SP-3164, A Novel Cereblon-Binding Protein Degrader, Shows Activity in Preclinical Lymphoma Models

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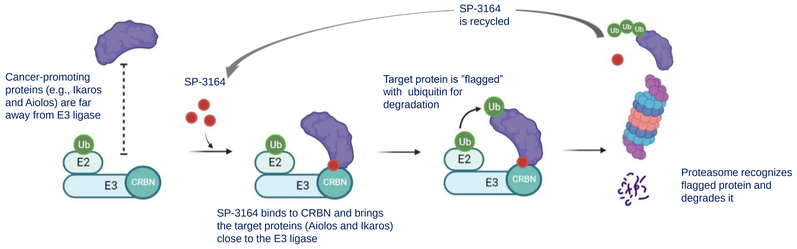
Introduction

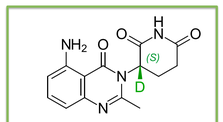
SP-3164, an oral, next-generation molecular glue, is currently in IND-enabling studies and is expected to enter the clinic in 2023. SP-3164 interacts with the cereblon (CRBN) component of a CRL4 E3 ligase, inducing recruitment and subsequent degradation of hematological transcription factors, Ikaros (IKZF1) and Aiolos (IKZF3) (Figure 1). Similar to other Ikaros and Aiolos degraders (e.g., lenalidomide and avadomide), SP-3164 has shown compelling activity in non-Hodgkin lymphomas (NHL) and it may have advantages over other molecular glues due to its unique characteristics.

Figure 1. SP-3164 mechanism of action



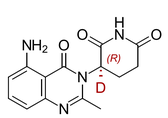
SP-3164 Development and Differentiation

- Most CRBN-binding degraders exist as a 1:1 mixture of interconverting (*S*)- and (*R*)-enantiomers, despite only the (*S*)-enantiomer having the desired anticancer and immunomodulatory activity.
- 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.
- By existing as the stabilized (*S*)-enantiomer, SP-3164 has the potential for an improved therapeutic profile compared to avadomide.



SP-3164
Deuterated (*S*)-avadomide

- ✓ Protein degradation
- ✓ Anticancer activity
- ✓ Immune stimulation



SP-3165
Deuterated (*R*)-avadomide

- No protein degradation
- No anticancer activity
- *Potentially* supports tumor growth

Objectives

- To validate SP-3164 as the active species of avadomide and demonstrate its activity in NHL models.
- To study SP-3164 antitumor activity in models of diffuse large B-cell lymphoma (DLBCL) in combination with standard of care agents.

Methods

- **Protein degradation**: DLBCL cells were cultured and treated with SP-3164, SP-3165, avadomide (AVA), or lenalidomide (LEN) for 6 hrs and Ikaros degradation assessed by western blot.
- ***In vitro* activity:** Cell lines were plated at previously determined optimal density into 96-well plates. Cells were treated with SP-3164 at a 9-point, 3.16-fold dilution (top concentration 50μM) for 96hrs or 168hr and viability assessed with CellTiterGlo®.
- **Pharmacokinetics (PK):** Mice (NOD/SCID, females) were administered AVA or SP-3164 by oral gavage; blood was collected at 5 time points (up to t = 5 hours) and plasma samples were analyzed by chiral LC/MS-MS.
- ***In vivo* efficacy:** Female NOD/SCID mice (n=10) were inoculated with $1X10^7$ WSU-DLCL2 (DLBCL) cells and treated with test agent when tumors reached ~175 mm^3. Single agent and combination regimens were studied in two separate studies.

Results

SP-3164 Induces Rapid and Efficient Ikaros Degradation

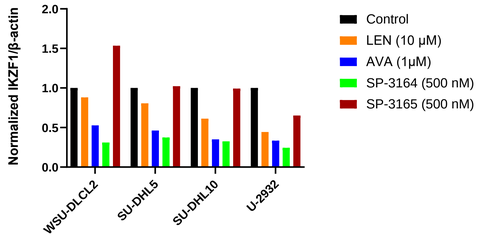


Figure 2. In DLBCL cell lines of different subtypes, SP-3164 (500 nM) resulted in greater Ikaros degradation at 6hr (compared to avadomide (1 μM) or lenalidomide (10 μM). By contrast, SP-3165 (500 nM) did not result in meaningful Ikaros depletion, validating it as the inactive enantiomer.

