CELLS WITH LEUKEMIA-ASSOCIATED *DNMT3A* MUTATIONS ARE MORE SENSITIVE TO CYTARABINE-INDUCED DNA DAMAGE

Olga Guryanova  |  Assistant Professor

Department of Pharmacology and Therapeutics
Acute myeloid leukemia (AML) is largely incurable

NCI SEER Cancer Statistics

6.8% 5-year survival 24.0%

intensive chemo, HSCT, better supportive care

5-10 months median survival in patients 60+ years old unfit for high-dose chemo

Döhner, Weisdorf & Bloomfield, NEJM 2015
Two-hit model of AML pathogenesis

Class I proliferation (JAK2, FLT3) → Myeloproliferative neoplasms (MPN)

Class II differentiation (RUNX1, CEBPA) → Myelodysplastic syndromes (MDS)

Other genes – epigenetic and chromatin modifier genes (DNMT3A, TET2, IDH1/2, EZH2, ASXL1), etc.

Acute myeloid leukemia (AML)
Recurrent DNA methyltransferase 3A (**DNMT3A**) mutations in AML

<table>
<thead>
<tr>
<th>Mutation</th>
<th>no./total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1</td>
<td>54/200 (27)</td>
</tr>
<tr>
<td>FLT3</td>
<td>56/200 (28)</td>
</tr>
<tr>
<td><strong>DNMT3A</strong></td>
<td>51/200 (26)</td>
</tr>
<tr>
<td>IDH1 or IDH2</td>
<td>39/200 (20)</td>
</tr>
<tr>
<td>NRAS or KRAS</td>
<td>23/200 (12)</td>
</tr>
<tr>
<td>RUNX1</td>
<td>19/200 (10)</td>
</tr>
<tr>
<td>TET2</td>
<td>17/200 (8)</td>
</tr>
<tr>
<td>TP53</td>
<td>16/200 (8)</td>
</tr>
<tr>
<td>CEBPA</td>
<td>13/200 (6)</td>
</tr>
<tr>
<td>WT1</td>
<td>12/200 (6)</td>
</tr>
<tr>
<td>PTPN11</td>
<td>9/200 (4)</td>
</tr>
<tr>
<td>KIT</td>
<td>8/200 (4)</td>
</tr>
</tbody>
</table>

Acute myeloid leukemia

modest DNA hypomethylation not associated with gene dysregulation

TCGA, 2013
Brunetti *et al.* 2017
OG lab research interests

1. modeling and mechanism

2. pre-leukemic hematopoiesis and clonal evolution

3. therapeutic response

Dnmt3a mut

NORMAL

CLONAL

HEMATOPOIESIS

DIAGNOSIS

REMISSION

RELAPSE
**DNMT3A** mutations are associated with advanced age and unfavorable outcome in AML

<table>
<thead>
<tr>
<th></th>
<th>DNMT3A WT</th>
<th>DNMT3A MUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis *</td>
<td>60 yrs</td>
<td>67 yrs</td>
</tr>
</tbody>
</table>

* * p < 0.001

Balasubramanian *et al.* 2018

Ley *et al.* 2010, Yan *et al.* 2011
Low-dose cytarabine – a treatment option for elderly AML patients

60+ years old, poor performance status

- Supportive care
- Low-dose cytarabine
- Investigational therapy (clinical trial)

Chain termination

Check point activation

- ATR
- Chk1

- G2→M checkpoint

Fork collapse

Apoptosis

- ATM
- Chk2
- p53

- Origin firing
Negative enrichment of the cell cycle-related signatures in cells expressing **DNMT3A<sup>R882</sup>**

**+/m vs WT stem&prog cells**

- NES -2.05
- FDR 0.001

**DNMT3A R882 vs WT (TCGA)**

- NES -1.70
- FDR 0.004

Guryanova et al. (2016) *Nat Med*

TCGA (2013) *NEJM*

Glass et al. (2017) *Cancer Discov*
DNMT3A directs therapeutic response to replication stress-inducing agents
Leukemia cell lines with **DNMT3A** mutations are more sensitive to replication stress inducers.

AML cell lines

![Diagram showing DNMT3A^mut and DNMT3A^wt](image)

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-562</td>
<td>ND</td>
</tr>
<tr>
<td>KU-812</td>
<td>16.12</td>
</tr>
<tr>
<td>SET-2</td>
<td>1.122</td>
</tr>
<tr>
<td>KO-52</td>
<td>1.303</td>
</tr>
</tbody>
</table>

**Transform of Fludarabine - 4 cell lines**

- Fludarabine, log(ug/ml)
- Viability, relative to untreated
- IC50: ND, 16.12, 1.122, 1.303

**Transform of Ara-C - 4 cell lines**

- Ara-C, log(uM)
- Viability, relative to untreated
- IC50: ND, 297.6, 465.2, 46.1, 165.8
DNMT3A-mutant cell lines treated with Ara-C accumulate markers of DNA damage and apoptosis.
Increased apoptosis in **DNMT3A**-mutant cell lines after Ara-C treatment

**Annexin V**

- **% Annexin V+DAPI- cells**
  - K-562
  - KU-812
  - SET-2
  - KO-52

<table>
<thead>
<tr>
<th></th>
<th>0h</th>
<th>4h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-562</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KU-812</td>
<td></td>
<td></td>
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<td>SET-2</td>
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<tr>
<td>KO-52</td>
<td></td>
<td></td>
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</tbody>
</table>

**Sub-G1**

- **% cells in sub-G1**
  - untreated
  - 0.5 uM
  - 5 uM

<table>
<thead>
<tr>
<th></th>
<th>untreated</th>
<th>0.5 uM</th>
<th>5 uM</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-562</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
</tbody>
</table>
Ara-C sensitivity correlates with nuclear structure changes in cells expressing DNMT3A variants.
Increased apoptosis after Ara-C exposure in cells expressing **DNMT3A** mutants

_AraC, 50µM_
Cells expressing mutant **DNMT3A** show persistent DNA damage signaling after Ara-C.

