Discovery of A Novel Menin-MLL Inhibitor for Potential Treatment of MLLr Leukemias and NPM1c AML

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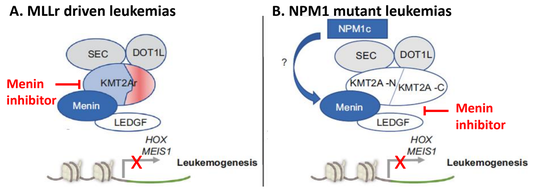
Abstract

Patients with MLL rearranged (MLLr) acute leukemias often have poor prognosis, and there is no targeted therapies available for this subtype of leukemias. The protein protein interaction (PPI) between MLLr and menin is critical for the pathogenesis of MLLr-driven leukemias. And it has been well demonstrated in both preclinic and clinic that blockade of this PPI could have therapeutic implications in the treatment of menin-MLL dependent leukemias.
Herein we report the discovery of BNM-1192, a small molecule menin inhibitor. BNM-1192 is a potent and highly selective menin inhibitor with optimized drug-like properties and showed excellent efficacy in MV4-11 xenograft mouse model. Furthermore, BNM-1192 has low risk in QTc prolongation given the fact it is an extremely weak hERG inhibitor with IC_{50} of greater than 100 μM. it also demonstrated favorable toxicological profile in preliminary tox studies .

Background

Rearrangement of the mixed lineage leukemia (MLL, also known as MLL1 or KMT2A) gene occurs in about 10% of acute leukemias, and is particularly prevalent in infant acute leukemias, accounting for up to ~70% of infant acute lymphocytic leukemia (ALL) cases. More than 80 partner genes are implicated in MLL fusions, and six main partner genes make up about 80% of cases, which include AF4, AF6, AF9, AF10, ENL and ELL. MLL fusion proteins enhance proliferation and block hematopoietic differentiation, ultimately driving the development of leukemia by dysregulation of the HOXA and MEIS1 genes. MLLr leukemia is one of the high-risk types of leukemia with aggressive nature, resistance to therapy, and high frequency of early relapse, and with a 5-year survival rate of only approximately 35%.

The interaction between menin and MLLr is critical to the pathogenesis of MLLr-driven leukemias. Recent studies also revealed the importance of the menin-MLL1 wild-type (wt) interaction in NPM1 mutant AML. And blocking the menin-MLL interaction has proved to be a viable therapeutic strategy for the treatment of MLLr associated acute leukemias and NMP1 mutant AML.



Adapted from Issa, G. C. et al., Leukemia, 2021, 35, 2482

BNM-1192 is a potent and selective menin inhibitor

Figure 1. left, Menin-MLL peptide cocrystal (PDB 4GQ6). Menin shown as ribbon in cyan, and MLL peptide as sticks in magenta. Right, docking pose of BNM-1192 binding to menin. BNM-1192 binds to the same menin pocket as MLL N-terminal peptide. And a hydrogen bond formed directly between small molecule and Glu363 of menin was revealed.

Table 1. Antiproliferative activities against leukemia cell lines.

	Cell lines	BNM-1192	SNDX-5613
IC_{50} (nM)	MV-4-11 (AF4 fusion)	3.5±1.2 (n=8)	9.2±3.0 (n=7)
	MOLM13 (AF9 fusion)	12	26
	OCI-AML3 (NPM1 mutant)	11	75
	HL-60 (MLL wild type)	>10000	~8000

Figure 2. Dose response curve. Left, targeted cell lines; right, HL-60, control cell line.

- BNM-1192 is very potent against leukemia cell lines with MLL-fusion protein and NPM1 mutant.
- ~1000-fold selectivity over control cell line, HL-60, observed.

