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

Heekyung Chung, PhD[1], Emily Creger[1], Lauren Sitts, MS[1], Kevin Chiu[1], Chi-Ching Mak, PhD[1], Sunil KC, PhD[1], Betty Tam, PhD[2], Gail Bucci[1], Josh Stewart[1], Timothy Phalen, PhD[1], Steven Cha, MD[1]

[1]*Samumed, LLC, San Diego, CA;* [2]*Formerly Samumed, LLC, San Diego, CA*

Poster #1377

Background

- Acute myeloid leukemia (AML) with the *FLT3* internal tandem duplication (*FLT3*-ITD) mutation accounts for ~25% of AML cases and is aggressive and prone to relapse[1]
- Aberrant activation of Wnt pathway signaling is associated with AML initiation and progression; it is also required for the self-renewal and survival of leukemic stem cells[2-4]
- CLKs regulate the activity of serine/arginine-rich splicing factors (SRSFs) that modulate spliceosome assembly, mRNA splicing, and subsequent gene expression[5,6]
- SM09419 is a novel, oral, small-molecule pan-CLK inhibitor that potently inhibits the Wnt signaling pathway[7]
- These studies examined the antitumor activity of SM09419 as a single agent and in combination with standard of care (SOC) in preclinical models of *FLT3*-ITD AML

Conclusions

- **SM09419 potently inhibited SRSF6 phosphorylation, Wnt signaling pathway activity, and cell proliferation and induced apoptosis in *FLT3*-ITD AML cell lines**
- **The strong *in vivo* antitumor effects observed in combination treatment suggest that SM09419 combined with current SOC therapies may provide clinical benefit by slowing or preventing relapse in patients with *FLT3*-ITD AML**
- **A Phase 1 study assessing the safety, tolerability, and pharmacokinetics of SM09419 in subjects with advanced hematologic malignancies is being initiated**

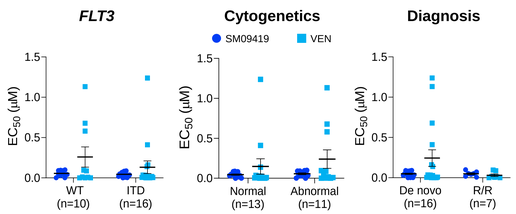
Results

Figure 1. SM09419 inhibited proliferation in AML cell lines and primary human AML cells regardless of *FLT3* mutation status, cytogenetics, and diagnosis

Cell Line	Mutations	EC_{50} (μM)	Average EC_{50} (μM)
MV-4-11	*FLT3*	0.049	0.200
MOLM-13	*FLT3*	0.144	
TF-1a	*TP53*, *NRAS*	0.281	
TF1	*TP53*, *NRAS*	0.116	
KG1a	*TP53*, *NRAS*, *MYC*^	0.124	
HL-60	*TP53*, *NRAS*	0.285	
Kasumi-1	*TP53*	0.133	
THP-1	*TP53*, *NRAS*	0.467	

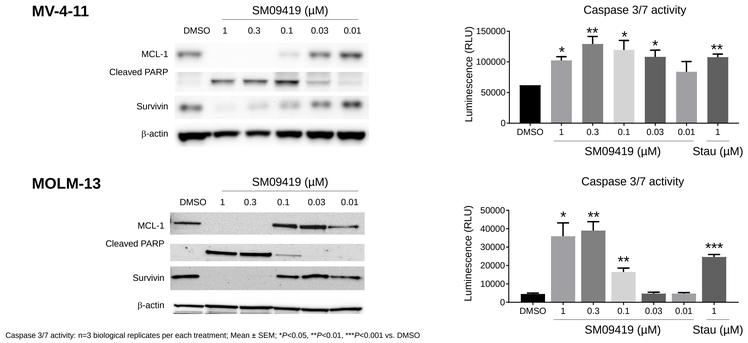
^ = Overexpression

Average EC_{50} in primary AML cells (N=26)
SM09419: 0.048 ± 0.0097 μM VEN: 0.181 ± 0.0361 μM



