Resolving the question of on- or off-target toxicity - a case study

Symposium on Streamlining Drug Discovery
San Diego, April 21\textsuperscript{st} 2017

Joachim Rudolph
April 21\textsuperscript{st}, 2017
Toxicity is the primary cause of attrition post pre-clinical candidate nomination


**Important role for medicinal chemists in addressing safety:**
- Strive for high target selectivity and optimal properties to decrease off-target effects
- Provide tool molecules to resolve question of on- versus off-target toxicity
p21-Activated Kinase 1 (PAK1) is Implicated in Breast Cancer

- Ser/Thr kinases in STE20 family
- Subdivided into two groups:

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Kinase Domain Homology (% to PAK1)</th>
<th>Kinase Domain Homology (% to PAK4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAK1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>PAK2</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>PAK3</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>PAK4</td>
<td>56</td>
<td>100</td>
</tr>
<tr>
<td>PAK5</td>
<td>54</td>
<td>86</td>
</tr>
<tr>
<td>PAK6</td>
<td>52</td>
<td>79</td>
</tr>
</tbody>
</table>

- PAK1 is amplified in 7.4% of all breast cancer (> 5 copies)
- Highest in poor prognosis luminal B subtype: ~15%
- Cell lines with PAK1 amplification are highly sensitive to PAK1 knockdown
Genentech PAK1 Program: The Road to a Lead Series

High-throughput screen: Low hit rates, non-selective leads
• Ligandability compromised by plasticity of the PAK1 ATP binding site

1st series: Screening hit to early lead

G3247 (HTS hit)
PAK1 $K_i$ 100 nM
PAK4 $K_i$ 49 nM
Promiscuous kinase inhibitor

G0236
PAK1 $K_i$ 18 nM
PAK4 $K_i$ 950 nM
Excellent kinase selectivity
Poor permeability, no cellular potency

2nd series: In-licensed from Afraxis

FRAX1036 (starting point)
PAK1 $K_i$ 22 nM
PAK4 $K_i$ 2,400 nM
pMEK IC$_{50}$ 179 nM
Excellent kinase selectivity
Low LLE, ion channel off-targets

Test conc. = 1µM
73 kinases
Hybridization of two Series with Goal to Increase Potency

Afraxis Series B

- FRAX1036
- PAK1 $K_i$ 22 nM
- LLE 2.8

Amino tails:
- Critical for solubility
- No interaction with protein residues (multiple X-ray structures)
- >100 analogs: no potency gain

Genentech Aminopyrazole Series

- G3878
- PAK1 $K_i$ 21 nM
- LLE 4.6

Amino tails:
- H-bond with Asp-393 backbone
- Critical potency driver

Opportunity to reach Asp-393 from Afraxis scaffold?
Vector Change of Amino Tail: 10x Potency Gain

**Series B**
- FRAX1036
- PAK1 $K_i$ 22 nM
- LLE 2.8

**Series C**
- G3586
- PAK1 $K_i$ 2 nM
- LLE 4.2

- Excellent pharmacological profile
- Poor permeability
- Poor exposure in mice ($F = 2\%$; 25 mg/kg PO)

Direct H-bond to Asp393 backbone
Discovery of G555: Reducing pKa Affords Improved Permeability and High Oral Bioavailability

<table>
<thead>
<tr>
<th></th>
<th>G3586</th>
<th>G555</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAK1 $K_i$ [nM]</td>
<td>2.1</td>
<td>4.4</td>
</tr>
<tr>
<td>p-MEK(S298) IC$_{50}$ [nM]</td>
<td>124</td>
<td>71</td>
</tr>
<tr>
<td>Microsomal stability (HRMDC)</td>
<td>7 / 37 / 76 / 26 / 20</td>
<td>12 / 32 / 50 / 22 / 24</td>
</tr>
<tr>
<td>MDCK A:B perm. [10$^{-6}$ cm/s] / Papp ratio</td>
<td>0.44 / 38</td>
<td>2.40 / 1.87</td>
</tr>
<tr>
<td>Aqueous solubility pH7.4 [µM]</td>
<td>34</td>
<td>15</td>
</tr>
</tbody>
</table>

Mouse PK study (mean blood conc., n=3)

- G3586, PO 25 mg/kg; F = 1.8%
- G3586, IV 2 mg/kg; CL = 21 mL/min/kg
- G555, PO 25 mg/kg; F = 79%
- G555, IV 2 mg/kg; CL = 24 mL/min/kg

Similarly excellent PK profile for G555 in cynomolgus monkey:
- F = 72%
- CL = 3.4 mL/min/kg

ACS Med Chem Lett 2015, 6, 1241-1246
G555: Improved Selectivity Profile

FRAX1036
PAK1 $K_i$ 22 nM
PAK4 $K_i$ 2,400 nM
pMEK $IC_{50}$ 179 nM

G555
PAK1 $K_i$ 4 nM
PAK4 $K_i$ 2,100 nM
pMEK $IC_{50}$ 71 nM

$2^\circ$ pharmacology profile (n=41; CERE)

