Targeting the Menin-MLL1 Interaction Site as a Treatment for Mixed Lineage Leukemia-rearranged (MLL-r) and NPM1c+ AML

Jerry McGeehan
Syndax Pharmaceuticals
Outline

- Overview of Mixed Lineage Leukemia-rearranged (MLLr)
- Review of the small molecule approach to inhibitor design
- In vitro characterization – activity and transcriptional effects
- In vivo characterization
  - Cell lines
    - PK/PD approach
    - s.c. xenografts with MLL cell lines
    - Disseminated models with MLL cell lines
  - Patient Derived Xenografts (PDX)
Introduction

- **MLL-r** is a rare, acute leukemia (ALL, AML) caused by spontaneous translocations at the MLL1 locus (11q23) generating oncogenic MLL-fusion proteins
  - Combined incidence ~4000+/yr with poor prognosis (5 year OS ~35%--40%)
  - *MLL*-rearrangements are found in approximately 5-10% of AML and ALL cases, but represent ~80% of infant leukemias
  - Targeting of MEN:MLL-fusion interaction in *MLL*-rearranged cells blocks cell proliferation. (Yokoyama et al 2005; Borkin et al., 2015)

- **NPM1c** mutations are found in about 25-30% of all adult AML
  - Therapeutic targeting of MEN:MLL1/MLL-fusion in *NPM1c* AML inhibits cell proliferation. (Kuhn et al., 2016)

- VTP-50469 was developed as a novel orally available MEN:MLL1 inhibitor to interrogate and validate the biology of menin-MLL inhibition
Potential use in multiple areas of unmet need beyond MLL-r

Potential Indications Include

- $\text{MLL}^{\text{PTD}}$ AML
- Ewing’s Sarcoma
- ER$^+$ Breast Cancer
- MLL-r+ Solid Tumors (CRPC)
- p53 Gain of Function Mutations

1. Leukemia. 2017 Jan;31(1):1-10
Menin-MLL interaction inhibitors should block the binding of all fusions to menin

## ‘MLL recombinome’ associated with different hematologic malignancies

<table>
<thead>
<tr>
<th>#</th>
<th>Direct TPG</th>
<th>Infant</th>
<th></th>
<th>Pediatric</th>
<th></th>
<th>Adult</th>
<th></th>
<th>Total</th>
<th></th>
<th>% of MLL-r</th>
<th></th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ALL</td>
<td>AML</td>
<td>Other</td>
<td>ALL</td>
<td>AML</td>
<td>Other</td>
<td>ALL</td>
<td>AML</td>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><strong>AFF1/AF4</strong></td>
<td>338</td>
<td>4</td>
<td>10</td>
<td>139</td>
<td>3</td>
<td>10</td>
<td>332</td>
<td>3</td>
<td>—</td>
<td>839</td>
<td>35.8</td>
</tr>
<tr>
<td>2</td>
<td><strong>MLLT3/AF9</strong></td>
<td>113</td>
<td>40</td>
<td>5</td>
<td>56</td>
<td>132</td>
<td>3</td>
<td>9</td>
<td>90</td>
<td>1</td>
<td>449</td>
<td>19.1</td>
</tr>
<tr>
<td>3</td>
<td><strong>MLLT1/ENL</strong></td>
<td>154</td>
<td>2</td>
<td>4</td>
<td>56</td>
<td>21</td>
<td>1</td>
<td>50</td>
<td>14</td>
<td>—</td>
<td>302</td>
<td>12.9</td>
</tr>
<tr>
<td>4</td>
<td><strong>MLLT10/AF10</strong></td>
<td>39</td>
<td>43</td>
<td>2</td>
<td>12</td>
<td>66</td>
<td>1</td>
<td>1</td>
<td>33</td>
<td>—</td>
<td>197</td>
<td>8.4</td>
</tr>
<tr>
<td>5</td>
<td><strong>PTD</strong></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>6</td>
<td>—</td>
<td>1</td>
<td>98</td>
<td>2</td>
<td>107</td>
<td>4.6</td>
</tr>
<tr>
<td>6</td>
<td><strong>ELL</strong></td>
<td>—</td>
<td>24</td>
<td>1</td>
<td>—</td>
<td>24</td>
<td>—</td>
<td>1</td>
<td>45</td>
<td>2</td>
<td>97</td>
<td>4.1</td>
</tr>
<tr>
<td>7</td>
<td><strong>MLLT4/AF6</strong></td>
<td>1</td>
<td>2</td>
<td>—</td>
<td>16</td>
<td>28</td>
<td>—</td>
<td>9</td>
<td>38</td>
<td>1</td>
<td>95</td>
<td>4.1</td>
</tr>
<tr>
<td>8</td>
<td><strong>EPS15</strong></td>
<td>16</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>—</td>
<td>4</td>
<td>5</td>
<td>—</td>
<td>38</td>
<td>1.6</td>
</tr>
<tr>
<td>9</td>
<td><strong>MLLT11/AF1Q</strong></td>
<td>1</td>
<td>13</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>23</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td><strong>no der(11)</strong></td>
<td>14</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>—</td>
<td>2</td>
<td>31</td>
<td>—</td>
<td>31</td>
<td>1.3</td>
</tr>
<tr>
<td>11</td>
<td><strong>6-Sep</strong></td>
<td>—</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>17</td>
<td>0.7</td>
</tr>
<tr>
<td>12</td>
<td><strong>MLLT6/AF17</strong></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>11</td>
<td>—</td>
<td>14</td>
<td>0.6</td>
</tr>
<tr>
<td>13</td>
<td><strong>9-Sep</strong></td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>6</td>
<td>—</td>
<td>13</td>
<td>0.6</td>
</tr>
<tr>
<td>14</td>
<td><strong>AFF3/LAF4</strong></td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td><strong>SUM</strong></td>
<td>692</td>
<td>160</td>
<td>24</td>
<td>313</td>
<td>339</td>
<td>19</td>
<td>415</td>
<td>373</td>
<td>10</td>
<td>2345</td>
<td>100.0</td>
</tr>
</tbody>
</table>

