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A Novel Inhibitor of FLT3 and its Drug-resistant Mutants with Superior Activity to Gilteritinib in MOLM-13 Preclinical Acute Myeloid Leukemia Xenograft Model

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BACKGROUND

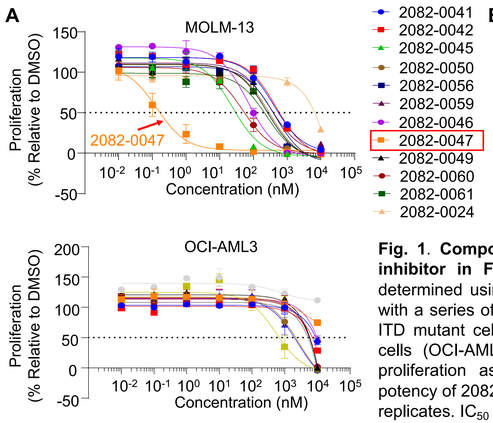
- Acute Myeloid Leukemia (AML) is the most common and deadly adult leukemia
- Fms-like tyrosine kinase 3 (FLT3) internal tandem duplication (FLT3-ITD) or activating domain mutations (FLT3-TKD) occur in ~30% of AML patients
- FLT3-ITD mutations confer a poor prognosis in AML patients
- Several FLT3 inhibitors including midostaurin and the second-generation inhibitor gilteritinib have been approved for marketing and improve outcome of AML patients with FLT3 mutations
- The use of FLT3 inhibitors has led to paths of resistance including the development of gate keeper mutations at the F691L site of FLT3
- F691L FLT3 mutations mediate resistance to all commercially approved FLT3 inhibitors pointing to a medically unmet need for this patient population

AIMS

- Given that acquisition tyrosine kinase domain (TKD) secondary mutations such as the gatekeeper F691L TKD mutation, or the emergence of additional mutations in alternative signaling pathways such as RAS/MAPK, it is critical to continue developing novel inhibitors capable of circumventing these resistance mechanisms.
- Herein we describe 2082-0047, a novel tyrosine kinase inhibitor with sub-nanomolar potency against FLT3 mutant AML, including TKD mutations. Compound 2082-0047 is a highly selective pan-FLT3 kinase inhibitor with better non-clinical efficacy, safety and tolerability compared to gilteritinib.

RESULTS

2082-0047 is potent against FLT3, including TKD mutations



B

BaF3 Cell line	2082-047 (nM)	Gilteritinib (nM)
ITD F691	4.70	18.49
ITD DH85H	0.61	10.26
ITD DH85Y	1.32	7.75
D835Y	1.40	7.42
D835H	0.32	2.05
ITD	0.36	3.81
GFP + IL-3	88.43	45.95

Fig. 1. **Compound screen identifies 2082-0047 as a potent inhibitor in FLT3 mutant AML cells. A)** Cellular IC_{50} was determined using MTS assay in MOLM-13 and OCI-AML3 cells with a series of compounds. Potent activity was evident in FLT3-ITD mutant cells (MOLM-13) with little activity in Flt3 wild type cells (OCI-AML3) suggesting Flt3 on target activity. **B)** MTS proliferation assay using Ba/F3 TKD cell lines to compare potency of 2082-47 and gilteritinib. Data represents four biological replicates. IC_{50} values were calculated using Graph Prism

2082-0047 potently inhibits FLT3 and suppresses bypass pathway by inhibiting IRAK4

A

Kinase	2082-047 IC_{50} (nM)
FLT3	0.37
FLT3 (D835Y)	0.11
FLT3 (ITD)	1.57
IRAK4	1.26
HPK1	9.5
JAK1	18.1
JAK2	21.6
JAK3	31.9
TYK2	24.9
LCK	56.5
SYK	47.6

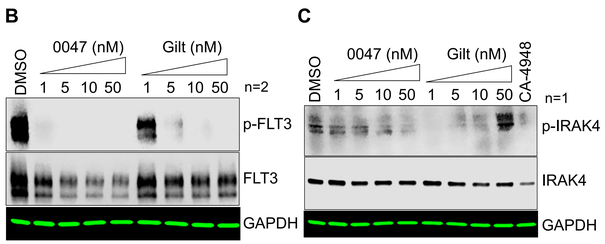
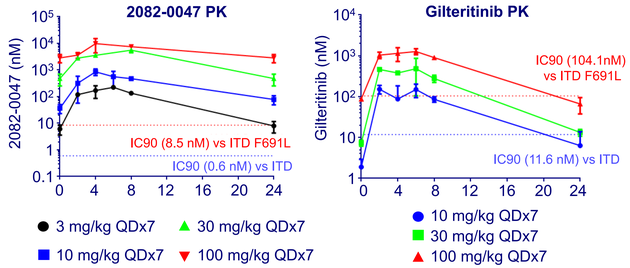