SP-3164 Exhibits Antiproliferative Effects Across NHL cell lines

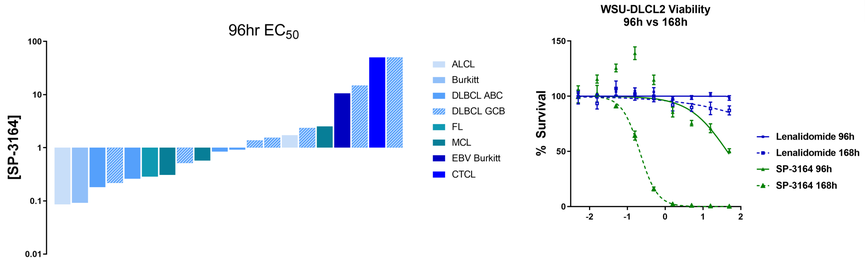


Figure 3. In a panel of lymphoma cancer cell lines representing various subtypes, SP-3164 demonstrated potent antiproliferative activity within 96hrs of dosing in 7 of 20 cell lines assayed (average EC50 <1uM, range 0.092-2.523 μM). In WSU DLCL2 cells, increased treatment (168 hr vs 96 hr) revealed increased sensitivity to SP-3164 (IC50 0.217μM).

SP-3164 PK Shows Exclusive Exposure to (*S*)-Enantiomer

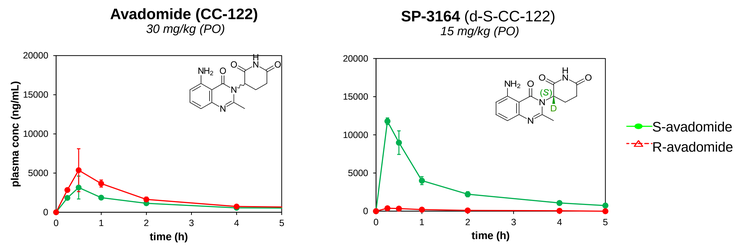
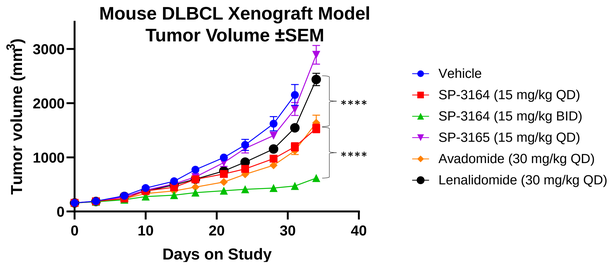


Figure 4. Mice were dosed with AVA (30 mg/kg) or SP-3164 (15mg/kg) and exposure to the (*S*)- and (*R*)-enantiomers was evaluated through a 5 hour period. SP-3164 showed minimal interconversion to the (*R*)-enantiomer and has a higher C_{max} and shorter $t_{1/2}$ compared to AVA.

SP-3164 Demonstrates Single-Agent Activity in DLBCL



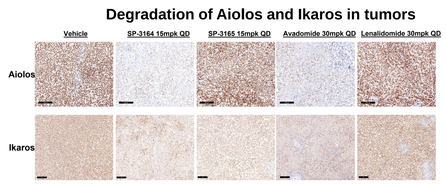


Figure 5. SP-3164 demonstrated pronounced antitumor activity as single agent outperforming lenalidomide and comparable to avadomide. SP-3165 lacked significant antitumor activity. Due to SP-3164's shorter half-life compared to avadomide, SP-3164 was studied BID resulting in the largest inhibitory effect (**** p≤ 0.0001). Tumor samples were collected at 1h, 3h, 6h, 8h, and 24h following the final dose of test agent and stained for Ikaros and Aiolos (6hr QD representative images shown).

SP-3164 Shows Synergistic Activity with rituximab in DLBCL

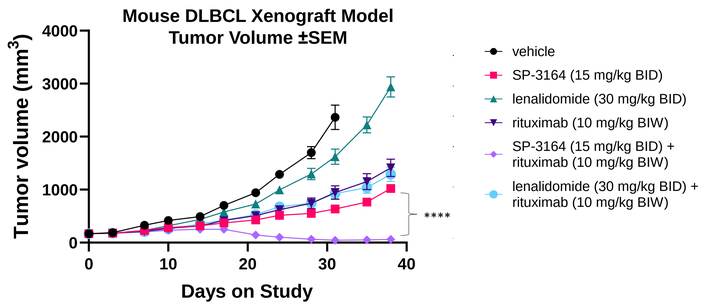


Figure 6. SP-3164's activity in combination with rituximab was compared to lenalidomide and rituximab in the WSU-DLCL2 DLBCL model. Mice were treated for 28 days and tumor volume was measured twice weekly. The combination of SP-3164 and rituximab resulted in sustained regressions with 50% of mice being tumor free, significantly better than approved regimen, lenalidomide and rituximab (****p ≤0.001).

Conclusions

- SP-3164 is a novel molecular glue that exists as the deuterium-stabilized, active (*S*)-enantiomer of avadomide and has compelling antitumor activity in NHL models.
- Preclinical studies validate SP-3164 as the active species and show that the (*R*)-enantiomer has no antitumor effects.
- In *in vivo* DLBCL studies, SP-3164 showed synergistic activity with rituximab (anti-CD20), resulting in tumor regressions and performing significantly better than the approved regimen, lenalidomide and rituximab.
- The presented data support clinical investigation of SP-3164 and a trial is planned for 2023.

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