Pre-clinical models for MDS

Peter D. Aplan MD
Senior Investigator
Genetics Branch
Disclosures

I receive royalties through the NIH Technology Transfer Office for the invention of NUP98-HOXD13 mice
Pre-clinical models of cancer

- *In vitro*
  - Purified components (eg, enzymes)
  - Cell culture (eg, organoids, co-culture)
  - MDS studies hampered by lack of cell lines

- *In vivo*
  - Model organisms (yeast, fly, fish, rodent, primate)
  - Mouse models
    - Xenograft of immunodeficient mice (MDS very difficult to engraft)
    - Genetic Engineered Mice (GEM)
## MDS GEMs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Author</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>Abdel-Wahab</td>
<td>J Exp Med, 2013</td>
</tr>
<tr>
<td>CREBBP</td>
<td>Rebel</td>
<td>PNAS, 2002</td>
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<td>DICER</td>
<td>Raaijmakers</td>
<td>Nature, 2010</td>
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<td>EVI1</td>
<td>Buonamici</td>
<td>JCI, 2004</td>
</tr>
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<td>NPM1</td>
<td>Grisendi</td>
<td>Nature, 2005</td>
</tr>
<tr>
<td>BCL2/NRAS</td>
<td>Omidvar</td>
<td>Cancer Res, 2007</td>
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<tr>
<td>RUNX1</td>
<td>Watanabe</td>
<td>Blood, 2008</td>
</tr>
<tr>
<td>SALL4B</td>
<td>Ma</td>
<td>Blood, 2006</td>
</tr>
<tr>
<td>S100A9</td>
<td>Chen</td>
<td>JCI, 2013</td>
</tr>
<tr>
<td>TRAF6</td>
<td>Starczynowski</td>
<td>Nat Med, 2010</td>
</tr>
<tr>
<td>TET2</td>
<td>Moran-Crusio</td>
<td>Cancer Cell, 2011</td>
</tr>
<tr>
<td>SRSF2</td>
<td>Smeets</td>
<td>Blood, 2018</td>
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<tr>
<td>U2AF1</td>
<td>Shirai</td>
<td>Cancer Cell, 2015</td>
</tr>
<tr>
<td>NUP98-HOXD13</td>
<td>Lin</td>
<td>Blood, 2005</td>
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</table>
NUP98 Translocations and Hematologic Malignancy

- NUP98 is fused to >30 different partner genes in patients with MDS, AML, CML, CMML, and T-ALL.
- Chimeric protein- NUP98 at amino terminus; partner at carboxy terminus.
- More common in children than adults (6-10% of pediatric AML pts have NUP98 fusions) (Bisio, Blood, 2014; Bolouri, Nat Med 2018).

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Partner gene</th>
<th>Homeo-domain?</th>
<th>Disease</th>
</tr>
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<tbody>
<tr>
<td>t(7;11)(p15;p15)</td>
<td>HOXA9, 11, 13</td>
<td>Yes</td>
<td>MDS, AML, CML</td>
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<tr>
<td>t(11;12)(p15;q13)</td>
<td>HOXC11, 13</td>
<td>Yes</td>
<td>MDS, AML</td>
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<tr>
<td>t(2;11)(q31;p15)</td>
<td>HOXD11, 13</td>
<td>Yes</td>
<td>MDS, AML, CML</td>
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<tr>
<td>t(1;11)(q23;p15)</td>
<td>PMX1(PRRX1)</td>
<td>Yes*</td>
<td>MDS, AML</td>
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<tr>
<td>t(9;11)(q34;p15)</td>
<td>PRRX2</td>
<td>Yes*</td>
<td>MDS, AML</td>
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<tr>
<td>t(11;20)(p15;q11)</td>
<td>TOP1</td>
<td>No</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>inv11(p15q22)</td>
<td>DDX10</td>
<td>No</td>
<td>MDS, AML</td>
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</table>
vanNHD13 mice develop MDS

NUP98-HOXD13 (NHD13) fusion

<table>
<thead>
<tr>
<th>CBCs (age 4-7 mos)</th>
<th>WBC (10^9/L)</th>
<th>NE (10^9/L)</th>
<th>Hb (g/dL)</th>
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<tbody>
<tr>
<td>NHD13 (n = 22)</td>
<td>1.8</td>
<td>0.44</td>
<td>11.9</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>6.5</td>
<td>1.4</td>
<td>14.2</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</table>

Overexpressed genes

Oas2, Ifit1, Ifi44, Hoxa9, Hoxa7, Pbx, Hoxc6,

*Interferon induced  *Homeodomain

Slape et al., JNCI, 2008.
Overexpression of *HOXA* cluster genes is a common finding in MDS and AML

- *HOXA9* was the single most differentially expressed gene in CD34+ cells from MDS patients with monosomy7 (Chen, Blood, 2004).
- Over 50% of AML patients showed overexpression (>5X nl BM) of *HOXA5/7/9 and MEIS1*. (Palmqvist, PLOS One, 2007).

