Donated Chemical Probe

Chemical Probe BAY-826
Tie/DDR Inhibitor

March, 2018

Sylvia Gruenewald, Lars Baerfacker
Tie/DDR probe BAY-826:

*Scientific rationale: Tie2 as an anti-cancer target*

**RTK Tie2 as a “classical” anti-angiogenesis target**

// Ang1/2-Tie2 signaling:  important role in angiogenesis and vessel maturation
// Tie2 inhibition:  impairs angiogenesis & reduces tumor growth
  shows combination benefit with anti-VEGFR therapy

**Tie2 as anti-tumor cell target beyond angiogenesis**

// Survival of Tie2-positive AML cells sustained through autocrine Ang1/Tie2 loop
// Tie2-positive hematological tumor cells may adhere to the bone marrow niche thus being protected from chemotherapy
// Activation of gliomal Tie2 increases tumorigenesis and invasive phenotype *in vivo*
// Tie2 activation in gliomal and brain tumor stem cells contributes to chemoresistance
// Infiltration of Tie2-expressing macrophages (TEMs) is implicated to promote angiogenesis and metastatic dissemination, potentially of relevance in various tumor indications incl. HCC.
Angiogenesis - Tie2 function is essential for development of embryonic vasculature, for maintenance of blood vessels and for the maturation of newly formed vessels

Comparison of the sTie2 secreting A375 melanoma xenografts with parental cells with and without treatment of PTK787/ZK22584


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Disease hypothesis based on literature data

**Tie/DDR probe BAY-826:**

**AML - Tie2 & Ang1 expressed in subset of AML blasts**

- **RT-PCR**
  - 1/2 No info on patient number of RT-PCR samples

- **Western Blot**
  - 1) No info on patient number of RT-PCR samples

**Blocking of Ang1-Tie2 signaling in Tie2+ but not Tie2- AML cells increases apoptosis**

1) No info on patient number of RT-PCR samples

**Glioma - Tie2 expression in neoplastic glial cells correlates with grade**

**Tie2 staining on human TMA sections**
- normal brain (NB), low grade astrocytoma (LGA), anaplastic astrocytoma (AA), and glioblastoma multiforme (GBM)

**Activation of gliomal Tie2 increases tumorigenesis and invasive phenotype in vivo**

- U251.vector or U251.Tie2 cells were injected with ECs into the brains of immuno-compromised mice
- Infiltrative/multifocal component of tumors in Tie2+/EC

**Books and journals**


Tie/DDR probe BAY-826:

Overall profile

- Pharmacology
  - Tie2 $K_D$ (KINOMEscan™): 1.6 nM
  - Tie2 $IC_{50}$ (in-house kinase assay): 0.45 nM
  - Tie2 $IC_{50}$ (HUVEC pTie2-ELISA): 1.3 nM
  - Tie1 $K_D$ (KINOMEscan™): 0.9 nM
  - DDR1 $K_D$ (KINOMEscan™): 0.4 nM
  - DDR2 $K_D$ (KINOMEscan™): 1.3 nM
  - VEGFR2 $K_D$ (KINOMEscan™): 1.6 μM
  - VEGFR3 $IC_{50}$ (in-house kinase assay): 0.44 μM
  - FGFR1/3 $IC_{50}$ (in-house kinase assay): >10 μM
  - PDGFR-β $IC_{50}$ (in-house kinase assay): >20 μM

- Molecular Properties
  - MW [g/mol]: 559
  - MWcorr [g/mol]: 490
  - TPSA [Å²]: 88
  - Rotatable bonds: 5

- PhysChem
  - Sw pH 6.5 [mg/L]: 1.8
  - log D (pH 7.5): 3.61

- In vitro PK
  - Clint [L/h/kg] | Fmax [%]
    - Human: 0.43 | 68
    - Mice: Rat: 1.91 | 54
    - Hep: Rat: 0.96 | 77

- Safety (LeadProfilingScreen, total # of assays:68)
  - Adenosine A3 (65 % inh.), Opiate κ (75% inh.), hERG (66 % inh.), Sodium Channel site2 (84% inh.)

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Tie/DDR probe BAY-826:

Negative control BAY-309

- **Molecular Properties**
  - MW [g/mol]: 519
  - MWcorr [g/mol]: 450
  - TPSA [Å²]: 64
  - Rotatable bonds: 5

- **PhysChem**
  - $S_{\text{pH}6.5}^{\circ}$ [mg/L]: 0.14
  - log D (pH 7.5): 3.71

- **Pharmacology**
  - Tie2 $K_D$: >20 μM

- **In vitro PK**
  - CaCo2
    - A-B [μm/s]
    - B-A [μm/s]
    - Ratio
    - No data (below detection limit)
Tie/DDR probe BAY-826:

