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

Symposium on Streamlining Drug Discovery
San Diego, April 21st 2017

Joachim Rudolph
April 21st, 2017

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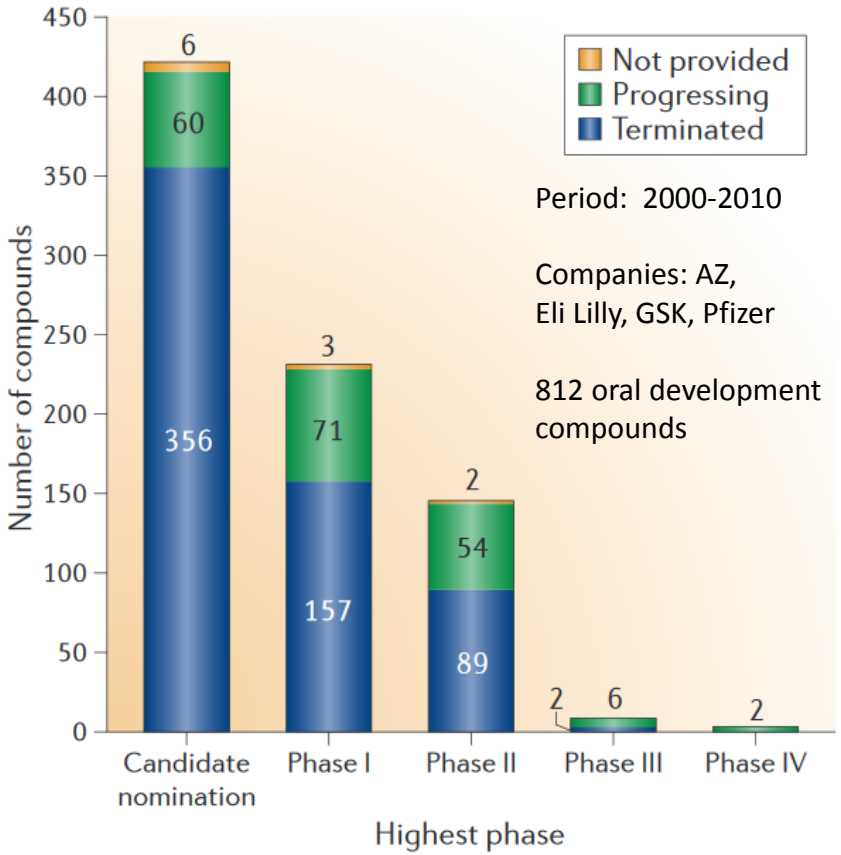
Toxicity is the primary cause of attrition post pre-clinical candidate nomination

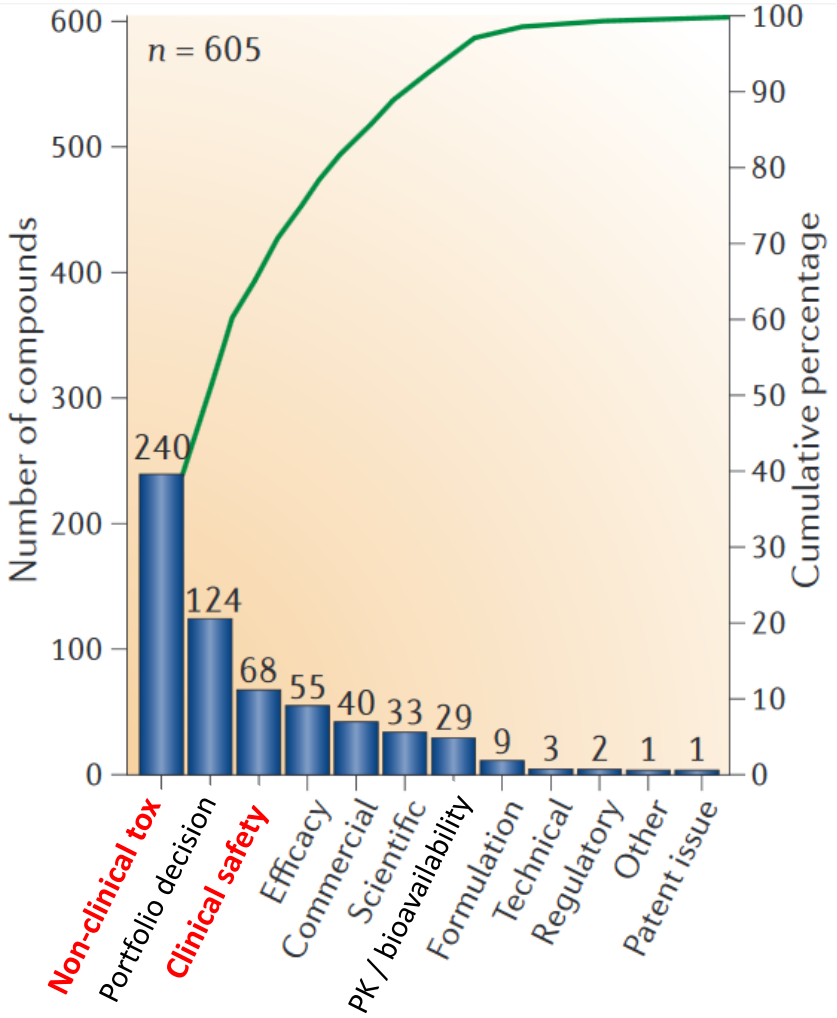


Period: 2000-2010

Companies: AZ, Eli Lilly, GSK, Pfizer

812 oral development compounds

Adapted from: Waring et al, An analysis of the attrition of drug candidates from four major pharmaceutical companies, *Nature Rev Drug Discov* **2015**, *14*, 475.



Important role for medicinal chemists in addressing safety:

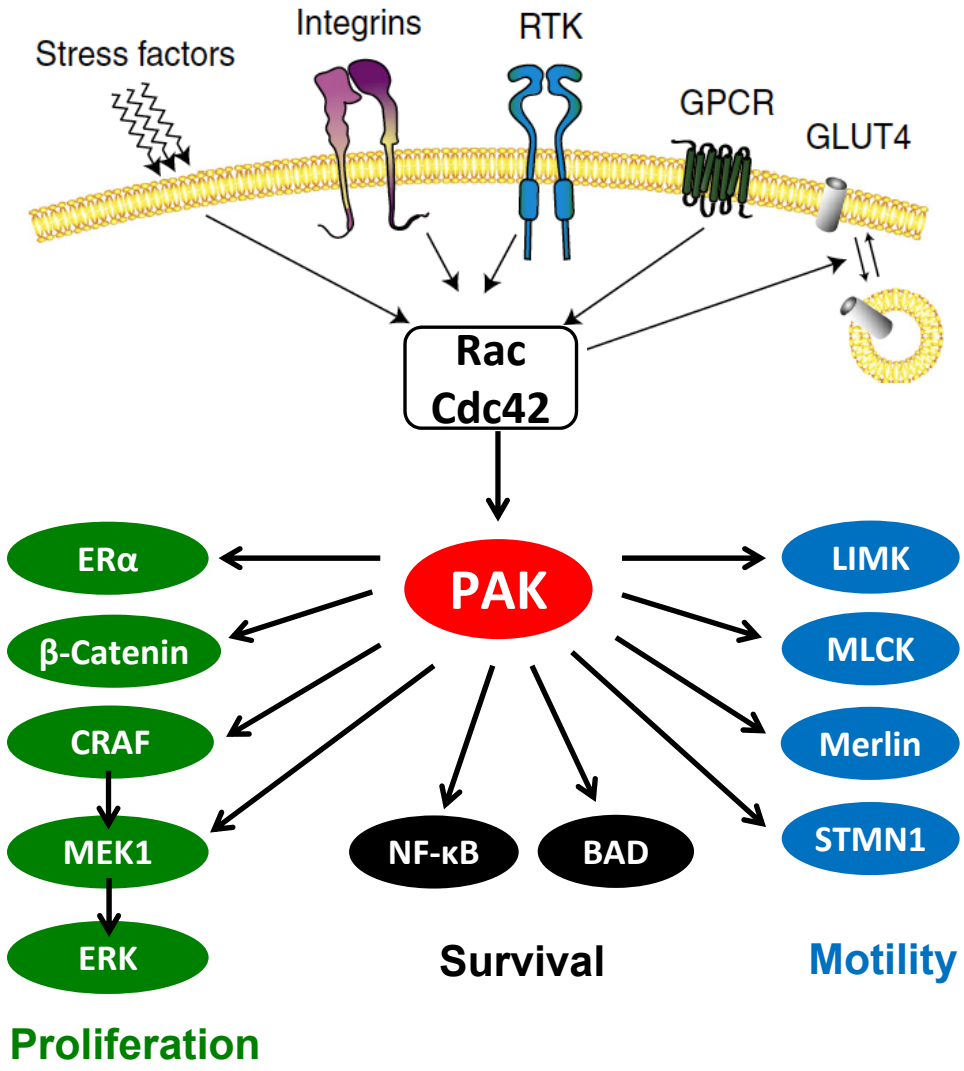
- Strive for high target selectivity and optimal properties to decrease off-target effects
- Provide tool molecules to resolve question of on- versus off-target toxicity

p21-Activated Kinase 1 (PAK1) is Implicated in Breast Cancer

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

	Isoform	Kinase Domain Homology (% to PAK1)	Kinase Domain Homology (% to PAK4)
Group 1	PAK1	100	
	PAK2	93	
	PAK3	96	
Group 2	PAK4	56	100
	PAK5	54	86
	PAK6	52	79

- 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



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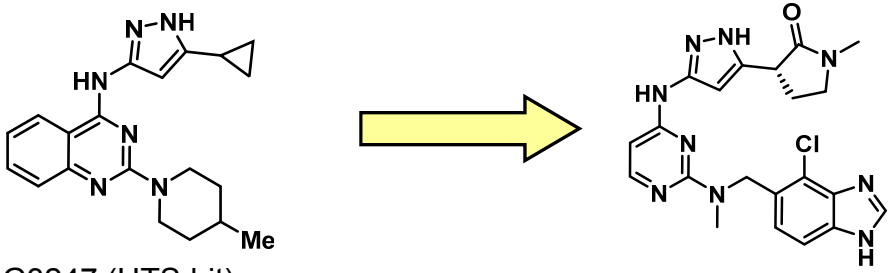
Genentech PAK1 Program: The Road to a Lead Series

High-throughput screen: Low hit rates, non-selective leads

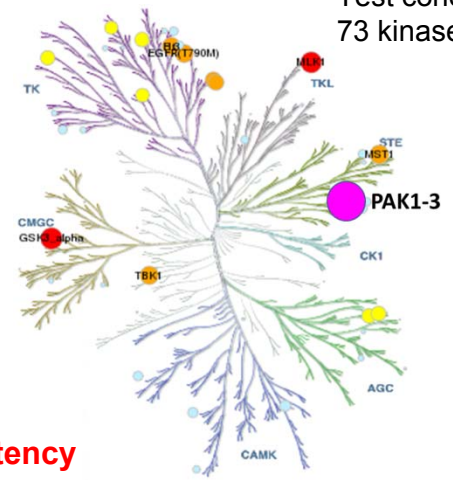
- Ligandability compromised by plasticity of the PAK1 ATP binding site

1st series: Screening hit to early lead



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

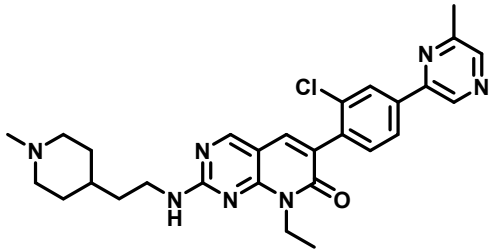
G0236
PAK1 K_i 18 nM
PAK4 K_i 950 nM
Excellent kinase selectivity
Poor permeability, no cellular potency



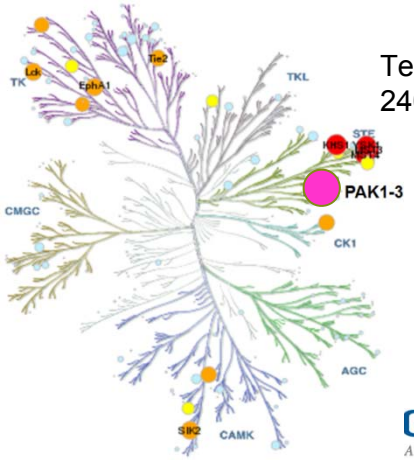
Test conc. = 1μM
73 kinases

ACS Med. Chem. Lett., **2015**, *6*, 711.

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. = 1 μM
240 kinases

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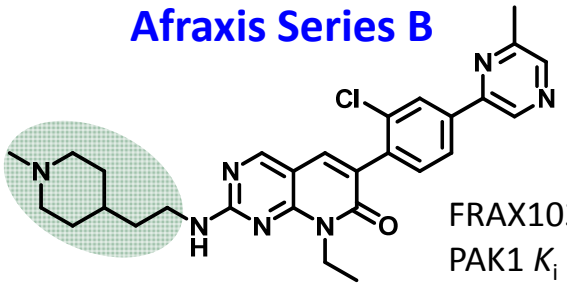
Hybridization of two Series with Goal to Increase Potency

Afraxis Series B

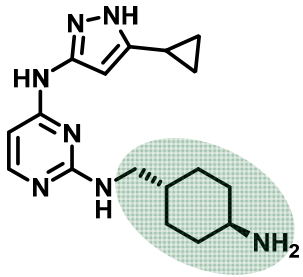


FRAX1036
PAK1 K_i 22 nM
LLE 2.8

Amino tails:

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

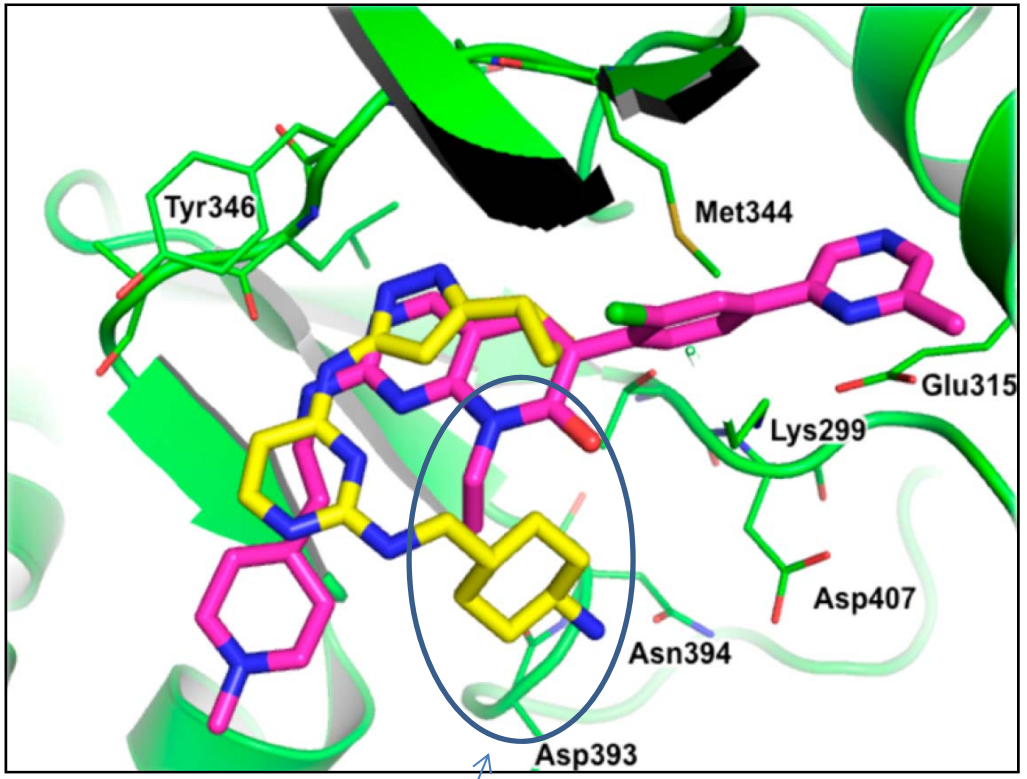
Genentech Aminopyrazole Series



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?

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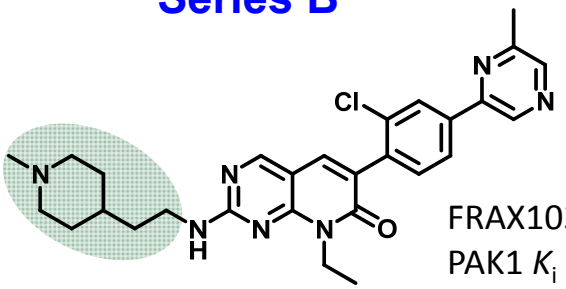
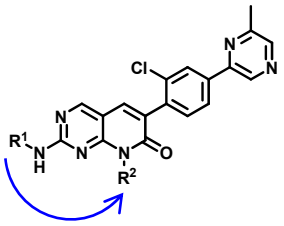
Vector Change of Amino Tail: 10x Potency Gain

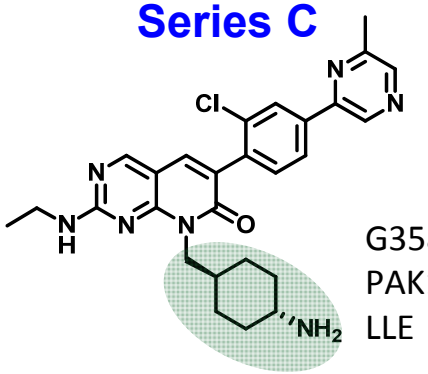
Series B



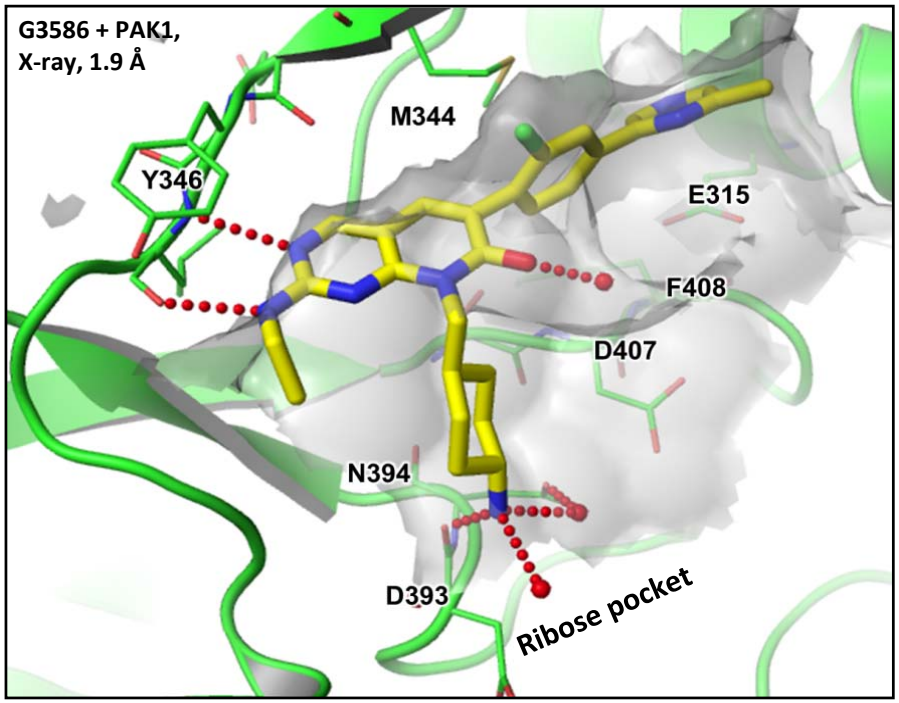
FRAX1036
PAK1 K_i 22 nM
LLE 2.8



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

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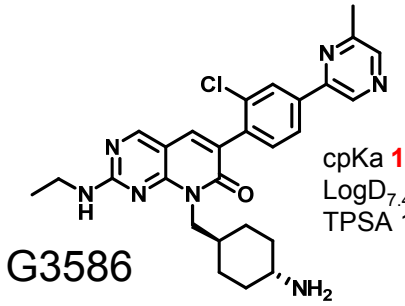
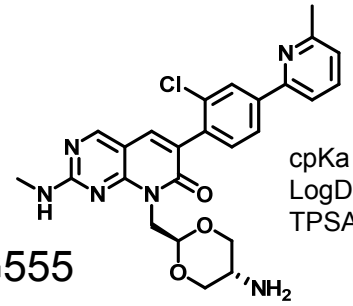
Discovery of G555: Reducing pKa Affords Improved Permeability and High Oral Bioavailability