|   |                | 876    | 671  | 798       |

Disruption of the Menin-MLLr interaction should have a direct, rapid global effect on the transcription profile in MLLr+ cells

- Decreasing cell proliferation
- Increasing cell differentiation
- Inducing apoptosis/cell death
Menin-MLL Inhibitors Target the High Affinity Binding Site of MLL1 (aa 9-13) on Menin

- Menin-MLLr interaction inhibitors derived through structure-based drug design in the high affinity MLL1 binding pocket
Menin-MLL Inhibitors Target the High Affinity Binding Site of MLL1 (aa 9-13) on Menin

- Inhibitors bind to a highly conserved binding pocket in menin

**Sequence:**

```
M-A-H-S-C-R-W-R-F-P-A-R-P-G-T-T-G-G-G-
```
Potent, Orally Active Menin Inhibitor (VTP-50469) Used to Interrogate Menin-MLL Biology

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VTP-50469</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding $K_i$ (nM)</td>
<td>0.104 **</td>
</tr>
<tr>
<td>Dissociation $t_{1/2}$ (min)</td>
<td>198</td>
</tr>
<tr>
<td>MV4;11 Cellular $IC_{50}$ (nM)</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>$t_{1/2}$ (h): Rat, Dog</td>
<td>4.1, 4.8</td>
</tr>
<tr>
<td>%F: Rat, Dog</td>
<td>61, &gt;100</td>
</tr>
</tbody>
</table>

