Abstract

Twelve bit tiff images were acquired using the Cytotoxicity assay: entry into clinical trials, and a Phase I trial design will be discussed. These data demonstrate that SL-801 is a promising clinical candidate that inhibits a clinically relevant target, and nuclear export characteristics and poor patient outcome. The anti-tumor effects of SL-801 were validated against solid and hematologic cancers, with GI values ≤100 nM in lymphomas, and 1-103 nM in prostate, and sarcoma lines. In addition, a 5-fold increase in active caspase-3 staining caused significant toxicities in Phase 1 malignancies. SL-801 is a novel small molecule that inhibits XPO1/CRM1, the principal nuclear export protein in eukaryotic cells, 100 nM in 230/240 (95.8%) cell lines. SL-801 sensitivity was validated in high content screening platform was used to evaluate the cytotoxicity of SL-801 against tumor cell lines was further validated in experimental models. The anti-tumor effects of SL-801 was tested against number of cell doubling. 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. Days of 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 clinically relevant target and supports the clinical development of a broad range of oncologic indications. The reversible binding of SL-801 to XPO1 may offer the potential to develop dosing schedules that enable recovery in normal tissue, while broadening the therapeutic index of this class of agents. IN-enzymatic work is underway to support entry into clinical trials, and a Phase I trial design will be discussed.

Materials and methods

Cytotoxicity assays: The Oncoscan™ 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 (Vysis, Batheal, IL). Cells were stained with 348-well plates, and SL-801 was added 24 hours after cell seeding. SL-801 was serially diluted 3.16-fold and assessed over 10 concentrations (31.6 pM – 1 M) at 96 hours. After a 72-hour incubation period, cells were fixed and stained with a nuclear dye to allow visualization of nuclear and a fluorescent-labeled anti-active caspase-3 antibody to analyze 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 InCellAnalyzer software and analyzed with Developer Toolbox 1.0 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/cellline) were used to correlate SL-801 sensitivity to genomic alterations. Xenografts: Make mice (n=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 antitumor effects of SL-801 was tested against a panel of 240 cell lines representing diverse cancer types to SL-801 treatment. ARH-77, human multiple myeloma (A), RPMI-226, multiple myeloma (B), MM.1S, multiple myeloma (C). Anti-tumor activity of SL-801 against solid and hematologic cancers in vivo

Figure 2: SL-801 was tested in 510 xenograft models of (A) ARH-77 multiple myeloma, (B) RPMI-226 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 cancer cell lines with GI50 values 10-100 nM in 100% (95.8%) cell lines and G50 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 oncoproteins and tumor suppressor genes.

• The anti-tumor effects of SL-801 were validated in vivo against 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.

• The in vitro and in vivo anti-tumor effects observed with SL-801 suggest that XPO1 activity in multiple malignancies contributes to neoplastic pathogenesis.