Settima edizione di

G.d.L. Biologia Cellulare Molecolare

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ASSOCIAZIONE ITALIANA EMATOLOGIA ONCOLOGIA PEDIATRICA

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...in Lab

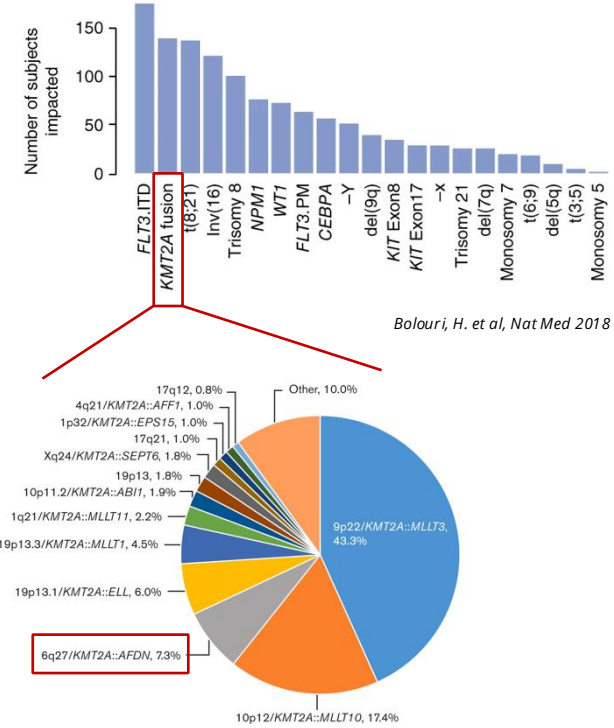
A PML nuclear body-driven degradation mechanism underlies arsenic trioxide sensitivity in KMT2A::AF6 acute myeloid leukemia

*Martina Pigazzi, **Sara Perpinello**, Ambra Da Ros, Maddalena Benetton, Giorgia Longo, Aniththa Thivakaran, Helmut Hanenberg, Jan-Henning Klusmann, Dirk Reinhardt, Franco Locatelli, and Claudia Tregnago*

Blood Cancer Discovery, *under revision*

Milano, 22 e 23 maggio 2026

KMT2A-rearranged pediatric AML

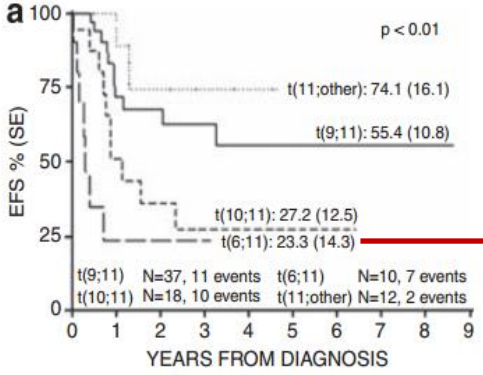


Bolouri, H. et al, Nat Med 2018

van Weelderen et al, Blood Adv 2024 (BFM)

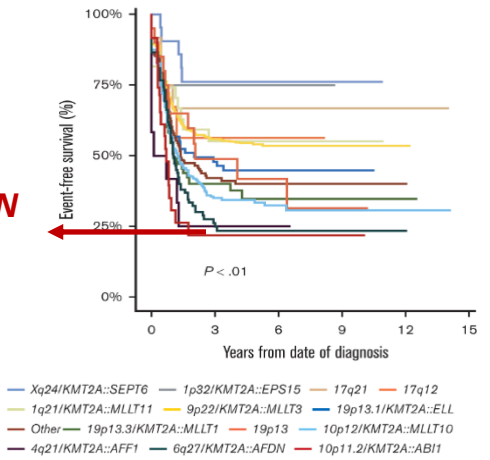
KMT2A-rearranged AML is among the most frequent cytogenetic lesions in pediatric AML and has been intensely studied for over two decades.

The fusion partner of KMT2A remains the strongest determinant of clinical behavior and prognosis



Pigazzi M. et al, Leukemia 2011

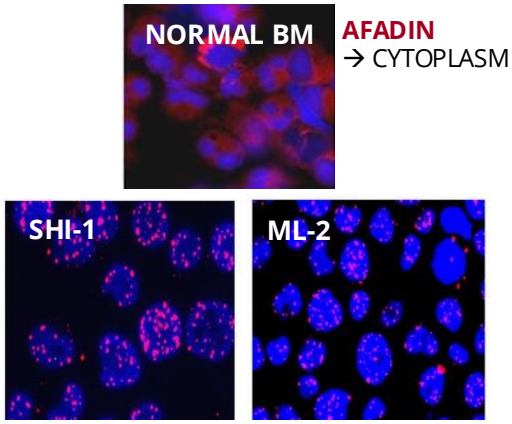
***KMT2A::AFDN* → worst outcome**



van Weelderen et al, Blood Adv 2024 (BFM)