Biochemical and cellular potency and selectivity

<table>
<thead>
<tr>
<th>Biochemical activity*</th>
<th>$K_D$ [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tie2</td>
<td>1.6 (0.5, IC$_{50}$)</td>
</tr>
<tr>
<td>Tie1</td>
<td>0.9</td>
</tr>
<tr>
<td>DDR1</td>
<td>0.4</td>
</tr>
<tr>
<td>DDR2</td>
<td>1.3</td>
</tr>
<tr>
<td>LOK</td>
<td>5.9</td>
</tr>
<tr>
<td>EPHB6</td>
<td>25</td>
</tr>
<tr>
<td>LYN</td>
<td>40</td>
</tr>
<tr>
<td>MERTK</td>
<td>66 (IC$_{50}$)</td>
</tr>
<tr>
<td>ABL1-non-phosphorylated</td>
<td>38</td>
</tr>
<tr>
<td>ABL1-phosphorylated</td>
<td>510</td>
</tr>
<tr>
<td>ABL1(T315I)-non-phosphorylated</td>
<td>100</td>
</tr>
<tr>
<td>ABL1(T315I)-phosphorylated</td>
<td>310</td>
</tr>
<tr>
<td>KIT</td>
<td>150 (&gt;20000, IC$_{50}$)</td>
</tr>
<tr>
<td>BRAF (V600E)</td>
<td>370</td>
</tr>
<tr>
<td>BRAF</td>
<td>730</td>
</tr>
<tr>
<td>RAF1</td>
<td>1400</td>
</tr>
<tr>
<td>VEGFR2</td>
<td>1600 (118, IC$_{50}$)</td>
</tr>
<tr>
<td>VEGFR3</td>
<td>439 (IC$_{50}$)</td>
</tr>
<tr>
<td>FGFR1</td>
<td>16200 (IC$_{50}$)</td>
</tr>
<tr>
<td>FGFR3</td>
<td>12800 (IC$_{50}$)</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>&gt;20000 (IC$_{50}$)</td>
</tr>
</tbody>
</table>

$K_D$ values determined by DiscoverX Corp. on the KINOMEscan platform

Summary of cellular NanoBRET™ assay data @SGC

<table>
<thead>
<tr>
<th>Kinase</th>
<th>BAY-826 IC50 (normed to Tie-2 data)*</th>
<th>Ratio of IC50s (BAY-309/BAY-826)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tie2</td>
<td>1.0</td>
<td>1604.7</td>
</tr>
<tr>
<td>DDR2</td>
<td>4.5</td>
<td>3056.1</td>
</tr>
<tr>
<td>DDR1</td>
<td>1.4</td>
<td>369.9</td>
</tr>
<tr>
<td>Tie1</td>
<td>8.1</td>
<td>23.4</td>
</tr>
<tr>
<td>STK10***</td>
<td>317.0</td>
<td>2.9</td>
</tr>
<tr>
<td>EPHB6</td>
<td>5822.8</td>
<td></td>
</tr>
</tbody>
</table>

* Dose response curves and derived IC$_{50}$ values, see backup section

// BAY-826 binds to Tie1, Tie2, DDR1 and DDR2 with a $K_D$ of ~ 1 nM
// BAY-826 is selective versus the known angiogenic RTKs VEGFR1/2/3, FGFR1/2/3/4 and PDGFR-α/β
// BAY-826 is equipotent vs DDR1/2 (and Tie1 kinase) in cellular assays and more than three orders of magnitude less active vs STK10 and EPHB6 kinase
// The neg. control BAY-309 reveals a sufficient cellular selectivity vs BAY-826 for all six kinases tested
### Tie/DDR probe BAY-826:

**Summary / conclusion**

<table>
<thead>
<tr>
<th>Probe criteria</th>
<th>Criteria</th>
</tr>
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<tbody>
<tr>
<td>Inhibitor/agonist potency: goal is &lt; 100 nM (IC50, Kd)</td>
<td>Surpasses criteria; biochemical assay (Tie-2) with IC50 0.45 nM;</td>
</tr>
<tr>
<td>Selectivity within target family: goal is &gt; 30-fold</td>
<td>Surpasses criteria; selectivity &gt; 100 fold vs all other angiogenic kinases, (# of kinases tested 453) albeit cellular equipotency vs DDR1/2 and Tie-1</td>
</tr>
<tr>
<td>Selectivity outside target family: describe the off-targets (which may include both binding and functional data)</td>
<td>Surpasses criteria; Promising LeadProfilingScreen: 4 out of 68 assays hit (all in μM potency range)</td>
</tr>
<tr>
<td>On target cell activity for cell-based targets: goal is &lt; 1 micromolar IC50/EC50</td>
<td>Surpasses criteria; functional cellular assays: pTie2-ELISA, HUVEC-cells with IC50 1.3 nM; NanoBRET™ assay with IC50 0.7 nM</td>
</tr>
<tr>
<td>On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture</td>
<td>n/a</td>
</tr>
<tr>
<td>Neg ctrl: in vitro potency – &gt; 100 times less; Cell activity – &gt;100 times less potent than the probe</td>
<td>Surpasses criteria; &gt; 10,000 times less in biochemical assay (Tie-2); &gt; 1,000 times less in cellular assay (DDR1/2, STK10, EPHB6)</td>
</tr>
</tbody>
</table>

We ask for acceptance of Tie/DDR inhibitor BAY-826 as chemical probe, accompanied by BAY-309 as negative control which may allow to selectively study the biology of Tie/DDR signaling in vitro and in vivo as it does not target other angiogenic kinases.
Tie/DDR probe BAY-826:

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Thank You
Tie/DDR probe BAY-826 & negative control BAY-309: NanoBRET™ assay data, SGC results

170829/170901 NanoBRET Tie2 Probe and Neg Ctrl

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