Kinase panel profile (n=230)

hERG $IC_{50}$ (patch clamp) = 0.48 μM

hERG $IC_{50}$ (patch clamp) > 10 μM

ACS Med Chem Lett 2015, 6, 1241-1246
PD and Efficacy Studies with G555: Moderate Knockdown and Dose-Limited Tumor Growth Inhibition

Mouse PK/PD (H292 NSCLC)

- G555 @ 10, 20, 30 mg/kg
- PD marker: pMEK S298

- Dose- and exposure-dependent reductions in pMEK

Mouse Efficacy (H292 NSCLC)

- 25 mg/kg BID = MTD
  - 59% TGI

- 25 mg/kg BID is max tolerated dose
  - Acute lethality at higher doses

Beth Blackwood, Diana Jakubiak
• Acute deaths at 40, 50 mg/kg (2-4 hrs after first dose)
• Observations: Mice hypoactive, cold to touch, blood dark and hard to draw, slow blood flow
• Brain/Plasma = 0.02 (unlikely CNS-driven lethality)
• Lethality $C_{\text{max}}$-driven: $C_{\text{max, unbound}}$ ~150 nM not tolerated (multiple studies)

Observations suggest cardiovascular toxicity $\rightarrow$ mechanistic safety studies
Mechanistic Safety Studies: G555 Causes Acute Cardiovascular Toxicity

**Isolated rat hearts (Langendorff)**

G555 perfused at 0.5, 1.5, 5, 10 µM

- Vasoconstriction and impaired relaxation of LV
- 2/5 hearts (5 µM): AV block and ventricular fibrillation, respectively

⇒ **Decline of left ventricle function, arrhythmia and heart failure; consistent with acute lethality**

**Mouse echocardiography**

Control and G555 dosed at 25 mg/kg QD (n=10)

Echo on Day 1 (2 hr), Day 6, and blood pressure on Day 7

- 5/10 animals survived after day 1 and 3/10 at end of study
- Decreased LV volume, stroke volume and cardiac output
- Slowed breathing; increased systolic and diastolic arterial pressure

⇒ **Severely decreased cardiac output and performance**

LV = left ventricle
AV node = atrioventricular node

Zoe Zhong, Jed Ross, Diana Jakubiak, Nicole Valle
Structural Diversification to Explore Question of On/Off-Target Toxicity

- G9479 causes acute lethality
- Same clinical signs at toxic doses: blood is dark and flowing slowly (consistent with failing heart); death ~0.5 – 4 hrs

**On-target toxicity?**
Three Diverse Chemotypes all Cause Acute Lethality in Mouse Tolerability Studies

Series C (lipophilic head, basic tail)

Series C (polar head, neutral tail)

Pfizer series

Aminopyridine

• Same clinical signs at toxic doses: blood is dark and flowing slowly (consistent with failing heart); death ~0.5 - 4 hrs
• Coincidental overlap of off-target activities? → next slide
**PAK1, 2, 3 are the Only Overlapping Kinase Targets**

Shown:
Kinases inhibited >75% (Invitrogen)
G555: 237 kinases (0.1 µM)
PD: 146 kinases (1 µM)
G9479: 96 kinases (1 µM)
Any hits >75% were tested against all three compounds

Similar analysis done for secondary pharmacology panel (CEREP): **No overlapping activities**
Cellular Potency Correlates with Minimally Toxic Concentrations Across Multiple Series

All PAK1 inhibitors shown were acutely toxic in mice at [1-4 x pMEK IC$_{50}$].
Structurally Closely Related PAK1 Active/Inactive Pair: Only Active Pair Member Causes Lethality in Mice

- **G1608**
  - PAK1: 0.011 µM
  - pMEK: 0.226 µM

- **G7702**
  - PAK1: 1.2 µM
  - pMEK: >30 µM

- C<sub>max</sub> 0.74 µM is toxic
- Lower concs. not tested

- C<sub>max</sub> 5.7 µM is safe
- Higher concs. not tested
**Acute CV Tox of PAK1/2 Inhibitors Likely “On-Target”**

<table>
<thead>
<tr>
<th>Supporting observations</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity present with chemically-diverse inhibitors</td>
<td>• Three chemotypes: Afraxis, aminopyridine, Pfizer all acutely lethal</td>
</tr>
<tr>
<td></td>
<td>No overlapping off-targets (CEREP, Kinases and others)</td>
</tr>
<tr>
<td>Toxicity absent with an inactive analog</td>
<td>• Active/inactive pair: only the active analog G1608 was toxic</td>
</tr>
<tr>
<td>+ve Relationship between target potency and toxicity</td>
<td>• Lethality correlated well with pMEK potency with 11 compounds (1-4x pMEK EC₅₀)</td>
</tr>
<tr>
<td></td>
<td>~300-fold potency range</td>
</tr>
<tr>
<td>Target expressed in tissue with toxicity</td>
<td>• PAK1 and 2 are widely expressed, including the cardiovascular system</td>
</tr>
<tr>
<td>Toxicity consistent with phenotype of KO mice?</td>
<td>• PAK1 KO – viable and fertile</td>
</tr>
<tr>
<td></td>
<td>• PAK2 KO – embryonic lethal (vasculature development; Chernoff, 2012);</td>
</tr>
<tr>
<td></td>
<td>PAK2 adult cKO – lethal (Chernoff, 2015)</td>
</tr>
<tr>
<td></td>
<td>Neither PAK2 kinase-dead KI nor PAK1/PAK2 double cKO generated to date</td>
</tr>
</tbody>
</table>

