple myeloma xenograft model, overall survival was significantly prolonged by daily oral administration of 125 mg/kg SL-801 for ten days. This dose and regimen were well tolerated, and 90% of SL-801-treated mice survived > 150 days, whereas median survival was 39.5 days in the vehicle-treated group ($p < 0.001$). Significant tumor growth inhibition was also observed in the NCI-H226 non-small cell lung cancer and 22RV1 prostate cancer xenograft models.

These data demonstrate that SL-801 is a promising clinical candidate that inhibits a novel, clinically validated target and support its clinical development in a broad range of oncologic indications. The reversible binding of SL-801 to XPO1 may offer the potential to develop dosing schedules to enable recovery in normal tissues, thus broadening the therapeutic index of this class of agents. IND-enabling work is underway to support entry into clinical trials, and a Phase I trial design will be discussed.

Materials and methods

Cytotoxicity assay:

The OncoPanel™ high content screening platform was used to evaluate the cellular response of 240 human cancer cell lines representing diverse cancer types to SL-801 treatment (Ricerca, Bothell, WA). Cells were seeded into 384-well plates, and SL-801 was added 24 hours after cell seeding. SL-801 was serially diluted 3.16-fold and assayed over 10 concentrations (31.8 pM – 1 μM or 318 pM – 10 μM). After a 72-hour incubation period, cells were fixed and stained with a nuclear dye to allow visualization of nuclei and a fluorescently labeled anti-active caspase-3 antibody to visualize apoptotic cells. Automated fluorescence microscopy was carried out using a GE Healthcare IN Cell Analyzer 1000, and images were collected with a 4X objective. Twelve bit tiff images were acquired using the InCell Analyzer 1000 3.2 and analyzed with Developer Toolbox 1.6 software. Two cell lines were removed from the analysis due to high coefficient of variance between replicate untreated samples.

Mutations analysis:

Publicly available data sets from the Broad-Novartis Cancer Cell Line Encyclopedia (http://www.broadinstitute.org/ccle) were used to correlate SL-801 sensitivity to genomic alterations.

Xenografts:

Male mice (n=8-10/group) were inoculated with cell lines intravenously or subcutaneously. SL-801 or vehicle (5% gum arabic) was administered to animals orally. Formulations for dosing were based on body weight at each administration day. Survival time or tumor volume was measured. Statistical analysis of survival time was performed using the log-rank test, and statistical analysis of tumor growth inhibition was performed using the t-test.

