PLX8394

BRAF-MAPK Paradox Breaker

CRUK Combinations Alliance
Plexxikon Inc.
March 23, 2016
### Plexxikon’s Development Pipeline

#### Compound

<table>
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<th>Compound</th>
<th>Target</th>
<th>Indication</th>
<th>Stage of Development</th>
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<tbody>
<tr>
<td>PLX4032 (vemurafenib)</td>
<td>BRAF</td>
<td>Adjuvant Melanoma</td>
<td>Pre-IND</td>
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<tr>
<td>PLX3397 (pexidartinib)</td>
<td>FMS</td>
<td>PVNS (TGCT)</td>
<td>Ph2</td>
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<tr>
<td>PLX3397 + RT + TMZ</td>
<td>FMS, KIT</td>
<td>Adjuvant GBM</td>
<td>Ph3</td>
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<td>PLX3397 + paclitaxel</td>
<td>FMS, KIT</td>
<td>Advanced Ovarian Cancer</td>
<td>Ph3</td>
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<td>PLX3397 + pembro</td>
<td>FMS</td>
<td>Melanoma, Solid Tumors</td>
<td>Pre-IND</td>
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<tr>
<td>PLX3397</td>
<td>KIT</td>
<td>KIT-mutant Melanoma</td>
<td>Ph3</td>
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<tr>
<td>PLX3397</td>
<td>FMS</td>
<td>Alzheimer’s/Imaging</td>
<td>Ph1</td>
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<td>PLX7486</td>
<td>TRK, FMS</td>
<td>Pain, Oncology</td>
<td>Ph2</td>
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<td>PLX9486</td>
<td>KIT-mutant</td>
<td>GIST, KIT-mutant tumors</td>
<td>Pre-IND, Ph2</td>
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<tr>
<td>PLX8394</td>
<td>BRAF</td>
<td>BRAF-mutant tumors</td>
<td>Ph2</td>
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<td>PLX51107 (2016)</td>
<td>BRD4</td>
<td>Leukemia</td>
<td>Ph1</td>
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<td>PLX73086 (AC708) (2016)</td>
<td>FMS</td>
<td>TGCT, non-oncology</td>
<td>Pre-IND</td>
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</tbody>
</table>
Clinically Actionable BRAF Mutations in Melanoma

Oncogenic BRAF mutation at V600 promotes RAS-independent MAPK signaling in melanoma (6.27.2002)

K507 (700 fold more active)

2005

2009

FDA approval 8.17.2011
PLX8394 – Rationale for Collaboration with Combinations Alliance

- Due to its paradox-breaking properties, PLX8394 may be the most combinable BRAFi inhibitor in clinical development.
- Plexxikon is developing PLX8394 as a single agent in niche indications, based on vemurafenib efficacy but desiring improved tolerability.
- Proposed combinations for the Combinations Alliance:
  - MEK inhibitors
  - Immunotherapies
    - Anti-PD1
    - Anti-PDL1
    - Anti-CTLA4
    - Other immune checkpoint inhibitors
  - EGFR inhibitors
  - Epigenetic modulators (HDACi, BRDi)
BRAF-mutant Glioblastoma Responding to Vemurafenib
Vemurafenib – Response and Resistance

Reactivation of MAPK pathway through acquired MEK1^{C121S} mutation

Before Therapy

Week 15

Week 23

Correlation between Clinical Response and Mode of Inhibition

Sorafenib binds preferably the “DFG-out” state of Raf

Vemurafenib binds preferably the “DFG-in” state of Raf
Paradoxical Activation of MAPK Pathway by BRAF Inhibitors in RAS-activated Cells

Inhibition

\[ \text{BRAF}^{\text{V600E}} \quad \text{GDP} \]

\[ \text{BRAF}^{\text{V600E}} \quad \text{pERK/pMEK} \]

**Phospho-Selectivity**

\[ \text{EC50 for RAS pathway activation (IPC-298, B9, HCT116)} \]

\[ \text{IC50 for BRAF pathway inhibition (A375, COLO829)} \]