**Graph:**
- **X-axis:** Time after treatment (0, 2, 4, 6, 8, 10, 12, 14 hours).
- **Y-axis:** Relative Density.
- **Legend:**
  - **AraC, 10µM (h):**
    - DNMT3A
    - p-Chk1
    - total Chk1
    - p-p53
    - total p53
    - GAPDH
  - **Graph lines:**
    - Vector
    - DNMT3A wt
    - DNMT3A mut

**Cell line:** U2OS
Accumulation of DNA damage marker $\gamma$H2A.X after Ara-C exposure in cells with **DNMT3A** mutations
Accumulation of DNA damage marker γH2A.X after Ara-C exposure in cells with DNMT3A mutations

**Graph:**
- **X-axis:** Untreated, 12h, 24h, 36h
- **Y-axis:** Normalized fluorescence
- **Legend:**
  - Vector
  - DNMT3A (wt)
  - DNMT3A (mut)

**Data Points:**
- Untreated: Low fluorescence
- 12h, 24h, 36h: Increasing fluorescence levels for each condition.
Accumulation of DNA damage after Ara-C exposure in cells with **DNMT3A** mutations
Accumulation of DNA damage in cells with DNMT3A mutations after continuous Ara-C exposure.

**Graph:**
- Y-axis: % Tail DNA
- X-axis: Time after treatment (hours)
- Lines:
  - Black: Vector
  - Blue: DNMT3A (wt)
  - Red: DNMT3A (mut)
- Legend:
  - Vector
  - DNMT3A (wt)
  - DNMT3A (mut)

**Explaination:**
U2OS cells were treated with Ara-C and monitored for DNA damage accumulation over different time periods. The graph shows a clear increase in tail DNA damage over time for both DNMT3A (wt) and DNMT3A (mut) compared to the Vector control. Statistical significance is indicated by asterisks: *** and **** for P < 0.001 and P < 0.0001, respectively.
Altered cell cycle profile after Ara-C treatment in cells with *DNMT3A* mutations

**U2OS**

- **% cells in G2 phase**
  - **0 h**
  - **4 h**
  - **8 h**

- **Ara-C, 10µM**

**8 h**

- **Vector**
- **DNMT3A (wt)**
- **DNMT3A (mut)**

---

**Cell number**

- **Dose**
  - **mut**
  - **wt**

- **treat**
  - **DNMT3Amut**
  - **DNMT3Awt**

**WT mut +/–**

**DNMT3Amut**

**Vector**

**DNMT3AWT**

**U2OS**

- **Ara-C, 10µM**

---

**DNA**

- **G1**
- **Sub-G1**
- **S**
- **G2**
Why do cells with **DNMT3A** mutations accumulate DNA damage after Ara-C?
No defect in HR or NHEJ in cells with DNMT3A mutations treated with Ara-C
DNMT3A mutant cells efficiently resolve Ara-C induced DNA damage.
Mouse leukemia with a *Dnmt3a* mutation is more sensitive to cytarabine *ex vivo*
Summary

- **DNMT3A**<sup>wt</sup>
  - DNA damage
  - **✓ DNA repair**
  - Apoptosis

- **DNMT3A**<sup>mut</sup>
  - DNA damage
  - **✓ DNA repair**
  - Apoptosis

**Ara-C**

Continuous exposure = continuous intravenous infusion
Ara-C sensitivity in $\text{DNMT3A}^{\text{mut}}$ setting:
Next steps

DNMT3A promotes recruitment of SPT-16 to DNA after torsional stress, through direct interaction; attenuated by DNMT3A mutations

Guryanova et al. (2016) Nat Med

DNMT3A detected at stalled replication forks 30’ after HU

Dungrawala et al. (2015) Mol Cell
Ara-C sensitivity in **DNMT3A<sup>mut</sup>** setting: Next steps

**Chromatin accessibility – ATAC-seq**

1. Cell preparation
2. Open chromatin
3. Targeting
4. Sequencing
5. Amplification
6. Tagmentation and purification

**Proteome at replication forks – iPOND** (isolation of proteins on nascent DNA)

1. EdU label nascent DNA
2. Crosslink protein-DNA
3. Biotin conjugate nascent DNA
4. Lyse and sonicate
5. Streptavidin purification
6. Elution, detection

**Replication fork dynamics – CldU/IdU labeling**

- Pulse CldU (red)
- Pulse IdU (green)
- Replication fork progression
- Latent origins
- Replication fork collapse
- Origin firing

**Differential sensitivity to cytarabine in vivo**

- **Dnmt3a<sup>wt</sup>**:
  - Flt3<sup>ITD</sup> : Npm1<sup>c</sup>
- **Dnmt3a<sup>mut</sup>**:
  - Flt3<sup>ITD</sup> : Npm1<sup>c</sup>

**% survival**

- **wt**: [Graph showing survival percentages]
- **mut**: [Graph showing survival percentages]
Thank you!

UF Health Cancer Center Collaborative Pilot Grant
NIH/NCI K99/R00 Pathway to Independence Career Development Award
**DNMT3A** R882 mutations disrupt tetramerization and attenuate cooperative DNA binding

*de novo* DNA methyltransferase

**Homotetramer**
- processive catalysis

**Homodimer**
- distributive catalysis

DNMT3A *wt*

DNMT3A R882mut

↓ processivity

Holz-Schietinger *et al.* 2012