Investigation of FHD-609, a potent degrader of BRD9, in preclinical models of acute myeloid leukemia (AML)

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Abstract

Acute myeloid leukemia (AML) is a complex disease with multiple sub-types, each characterized by unique clinical and molecular features, driving the need to develop targeted therapies which exploit specific vulnerabilities. Bromodomain-containing protein 9 (BRD9) is a component of the ncBAF chromatin remodeling complex and has been recently indicated as a strong dependency in AML (Weisberg et al 2022). It has been shown that inhibitors of BRD9 induce growth inhibition and expression of apoptotic makers in AML cell lines (Hohmann et al 2016; Zhou et al 2021). FHD-609 is a potent and selective BRD9 degrader that entered clinical trials for Synovial Sarcoma and SMARCB1 loss cancers. Herein, we profiled the in vitro and in vivo anti-proliferative effects of FHD-609 in AML cells. In addition, we set out to explore the MOAs and predictive biomarkers that are associated with BRD9 dependency in AML.

Key results

- FHD-609 potently degrades BRD9 in AML cells and led to a strong anti-proliferative effect on a subset of AML cell lines in vitro
- IRF8 high expression is a potential predictive biomarker that is associated with AML sensitivity to FHD-609
- FHD-609 treatment led to significant closing of chromatin with IRF8 motifs, and reduction of IRF8 protein levels in AML cells sensitive to FHD-609
- FHD-609 treatment demonstrated strong anti-tumor growth in both IRF8 high CDX and PDX models
- Given these findings, future work evaluating the role of BRD9-targeting agents should consider exploring their utility in the IRF8-high expressing AML subpopulation

Figure 1. A subset of AML cell lines are sensitive to FHD-609, a potent and selective BRD9 degrader

A.

AML cell line sensitivity to FHD-609	AML cell line name
Sensitive	**EOL-1, MV411, MOLM-13, MOLM-14, OCI-AML-2, OCI-AML-4, NOMO-1, ML-2, SHI-1,** OCI-AML-3, KO-52, HNT-34, NKM-1, Kasumi-1, KO-52, SET-2
Not Sensitive	AML-193, Kasumi-6, **THP-1**, HL60, CMK11-5, GDM-1, SKM-1, HEL92.1.7, KG-1, OCI-AML-5, OCI-M1, TF-1, HEL, CMK115, KG-1a, P31/FUJ, OCI-M2, U937, **SHI1**, M07E, NB4, MUTZ8, Kasumi-2

A) A panel of 39 AML cell lines representative of broad genetic backgrounds were treated with FHD-609 for 10 days. CTG assays were performed at end-point, and a threshold for sensitivity was set at IC50<20nM and growth inhibition >50%.

Figure 1. cont'd

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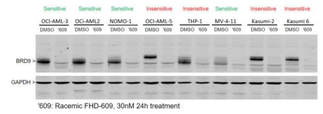

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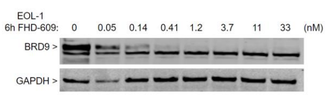

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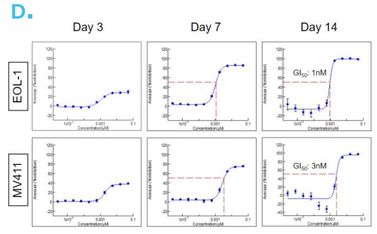

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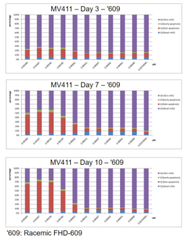
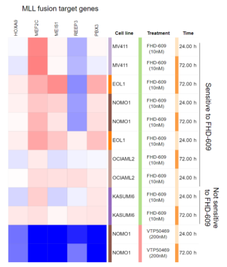


B) Western blot showing potent degradation of BRD9 following treatment with 30nM racemic FHD-609 in select AML cell lines. C) EOL-1 AML cell line treated with a dose-response of FHD-609 showing potent degradation of BRD9 in the low nanomolar range. D) Growth curves of EOL-1 and MV411 MLLr AML cell lines treated for 3, 7, and 14 days with racemic FHD-609. E) Dose-dependent increase in the proportion of apoptotic cells following 3-, 7-, and 10-day treatment with racemic FHD-609 in MLLr-AML cell line MV411

Figure 2. Identification of IRF8 as a strong individual predictor for AML cell line sensitivity to FHD-609

A.



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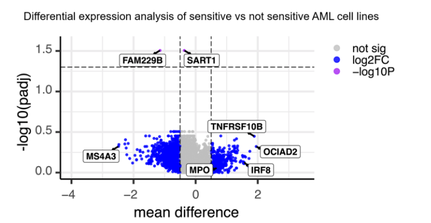

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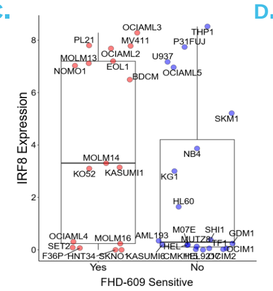

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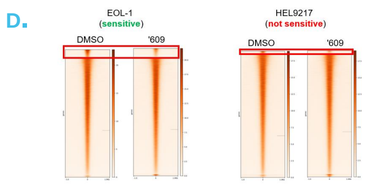


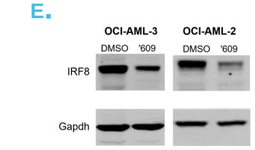
Enriched Motifs down for FHD-609 in EOL-1 at 24h	p-value
PU.1:IRF8	1e-127
IRF8	1e-98
PU.1-IRF	1e-65
E2A	1e-61
IRF3	1e-59