G3586

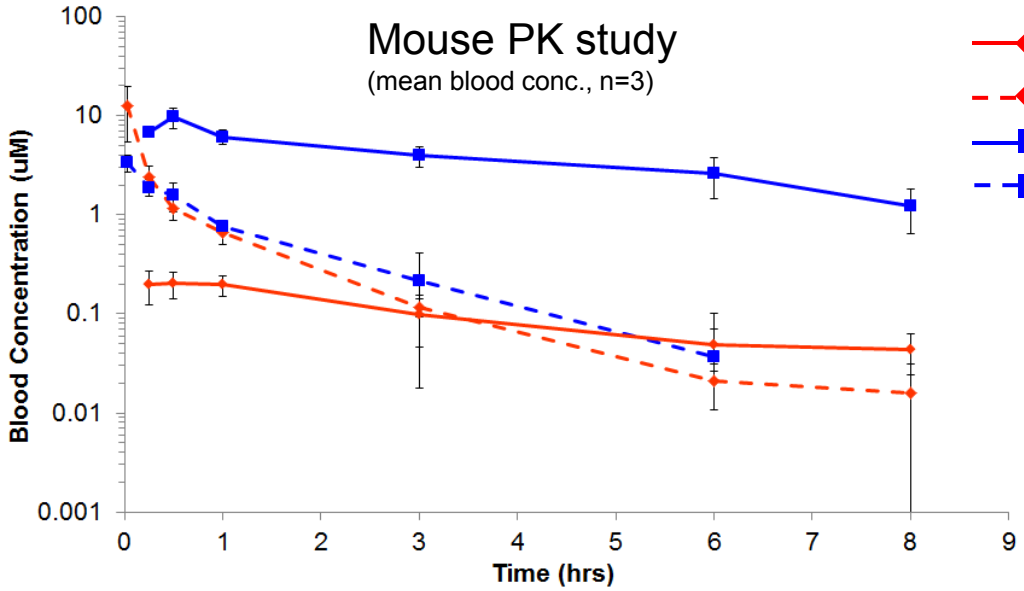
cpKa **10.4**
$LogD_{7.4}$ 1.0
TPSA 111



G555

cpKa **7.7**
$LogD_{7.4}$ 2.5
TPSA 117

PAK1 K_i [nM]	2.1	4.4
p-MEK(S298) IC_{50} [nM]	124	71
Microsomal stability (HRMDC)	**7** / **37** / **76** / **26** / **20**	**12** / **32** / **50** / **22** / **24**
MDCK A:B perm. [10^{-6} cm/s] / Papp ratio	**0.44 / 38**	**2.40 / 1.87**
Aqueous solubility pH7.4 [µM]	34	15



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

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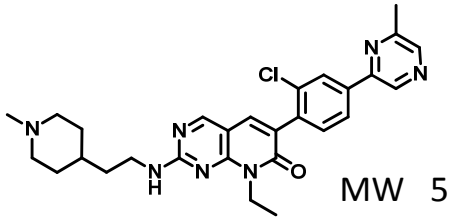
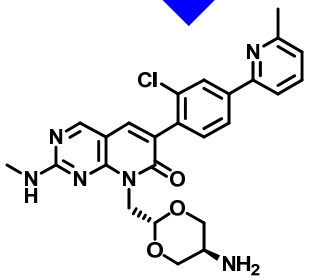
G555: Improved Selectivity Profile



MW 518
cLogP 4.9
cpKa 9.6

FRAX1036

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

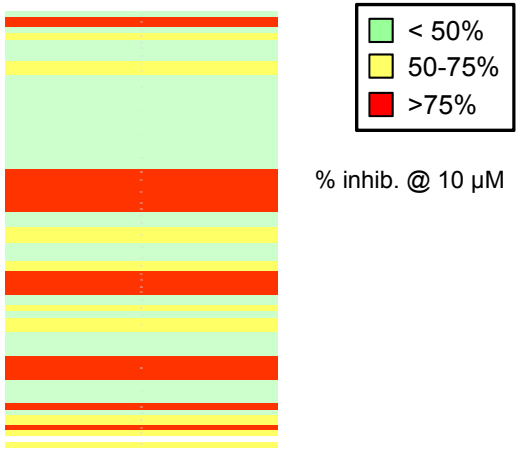


MW 493
cLogP 2.9
cpKa 7.7

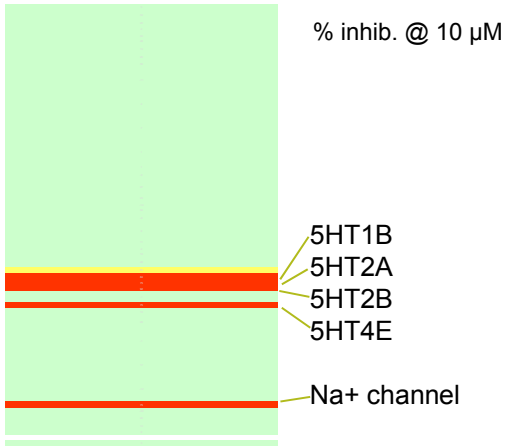
G555

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

2° pharmacology profile (n=41; CEREP)

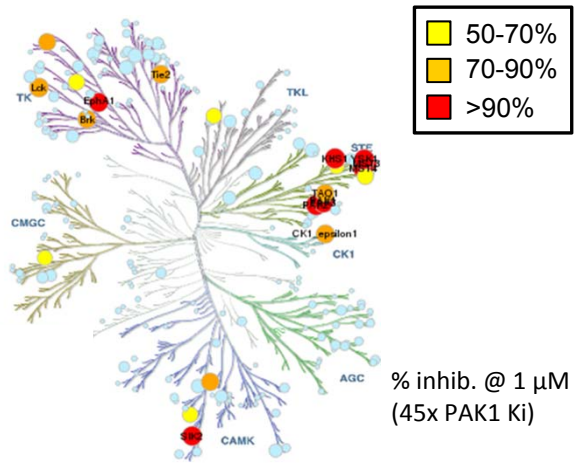


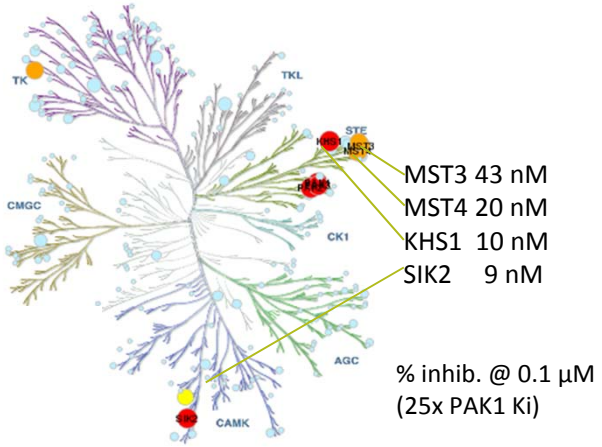
hERG IC_{50} (patch clamp) = 0.48 µM



hERG IC_{50} (patch clamp) > 10 µM

Kinase panel profile (n=230)





