SP-3164, a novel molecular glue degrader with activity in preclinical models of multiple myeloma

#6253

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Introduction

SP-3164, an oral, next-generation molecular glue, is currently in IND-enabling studies and is expected to enter the clinic in 2023. SP-3164 interacts with the cereblon (CRBN) component of a CRL4 E3 ligase, inducing recruitment and subsequent degradation of hematological transcription factors, Ikaros (IKZF1) and Aiolos (IKZF3) (Figure 1). SP-3164 uses deuterium to stabilize the (*S*)-enantiomer of avadomide (CC-122), an extensively studied clinical compound, preventing interconversion to the undesired (*R*)-enantiomer (Figure 2). SP-3164 has demonstrated superior single agent and combination activity in mouse models of multiple myeloma compared to approved molecular glues.

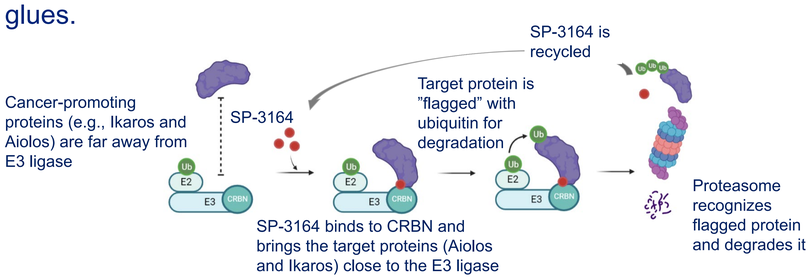

Figure 1. SP-3164's mechanism of action

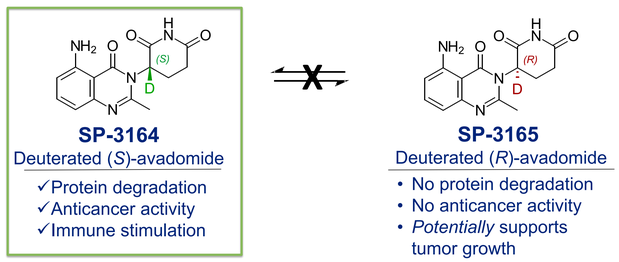

Figure 2. SP-3164 is the stabilized active enantiomer of avadomide

Objectives

- To demonstrate SP-3164's protein degradation effects and validate it as the active anticancer enantiomer of avadomide
- To study SP-3164 antitumor activity in models of multiple myeloma as a single agent and in combination with approved agents

Methods

- **In vitro viability:** Cell lines were plated and treated with SP-3164 (9 concentrations) for 96 hrs and viability assessed with CellTiterGlo®
- **Protein degradation and caspase-3 cleavage**: MM.1s cells were cultured and treated with SP-3164 for 6, 48, or 96 hrs and Ikaros and c-caspase-3 levels assessed by western blot.
- ***HiBit-IKZF3 degradation:*** . HiBiT-IKZF3 MM1.S CRISPR cell lines were treated and Ikaros levels monitored at 2-, 4-, and 24-hrs post treatment.
- **Flow cytometry**: Cells were treated with SP-3164 for 72 hrs, stained for annexin v and 7-actinomyacin D, and analyzed by flow cytometry.
- ***In vivo* efficacy:** Female NOD/SCID mice (n=10) were inoculated with 1X10^6 NCI-H929 (MM) cells and treated with test agent(s) when tumors reached ~100 mm^3.

Results

SP-3164 Induces Rapid and Efficient Ikaros and Aiolos degradation in PBMCs

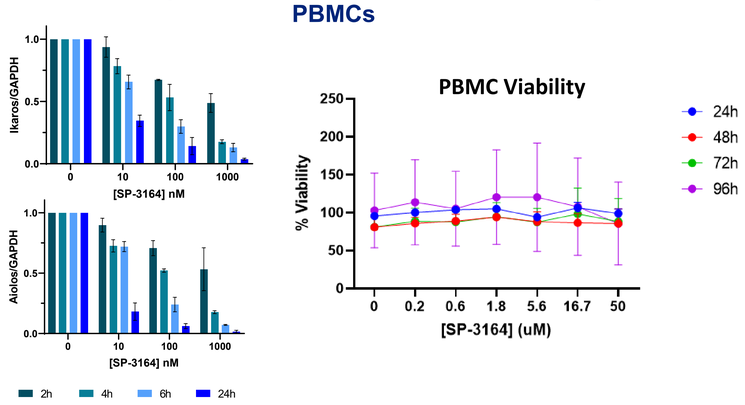

Figure 2. A) SP-3164 (10, 100, 1000 nM for 2, 4, 6, and 24 h) treatment resulted in potent time- and dose-dependent degradation of Ikaros and Aiolos in PBMCs. B) PBMC viability is unaffected through 96hrs of SP-3164 treatment.

