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

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 #3913

Background

- Acute myeloid leukemia (AML) with *TP53* mutation (*TP53*mut) accounts for ~13% of AML cases and is an aggressive, treatment-resistant subtype of cancer with dismal prognosis and limited therapeutic options[1]
- Aberrant activation of the Wnt signaling pathway is associated with AML initiation and progression; it is also required for the self-renewal and survival of leukemic stem cells, making Wnt signaling inhibition a potential therapeutic modality for adverse AML[2-4]
- CLKs regulate the activity of serine/arginine-rich splicing factors (SRSFs) that modulate spliceosome assembly, mRNA splicing, and gene expression[5,6]
- SM09419 is a novel, oral, small-molecule pan-CLK inhibitor that potently inhibits the Wnt pathway[7]
- These studies examined the antitumor activity of SM09419 in preclinical models of *TP53*mut AML

Conclusions

- **SM09419 demonstrated potent *in vitro* antileukemic effects and inhibited SRSF activation and Wnt pathway signaling in *TP53*mut AML cells**
- **SM09419 had strong *in vivo* antileukemic activity as a single agent and in combination with VEN and/or AZA; SM09419 was well tolerated, except in the triplet combination**
- **These results suggest that SM09419 is a potential treatment for hard-to-treat subtypes such as *TP53*mut 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 *TP53* mutation status, cytogenetics, or diagnosis

Cell Line	Mutations	EC_{50} (μM)	Average EC_{50} (μM)
HL-60	*TP53*, *NRAS*	0.285	0.234
TF-1a	*TP53*, *NRAS*	0.281	
TF1	*TP53*, *NRAS*	0.116	
KG1a	*TP53*, *NRAS*, *MYC*^	0.124	
Kasumi-1	*TP53*	0.133	
THP-1	*TP53*, *NRAS*	0.467	

^ = Overexpression

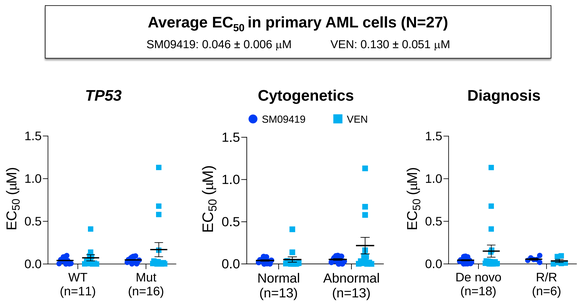
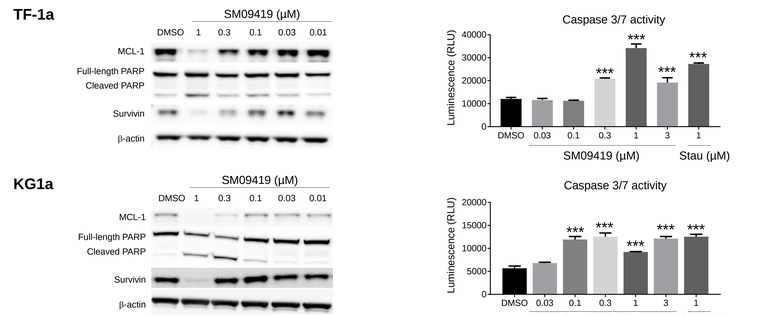
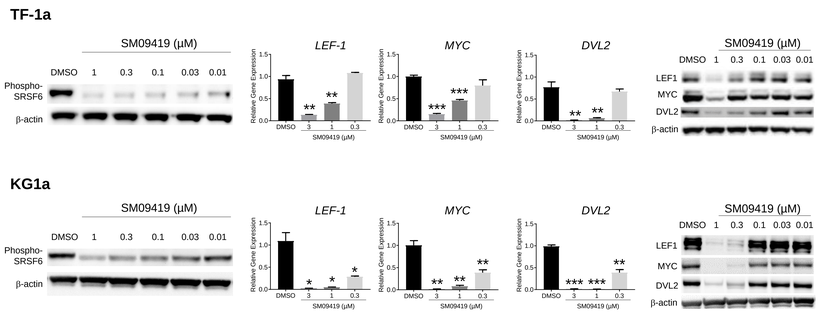


Figure 2. SM09419 induced apoptosis in *TP53*mut AML cells



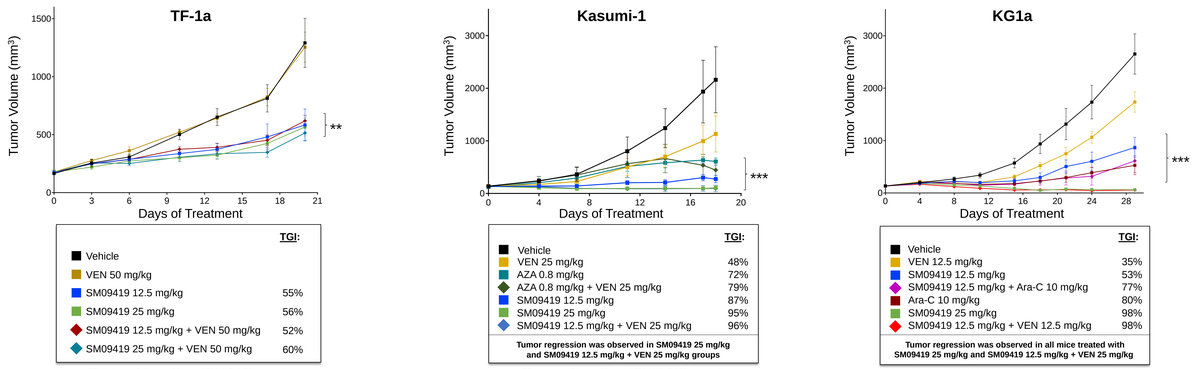
Caspase 3/7 activity: n=3 biological replicates; Mean ± SEM; ***P<0.001 vs. DMSO

