SM09419, a Novel, Small-Molecule CDC-like Kinase (CLK) Inhibitor, Demonstrates Strong Inhibition of the Wnt Signaling Pathway and Antitumor Effects in Mantle Cell Lymphoma Models

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Background

- Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma (NHL) that accounts for ~7% of all NHL in the U.S. and is associated with chemoresistance and relapse.
- MCL is associated with aberrant activation of the Wnt signaling pathway, which plays a key role in the survival and maintenance of MCL-initiating cells.1,2
- CLKs regulate the activity of serine/arginine-rich splicing factors (SRSFs) that modulate spliceosome assembly, mRNA splicing, and subsequent gene expression.3,4
- SM09419 is a novel, oral, small-molecule pan-CLK inhibitor in development for the treatment of hematologic malignancies.
- These studies examined the antitumor activity of SM09419 in preclinical models of MCL.

Methods

In vitro assays:
- CLK inhibition was assessed by Thermo Fisher Z’22
- Wnt pathway inhibition was assessed by a luciferase reporter assay in SW480 colon cancer cells.
- Gene expression after 24 hours of exposure to vehicle or SM09419 was measured by qRT-PCR using TaqMan® primers and normalized to GAPDH expression.
- Cell proliferation was measured by the CellTiter Blue® assay in duplicate (Fig. 3).
- Apoptosis in MCL cells treated with vehicle, SM09419, or staurosporine (Stau) for 48 hours was assessed by Western blot (PARP cleavage and expression of apoptosis regulators) and the Caspase-3/7 assay kit (Fig. 3).

In vivo assays:
- Cell line-derived xenografts: SCID mice were implanted with REC-1 or Jeko-1 cells in the right flank and randomized into treatment groups when tumors reached ~100 mm³.
- Patient-derived xenografts (PDX) from Dana Farber Cancer Institute: NSG mice were intravenously injected with patient-derived MCL cells and randomized into treatment groups when CD45+CD19+ cells reached the indicated percent in peripheral blood. Mice were treated with vehicle or SM09419 for the indicated days.
- Survival: Vehicle, survival weight, and percent of CD45+CD19+ cells in blood, bone marrow, and spleen were calculated relative to vehicle (Fig. 5).
- Tolerability was determined by average bodyweight change from baseline (<15% loss considered well tolerated).
- Effects of SM09419 on SRSF phosphorylation and Wnt pathway-related gene and protein expression in MCL cell lines were measured by Western blot (Fig. 2 and Fig. 4).

Results

- Figure 1. SM09419 potently inhibited activity of all CLKs and the Wnt signaling pathway

Conclusions

- Potent SM09419-mediated reduction of SRSF6 phosphorylation and Wnt pathway gene and protein expression demonstrates a novel mechanism for inhibition of the Wnt pathway in MCL.
- SM09419 had strong in vitro and in vivo antileukemic activity as a single agent; this suggests that SM09419 may provide a clinical benefit for patients with treatment-resistant or refractory MCL.
- A Phase 1 study assessing the safety, tolerability, and pharmacokinetics of SM09419 in subjects with advanced hematologic malignancies is being initiated.

References


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