A Novel Cereblon-Binding Molecular Glue, SP-3164, Shows Preclinical Activity In Non-Hodgkin Lymphomas

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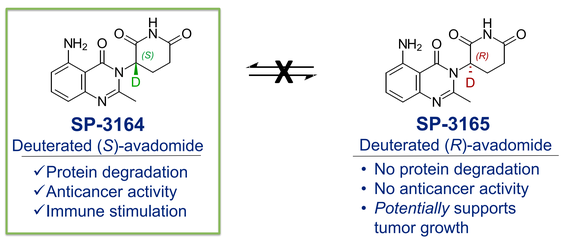
INTRODUCTION

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). SP-3164 incorporates deuterium to stabilize the (*S*)-enantiomer of avadomide (CC-122), an extensively studied clinical drug candidate, preventing interconversion to the undesired (*R*)-enantiomer. SP-3164 has shown compelling activity in preclinical models of Non-Hodgkin lymphomas (NHL) and may have advantages over other molecular glues.



OBJECTIVE

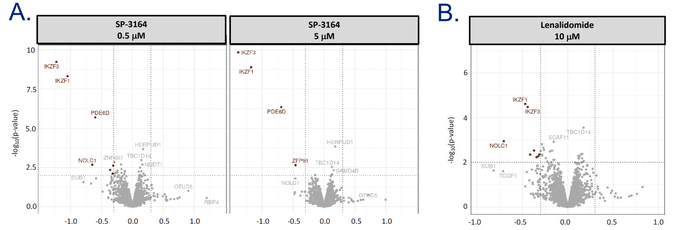
To characterize SP-3164's protein degradation and anticancer activity and demonstrate its therapeutic potential in NHLs, including follicular lymphoma (FL).

METHOD(S)

- **Quantitative proteomics:** Jeko-1 cells were treated for 4 hrs with SP-3164 (0.5 μM or 5 μM) or lenalidomide (10 μM). Protein abundance was determined by LC-MS-based protein quantification.
- **Protein degradation**: PBMCs were treated with SP-3164 for 6 hrs and Aiolos degradation was assessed by western blot.
- ***In vitro* viability**: PBMCs were plated and treated with SP-3164 (9 concentrations) for 96 hrs and viability assessed with CellTiterGlo®
- **Apoptosis/degradation**: JeKo-1 cells were treated with SP-3164 for 48 hours and stained for cleaved caspase-3 and Ikaros and analyzed by flow cytometry. Data shown is relative to DMSO control.
- **Cytokine secretion:** Human T cells, isolated from healthy donor PBMCs (n=6), were treated with a 10-point dose-response curve of SP-3164 or SP-3165 (d-R-CC-122) for 6 hrs. Soluble cytokine targets were measured in media using the Meso Scale Discovery assay system.
- ***In vivo* efficacy:** Female CB17/SCID mice (n=10) were inoculated with 5X10^6 DOHH-2 (FL) cells and treated with test agent(s) when tumors reached ~110 mm^3. Tumor samples were collected at 2 hrs after the last dosing and stained for Ikaros and Aiolos.

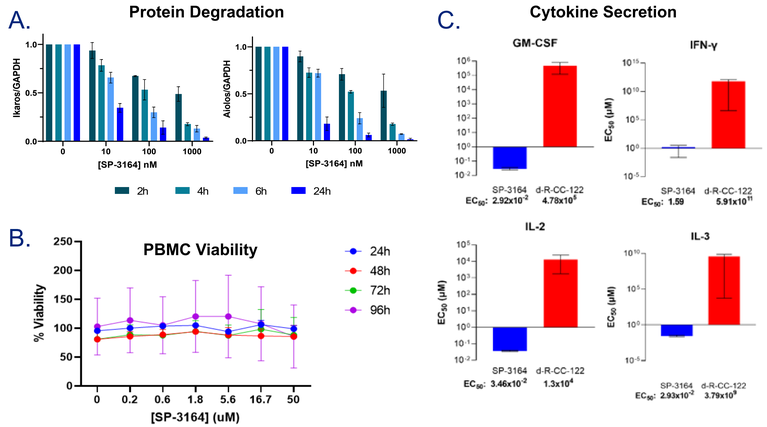
RESULTS

Quantitative Proteomics Reveals Potency of SP-3164-Induced Degradation



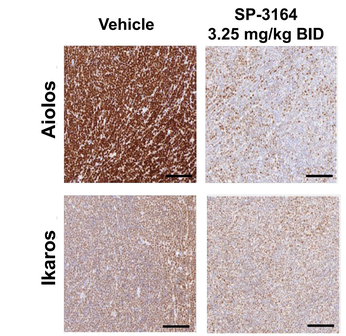
Four treatment of SP-3164 treatment (0.5 μM and 5 μM) resulted in 2-fold greater degradation of neosubstrates Ikaros (IKZF1) and Aiolos (IKZF3) in Jeko-1 cells (A) compared to lenalidomide (10 mM) (B)