ACS Med Chem Lett **2015**, *6*, 1241-1246

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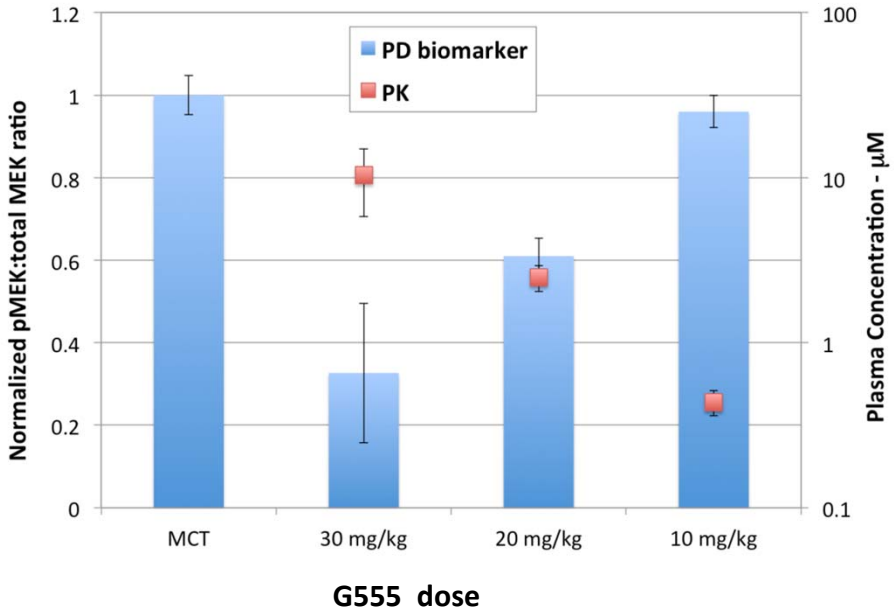
PD and Efficacy Studies with G555: Moderate Knockdown and Dose-Limited Tumor Growth Inhibition

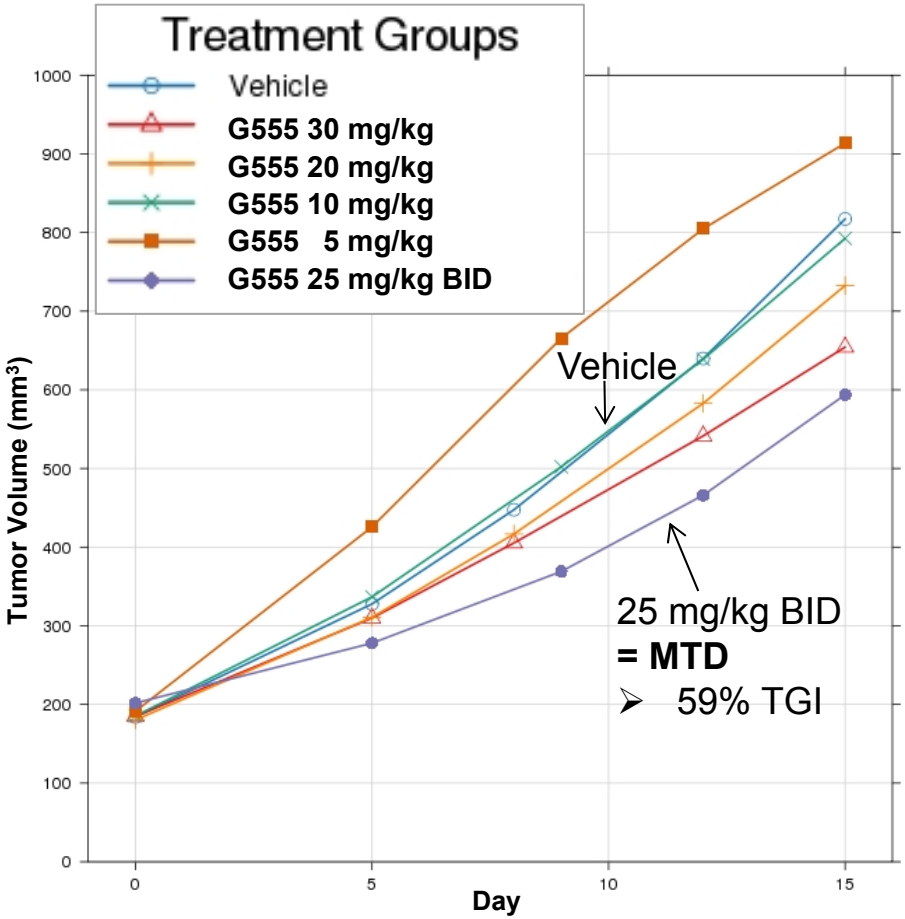
Mouse PK/PD (H292 NSCLC)

- 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 is max tolerated dose

➢ **Acute lethality at higher doses**

Beth Blackwood, Diana Jakubiak

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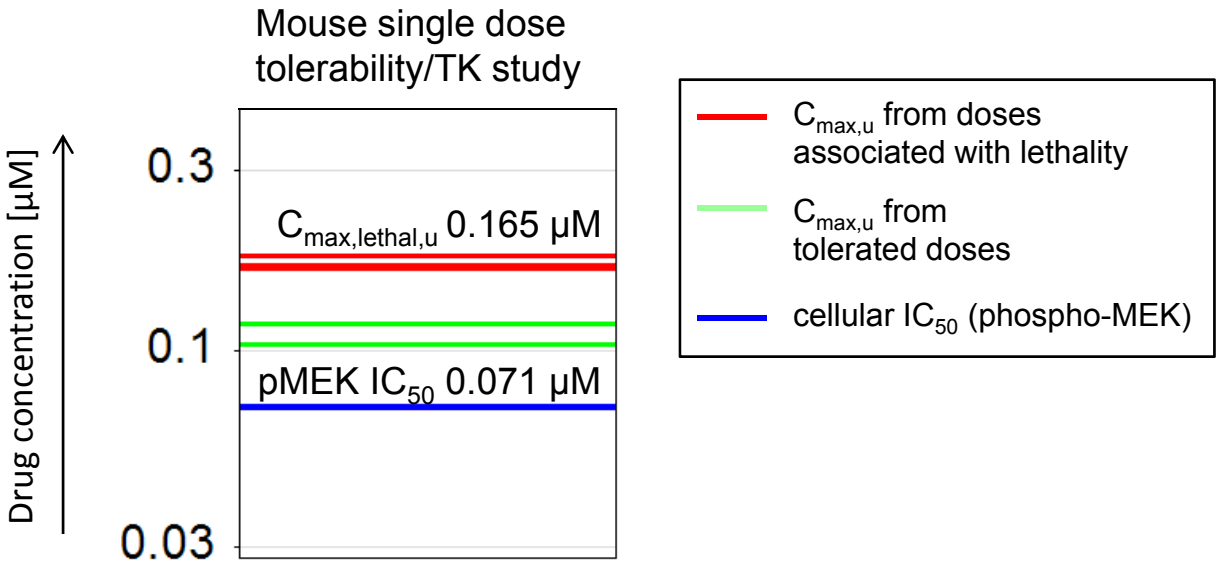
G555 In Vivo Studies: Narrow Therapeutic Window

- 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_{max}-driven: $C_{max,\ unbound}$ ~150 nM not tolerated (multiple studies)