Focus on t(6;11)KMT2A::AFDN biology

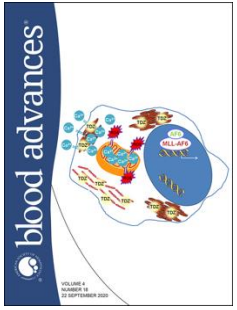
1) KMT2A fusion **re-localizes Afadin from a cytoplasmic protein to nuclear puncta** where it supports the leukemogenic KMT2A-driven transcriptional program



RAS hyperactivation
→ **TIPIFARNIB**

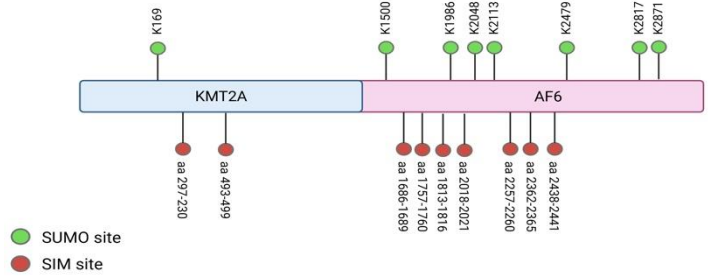
Manara E. et al, Blood 2014

Cytoskeletal alteration
→ **THIORIDAZINE**

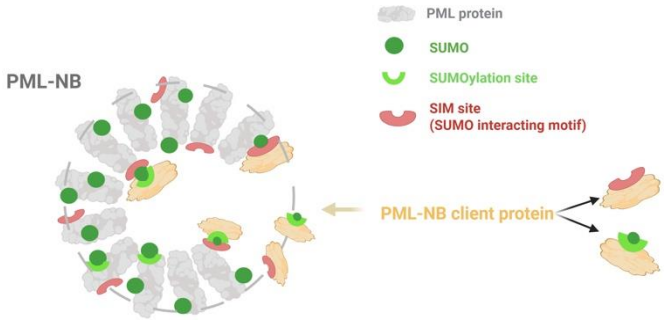


Tregnago C. et al, Blood Adv 2020

2) KMT2A::AFDN chimeric protein contains **SUMO-interacting motifs**: 8 SUMOylation and 9 SIM sites



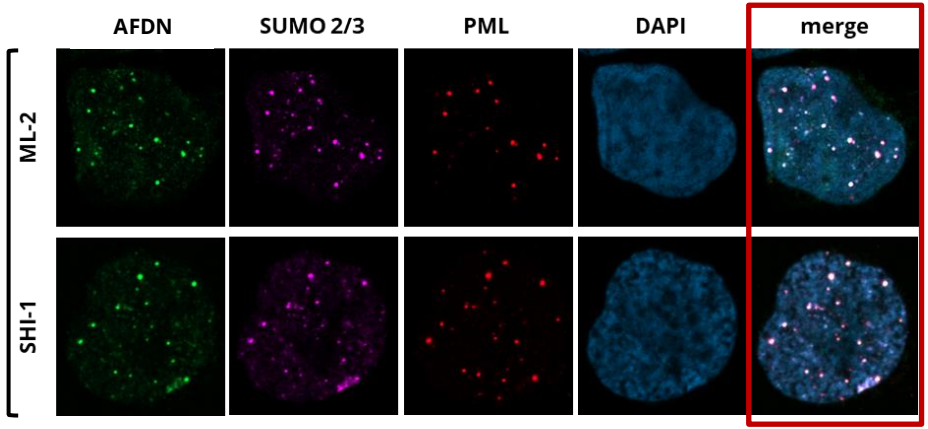
KMT2A::AFDN localizes in PML-NBs



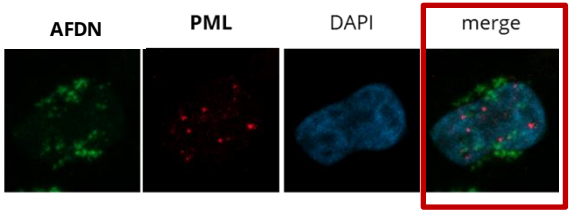
PML nuclear bodies are foci composed of SUMO-modified proteins, acting as regulatory platforms that coordinate genome stability, protein degradation and transcriptional control