Enriched Motifs down for FHD-609 in HEL9217 at 24h	p-value
NFE2L2	1e-6
CHOP	1e-5
BACH1	1e-5
ATF4	1e-4
NF-E2	1e-4

A) Heatmap of a subset of MLL fusion target genes in AML cell lines treated with 10nM FHD-609 or 200nM VTP50469 (Menin inhibitor) for 24 or 72h. B) Differential expression analysis of FHD-609 sensitive versus not sensitive AML cell lines identifies high expression of IRF8 as a top predictor of sensitivity. C) AML cell lines with high expression of IRF8 are generally more sensitive to FHD-609, while IRF8-low AML cell lines are generally less sensitive D) ATACseq was performed in EOL-1 (FHD-609 sensitive) and HEL9217 (not FHD-609 sensitive) cell lines treated with DMSO or 30nM racemic FHD-609 for 24h. IRF8 is the top motif enriched in the FHD-609-treated sensitive AML cell line EOL-1 and there are no significant enrichments in the insensitive HEL9217 cell line.

Figure 2. cont'd

E.



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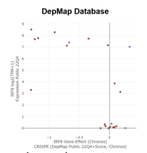
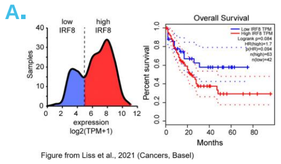


E) FHD-609 treatment (30nM for 72h) reduced IRF8 protein levels in sensitive AML cell lines. F) The majority of IRF8-high expressing AML cell lines in the Achillies CRISPR DepMap database depend on IRF8 for survival

Figure 3. A subset of AML patients with diverse genetic backgrounds have high IRF8 expression

A.



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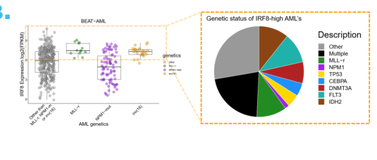
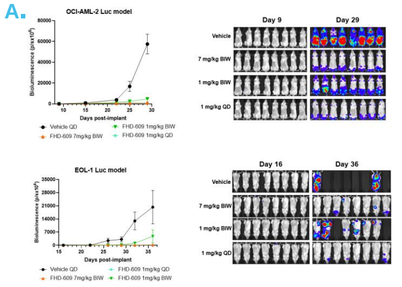


Figure from Liss et al., 2021 (Cancers, Basel)

A) From Liss et al., 2021 (Cancers, Basel). High expression of IRF8 in AML patients is associated with poor overall survival. B) In both TCGA-AML and BEAT-AML datasets there is subset of patients with high IRF8 expression. IRF-8 high AML have mixed genetic backgrounds, but almost all MLL-r (5% AML) and inv(16) AML (CBFB-MYTH1; 7% AML) have high IRF8.

Figure 4. CDX and PDX of IRF8-high AML cells show strong response to FHD-609

A.



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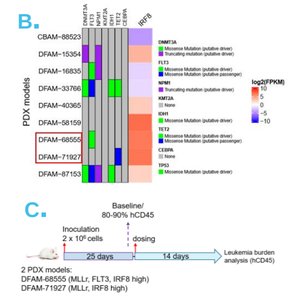

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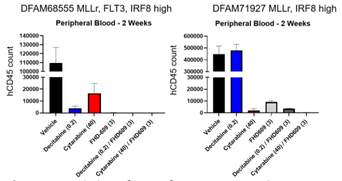

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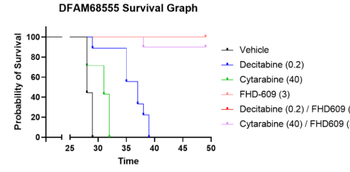

A) DX models of Luciferase-tagged OCI-AML-2 and EOL-1 cell lines show reduced tumor burden with FHD-609 treatment compared to vehicle. B) Genomic profiling from Dana Farber Cancer Institute of AML PDX models, showing IRF8 expression and genetic mutation background. C) Experimental design. A total of 3 AML PDX cell lines were used to inoculate mice and allowed to expand for 25 days. Dosing began on the 25th day for 14 days, and hCD45 was measured by flow cytometry. FHD-609 dosed BIW, Decitabine and Cytarabine dosed 5 days on, 2 days off, Vehicle was dosed QD. D) Peripheral blood was collected 2 weeks following the first dose and total hCD45 was measured by flow cytometry E) Survival curve for AML PDX model DFAM68555 (MLLr, FLT3, IRF8 high).

References

(1) Weisberg E, et al., BRD9 degraders as chemosensitizers in acute leukemia and multiple myeloma. Blood Cancer J. 2022 Jul 19;12(7):110. doi: 10.1038/s41408-022-00704-7. PMID: 35853853 **(2)** Hohmann AF, et al., Sensitivity and engineered resistance of myeloid leukemia cells to BRD9 inhibition. Nat Chem Biol. 2016 Sep;12(9):672-9. doi: 10.1038/nchembio.2115. Epub 2016 Jul 4. PMID: 27376689 **(3)** Zhou L, et al., Targeting BRD9 by I-BRD9 efficiently inhibits growth of acute myeloid leukemia cells. Transl Cancer Res. 2021 Jul;10(7):3364-3372. doi: 10.21037/tcr-21-42. PMID: 35116642 **(4)** Liss F, et al., IRF8 Is an AML-Specific Susceptibility Factor That Regulates Signaling Pathways and Proliferation of AML Cells. Cancers (Basel). 2021 Feb 12;13(4):764. doi: 10.3390/cancers13040764. PMID: 33673123