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

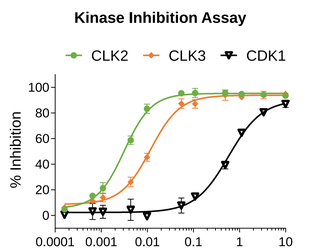
- Mantle cell lymphoma (MCL) is a type of B-cell non-Hodgkin lymphoma (NHL) that accounts for ~7% of all NHL in the U.S. and is associated with chemoresistance and relapse[1]
- MCL is associated with aberrant activation of the Wnt signaling pathway, which plays a key role in the survival and maintenance of MCL-initiating cells[2,3]
- CLKs regulate the activity of serine/arginine-rich splicing factors (SRSFs) that modulate spliceosome assembly, mRNA splicing, and subsequent gene expression[4,5]
- SM09419 is a novel, oral, small-molecule pan-CLK inhibitor in development for the treatment of hematological malignancies
- These studies examined the antitumor activity of SM09419 in preclinical models of MCL

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**

Results

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



	CLK2	CLK3	CLK1	CLK4	CDK1
IC_{50} (μM)	0.005	0.019	0.020	0.002	0.72

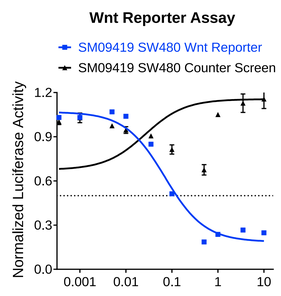
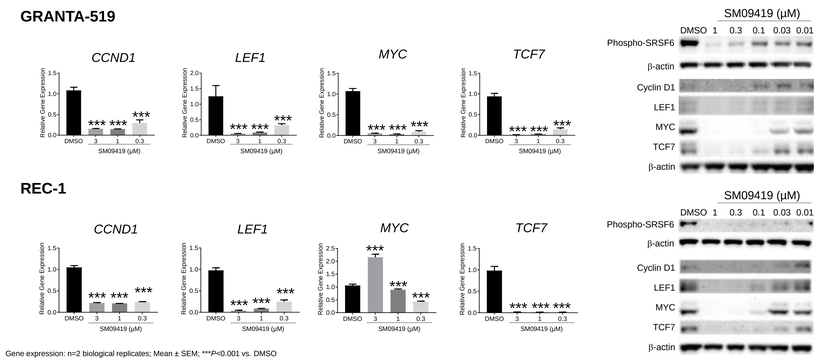


Figure 2. SM09419 significantly inhibited SRSF6 phosphorylation and Wnt pathway-related gene and protein expression in MCL cell lines



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

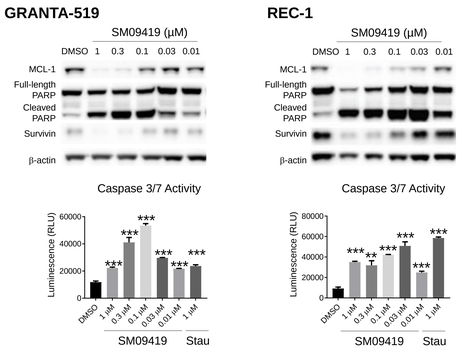
Figure 3. SM09419 strongly impaired proliferation and induced apoptosis in MCL cell lines

A.

Cell Line	Mutations	EC_{50} (μM)	Average EC_{50} (μM)
REC-1	*TP53, CCND1^*	0.03	0.102
JVM-2	*CCND1^*	0.236	
JeKo-1	*TP53*	0.021	
GRANTA-519	*CDKN2B*	0.111	
Mino	*TP53, NRAS*	0.11	

^ = Overexpression

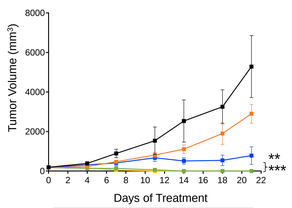
B.