→**client proteins of NBs recruited through SUMO-SIM interactions**

KMT2A::AFDN-rearranged AML

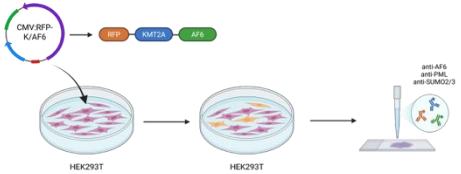


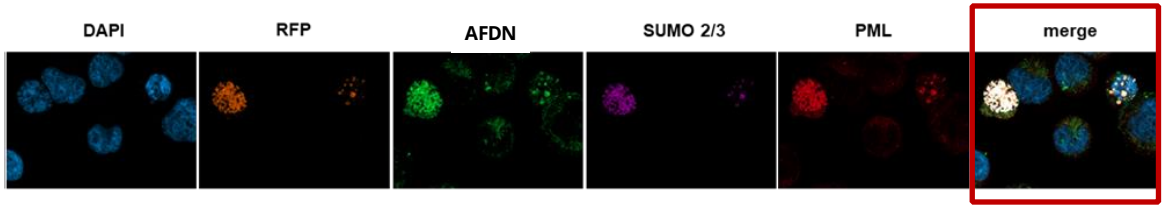
non KMT2A::AFDN



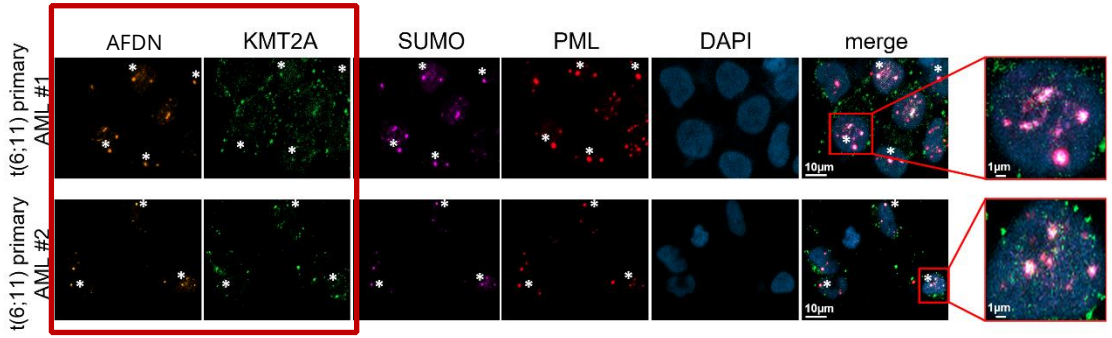
PROOF OF CONCEPT:

Transfection of HEK293T **with K/AFDN** protein fused with **RFP reporter gene**





VALIDATION IN PRIMARY KMT2A::AFDN AML



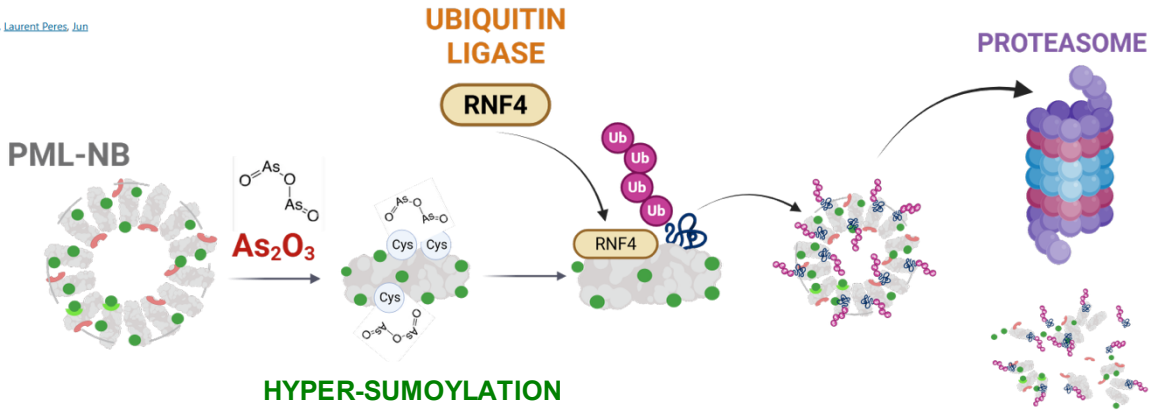
AFDN, KMT2A, and PML co-localize in presence of KMT2A::AFDN

PML and Arsenic Trioxide (ATO) in KMT2A::AFDN AML

Arsenic degrades PML or PML–RARα through a SUMO-triggered RNF4/ubiquitin-mediated pathway

Valérie Lallemand-Breitenbach, Marion Jeanne, Shirine Benhenda, Rihab Nasr, Ming Lei, Laurent Peres, Jun Zhou, Jun Zhu, Brian Raught & Hugues de Thé

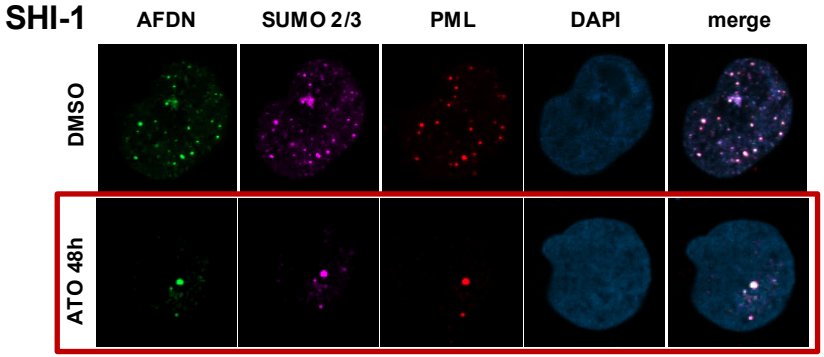
Nature Cell Biology **10**, 547–555 (2008) | Cite this article