Fig. 2. **A)** Cell-free Enzyme Inhibition assay (Reaction Biology) was conducted to determine potency and selectivity against 22 kinases. Reactions were done using 5μM ATP and 2082-0047 was tested in 10-dose IC_{50} mode with a 3-fold serial dilution starting at 0.3 μM and control (Staurosporine) was tested with 4-fold serial dilution starting at 20 μM. **B/C)** Immunoblot analysis was performed to validate potent inhibition of FLT3 phosphorylation (B) and IRAK4 phosphorylation (C) after treatment with 2082-0047 or gilteritinib in human FLT3 mutant AML cell line MV4-11. CA-4948 was used as an IRAK4 inhibitor positive control. Cells were serum starved and treated for 30 minutes.

2082-0047 pharmacokinetics pharmacological profile

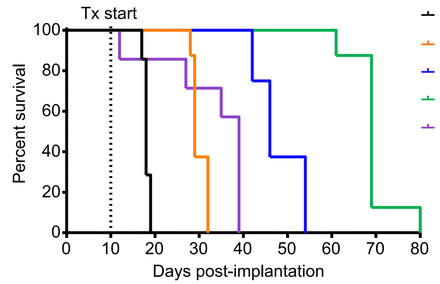


Pharmacological properties:

- 2082-0047 demonstrates stability in S9 liver fractions and hepatocytes; no CYP inhibition nor induction of different species; stable in plasma, simulated gastric fluid, simulated intestinal fluid; not mutagenic in AMES test; high oral bioavailability (F_{abs} in mice 78%)
- Inhibits FLT3 and all FLT3 mutations with 10-100x efficacy over protein adjusted IC90 concentrations and 10x safety therapeutic window vs gilteritinib, which does not safely cover all FLT3 mutations in therapeutic doses

Fig. 3. Repeat dose PK studies conducted for 2082-0047 and gilteritinib using male CD-1 mice. Mice were administered 2082-0047 or giltereitinib orally every day for seven days.

2082-0047 superior *in vivo* activity to gilteritinib

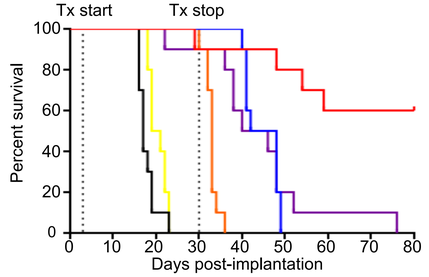


Arms	p value
Vehicle vs 3 mg/kg	< 0,0001
Vehicle vs 10 mg/kg	< 0,0001
Vehicle vs 20 mg/kg	< 0,0001
Vehicle vs Gilteritinib	0,0054
Gilteritinib vs 3 mg/kg	0,0428
Gilteritinib vs 10 mg/kg	0,0002
Gilteritinib vs 20 mg/kg	0,0002
3 mg/kg vs 10 mg/kg	< 0,0001
3 mg/kg vs 20 mg/kg	< 0,0001
10 mg/kg vs 20 mg/kg	< 0,0001

Log-rank (Mantel-Cox)

Fig. 4. MOLM-13 disseminated model. 1E6 MOLM-13 cells were injected intravenously into female NOD/SCID immunocompromised mice (N=10/arm). Treatment was initiated at 10 days post-implant (dotted black line).

2082-0047 synergizes with the BCL2 inhibitor, venetoclax



Arms	p value
Vehicle vs 3 mg/kg	< 0,0001
Vehicle vs 10 mg/kg	< 0,0001
3 mg/kg vs 3 mg/kg combo	< 0,0001
10 mg/kg vs 10 mg/kg combo	< 0,0001

Log-rank (Mantel-Cox)

Fig. 5. MOLM-13 systemic model. 1E7 MOLM-13 cells were injected intravenously into female NOD/SCID immunocompromised mice (N=10/arm). Treatment was initiated 3 days post-implant (dotted green line) post implant and treatment stopped at day 30 (dotted black line).

CONCLUSIONS

- 2082-0047 exhibits nanomolar potency against FLT3 WT and TKD mutations and potentially suppresses IRAK4.
- 2082-0047 shows 400-1000x selectivity against other kinase targets and >1000x selectivity against other targets from safety panel.
- 2082-0047 has favorable pharmacokinetics, ADME and safety profile where oral administration of up to 150 mg/kg for 14 days was tolerated in dogs
- 2082-0047 shows superior survival in aggressive disseminated MOLM13 AML model versus the best-in-class FLT3 inhibitor, gilteritinib and synergizes with the BCL-2 inhibitor, venetoclax *in vivo*.
- Collectively, our data support further IND-enabling studies and clinical development of this agent

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