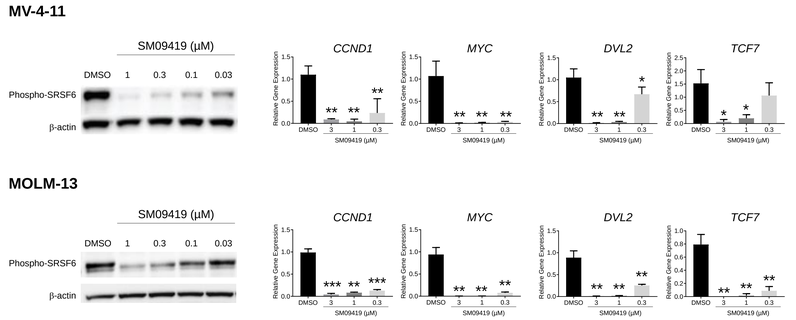
QD dosing for all treatments; n=5/group; *P<0.05, ***P<0.001 vs. vehicle; SM09419 was well tolerated in all groups

Figure 2. SM90419 induced apoptosis in *FLT3*-ITD AML cells



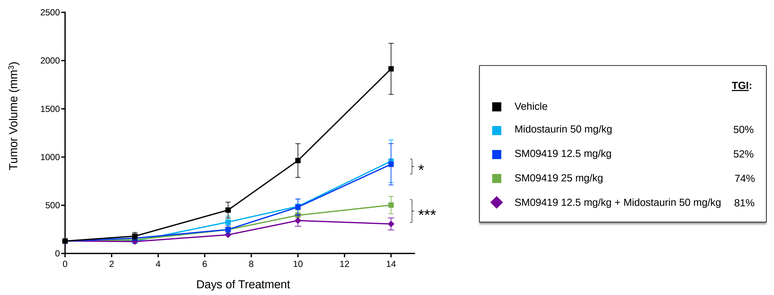
Caspase 3/7 activity: n=3 biological replicates per each treatment; Mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 vs. DMSO

Figure 3. SM09419 dose-dependently inhibited SRSF6 phosphorylation and Wnt pathway-related gene expression in *FLT3*-ITD AML cells



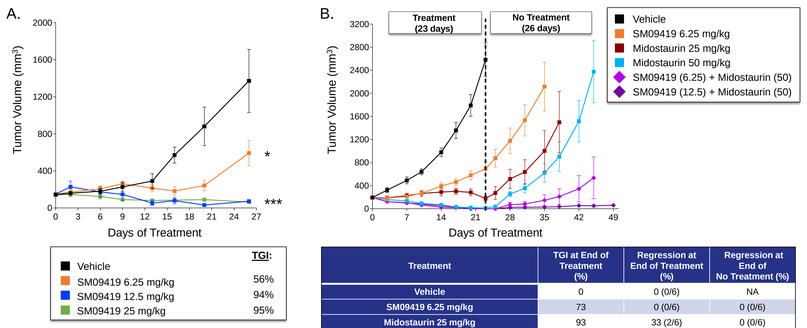
Gene expression: n=2 biological replicates; Mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 vs. DMSO

Figure 4. SM09419 + Midostaurin was more efficacious than single agent treatment in MOLM-13 xenografts



QD dosing for all treatments; n=5/group; *P<0.05, ***P<0.001 vs. vehicle; SM09419 was well tolerated in all groups

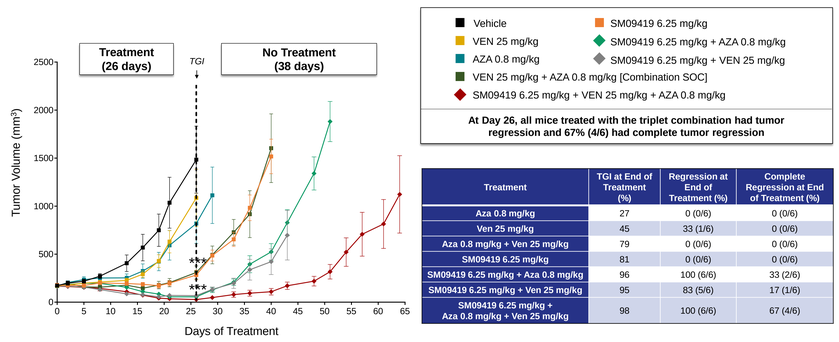
Figure 5. SM09419 was active as a single agent (A) and SM09419 + Midostaurin maintained tumor regression after treatment discontinuation (B) in MV-4-11 xenografts