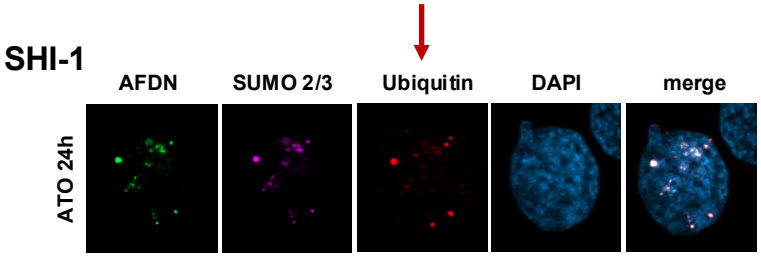


- KMT2A::AFDN → PML bodies
- PML in APL → target of ATO

if the KMT2A::AFDN chimera localizes to PML nuclear bodies, could ATO degrade it as well?

ATO treatment target PML and KMT2A::AFDN

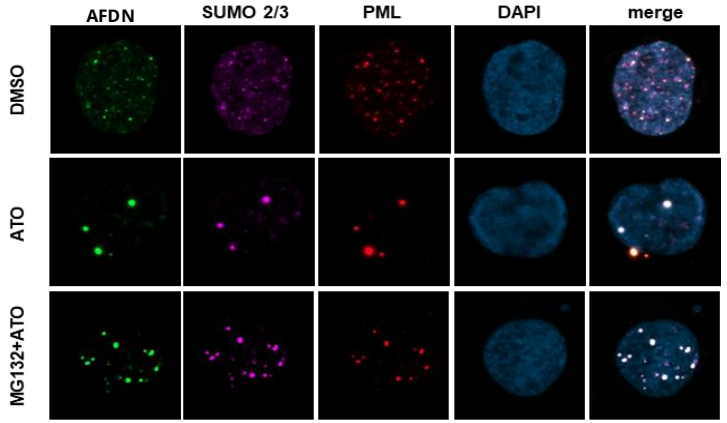




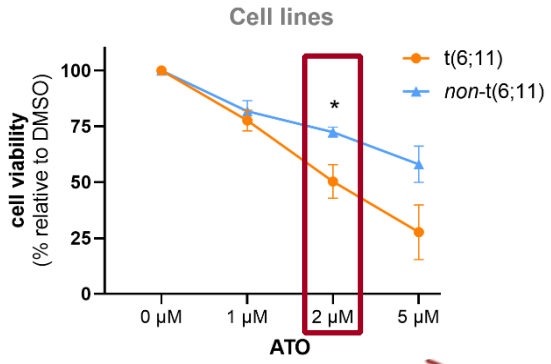
After ATO exposure PML-NBs and KMT2A::AFDN are degraded

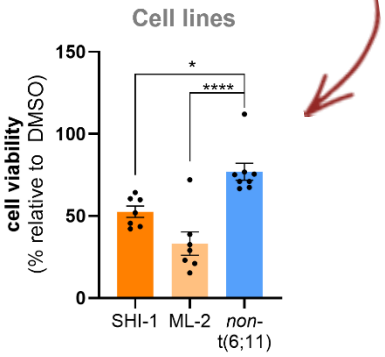
MG132 rescues ATO-treated cells by inhibiting proteasome-mediated degradation of PML

