SOHO ANNUAL MEETING 2024

DOUBLE MUTANT *DNMT3A* INCREASES METHYLATION DAMAGE IN ACUTE MYELOID LEUKEMIA BY INHIBITION OF TDG FUNCTION

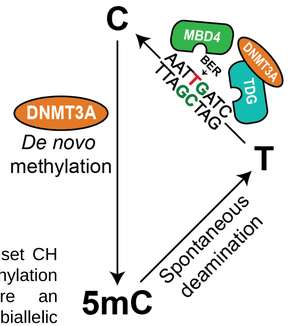
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INTRODUCTION

DNMT3A is an essential *de novo* DNA methyl transferase, commonly mutated in acute myeloid leukemia (AML) and clonal hematopiesis (CH). Methylated cytosines are at risk of C-to-T transitions through spontaneous deanimation, also referred to as **methylation damage**. **The DNA repair glycosylases MBD4** and **TDG** safeguard the cell against methylation damage.



Congenital loss of MBD4 predisposes to early onset CH and AML marked by extremely high levels of methylation damage (1). MBD4-deficient individuals share an evolutionary path towards AML, characterized by biallelic *DNMT3A* mutation, followed by a mutation in either *IDH1* or *IDH2*.

In MBD4-deficient AML patients, **TDG does not compensate for MBD4 loss**. Previous investigation suggests that **DNMT3A and TDG interact**, thereby positively influencing the TDG repair capacity (2).

Does mutation of DNMT3A negatively impact the capacity of TDG to repair methylation damage?

AIMS

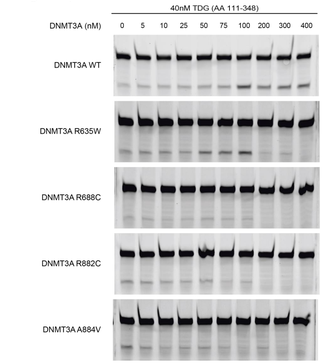
1. To investigate the impact of common *DNMT3A* mutations on TDG function to learn **whether methylation damage repair is impaired**.
2. To translate our findings to the **general AML population**:
   - Investigate differences in the **driver landscapes** of single (SM) versus double mutant (DM) *DNMT3A* AML.
   - Investigate potential **survival differences** between SM and DM *DNMT3A* AML.
3. Investigate whether **overall methylation damage** is increased in DM *DNMT3A* AML.

RESULTS

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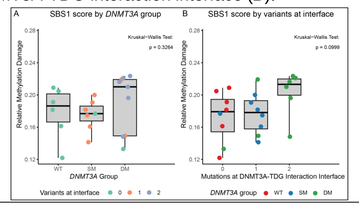
Glycosylase assay

- Wildtype DNMT3A potentiates TDG activity.
- Mutant DNMT3A conferred weaker TDG repair capacity and inhibition was observed at higher concentrations, indicating that mutant DNMT3A impacts methylation damage repair.



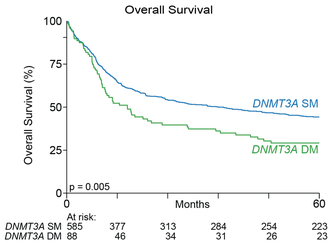
Methylation damage by *DNMT3A* status

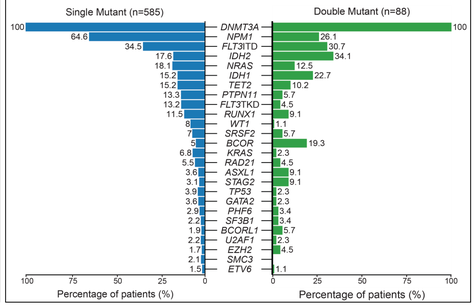
- WGS of primary AML samples uncovered a **genome-wide** increase in methylation damage when comparing DM vs SM (*A*), which becomes even more prominent when *DNMT3A* mutations are positioned at the DNMT3A-TDG interaction interface (*B*).



DNMT3A mutational status and patient outcome

- The driver landscape differs between *DNMT3A* SM and DM:
  - SM: enrichment for *DNMT3A* R882 hotspot and mutated *NPM1*.
  - DM: 10-fold increase in the *IDH2* R172 hotspot mutation.
- DM patients had a significantly worse overall survival.





METHODS

SCAN ME

- TDG repair activity was assessed in the presence of varying concentrations of recombinant wildtype or mutant DNMT3A (R635W, R688C, R882C, A884V) in vitro using **glycosylase assays.**
- **Whole-genome sequencing** (WGS) was performed on diagnostic bone marrow aspirates from 22 AML patients (6 *DNMT3A* WT, 7 SM and 9 DM patients) to assess methylation damage.
- **Alphafold 2.0** was used to model the interaction between DNMT3A and TDG.
- Differences in survival and mutational spectrum was assessed in our AML cohort comprising 1,872 *DNMT3A* WT, 585 SM and 88 DM patients.

CONCLUSIONS

Double mutant *DNMT3A*, a subgroup comprising ±4% of the complete AML population**, is linked to increased methylation damage, diverging driver landscape** and **unfavorable overall survival**.

- Wildtype DNMT3A potentiates TDG activity.
- Mutant DNMT3A impairs TDG activity.
- *DNMT3A* mutational status is correlated with patient outcomes.
- *DNMT3A* DM patients experience more methylation damage, especially when mutations are predicted to be located at the TDG-DNMT3A interaction interface.

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REFERENCES

1 Sanders MA, Chew E, Flensburg C, Zeilemaker A, Miller SE, Al Hinai AS, et al. MBD4 guards against methylation damage and germ line deficiency predisposes to clonal hematopoiesis and early-onset AML. Blood. 2018;132(14):1526-34.

2 Li YQ, Zhou PZ, Zheng XD, Walsh CP, Xu GL. Association of Dnmt3a and thymine DNA glycosylase links DNA methylation with base-excision repair. Nucleic Acids Res. 2007;35(2):390-400.