Accepted as poster #1377 at the Annual Meeting for the American Society of Hematology, San Diego, California, December 7-10, 2019

SM09419, a Novel, Small-Molecule CDC-like Kinase (CLK) Inhibitor, Demonstrates Strong Inhibition of the Wnt Signaling Pathway and Antitumor Effects in FMS-like Tyrosine Kinase 3 (*FLT3*)-Mutant Acute Myeloid Leukemia Models

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Background: Acute myeloid leukemia (AML) with the *FLT3* internal tandem duplication (*FLT3*-ITD) mutation accounts for ~25% of all AMLs, carries a poor prognosis, and is prone to relapse despite targeted therapy. *FLT3* mutations are associated with aberrant activation of the Wnt signaling pathway, which itself is implicated in AML initiation/progression and is required for the self-renewal and survival of leukemic stem cells. CLKs regulate the activity of serine/arginine-rich splicing factors (SRSFs) that modulate spliceosome assembly, mRNA splicing, and gene expression. SM09419 is a novel, oral, small-molecule pan-CLK inhibitor that potently inhibits the Wnt pathway. These studies examined the antitumor activity of SM09419 as a single agent and in combination with targeted and standard therapies in preclinical models of *FLT3*-ITD AML.

Methods and Results: In MV-4-11 and MOLM-13 AML cells carrying the *FLT3*-ITD mutation, SM09419 dose-dependently inhibited SRSF6 phosphorylation and potently suppressed expression of Wnt pathway-related genes (*CCND1*, *MYC*, *TCF7*, *DVL2*). The effect on cell proliferation was tested in 8 AML cell lines with varying mutation profiles as well as 26 different leukapheresis-derived primary human AML cells. Proliferation was strongly impaired by SM09419 across all tested cell lines (average EC_{50}=0.2 $\pm$ 0.048 µM]); MV-4-11 and MOLM-13 cells had EC_{50} of 0.049 and 0.144 µM, respectively. SM09419 also potently inhibited proliferation in all primary AML cells (average EC_{50}=0.048 $\pm$ 0.0097 µM) regardless of *FLT3* mutation status, cytogenetics, or AML diagnosis (*de novo* or relapsed/refractory). SM09419 also induced apoptosis in MV-4-11 and MOLM-13 cells, increasing caspase 3/7 activation and PARP cleavage while reducing survivin and MCL-1 expression relative to vehicle.

In vivo antitumor effects and tolerability of oral SM09419 (QD) alone or combined with either midostaurin (*FLT3* inhibitor) or venetoclax (*BCL2* inhibitor) and/or azacitidine were assessed in *FLT3*-ITD xenograft models (n=5–6/group). In MOLM-13 xenografts, SM09419 (12.5 and 25 mg/kg) induced strong tumor growth inhibition (TGI) vs. vehicle at Day 14 (TGI 52% [P<0.05] and 74% [P<0.001], respectively). Midostaurin (50 mg/kg) induced significant TGI vs. vehicle (50%, P<0.05), which was increased when administered in combination with 12.5 mg/kg SM09419 (81%, P<0.001). In MV-4-11 xenografts, single-agent SM09419 (6.25, 12.5, and 25 mg/kg) induced significant TGI vs. vehicle (56% [P<0.05], 94%, and 95% [P<0.001], respectively) at Day 26 with tumor regression in all mice dosed at 12.5 mg/kg and 25 mg/kg. In a

subsequent experiment, midostaurin (50 mg/kg) alone and combined with 6.25 mg/kg SM09419 for 23 days induced tumor regression in MV-4-11 xenografts (100% TGI vs. vehicle, $P<0.0001$). After treatment discontinuation, tumor regression was maintained in all mice (6/6) treated with the combination for 26 days, whereas tumor regrowth was immediately observed in midostaurin-treated mice. In another MV-4-11 xenograft study, the combination of 6.25mg/kg SM09419 with azacitidine (0.8 mg/kg QD) and/or venetoclax (25 mg/kg QD) induced significant TGI (95–98% vs. vehicle, $P<0.001$) with tumor regression at Day 26. Azacitidine + venetoclax induced 79% TGI ($P<0.001$), but no tumor regression was observed. The triple combination induced tumor regression in all mice and complete regressions in 4/6 mice (67%); it had a greater effect on slowing tumor regrowth after treatment discontinuation vs. a single agent or doublet. SM09419 alone or in combination was well tolerated in these xenograft models based on body weight measurements.

Conclusion: In summary, SM09419 potently inhibited SRSF6 phosphorylation and Wnt signaling pathway activity and induced apoptosis in *FLT3*-ITD cell lines. It also inhibited proliferation in cell lines and primary AML cells regardless of *FLT3* status. The strong *in vivo* antitumor effects observed as combination treatment suggest that SM09419 combined with standard therapies may provide a clinical benefit by slowing or preventing relapse in AML with a marker of poor prognosis such as *FLT3*-ITD. A Phase 1 study assessing safety, tolerability, and pharmacokinetics of SM09419 in subjects with advanced hematologic malignancies is being initiated.