- Used in vitro and in vivo to define the scope of Menin-MLL inhibitor biology

** Equipotent on mouse menin
VTP-50469 inhibits the proliferation of multiple MLL-r harboring cells along

- Similar IC\textsubscript{50} across multiple MLL-r harboring cells

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>LINEAGE</th>
<th>GENETIC LESION</th>
<th>DAY</th>
<th>IC\textsubscript{50} nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-60</td>
<td>AML</td>
<td>NONE</td>
<td>12</td>
<td>&gt;&gt;2000</td>
</tr>
<tr>
<td>OCI-AML3</td>
<td>AML</td>
<td>NPM1\textsuperscript{c+}</td>
<td>5</td>
<td>27 +/-10</td>
</tr>
<tr>
<td>MOLM13</td>
<td>AML</td>
<td>MLL-AF9</td>
<td>12</td>
<td>13 +/-9</td>
</tr>
<tr>
<td>THP1</td>
<td>AML</td>
<td>MLL-AF9</td>
<td>12</td>
<td>37 +/-21</td>
</tr>
<tr>
<td>NOMO1</td>
<td>AML</td>
<td>MLL-AF9</td>
<td>14</td>
<td>16 +/-9</td>
</tr>
<tr>
<td>ML2</td>
<td>AML</td>
<td>MLL-AF6</td>
<td>14</td>
<td>20 +/-10</td>
</tr>
<tr>
<td>EOL1</td>
<td>AML</td>
<td>MLL-PTD</td>
<td>14</td>
<td>20 +/-10</td>
</tr>
<tr>
<td>REH</td>
<td>B-ALL</td>
<td>NONE</td>
<td>4</td>
<td>&gt;&gt;2000</td>
</tr>
<tr>
<td>KOPN8</td>
<td>B-ALL</td>
<td>MLL-ENL</td>
<td>4</td>
<td>15 +/-10</td>
</tr>
<tr>
<td>HB11;19</td>
<td>B-ALL</td>
<td>MLL-ENL</td>
<td>4</td>
<td>36 +/-12</td>
</tr>
<tr>
<td>MV4;11</td>
<td>B-ALL</td>
<td>MLL-AF4</td>
<td>4</td>
<td>17 +/-10</td>
</tr>
<tr>
<td>SEMK2</td>
<td>B-ALL</td>
<td>MLL-AF4, AF4-MLL</td>
<td>4</td>
<td>27 +/-11</td>
</tr>
<tr>
<td>RS4;11</td>
<td>B-ALL</td>
<td>MLL-AF4, AF4-MLL</td>
<td>4</td>
<td>25 +/-12</td>
</tr>
</tbody>
</table>
Menin inhibition in MV4;11 cells has acute treatment effects which are differentiated from DOT1L inhibition
RS4;11 cells treated for 72h with VTP-50469 show significant changes in the transcription program

- Strong down regulation of the HOX genes and factors supporting proliferative phenotype
- Upregulation of genes leading to differentiated immune phenotype
VTP-50469 dissociates MEN from nuclear complexes in cells

Glycerol gradient (10%-20%) fractionation of nuclear extracts, 300mM NaCl

- Identical fractionation results obtained from RS4;11 (MLL-AF4), ML-2 (MLL-AF6) and OCI-AML3 (NPM1c+) cells

Andrei Krivtsov
VTP-50469 treatment evicts both Menin and DOT1L from Chromatin
In vivo studies with VTP-50469

- PK/PD model development
- Antitumor efficacy in s.c. xenografts (MV4;11) by oral administration
- Survival (K-M) benefit in disseminated leukemia (MV4;11) by oral administration
- Survival (K-M) and leukemic burden in multiple Pediatric Derived Xenografts (PDXs) by oral administration
  - Compound administered orally at MTD (NCI/PPTC)
  - Compound administered in feed (DFCI)
Steady-state infusion of VTP-50469 can be used to define PK/PD in vivo using MV4;11 s.c. tumors in nu Rats

- Implant MV4;11 cells s.c. and grow to 200-300 mm$^3$
- Implant Alzet 7-day mini-pumps containing VTP-50469 (0.8, 4, 20 mg/ml) contralaterally
- Measure changes in tumor size over 3-4 days and in target transcripts at sacrifice
- Measure blood levels to establish PK/PD relationship

- Clear PK/PD for changes in tumor size and MEIS1 transcripts
- PK/PD findings should be translatable to human studies
VTP-50469 Causes Complete Regression of MV4;11 s.c. Tumors in nu-Mice and nu-Rats

- Exposures indicate that robust tumor regression seen with 4 h drug levels in excess of plasma IC$_{50}$
- Regression also seen with MOLM-13 (AF-9), SHI-1 (AF-6), KOPN-8 (ENL)
VTP-50469 Provides a Significant Dose-Dependent Survival Benefit (K-M) in Disseminated MV4;11 Tumors

- Engraft MV4;11-luc+ cells given i.v.
- Animals randomized at d5 by BLI
- VTP-50469 administered orally twice a day (15, 30, 60 mpk bid) for 28 days
- Survival monitored until d74