SP-3164 Induces Rapid and Efficient Ikaros Degradation and Induces Caspase Cleavage in MM1.S Cells

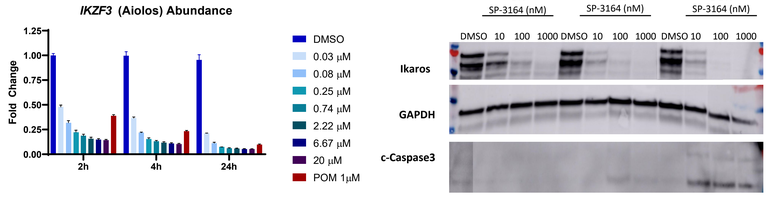

Figure 3. A) 30nM SP-3164 degrades 50% of Aiolos as early as 2 hrs in the HiBit-IKZF3 MM1.s CRISPR cell line. B) SP-3164 (10, 100, 1000 nM for 6, 48, and 96 h) treatment resulted in potent time- and dose-dependent degradation of Ikaros in MM1.S cells. Evidence of induction of apoptosis is demonstrated by cleaved caspase 3 at 96 hrs.

In Vitro Single-Agent Anti-proliferative Activity of SP-3164 in Multiple Myeloma

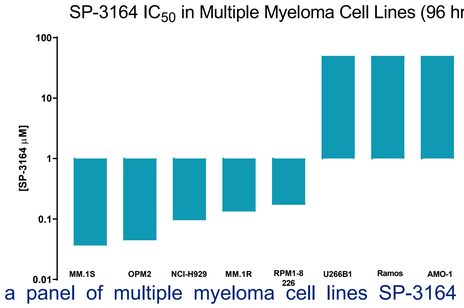

Figure 4 . In a panel of multiple myeloma cell lines SP-3164 demonstrated potent antiproliferative activity within 96 hrs of dosing in 5 of 8 cell lines assayed (Average IC_{50} 96 nM, range 0.04 μM-0.17 μM)

SP-3164 Shows Single-Agent Activity in Multiple Myeloma

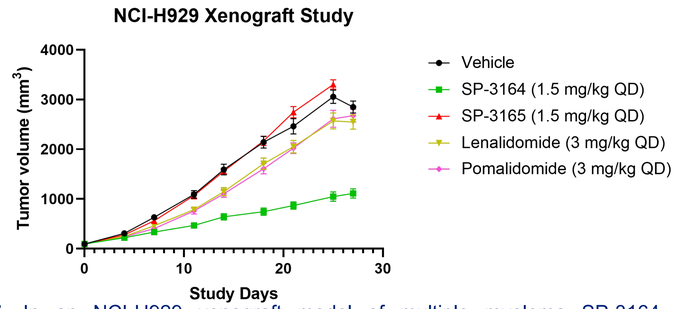

Figure 5. In an NCI-H929 xenograft model of multiple myeloma SP-3164 demonstrated pronounced antitumor activity as single agent outperforming lenalidomide and pomalidomide. SP-3165 lacked significant antitumor activity.

SP-3164 Synergizes with Standard of Care Agents in Multiple Myeloma

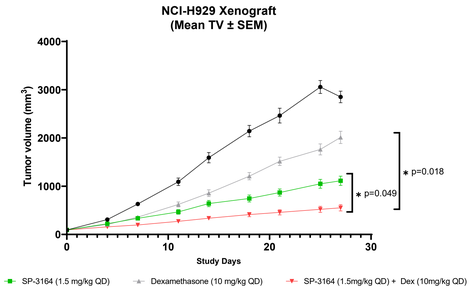

Figure 6. Combination of SP-3164 (1.5mg/kg QD) with dexamethasone (10mg/kg QD) resulted in a significant increase in tumor growth inhibition compared to either SP-3164 or dexamethasone as a single agent.

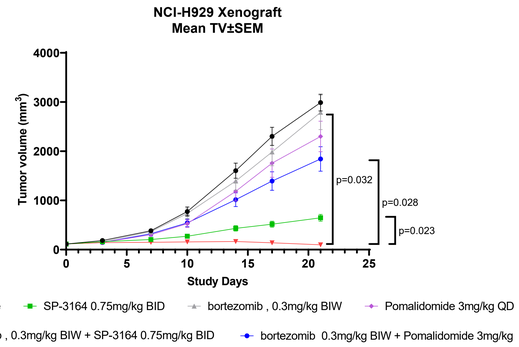

Figure 7. The combination of SP-3164 and bortezomib performed resulted in sustained regressions in 50% of treated mice and performed significantly better than SP-3164 or bortezomib alone and the combination of pomalidomide and bortezomib.

Conclusions

- SP-3164, the deuterium-stabilized, active (*S*)-enantiomer of avadomide, is a novel molecular glue that shows compelling antitumor activity in MM models
- SP-3164 potently and efficiently degrades Ikaros and Aiolos in PBMCs. In cancer cells, SP-3164 degrades Ikaros and Aiolos, induces apoptosis and reduces viability *in vitro*
- *In vivo*, SP-3164 showed significant activity as a single agent and in combination with dexamethasone
- *In vivo* combination of SP-3164 with bortezomib resulted in tumor regressions and performed significantly better than pomalidomide and bortezomib

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