VTP-50469 is a novel, orally-available Menin-MLL1 inhibitor effective against MLL-rearranged and $NPM1c^+$ leukemia

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Introduction:

- **MLL**-rearrangements are found approximately 5-10% of AML and B-ALL cases, also >70% of infant leukemias (Krivtsov and Armstrong 2007). **NPM1c** mutations are found in about 25-30% of all adult AML (Ley T et al., 2013).

- Therapeutic targeting of MEN:MLL1/MLL-fusion interaction in **MLL**-rearranged and **NPM1c** AML inhibits cell proliferation. (Yokoyama et al 2005; Borkin et al., 2015; Kuhn et al., 2015)

- Currently available MEN:MLL interaction inhibitors have modest drug like properties. Therefore, VTP-50469 was developed as a novel orally available MEN:MLL1 inhibitor.
VTP-50469 selectively kills cell lines with *MLL*-rearrangements and *NPM1c* mutations

**CellTiter-Glo assay**

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>FUSION</th>
<th>IC₅₀ nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV4;11</td>
<td>MLL-AF4</td>
<td>17</td>
</tr>
<tr>
<td>SEM-K2</td>
<td>MLL-AF4, AF4-MLL</td>
<td>27</td>
</tr>
<tr>
<td>RS4;11</td>
<td>MLL-AF4, AF4-MLL</td>
<td>25</td>
</tr>
<tr>
<td>MOLM-13</td>
<td>MLL-AF9</td>
<td>13</td>
</tr>
<tr>
<td>KOPN-8</td>
<td>MLL-ENL</td>
<td>15</td>
</tr>
<tr>
<td>HB11;19</td>
<td>MLL-ENL</td>
<td>36</td>
</tr>
<tr>
<td>REH</td>
<td>NONE</td>
<td>&gt;&gt;2000</td>
</tr>
<tr>
<td>HL-60</td>
<td>NONE</td>
<td>&gt;&gt;2000</td>
</tr>
</tbody>
</table>

**Colony forming assay in semi-solid media**

- OCI-AML3 (NPM1c+), day 6
  - IC₅₀ 18nM

- Mv4;11 (MLL-AF4)
  - RS4;11 (MLL-AF4)
  - MOLM-13 (MLL-AF9)
  - KOPN-8 (MLL-ENL)
  - HB11;19 (MLL-ENL)
  - REH, HL-60 (NONE)
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
VTP-50469 treatment leads to MEN loss from TSS in *MLL*-rearranged cell lines

RS4;11, 2 days

MOLM13, 5 days
Treatment with VTP-50469 suppresses MLL-fusion target and DOT1L inhibitor sensitive genes.

VTP-50469 treatment changes expression of MLL-target and DOT1L inhibitor sensitive genes faster as compared to EPZ4777.
VTP-50469 treatment reduces leukemia burden in PDX models of MLL-r and NPM1 mutant leukemia

MLL-r B-ALL (n=3) and AML (n=2); NPM1c+ AML (n=4)
Conclusions:

• VTP-50469 specifically inhibits proliferation of cell lines carrying MLL-rearrangements or NPM1c+ mutations with an IC$_{50}$<40 nM.

• VTP-50469 facilitates dissociation of MEN from high molecular weight complexes and leads to eviction of MEN and reverses MLL-fusion driven gene expression.

• Treatment of MLL-r and NPM1c+ PDX models with VTP-50469 leads to differentiation and significant reduction of leukemia burden.

• Similar results in MLL-r B-ALL PDX presented by our collaborators Richard Lock, Malcolm Smith in abstract #3187