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



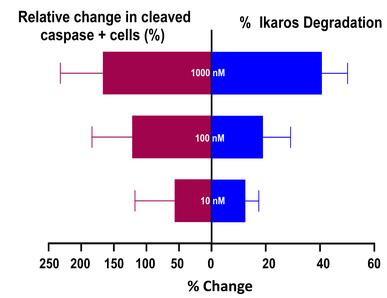
A) SP-3164 (10, 100, 1000 nM for 2, 4, 6, and 24 hrs) treatment resulted in potent time- and dose-dependent degradation of Ikaros and Aiolos in PBMCs. B) PBMC viability is unaffected through 96 hrs of SP-3164 treatment. C) SP-3164 potently induces secretion of immunomodulatory cytokines, GM-CSF, IFN-g, IL-2, and IL-3. d-R-CC-122 (SP-3165) does not induce cytokine secretion at clinically relevant concentrations.

SP-3164 Induces Degradation *In Vivo*



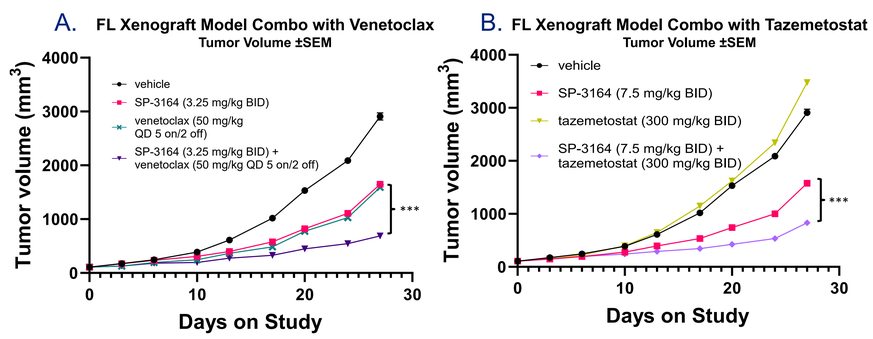
IHC analysis of DOHH2 xenograft tumors showed SP-3164 treatment resulted in Ikaros and Aiolos degradation in vivo.

Apoptosis Correlates with Ikaros Degradation



JeKo-1 apoptosis, as measured by cleaved caspase 3 expression, correlated with extent of Ikaros degradation at 48 hrs.

SP-3164 Demonstrates Potent *In Vivo* Anti-tumor single agent and commination activity



*SP-3164's activity was studied in a mouse model of follicular lymphoma (FL, DOHH2) as a single agent and in combination. A) With venetoclax, SP-3164 showed improved tumor growth inhibition (TGI) compared to either agent alone. B) With tazemetostat (TAZ), SP-3164 performed better than the approved agent, which had no effect. In combination, SP-3164 sensitized cells to TAZ resulting in improved TGI. (***p ≤0.001)*

CONCLUSIONS

- SP-3164, the deuterium-stabilized (*S*)-enantiomer of avadomide, is a novel molecular glue with compelling antitumor activity in NHL models.
- SP-3164 is more potent than lenalidomide, inducing 2-fold more degradation of neosubstrates 1/10 the dose.
- Quantitative proteomics reveal a PDE6 as neo-substrate unique to SP-3164 when compared to lenalidomide.
- *In vitro* immunomodulation studies validate SP-3164 as the active species and that the (*R*)-enantiomer has minimal effects.
- SP-3164 potently and efficiently degrades Ikaros and Aiolos in PBMCS in a time- and dose dependent manner.
- In cancer cells, SP-3164 degrades Ikaros and Aiolos, induces apoptosis, and reduces viability *in vitro*.
- In *in vivo* FL studies, SP-3164 showed compelling single agent TGI and when combined with venetoclax or tazemetostat resulted in more pronounced TGI.
- The preclinical data support the clinical evaluation of SP-3164 in lymphomas. A Phase 1 clinical trial is planned to start in H2 2023.

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