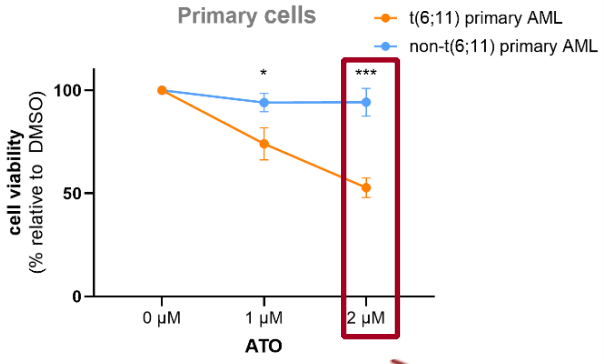
RESCUE →

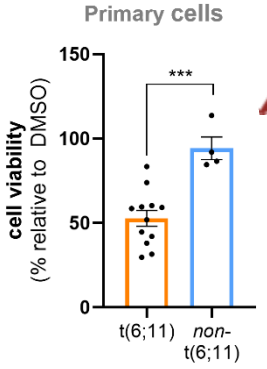


KMT2A::AFDN cells are sensitive to ATO

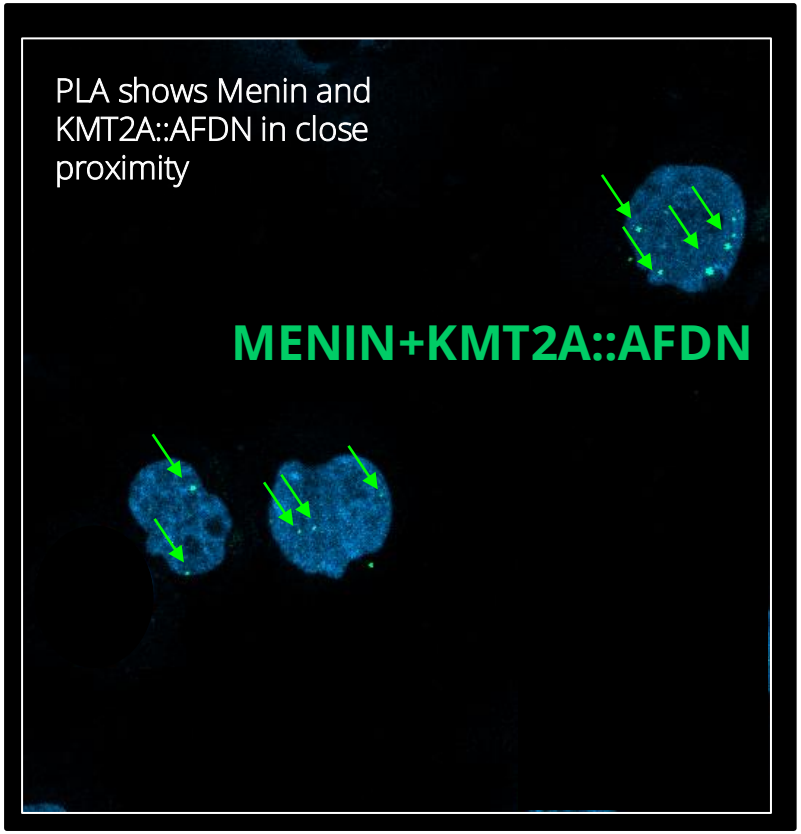


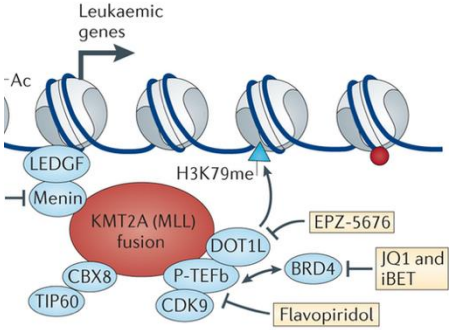




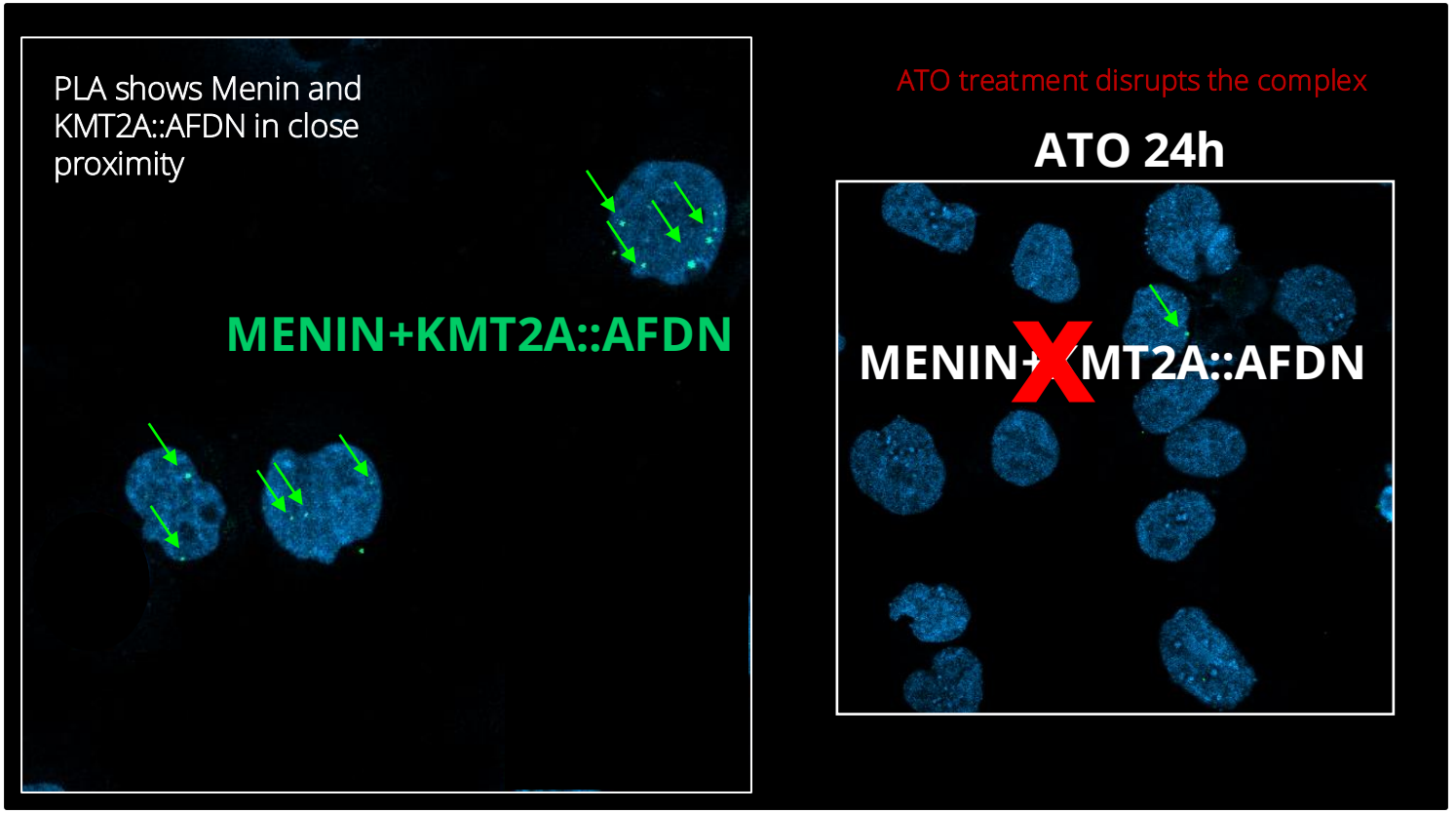


PLA (proximity ligation assay) for AFDN and MENIN