Observations suggest cardiovascular toxicity → mechanistic safety studies

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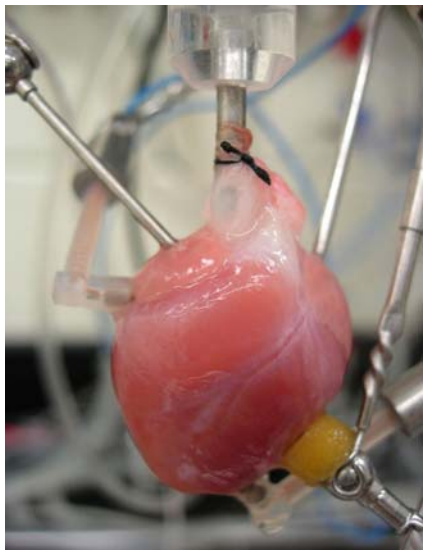
Mechanistic Safety Studies: G555 Causes Acute Cardiovascular Toxicity

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**

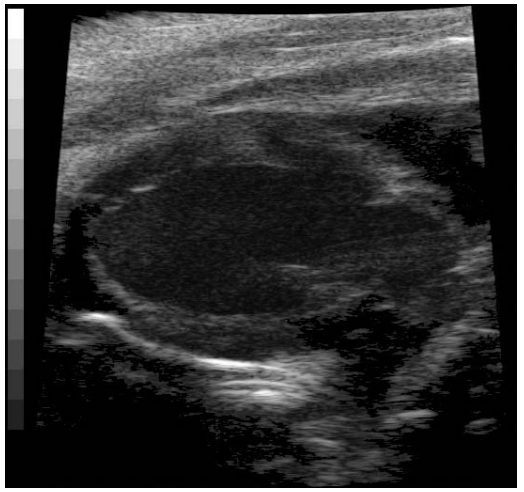


Mouse echocardiography

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

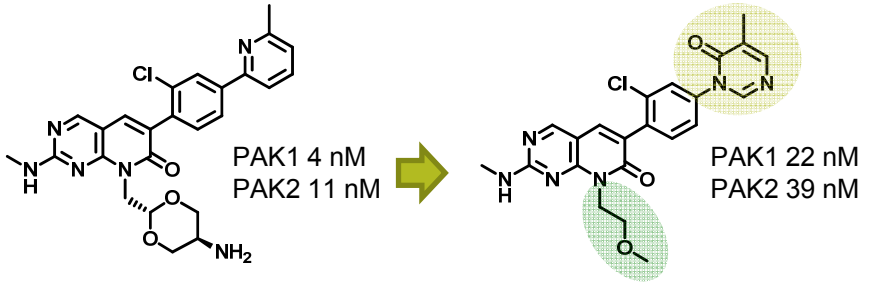
⇒ **Severely decreased cardiac output and performance**

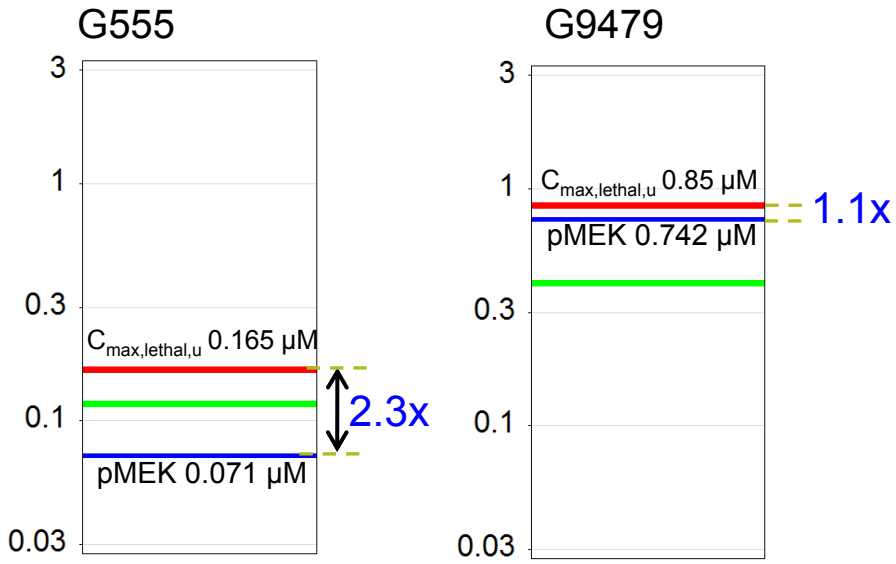


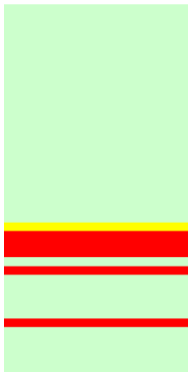
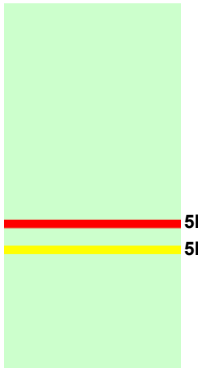
LV = left ventricle
AV node = atrioventricular node

Zoe Zhong, Jed Ross, Diana Jakubiak, Nicole Valle

Structural Diversification to Explore Question of On/ Off-Target Toxicity





	G555	G9479
$LogD_{7.4}$	2.5	1.7
cpKa	7.7	4.7
CEREP % inhib. @ 10 µM (42 targets) < 50% 50-75% >75%		
Invitrogen Kinase panel	2/96 > 75% @ 1 µM	6/237 > 75% @ 1 µM

- 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?**

$C_{max,lethal,u}$: lowest $C_{max,u}$ associated with lethality
$C_{max,safe,u}$: highest $C_{max,u}$ from tolerated dose
Cellular IC_{50} (phospho-MEK)
Window between pMEK and lowest $C_{max,lethal,u}$

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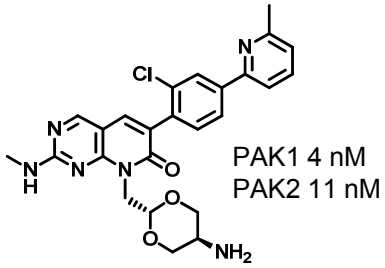
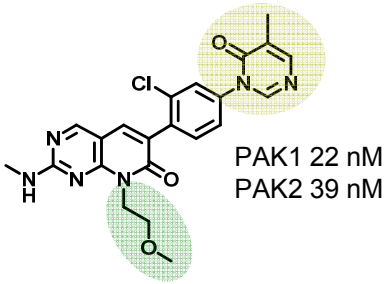
Three Diverse Chemotypes all Cause Acute Lethality in Mouse Tolerability Studies

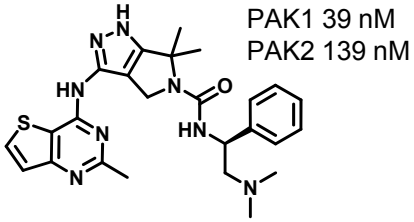
Series C
(lipophilic head, basic tail)



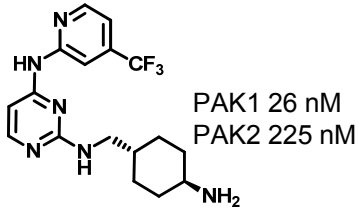
Series C
(polar head, neutral tail)