Activation

\[ \text{Activated RAS (RAS}^{\text{mut or RTK}}) \]

\[ \text{GTP} \quad \text{CRAF} \quad \text{BRAF} \]

\[ \text{IC50} = 0.04 \mu\text{M} \]

\[ \text{EC50} = 0.5 \mu\text{M} \]

\[ \text{pERK/pMEK} \]
Studies on the RAF Inhibitor Paradox

5 different RAF inhibitors all activate the MEK/ERK Pathway

<table>
<thead>
<tr>
<th>pMEK</th>
<th>μM</th>
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<tbody>
<tr>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>lysates</td>
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<td>Sorafenib</td>
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MEK/ERK Signaling

RAFi

ATP
Paired Biopsy Data – Resistant Tumors Usually Re-Activate the MAP Kinase Pathway

Vemurafenib-resistant tumor

- pERK (40X)
- Ki-67 (40X)

Mutation Status:
- BRAF V600E
- NRAS Q61K
- BRAF V600E
- NRAS No Mutation

Trunzer et al. J Clin Oncol. 2013
BRAF Inhibitor Resistance Mechanisms

- Aberrant splicing of BRAF
- Elevated expression of CRAF, COT1, mutant BRAF
- Activating mutations in NRAS, MEK1/2, AKT1 or BRAF
- Lost of PTEN (activation of PI3K), Lost of NF1 (activation of RAS)
- Activation of RTKs (PDGFRβ, IGF-1R, EGFR)
- Microenvironment (e.g. stromal derived HGF)

Possibly respond to PLX8394

Rizos et al., Clin Cancer Res 2014
Progression of RAS-Mutant Leukemia during RAF Inhibitor Treatment

Callahan et al. NEJM 2012
Abdel-Wahab et al. Cancer Discovery 2014
Progression of RAS-Mutant Metastatic CRC during RAF/MEK Inhibitor Treatment

Paradoxical activation can be seen in other premalignant lesions even with RAFi-MEKi combined treatment.

Andrews, et al., Cebon et al, JCO 2013
Rapid cSCC emergence on RAF inhibitors

Majority of tumors have RAS mutations

- Median time to first incidence 8 weeks (range 2–36)
- Each dot represents one patient:
  
  # weeks to development of first cuSCC/KA lesion

- 21/35 (60%) of samples have RAS mutations

Su et al. NEJM 2012
PLX8394: Next Generation BRAF Inhibitor
Paradox Breaker (PB)

• **Opportunity:**
  Avoiding paradoxical MAPK pathway activation

• **Hypothesis:**
  PLX8394 as a ‘paradox breaker’ might
  - Improve response of some V600 tumors
  - Delay resistance (1st gen compounds enable pathway re-ignition)
  - Reduce toxicities such as skin lesions
  - Combine better with Immunotherapy

• **Status:**
  Currently in clinical development
RAF inhibitors that evade paradoxical MAPK pathway activation

Chao Zhang¹, Wayne Spevak¹, Ying Zhang¹, Elizabeth A. Burton¹, Yan Ma¹, Gaston Habets¹, Jiazhong Zhang¹, Jack Lin¹, Todd Ewing¹, Bernice Matusow¹, Garson Tsang¹, Adhirai Marimuthu¹, Hanna Cho¹, Guoxian Wu¹, Weiru Wang¹, Daniel Fong¹, Hoa Nguyen¹, Songyuan Shi¹, Patrick Womack¹, Marika Nespi¹, Rafe Shellooe¹, Heidi Carias¹, Ben Powell¹, Emily Light¹, Laura Sanftner¹, Jason Walters¹, James Tsai¹, Brian L. West¹, Gary Visor¹, Hamid Rezaei¹, Paul S. Lin¹, Keith Nolop¹, Prabha N. Ibrahim¹, Peter Hirth¹ & Gideon Bollag¹

Oncogenic activation of BRAF fuels cancer growth by constitutively promoting RAS-independent mitogen-activated protein kinase (MAPK) pathway signalling¹. Accordingly, RAF inhibitors have brought substantially improved personalized treatment of metastatic melanoma²⁻⁵. However, these targeted agents have also revealed an unexpected consequence: stimulated growth of certain cancers⁶⁻⁹. Structurally diverse ATP-competitive RAF inhibitors can either inhibit or paradoxically activate the MAPK pathway, depending on the MAPK pathway in cells bearing oncogenic RAS or elevated upstream receptor signalling¹⁰⁻¹². This paradox can promote cellular proliferation and manifest clinically with progression of cutaneous squamous cell carcinomas (cuSCC) and keratoacanthomas, sometimes within weeks of therapy initiation⁶⁻¹⁵. These paradox-induced skin tumours have an uncharacteristically high incidence of RAS mutations⁶⁻¹⁶, raising the concern that the same mechanism might accelerate progression of other RAS-driven cancers. Recent case reports of increased incidence of prim-