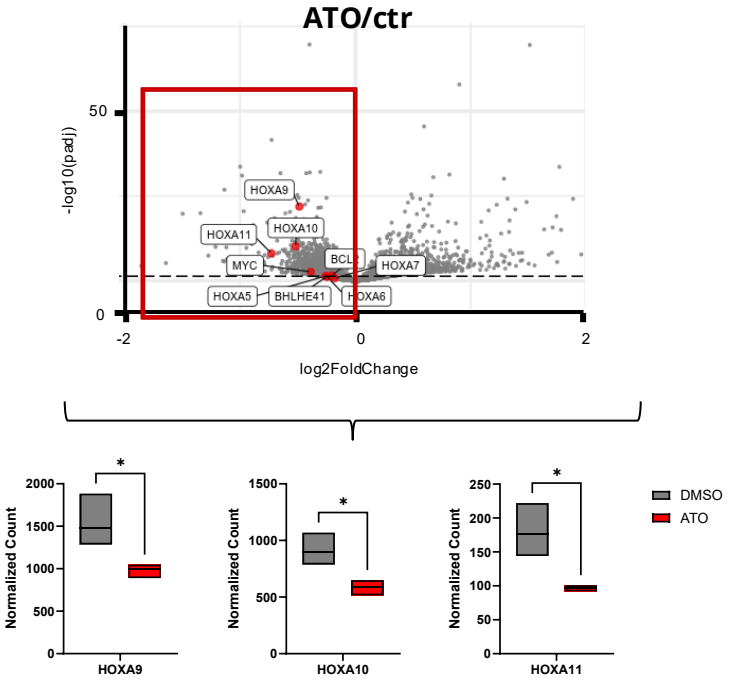


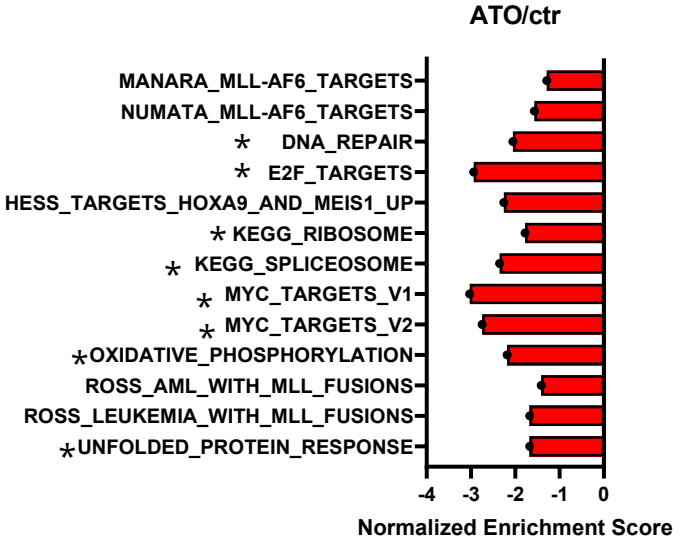


PLA (proximity ligation assay) for AFDN and MENIN

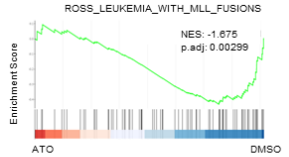


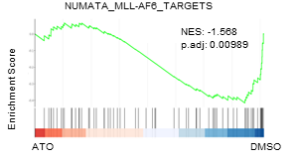
ATO treatment and KMT2A::AFDN gene expression