Figure 3. SM09419 dose-dependently inhibited SRSF6 phosphorylation and Wnt pathway-related gene and protein expression in *TP53*mut AML cells



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

Figure 4. SM09419 demonstrated strong antileukemic effects as a single agent and in combination with Venetoclax, Azacytidine, or Cytarabine in AML xenografts

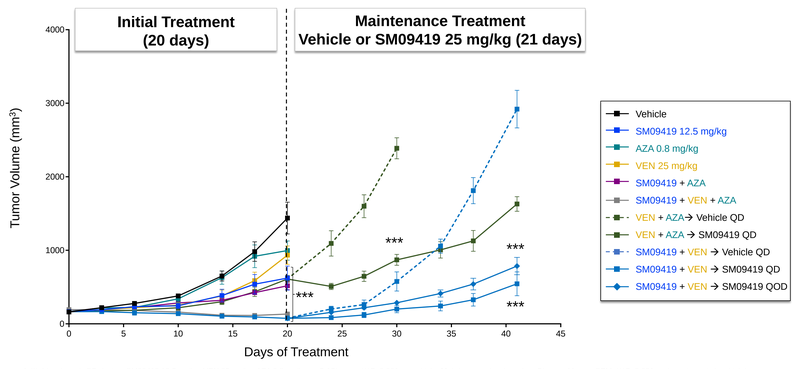


QD dosing; n=5-6/group; Mean ± SEM; **P<0.01 vs. vehicle
SM09419 was well tolerated in all groups

QD dosing; n=5/group; Mean ± SEM; ***P<0.001 vs. vehicle
SM09419 was well tolerated in all groups

SM09419 and VEN dosed QD, Ara-C dosed 5x/week; n=4-5/group; Mean ± SEM
***P<0.001 vs. vehicle. SM09419 was well tolerated in all groups

Figure 5. SM09419 + Venetoclax as initial treatment induced tumor regression and SM09419 as maintenance treatment slowed tumor regrowth in KG1a xenografts



Initial treatment: QD dosing; SM09419 12.5 mg/kg, VEN 25 mg/kg, AZA 0.8 mg/kg; n=5-15/group; ***P<0.001 vs. vehicle; **Maintenance treatment:** n=5/group; Mean ± SEM; ***P<0.001 vs. [counterpart → vehicle]
Line plot was discontinued when first mouse in group was euthanized due to tumor volume (>3000 mm³)

Initial Treatment	TGI *Day 20 (%)*	Regression *Day 20 (%)*	Maintenance Treatment	TGI *Day 30 (%)*	TGI *Day 41 (%)*
AZA	31	0 (0/5)	NA	NA	NA
VEN	35	0 (0/5)	NA	NA	NA
SM09419	57	0 (0/5)	NA	NA	NA
SM09419 + AZA	64	0 (0/5)	NA	NA	NA
VEN + AZA	58	0 (0/10)	Vehicle	0	NA
			SM09419	63	
SM09419 + VEN	95	80 (12/15)	Vehicle	0	0
			SM09419 QD	66	80
			SM09419 QOD	51	72
SM09419 + VEN + AZA	91	50 (5/10)	NA	NA	NA

SM09419 + VEN + AZA was not tolerated in 33% of mice due to GI toxicity
SM09419 alone or as a doublet combination was well tolerated

Methods

In vitro assays:

- Cell proliferation in 6 *TP53*mut AML cell lines and 27 primary leukapheresis-derived human AML cell samples (Champions Oncology) treated with vehicle, SM09419, or Venetoclax was assessed using the CellTiter-Blue® assay and CellTiter-Glo® assay in 4 replicates, respectively. Differences in *TP53* mutation status, cytogenetics, or AML diagnosis (de novo or relapsed/refractory [R/R]) were directly compared in primary human AML cells (**Fig. 1**)
- Apoptosis in AML 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-Glo® 3/7 assay kit (**Fig. 2**)
- Effects of SM09419 on SRSF phosphorylation and Wnt pathway-related protein expression in AML cell lines were measured by Western blot after 48 hours of treatment (**Fig. 3**)
- Gene expression in AML cell lines after 24 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: SCID mice were implanted with TF-1a, Kasumi-1, or KG1a cells in the right flank and randomized into treatment groups when tumors reached ~100-200 mm^3. Mice were orally treated with vehicle, SM09419, Venetoclax (VEN), Azacitidine (AZA), Cytarabine (Ara-C), or combinations of these drugs for indicated doses and times (**Fig. 4** and **Fig. 5**)
  - 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 (<15% loss considered well tolerated)

References

1. Short NJ, et al. *Lancet.* 2018.
2. Ysebaert L, et al. *Leukemia.* 2006.
3. Lane WW, et al. *Blood.* 2011.
4. Wang Y, et al. *Science.* 2010.
5. Colwill K, et al. *EMBO J.* 1996.
6. Long JC, et al. *Biochem J.* 2009.
7. Chung H, et al. *61st ASH Annual Meeting & Exposition*. 2019. Abstract 4059.

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