Suggests that enforced expression of *HOXA* cluster genes might be a common pathway for MDS/AML.
Is MDS transplantable?

NHD13 + Donor (CD45 Ly5.2) NHD13 − (WT)

± WT competitor BM (CD45 Ly5.1)

1000rad

1000rad

Recipient mice (CD45 Ly5.1)

Assay for hematologic engraftment and differentiation.

**Cell number**

1x10⁶ NHD13 or WT (Ly5.2) + 1x10⁵ WT competitor (Ly5.1)/ mouse

1x10⁵ NHD13 or WT (Ly5.2) + 1x10⁶ WT competitor (Ly5.1)/ mouse
Peripheral blood cytopenias and normocellular BM = ineffective hematopoiesis

Mice transplanted with $1 \times 10^6$ Donor (Ly5.2) and $1 \times 10^5$ WT competitor (Ly5.1) cells

<table>
<thead>
<tr>
<th></th>
<th>HGB (g/dL)</th>
<th>MCV (fL)</th>
<th>PLT (K/uL)</th>
<th>WBC (K/uL)</th>
<th>Polys (K/uL)</th>
<th>BM cellularity</th>
<th>% Ly5.2</th>
</tr>
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<tr>
<td>6 week</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>WT e6/5</td>
<td>13.37 ± 0.29</td>
<td>48.43 ± 0.28</td>
<td>813.0 ± 34.9</td>
<td>8.27 ± 0.49</td>
<td>2.09 ± 0.16</td>
<td></td>
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</tr>
<tr>
<td>NHD13 e6/5</td>
<td>11.53 ± 0.26</td>
<td>55.70 ± 0.67</td>
<td>932.8 ± 89.9</td>
<td>2.36 ± 0.15</td>
<td>0.51 ± 0.06</td>
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<tr>
<td>16 week</td>
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</tr>
<tr>
<td>WT e6/5</td>
<td>13.27 ± 0.27</td>
<td>45.80 ± 0.12</td>
<td>864.3 ± 3.2</td>
<td>12.00 ± 1.55</td>
<td>1.80 ± 0.33</td>
<td>6.53X10^7 ± 0.7X10^7</td>
<td>80.49 ± 1.73</td>
</tr>
<tr>
<td>NHD13 e6/5</td>
<td>8.40 ± 1.57</td>
<td>57.13 ± 2.37</td>
<td>540.3 ± 259.1</td>
<td>3.70 ± 0.75</td>
<td>0.53 ± 0.18</td>
<td>5.33X10^7 ± 0.5X10^7</td>
<td>82.87 ± 2.57</td>
</tr>
</tbody>
</table>
NHD13 mice as an *in vivo* model for MDS therapy

**CENTRAL HYPOTHESIS:** GEM models can provide data to inform clinical trials.

1. TGFβ trap—ACE536/Luspatercept
2. DNA methyltransferase inhibitors
3. Hematopoietic Stem Cell Transplant
NHD13 mice provide *in vivo* proof of principle for ACE-536 (Luspatercept)

**Early stage: 6 months old**

- RBCs (1 x 10^6 cells µL^-1)
  - WT + TBS
  - MDS + TBS
  - MDS + RAP-536

- Hgb (gm dl^-1)
  - WT + TBS
  - MDS + TBS
  - MDS + RAP-536

**Time (months)**

- RBC (x10^6 μL^-1)
  - MDS + TBS
  - MDS + RAP-536

**Pie charts**

- WT + TBS: 26% Erythroid precursors
- MDS + TBS: 48% Erythroid precursors
- MDS + RAP-536: 32% Erythroid precursors

*Suragani et al., Nat Med, 2014*
Efficacy of DNMT1i decitabine (DAC) in MDS model

Harvest BM

Mix WT and MDS BM

Transplant

Survival MDS/WT chimeric mice

% Survival

Log Rank test

P=0.004

Weeks post transplant

7291 BM Saline

7294 BM

Collaborator: J.P. Issa (Temple)
Conclusion: 1000 cGy XRT (myeloablative in C57Bl6 mice) can induce long-term remission, but was not curative.