n=3 t(6;11)-r primary AML+ML2
Pre-post ATO 12h and 48h



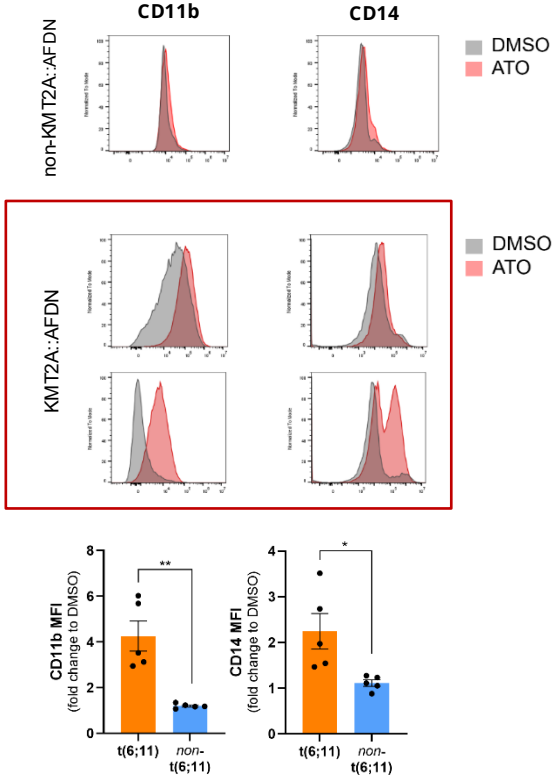


* Genesets downregulated upon Menin inhibition
(*Fiskus et al, Blood Cancer Journal 2023*)

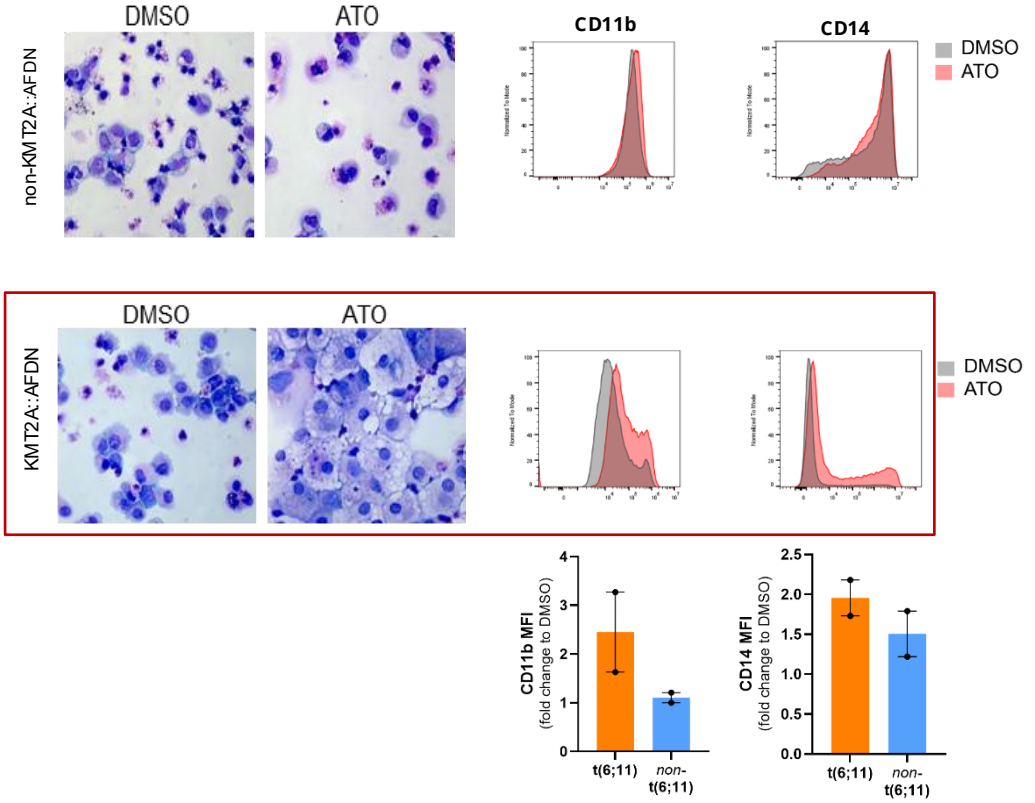
ATO treatment, by inducing KMT2A::AFDN degradation, promotes downregulation of genes and genesets related to KMT2A fusions