Zhang et al., Nature 526, 523-586 (22 October 2015).
From Hit to Development Candidate
Discovery of selective RAF kinase inhibitors

PLX8394

Vemurafenib

Nature 2010, 2015
Next-Gen BRAF Inhibitors Overcome Paradoxical MAPK Pathway Activation

1st-generation BRAFi induce BRAF-CRAF Heterodimers, PLX8394 does not

Dimerization In B9 Cells

Zhang et al. Nature 2015
‘Paradox Breaking’ property can be transferred to another chemical series

Zhang et al. Nature 2015
PLX8394 Is Active Against Vemurafenib-Resistant Cells

From Poulidakos, Rosen, Solit, Nature 2011

Resistance to BRAF^{V600E} melanoma

In vitro and in human patients

Mediated by alternatively spliced BRAF^{V600E}

 Constitutive dimers are resistant to Vemurafenib

Viability

Apoptosis

From Poulikakos, Rosen, Solit, Nature 2011

Resistance to BRAF^{V600E} melanoma

In vitro and in human patients

Mediated by alternatively spliced BRAF^{V600E}

 Constitutive dimers are resistant to Vemurafenib
PLX8394 inhibits ERK activation in BRAF\textsuperscript{mut}/NRAS\textsuperscript{mut} co-expressing melanoma cells

Re-activation of the ERK signaling pathway and development of acquired resistance are sometimes mediated by acquired mutations in NRAS (or selection of a small population of cells co-expressing mutant BRAF and NRAS)
Effective against BRAF Kinase Fusions in vivo

>60% of pediatric astrocytoma caused by BRAF-fusion; paradoxical activation & BRAFi resistance
Frequency of BRAF mutations

Multiple Potential Indications

- Hairy Cell Leukemia, Papillary Craniopharyngiomas
- Melanoma
- Papillary and Anaplastic Thyroid Cancer
- Langerhans Cell Histiocytosis, Erdheim-Chester Disease
- Serous Ovarian Cancer
- Astrocytoma/Glioblastoma
- Cholangiocarcinoma
- Colorectal Cancer
- Non Small Cell Lung Cancer
- Bladder Cancer
- Prostate Cancer
- Multiple Myeloma
- Breast Cancer
- GIST, Gastric Cancer, Barrett’s Esophageal Cancer
- Head and Neck Cancers

~100%
30-80%
8-30%
<8%
PLX120-03: PLX8394 Phase I Trial
STUDY DESIGN

• Part 1: “3+3” Dose escalation phase (n=up to 42)
  – Patients with advanced solid tumors refractory to standard therapy or no standard therapy exists or considered appropriate by the investigators

• Part 2: Extension cohort phase (n=65) at RP2D
  – Metastatic Melanoma
  – Papillary thyroid carcinoma (PTC)
  – Anaplastic thyroid carcinoma (ATC)
  – NSCLC, colorectal carcinoma (CRC) and other BRAF mutated malignancies
Study Design

Dose-Escalation Cohorts (Part I)

- Dose Cohort 1
  - n=3–6

- Dose Cohort 2
  - n=3–6

- Dose Cohorts 3–7a
  - n=3–6/cohort

Safety and Tolerability Data

Extension Cohorts (Part 2)

- MTD/MAD RP2D

- Advanced Unresectable BRAF-Mutated Cancers

- Melanoma
  - Pretreated Melanoma
    - n=10–15

- Non-Melanoma Solid Tumors
  - n≈50
    - Papillary thyroid carcinoma
      - n=10–15
    - Anaplastic thyroid carcinoma
      - n=10–15
    - Other
      - n=15–20

a Potential, depending on the safety and tolerability data.

b For example, colorectal cancer, nonsmall-cell lung cancer, cholangiocarcinoma, histiocytosis (e.g., LCH, ECD).