Anti-tumor activity of SL-801 against cancer cell lines *in vitro*

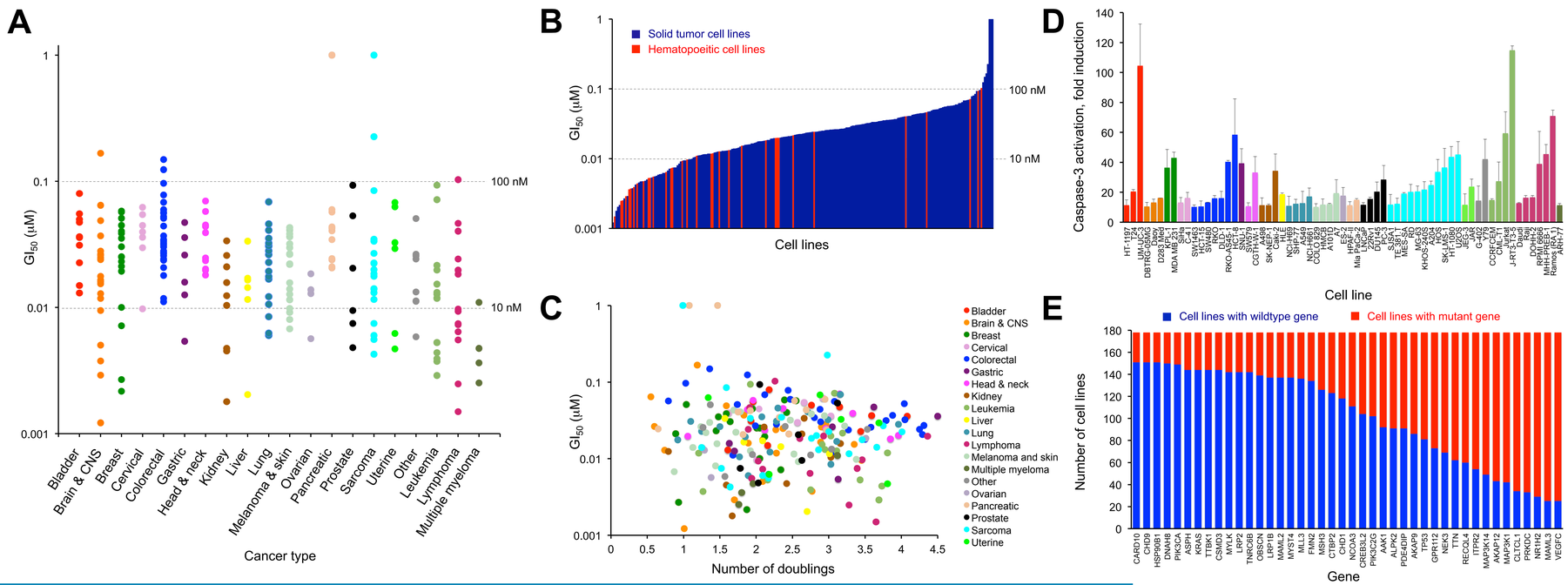


Figure 1: The *in vitro* anti-tumor effects of SL-801 was tested against a panel of 240 cell lines. **(A)** GI_{50} values are shown for each cell line and are grouped by cancer type. **(B)** Cell lines are ranked by GI_{50} values. *Blue*, cell lines of solid tumor origins; *red*, cell lines of hematopoeitic origins. **(C)** GI_{50} values for each cell line are plotted against number of cell doublings. **(D)** Apoptosis was measured using an antibody against cleaved caspase-3. Data are shown for cell lines in which caspase-3 activation was induced at least 10-fold over that in untreated cells at 100 nM SL-801. **(E)** Out of the cell lines tested, 178 cell lines with SL-801 GI_{50} values < 100 nM had gene mutation data available on the Broad-Novartis Cancer Cell Line Encyclopedia. *Blue*, cell lines in which the gene of interest is wildtype; *red*, cell lines in which the gene of interest has a mutation leading to an amino acid change(s) at the protein level. Genes in which at least 15% of cell lines express the mutant protein are shown.

