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SM09419, a Novel, Small-Molecule CDC-like Kinase (CLK) Inhibitor, Demonstrates Strong Inhibition of the Wnt Signaling Pathway and Antitumor Effects in Tumor Protein p53 (*TP53*)-Mutant Acute Myeloid Leukemia Models

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Background: Acute myeloid leukemia (AML) with *TP53* mutation makes up ~13% of AML cases and is an aggressive, treatment-resistant subtype with dismal prognosis and limited therapeutic options. Aberrant activation of the Wnt signaling pathway is associated with AML initiation/progression and is required for the self-renewal and survival of leukemic stem cells, making Wnt signaling inhibition a potential therapeutic modality for adverse AML. 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 in a Wnt signaling reporter assay. The purpose of these studies was to examine the antitumor activity of SM09419 as a single agent and in combination with standard therapies in preclinical models of *TP53* mutant (*TP53mut+*) AML.

Methods and Results: In TF-1a and KG-1a AML cells with *TP53* mutations, SM09419 dose-dependently inhibited SRSF6 phosphorylation and potently suppressed expression of Wnt-related genes (*LEF1, MYC, DVL2*) and proteins vs. vehicle. The effect of SM09419 on cell proliferation was tested in 6 *TP53mut+* AML cell lines. Proliferation was strongly impaired by SM09419 across all cell lines (EC_{50}=0.23 $\pm$ 0.056 µM). SM09419 significantly induced apoptosis in TF-1a and KG-1a cells, increasing caspase 3/7 activation and PARP cleavage while reducing survivin and MCL-1 expression relative to vehicle. In addition, SM09419 potently inhibited cell proliferation when tested in 27 leukapheresis-derived human primary AML cell lines (EC_{50}=0.046 $\pm$ 0.0061 µM) regardless of *TP53* status, cytogenetics, or AML diagnosis (*de novo* or relapsed/refractory).

In vivo antitumor effects and tolerability of oral SM09419 (QD) alone or combined with cytarabine (Ara-C), venetoclax (VEN), or azacytidine (AZA) were assessed in mice bearing *TP53mut+* flank xenografts (n=5–15/group). In TF-1a xenografts, SM09419 (12.5 and 25 mg/kg) induced significant tumor growth inhibition (TGI) vs. vehicle at D20 (55–56% TGI [P<0.01]). VEN (50mg/kg) was not effective (3% TGI) and combining VEN with SM09419 had no additional benefit (52%–60% TGI). In Kasumi-1 xenografts, SM09419 (12.5 and 25 mg/kg), AZA (0.8 mg/kg), and VEN (25 mg/kg) induced TGI vs. vehicle of 87%, 95% (both P<0.0001), 72% (P<0.001), and 48% (NS), respectively at D18. SM09419 25 mg/kg alone induced tumor regression in 40% (2/5) of the mice. SM09419 (12.5 mg/kg) + VEN induced greater TGI vs. vehicle (96%, P<0.0001) with tumor regression in 80% (4/5) of the mice, while AZA + VEN induced 79% TGI (P<0.001) with no tumor regression. In KG-1a xenografts, single-agent

SM09419 (12.5 and 25 mg/kg) and Ara-C (10mg/kg) induced significant TGI vs. vehicle (53%, 98%, and 80% [P<0.001], respectively) at D28 but VEN (12.5mg/kg) did not (35% TGI). The combination of SM09419 (12.5 mg/kg) + VEN (12.5 mg/kg) improved TGI (98%) vs. vehicle. Tumor regression was seen in all mice with single-agent SM09419 (25 mg/kg) and 12.5 mg/kg + VEN. In another KG-1a xenograft study, mice were treated with combinations of SM09419 (12.5 mg/kg), AZA (0.8 mg/kg), and VEN (25 mg/kg) for 20 days followed by 21 days of SM09419 (25 mg/kg) or vehicle maintenance in some groups. SM09419 + VEN, SM09419 + AZA, and AZA + VEN induced TGI of 95%, 64%, and 58%, respectively (all P<0.0001), with 80% (12/15) regression in SM09419 + VEN. The triplet induced 91% TGI but was not well tolerated due to GI toxicity. In the maintenance phase, SM09419 given QD or QOD greatly slowed tumor regrowth vs. vehicle at D41 in mice previously treated with SM09419 + VEN (80% and 72% TGI [P<0.001], respectively). SM09419 QD maintenance therapy also slowed tumor regrowth following AZA + VEN (P<0.0001). SM09419 alone and in combination (except with AZA + VEN) was well tolerated in all tested xenografts.

Conclusion: In summary, SM09419 potently inhibited SRSF phosphorylation and Wnt pathway signaling and induced apoptosis in *TP53mut*+ AML cell lines. It also inhibited proliferation in cell lines and primary AML cells regardless of *TP53* status. Strong *in vivo* antileukemic effects were observed with SM09419 as a single agent or in combination with other AML therapies, suggesting that it is a potential treatment for hard-to-treat AML subtypes such as *TP53mut*+ AML. A Phase 1 study assessing safety, tolerability, and pharmacokinetics of SM09419 in subjects with advanced hematologic malignancies is being initiated.