ATO triggers KMT2A::AFDN AML differentiation

AML cell lineS
6 DAYS

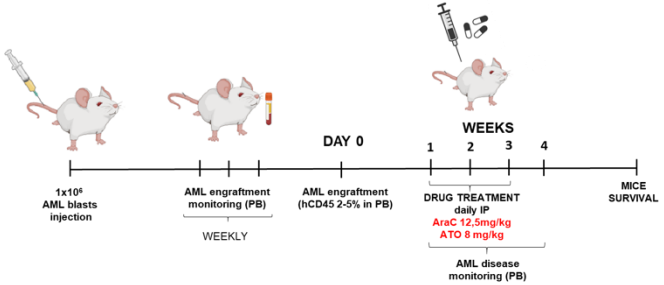


Primary AML
14 DAYS

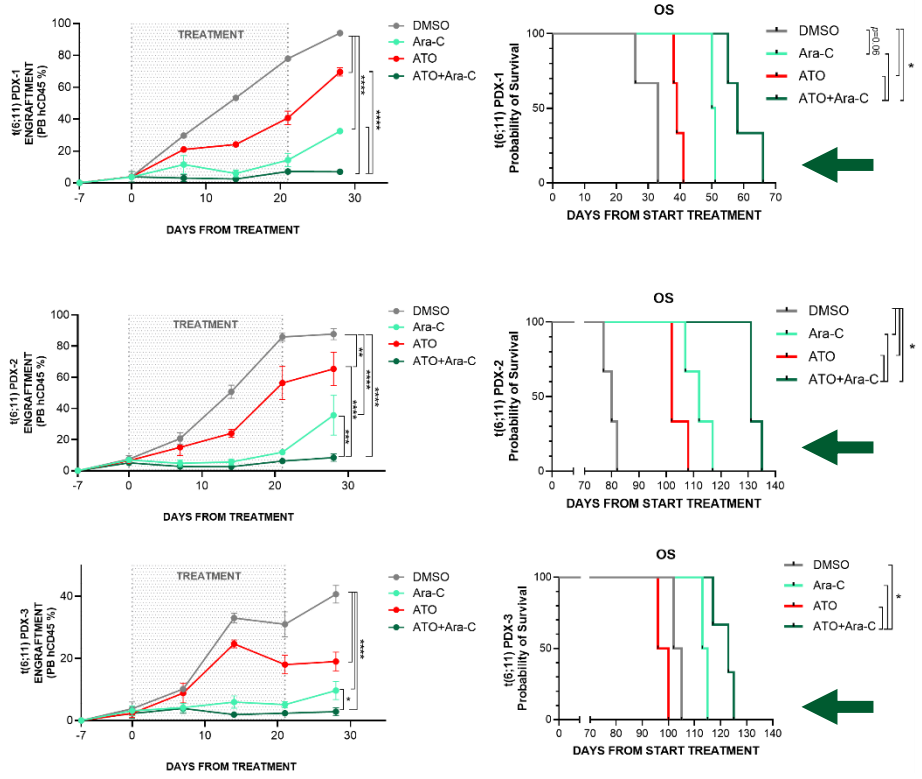


ATO + AraC in KMT2A::AFDN PDX models

In vivo experimental setting

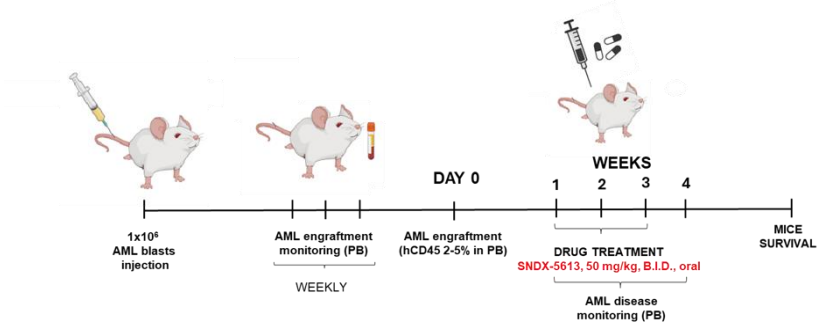


n=3 KMT2A::AFDN PDX models

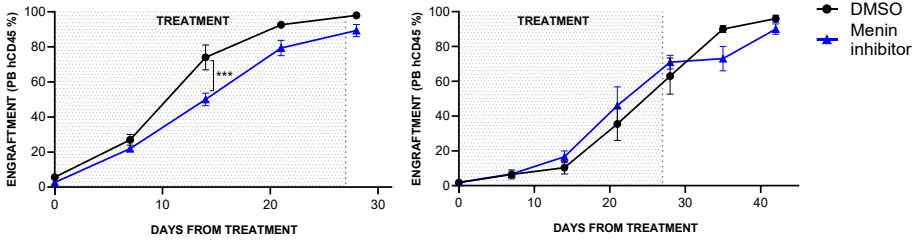


Combination of ATO with low-dose AraC reduces leukemic burden and prolongs AML-PDXs overall survival

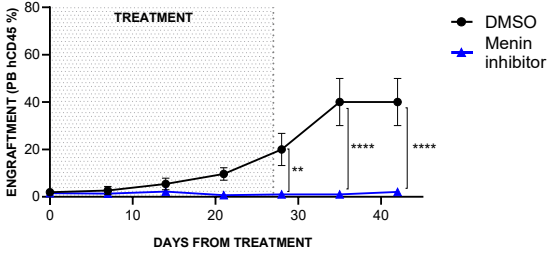
Menin inhibitor in KMT2A::AFDN PDX models and ATO



KMT2A::AFDN PDX models



KMT2A::MLLT3 PDX model



✓ KMT2A::AFDN PDX models do not respond to Menin inhibitors

Conclusions

- **KMT2A::AFDN chimera localizes in PML-NB**, uncovering a previously unrecognized layer of regulation for this leukemia subtype.
- **KMT2A::AFDN sensitivity to ATO suggests a mechanistic parallel with APL biology**, expanding the therapeutic relevance of ATO beyond PML::RARα-pos leukemia.
- **ATO** not only destabilizes the fusion protein but also **dismantles its transcriptional program**, promoting differentiation.

These results suggest that pharmacologic **degradation of KMT2A::AFDN with arsenic trioxide represents a feasible and targeted therapeutic strategy.**

Acknowledgements

Pigazzi Lab

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Carmelo Rizzari
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