Anti-tumor activity of SL-801 against solid and hematologic cancers *in vivo*

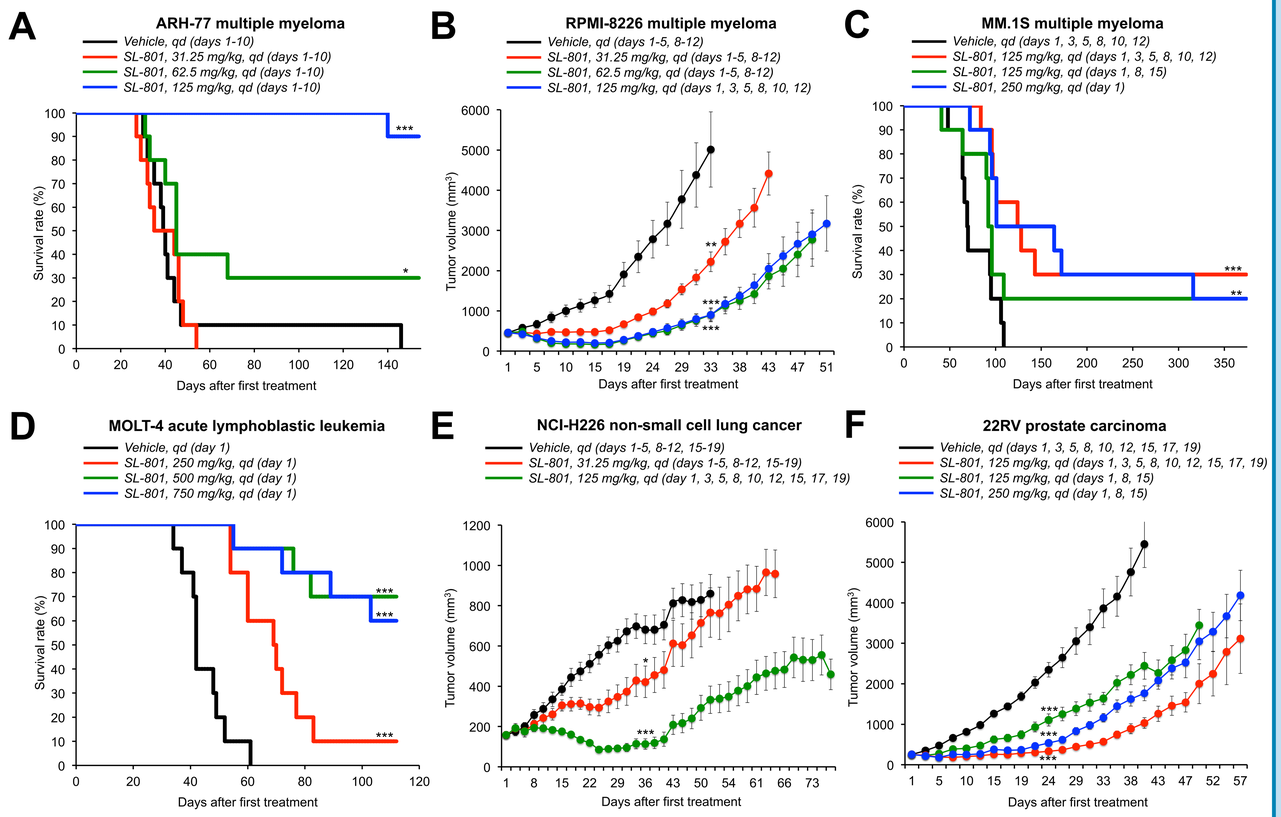


Figure 2: SL-801 was tested in SCID xenograft models of **(A)** ARH-77 multiple myeloma, (B) **RPMI**-8226 multiple myeloma, **(C)** MM.1S multiple myeloma, **(D)** MOLT-4 acute lymphoblastic leukemia, **(E)** NCI-H226 non-small cell lung cancer, and **(F)** 22RV prostate cancer. SL-801 or vehicle (5% gum arabic) was administered to animals orally once daily as indicated in the legends. Survival time or tumor volume was measured. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Conclusions

- The anti-tumor effects of SL-801 were investigated *in vitro* against a panel of 240 cancer cell lines.
  - SL-801 was broadly cytotoxic in both solid and hematologic cancers, with GI_{50} values ≤ 10 nM in 51/240 (21.3%) cell lines and GI_{50} values ≤ 100 nM in 230/240 (95.8%) cell lines.
  - Sensitivity to SL-801 was independent of cell growth rate.
  - At 100 nM SL-801, caspase-3 activation was induced greater than 10-fold in several cell lines.
  - SL-801 was cytotoxic towards solid and hematologic cancer cell lines regardless of mutation status of key oncogenes and tumor suppressor genes.
- The anti-tumor effects of SL-801 were validated *in vivo* in several SCID xenograft models.
  - SL-801 significantly extended overall survival in the ARH-77 and MM.1S multiple myeloma and MOLT-4 acute lymphoblastic leukemia xenograft models.
  - SL-801 significantly decreased tumor volume in the RPMI-8226 multiple myeloma, NCI-H226 non-small cell lung cancer, and 22RV prostate carcinoma xenograft models.
- The *in vitro* and *in vivo* anti-tumor effects observed with SL-801 suggest that XPO1 activity in multiple malignancies contributes to neoplastic pathogenesis.
- IND-enabling efforts are underway to support initial clinical trials in both solid and hematologic cancers in early 2016.

J. Chen: Stemline - employment, equity ownership; P. McDonald: Stemline – employment, equity ownership; J. Schwartz: Stemline – employment, equity ownership; R. Schneider: Stemline – employment; K. Sakakibara: CanBas – employment, equity ownership; N. Saito: CanBas – employment, equity ownership; T. Sato: CanBas – employment, equity ownership, patents & royalties; T. Kawabe: CanBas – employment, equity ownership, patents & royalties; C. Brooks: Stemline - employment, equity ownership, patents & royalties