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

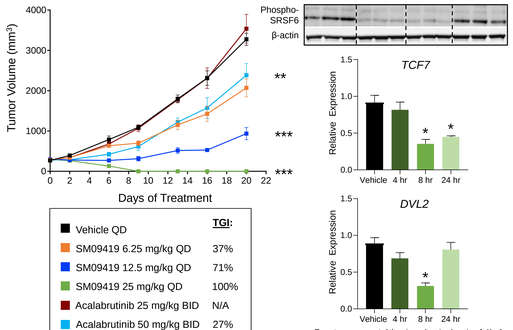
Figure 4. SM09419 potently inhibited tumor growth and induced tumor regression in MCL xenografts

A. **REC-1 Xenografts**



	TGI:
Vehicle QD	
SM09419 6.25 mg/kg QD	45%
SM09419 12.5 mg/kg QD	88%
SM09419 25 mg/kg QD	100%
SM09419 50 mg/kg QD	100%

B. **JeKo-1 Xenografts and Target Engagement in JeKo-1 Tumors**



	TGI:
Vehicle QD	
SM09419 6.25 mg/kg QD	37%
SM09419 12.5 mg/kg QD	71%
SM09419 25 mg/kg QD	100%
Acalabrutinib 25 mg/kg BID	N/A
Acalabrutinib 50 mg/kg BID	27%

Target engagement: Athymic nude mice bearing JeKo-1 tumors were administered a single dose of SM09419 (25 mg/kg, PO; n=9) or vehicle (n=3)

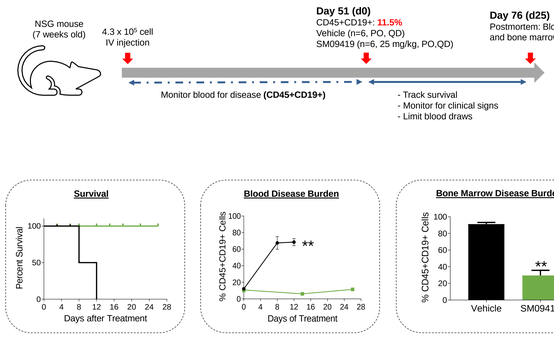
n=5/group; Mean ± SEM; **P*<0.05, ***P*<0.01, ****P*<0.001 vs. vehicle; SM09419 was well tolerated in all groups

Figure 5. SM09419 increased survival and reduced disease burden in two PDX models of MCL

A.

DFBL-91438

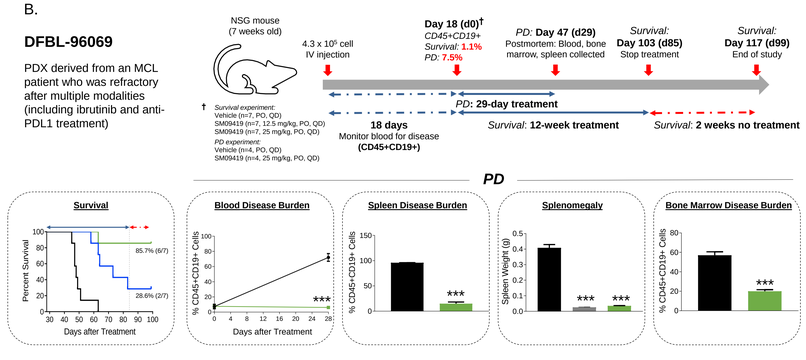
PDX derived from an MCL patient who was progressive after 8 different treatments (including ibrutinib treatment)



B.

DFBL-96069

PDX derived from an MCL patient who was refractory after multiple modalities (including ibrutinib and anti-PDL1 treatment)



QD dosing; Mean ± SEM; ***P*<0.01, ****P*<0.001 vs. vehicle; SM09419 was well tolerated in both PDX models

Methods

In vitro assays:

- CLK inhibition was assessed by Thermo Fisher Z'-LYTE™ and LanthaScreen kinase assays (**Fig. 1**)
- Wnt pathway inhibition was assessed by a luciferase reporter assay in SW480 colon cancer cells (**Fig. 1**)
- Effects of SM09419 on SRSF phosphorylation and Wnt pathway-related protein expression in MCL cell lines were measured by Western blot (**Fig. 2** and **Fig. 4**)
- Gene expression after 24 hours of exposure to vehicle or SM09419 was measured by qRT-PCR using TaqMan® primers and normalized to GAPDH expression (**Fig. 2** and **Fig. 4**)
- 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-Glo® 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 ~100-200 mm^3. Mice were orally treated with vehicle, SM09419, or Acalabrutinib for 20-21 days. Tumor growth inhibition (TGI) was calculated relative to vehicle (**Fig. 4**)
- 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, spleen 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)

References

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