Highly significant survival benefit at all doses vs Vehicle by K-M analysis (p<0.001)
Most effective doses have exposures >>pIC₅₀ at 4 hr post dose (30, 60 mg/kg)
At sacrifice (d74), 7 of 9 animals in the 60 mg/kg cohort have ≤0.01% MV4;11 cells in their bone marrow
**PPTC - Pediatric ALL Patient Derived Xenografts (PDX)**

**Profound Effect of Menin Inhibitor on Survival**

- Animals engrafted (8 PDXs) and randomized when blasts >1% in PB
  - At event when blasts >25% in PB
- Animals treated by oral gavage for 28 days at the MTD
  - 120 mg/kg BID
- Highly significant increase in survival by K-M analysis
  - 6 of 7 MLL-r leukemias
  - No effect in Ph+ leukemia (ALL-56)
- Two (2) animals in MLL-2 group survived to 328 d
  - First time the PPTC has observed a “cure” with single agent treatment
BM taken from endosteal and central sections of femurs (L, R) at randomization, in vehicle treated animals at event (25% blast in PB) and from VTP-50469 treated animals at Day 28

>100x reduction in MLLr+ cells in BM after treatment; no effect in non-MLLr (ALL-56)
VTP-50469 Formulated in Feed is Bioavailable and Can Achieve Plasma Levels in Excess of pIC$_{50}$

- Concentration-related increase in plasma levels of drug
- VTP-50469 plasma levels $\gg$ pIC$_{50}$ over 24 hr in 0.10% strength
- High dose strength chosen for PDX studies
DFCI - Pediatric ALL and AML Patient Derived Xenografts (PDX) Significant Reduction in MLLr Leukemia

1-10% PB Leukemia

- No weight loss or changes in CBC at 28 days
- Increased survival in MLL-ALL
- No activity in other AML/ALL

PDX (n=9)

MLL-r B-ALL (n=3) and AML (n=2); NPM1c+ AML (n=4)
DFCI - Treatment with VTP50469 reduces leukemia burden in NPM1c+ AML PDX models

- Compound administered in feed for 28d significantly reduced leukemic burden in whole blood and BM at sacrifice

- **NPM1c+ AML patient population is ~4 fold larger than MLLr+ patient population and represents another potential indication**
PDX data show VTP-50469 has strong anti-leukemic efficacy in both MLLr and NPM1c AML with robust reduction leukemic burden post treatment.
A potent, orally active Menin-MLL interaction inhibitor, VTP-50469, was developed using structure-based drug design.
Menin inhibitors demonstrate potent anti-proliferative activity across the range of MLL fusions and also in NPM1c+ AML.
Menin inhibitors exert broad effects on the transcriptional landscape in MLLr+ cells.
Menin inhibitors have strong anti-tumor effects in both s.c. and disseminated models of disease.
VTP-50469 provided profound survival advantage in PDX models of MLLr (AML, ALL) and NPM1c+ AML.
Acknowledgements

Children’s Cancer Inst. (Sydney)
Richard B. Lock
Kathryn Evans
Tara Pritchard

RTI International, Research
Stephen W. Erickson
Yuelong Guo

National Cancer Institute (PPTC)
Malcolm A. Smith.
Beverly A. Teicher

Dana Farber Cancer Institute
Scott A. Armstrong
Andrei V. Krivtsov
Jayant Y. Gadrey
Hannah Uckelmann
Benjamin K. Eschle
Sayuri Kitajima

Vitae Pharmaceuticals Biology
Deepak Lala
Yi Zhao
Yuri Bukhtiyarov
Joan Guo
Meng Shi
Joel Cassel
Paul Noto
Barbara Kruk
Bethany Algayer
Kerri Lipinski
Geeta Kandpal

Structural Biology
Brian McKeever
Joe Chen
Becky van Orden

Vitae Pharmaceuticals Chemistry
David Claremon
Shankar Venkatraman
Cheng-Guo Dong
Steven Lotesta
Lanqui Jia
Zhenrong Xu
Jing Yuan
Larry Dillard
Bob Simpson

CROS
BioDiscovery (Irvine CA)
MI Bioreserch (Ann Arbor MI)

SYNDAX
Briggs Morrison