BNM-1192 leads to menin protein degradation

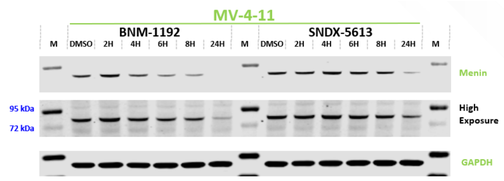


Figure 3. Western blot to determine menin protein. MV-4-11 cells were treated with BNM-1192 and SNDX-5613 at 10 μM for 2, 4, 6, 8, 24 hours, respectively, with DMSO as the control. Transfer: iBlot P0-9min; Sample: cell lysate; Total Protein: 30ug (BCA) 4-12% BT Gel & MOPS; M: Marker (Beyotime# P0069)

BNM-1192 demonstrated decent PK properties

Table 2. Cross-species PK

PK parameters	Mouse	Rat	Dog
CL (mL/min/kg)[a]	64	90	22
$t_{1/2}$ (h)[a]	1.6	2.4	3.9
Vss (L/kg)[a]	4.5	13	3.8
AUC_{0-24} (ng*h/mL)[b]	1272	272	6186
F (%)[b]	50	16 (175[c])	79

[a] iv 1mg/kg; [b] po 10 mg/kg; [c] po 100 mg/kg;

- Good to excellent bioavailability in mouse and dog; improved exposure at higher dose for rat.

BNM-1192 showed excellent efficacy in MV-4-11 xenograft mouse model

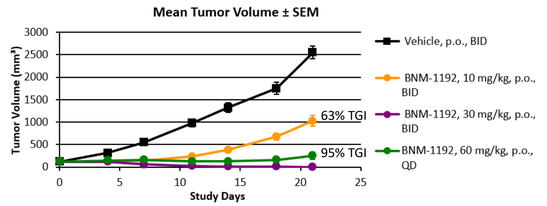


Figure 4. MV-4-11 xenograft mouse model. Mice were administered orally either vehicle or compound for 21 days dosed QD or BID as indicated.

Table 3. PK of mice with tumor burden

Dose (mg/kg)	C_{max} (ng/mL)	C_{8h} (ng/mL)	AUC_{0-24} (ng.hr/mL)
10	68	7.2	250
30	564	43	1680
60	1575	154	7166

- BNM-1192 showed dose-dependent efficacy
- BNM-1192 at 30 mg/kg BID resulted in tumor regression
- BNM-1192 at 60 mg/kg QD also showed good tumor inhibition (95%).
- Duration of coverage above IC_{50} (IC_{90}) seems more important than exposure for efficacy

BNM-1192 has low risk in QTc prolongation

Table 4. *In vitro* Early safety data

	BNM-1192	SNDX-5613
hERG (IC_{50}, μM)	>100	9.6
Mini Ames (TA98/TA100/TA1535/TA1537; WP2 uvrA (pKM 101)	Negative	ND

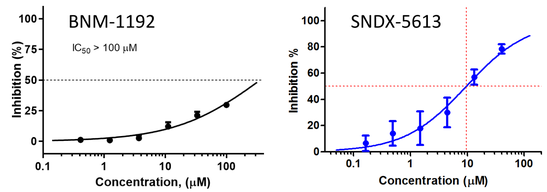


Figure 5. Concentration-dependent blockade of hERG channel. Left, BNM-1192; right, SNDX-5613.

BNM-1192 showed favorable tox profile

- A 7-day preliminary tox studies in rat was done
- No significant findings
- The high dose, 1000 mg/kg, identified as the HNSTD
- >500-fold safety margin based on exposure (AUC)

Conclusions

- BNM-1192 is a low nanomolar menin inhibitor. As high as 1000-fold selectivity was observed for targeted cell lines over mechanistically irrelevant cell line.
- BNM-1192 resulted in tumor regression at 30 mg/kg, BID in MV-4-11 xenograft mouse model. And QD dosing is a promising alternative dosing regimen.
- BNM-1192 is an extremely weak hERG inhibitor, indicating very low potential in QTc prolongation.
- BNM-1192 demonstrated favorable profile in early safety and toxicology.
- IND-enabling studies of BNM-1192 is ongoing, and IND application is expected in early 2023.