Open Questions:
- Induce GVT with minimal GVHD
  - Donor lymphocyte infusions (DLI) post-transplant
  - Transplant with specific T-cell subsets (Tregs)

GVHD: graft versus host disease; GVT: graft versus tumor
Summary

- NHD13 mice develop a highly penetrant form of MDS which recapitulates the key features of human MDS.
- NHD13 hematopoietic cells outcompete WT cells \textit{in vivo}.
- Treatment of NHD13 mice with DNMTi (DAC) leads to increased survival and normalization of blood counts.
- Myeloablative doses of IR lead to increased survival, but ultimately fail due to persistence of radio-resistant MDS.
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  - Li Li

- John Denu (U. Wisc)

- JP Issa (MD Anderson/Temple)
NHD13 BM cells outcompete WT cells
Mice transplanted with $1 \times 10^5$ Donor (Ly5.2) and $1 \times 10^6$ WT competitor (Ly5.1) cells

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<td>0.87 ± 0.13</td>
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Peripheral Blood engraftment

* AML at 48+ wks

BM engraftment

* 73.6%

* 6.0%
Case Report

- Initial dx of B-lineage ALL at 4 yrs of age, \textit{TEL-AML1} fusion.
- Isolated CNS relapse 6 mos off rx
- Reinduction with HD ARA-C, VP-16, VCR, L-ASP, CTX, DOX and CNS XRT; continuation chemorx x 2 yrs.
- Thrombocytopenia 9 mos off rx, MDS->AML M6
- \textit{t(2;11)(q31;p15)} (no \textit{TEL/AML1} fusion)
- NUP98-HOXD13 fusion gene
Variable responses to DAC

MDS/CD45.2 cells (PB)

- Saline Tx
- DAC Tx

Weeks Post Transplant

Complete Remission

FSC-H

CD45.2

Mac-1

Gr-1

Good response

Poor response

93%

7%

14%

86%
DNA methyltransferase 1 inhibitors (DNMT1i) and MDS

• 2 of 3 FDA approved agents for MDS are DNMT1i.

• At diagnosis, irrespective of blast percentage, a large fraction (>80%) of hematopoiesis is derived from the MDS clone.

  ➢ After successful therapy, majority of hematopoiesis derived from normal hematopoietic precursors.

  ➢ Thus, pre/post treatment analysis of DNMT1i effect may be studying vastly different cell populations.

Walter, et al., NEJM, 2012  
Saunthararajah, ASH, 2013
Demethylation of MDS gene set with DAC treatment

- Bisulfite pyrosequencing in sorted cells:
  - At CpG Islands in MDS-associated genes.

Collaborator: JP Issa
Novel DNMT1 inhibitors

- T-dCyd and 5’-aza-T-dCyd—thiol substituted cytosine analogs
- Potent DNMT1i *in vitro*
- Effective in *NHD13* MDS model

![Graph showing percent survival over weeks post-transplant](image)

- Both agents now in phase I clinical trial

Collaborators: Michael Difilipantonio, Jim Doroshow, NCI NExT

[https://clinicaltrials.gov/ct2/show/NCT02423057](https://clinicaltrials.gov/ct2/show/NCT02423057)

Unpublished
Generation of chimeric mice with MDS

Harvest BM

NHD13 Tg Donor (C57/BL6, CD45.2)

Wild type Donor (B6, CD45.1)

(MDS/CD45.2)

Mix

Inject 1X10^6 BMC + 1X10^5 WT BMC

Recipient (B6 Ly5.1)

Transplantation 1000rad

% Engraftment

CD45.2 cells in PB

- Macrocytosis
- Anemia
- Neutropenia

CBCs
HOX genes and hematopoiesis

1) *HOXA7-11* often co-regulated="HOXA cluster".

2) "HOXA cluster" genes expressed in HSPCs, and down-regulated as cells mature.

(Argiropoulos and Humphries, Oncogene, 2007)
Increased survival and hematologic improvement in chimeric MDS mice treated with DAC

### Treatment schedule

- **BMT**: 0
- **Cycle 1**: 5
- **Cycle 2**: 10
- **Cycle 3**: 15
- **Repeated until endpoint**: 20, 25, 30, 35

- **Weekly Assessment**:
  - **Day 0**: PB assessment – FACS and CBC
  - **Day 5**: 0.018 mg DAC/mouse/day x5 days

### Survival

- **MDS/WT chimeric mice**

- **Log Rank test**: P=0.004

### Average Hemoglobin

- **Weeks post transplant (PT)**: 6, 14, 19, 26, 31

- **Saline**: n=5
- **DAC**: n=7

- **Saline**: 12, 10, 8, 6, 4
- **DAC**: 12, 10, 8, 6, 4

- **Tx**: Treatment
Increased survival and hematologic improvement in chimeric MDS mice treated with DAC

Treatment schedule

PB assessment – FACS and CBC
0.018 mg DAC/mouse/day x5 days

Survival
MDS/WT chimeric mice

Log Rank test
P=0.004

Average Hemoglobin

Weeks post transplant (PT)