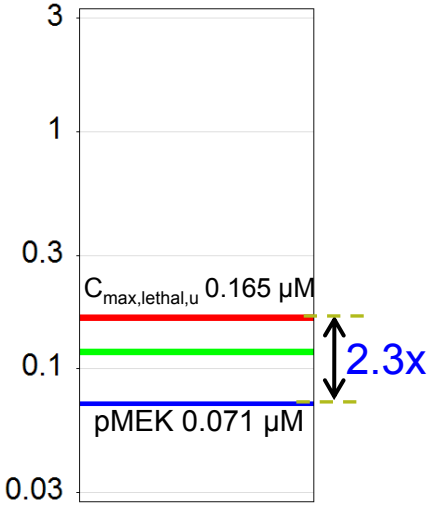
Pfizer series



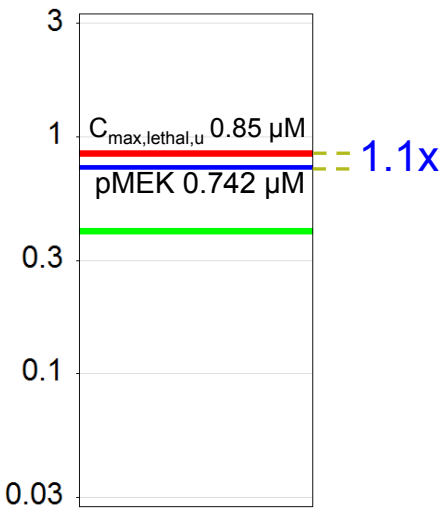
Aminopyridine



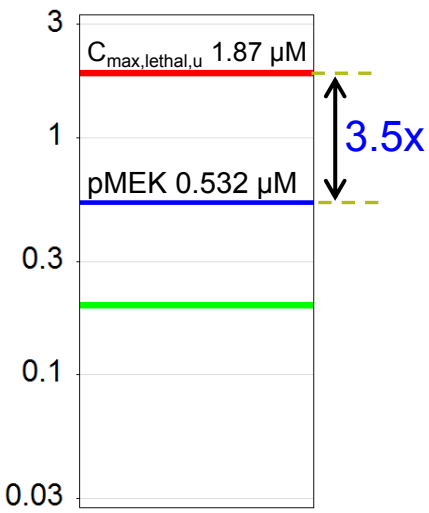
G555



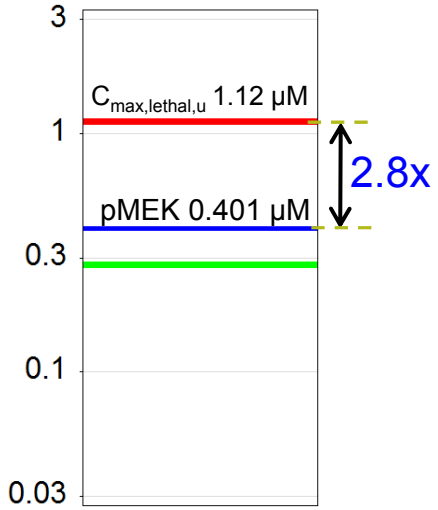
G9479



PD325901



G3468



- 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

$C_{max,lethal,u}$: lowest $C_{max,u}$ associated with lethality
$C_{max,safe,u}$: highest $C_{max,u}$ from tolerated dose
pMEK IC_{50}
Window between pMEK and lowest $C_{max,lethal,u}$

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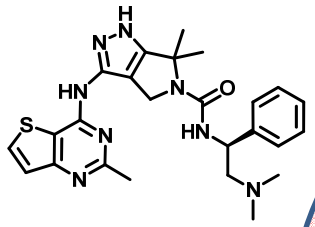
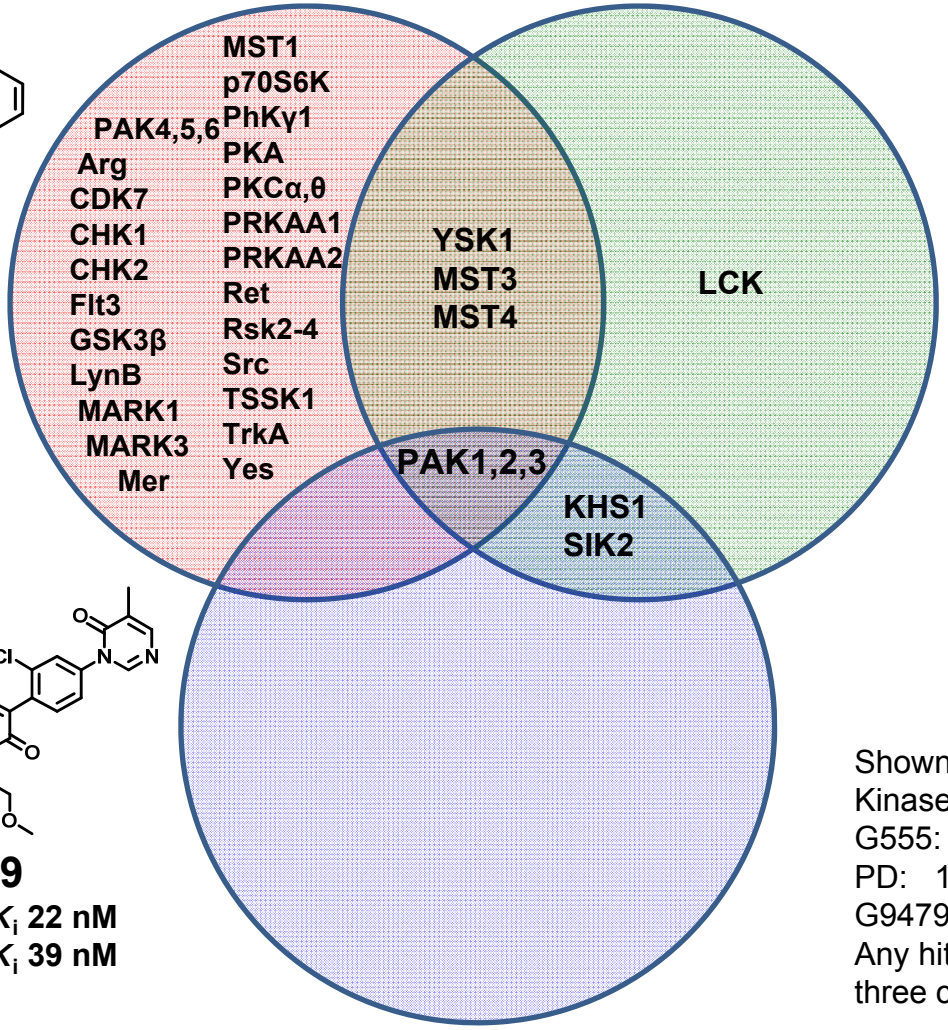
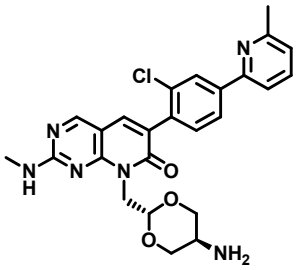
PAK1, 2, 3 are the Only Overlapping Kinase Targets