Treatment	TGI at End of Treatment (%)	Regression at End of Treatment (%)	Regression at End of No Treatment (%)
Vehicle	0	0 (0/6)	NA
SM09419 6.25 mg/kg	73	0 (0/6)	0 (0/6)
Midostaurin 25 mg/kg	93	33 (2/6)	0 (0/6)
Midostaurin 50 mg/kg	100	100 (6/6)	0 (0/6)
SM09419 6.25 mg/kg + Midostaurin 25 mg/kg	100	100 (6/6)	67 (4/6)
SM09419 6.25 mg/kg + Midostaurin 50 mg/kg	100	100 (6/6)	100 (6/6)

QD dosing for all treatment; Mean ± SEM; **A.** n=5 mice/group; *P<0.05, ***P<0.001 vs. vehicle
B. n=6 mice/group; Line plot was discontinued when first mouse in each group was euthanized due to tumor volume (>2000 mm³); SM09419 was well tolerated in all groups

Figure 6. SM09419 + AZA ± VEN induced tumor regression in MV-4-11 xenografts; the triplet combination had the strongest inhibitory effect on tumor regrowth after discontinuation



At Day 26, all mice treated with the triplet combination had tumor regression and 67% (4/6) had complete tumor regression

Treatment	TGI at End of Treatment (%)	Regression at End of Treatment (%)	Complete Regression at End of Treatment (%)
Aza 0.8 mg/kg	27	0 (0/6)	0 (0/6)
Ven 25 mg/kg	45	33 (1/6)	0 (0/6)
Aza 0.8 mg/kg + Ven 25 mg/kg	79	0 (0/6)	0 (0/6)
SM09419 6.25 mg/kg	81	0 (0/6)	0 (0/6)
SM09419 6.25 mg/kg + Aza 0.8 mg/kg	96	100 (6/6)	33 (2/6)
SM09419 6.25 mg/kg + Ven 25 mg/kg	95	83 (5/6)	17 (1/6)
SM09419 6.25 mg/kg + Aza 0.8 mg/kg + Ven 25 mg/kg	98	100 (6/6)	67 (4/6)

QD dosing for all treatment; n = 5-6 mice/group; Mean ± SEM; ***P<0.001 vs. vehicle
Line plot was discontinued when first mouse in each group was euthanized due to tumor volume (>2000 mm³); SM09419 was well tolerated in all groups

Methods

In vitro assays:

- Cell proliferation in 8 AML cell lines and 26 primary leukapheresis-derived human AML cell samples (Champions Oncology) treated with vehicle, SM09419, or Venetoclax was assessed using the CellTiter-Blue® assay or CellTiter-Glo® assay, respectively (**Fig. 1**)
- Apoptosis in AML cells treated with vehicle, SM09419, or staurosporine (Stau) for 24 hours was assessed by Western blot (PARP cleavage and expression of apoptosis regulators) and the Caspase-Glo® 3/7 assay kit (**Fig. 2**)
- SRSF phosphorylation in AML cells treated with vehicle or SM09419 for 1 hour was measured by Western blot (**Fig. 3**)
- Gene expression in AML cell lines after 16 hours of exposure to vehicle or SM09419 was measured by qRT-PCR using TaqMan® primers and normalized to GAPDH expression (**Fig. 3**)

In vivo assays:

- Cell line-derived xenografts: Nude mice were implanted with MOLM-13 or MV-4-11 cells in the right flank and randomized into treatment groups when tumors reached ~100-200 mm³. Mice were orally treated with vehicle, SM09419, Midostaurin, Azacitidine (AZA), Venetoclax (VEN), or combinations of these drugs for indicated doses and times (**Figs. 4-6**)
  - Tumor growth inhibition (TGI) was calculated relative to vehicle
  - Tumor regressions were assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines: 30%-100% reduction in tumor volume relative to the start of the study
  - Tolerability was determined by average bodyweight change from baseline (<10% loss considered well tolerated)

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All authors are employees, shareholders, or consultants of Samumed, LLC. Other disclosures are listed in the published abstract.

9360 Towne Centre Drive, San Diego, CA 92121
info@samumed.com