**Hypothesis:**

PAK2 is the driver of toxicity, either alone or in combination with PAK1

*J Med Chem* 2016, 59, 5520-5541
• PAK1/PAK2 kinase domain homology: 93%
• 2 residue differences in ATP binding site; similar residues and difficult to target

Path Forward for a PAK2-Sparing PAK1 Inhibitor?

• For ~2400 ATP-competitive inhibitors made, PAK1 vs PAK2 selectivity never obtained
Path Forward for a PAK2-Sparing PAK1 Inhibitor?

Allosteric inhibitor with PAK1 vs PAK2 selectivity described by Novartis*

NVS-PAK1-1
PAK1 $K_i$: 0.021 µM
PAK2 $K_i$: 1.3 µM (62x)
PAK4 $K_i$: >2.9 µM (>136x)
pMEK IC$_{50}$: 1.1 µM
Prolif. assays (various cell lines): EC$_{50}$ > 10 µM

• In line with Novartis’ data, poor cellular potency
• In cell lines dependent on and expressing both isoforms, a dual PAK1/2 inhibitor will likely be necessary for cellular efficacy
• No cancer cell lines identified that are dependent on and express only PAK1

*(a) ACS Fall 2013; (b) ACS Med. Chem. Lett. 2015, 6, 776
Conclusions

- Simultaneous inhibition of PAK1 and PAK2 provides cellular efficacy but is associated with severe cardiovascular toxicity

- Selective PAK1 inhibition is insufficient to promote cellular efficacy, but presumably safe (based on PAK1 knock-out experiments)

  - No path forward for PAK1 inhibitor program

Important lesson learned:

- Certain kinases have been implicated in cardiovascular functions but kinase related CV toxicity of the acuteness and severity observed here is uncommon; watch your preoccupation “Acute cardiac toxicity = ion channel”
On-target Toxicity – Generating Evidence

Strong set of tool molecules
- Sufficient selectivity
- Structurally diverse (best: distinct series)
- Varying cellular potencies
- Active / inactive pairs

- Consider enabling technologies if drug exposure is an issue

Target expression knowledge
- For on-target toxicity, target must be expressed in tissue with toxicity

Genetic rodent models
- Embryonic knock-out
- Conditional knock-out
- Embryonic function-deficient knock-in
- Conditional function-deficient knock-in
Acknowledgments

Chemistry
Chudi Ndubaku
James Crawford
Joy Drobnick
Lewis Gazzard
Wendy Lee
Simon Mathieu
Joachim Rudolph
Haiming Zhang
Xianrui Zhao
Dan Burdick
Thuy Tran
Shumpei Wang
Qi Chao, Ping Dong, David Favor and colleagues at ChemPartner
Dan Sutherland
Wendy Young

Bioinformatics
Anneleen Daemen
Florian Gnadt
Peter Haverty
Gerard Manning

Biochemical Pharmacology
Christopher Heise
Maureen Beresini
Kevin Clark
Tony Giannetti
Bob Mintzer
Keith Pitts
Sreema Ramaswamy

Discovery & Translational Oncology
Klaus Hoeflich
Tom O’Brien
Beth Blackwood
Marcia Belvin
Jennifer Epler
Lori Friedman
Christy Ong
Diana Wadley-Jakubiak
Amy Young
Wei Zhou

Pathology
Sarah Gierke
Adrian Jubb
Hartmut Koeppen
Karen Lyle

Safety Assessment
Zoe Zhong
Gary Cain
Carolina Chou
Mike Flagella
Paula Katavolos
Rama Pai
Dolo Diaz

Clinical
Jen Lauchle

Protein Chemistry & Structural Biology
Weiru Wang
Mary Coons
Yvonne Franke
Anjela Oh
Lionel Rouge
Christine Tam
Jiansheng Wu
Shanghai ChemPartner

Molecular Biology
Meredith Dempsey
Leisa Johnson
Melissa Junittle
Lee Nguyen
Amy Rappaport

Diagnostics, Biomarkers
Marcin Kowanetz
Max Ma
Maike Schmidt
Tim Wilson
Peng Yue

Legal
Brian Buckwalter
Alex Andrus
Jori Mandelman

BD, Alliance Management
Nicholas Galli
Kinney Horn
Mark Rowen
Zhenhai Shen

Other
David Campbell (Afraxis)
Sergio Duron (Afraxis)
Carmine Stengone (Afraxis)
Jonathan Chernoff (Fox Chase)
François Diederich (ETH)