PD325901
PAK1 K_i 39 nM
PAK2 K_i 139 nM





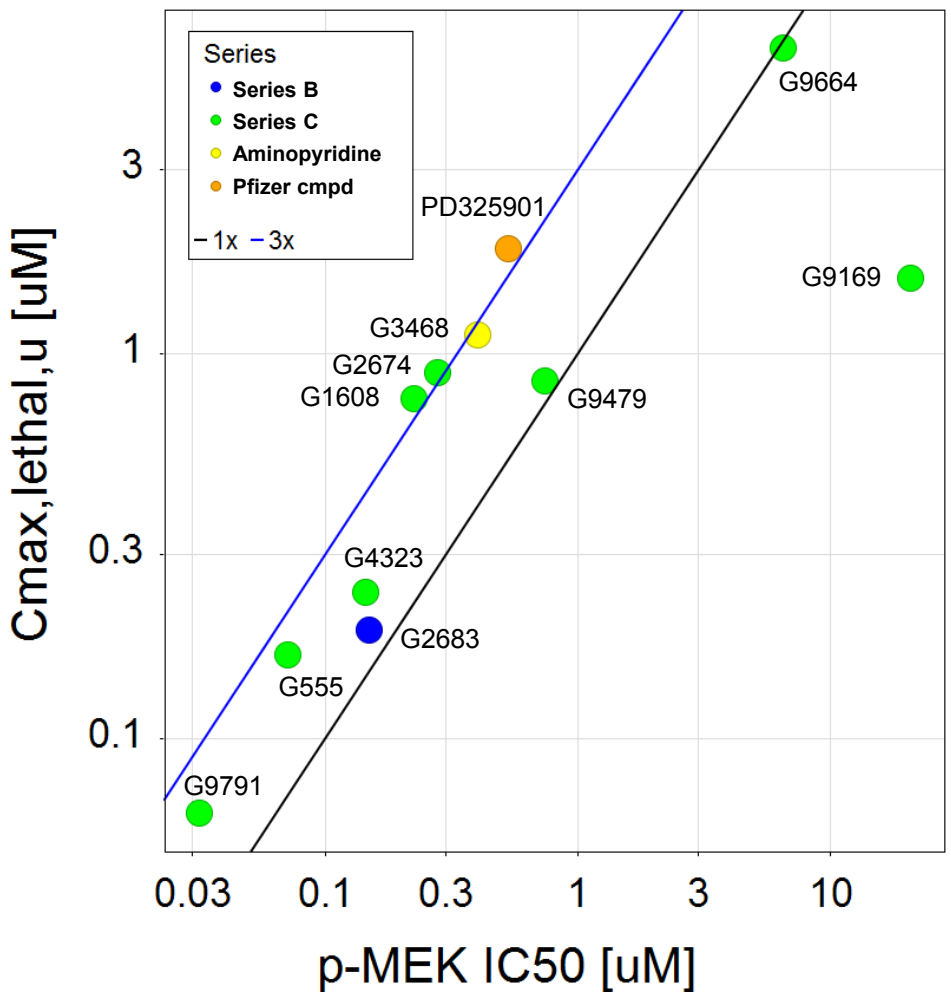
G555
PAK1 K_i 4 nM
PAK2 K_i 11 nM

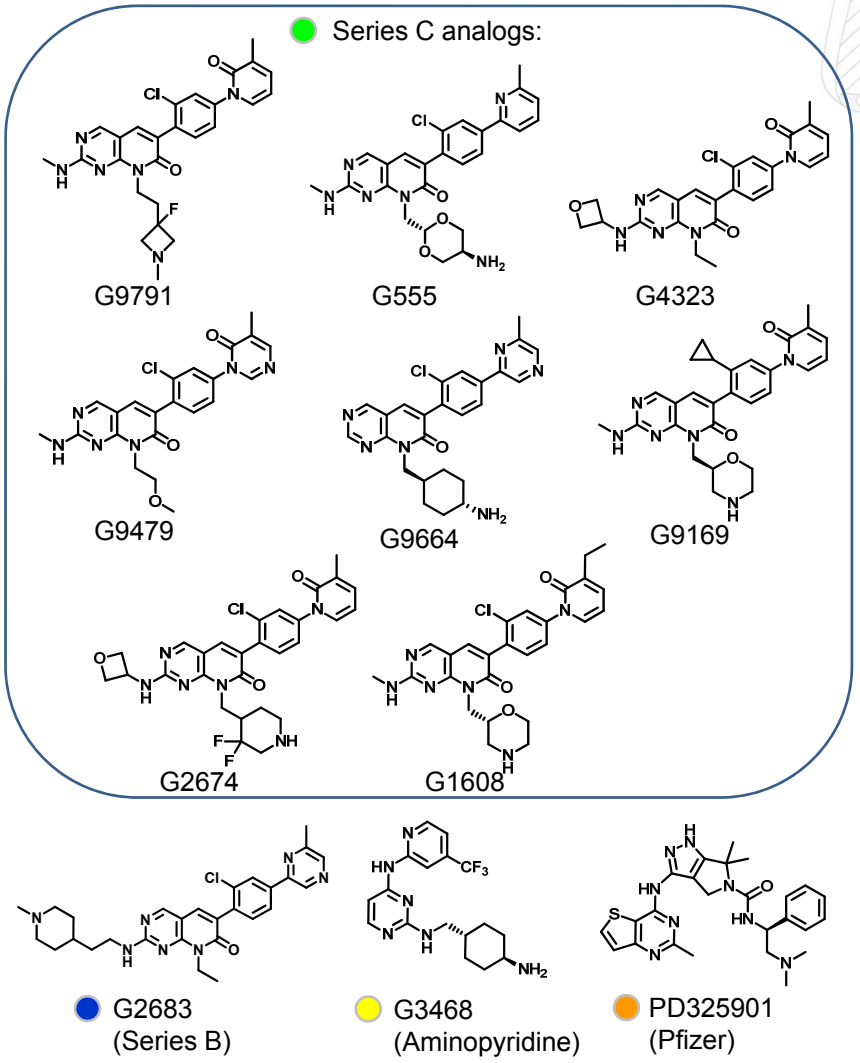
G9479
PAK1 K_i 22 nM
PAK2 K_i 39 nM

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}]

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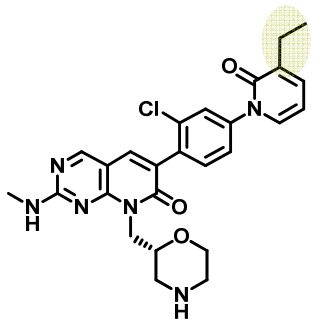
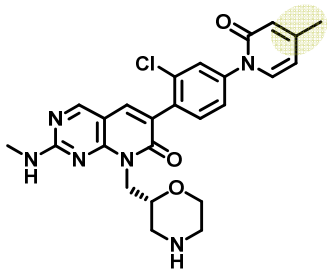
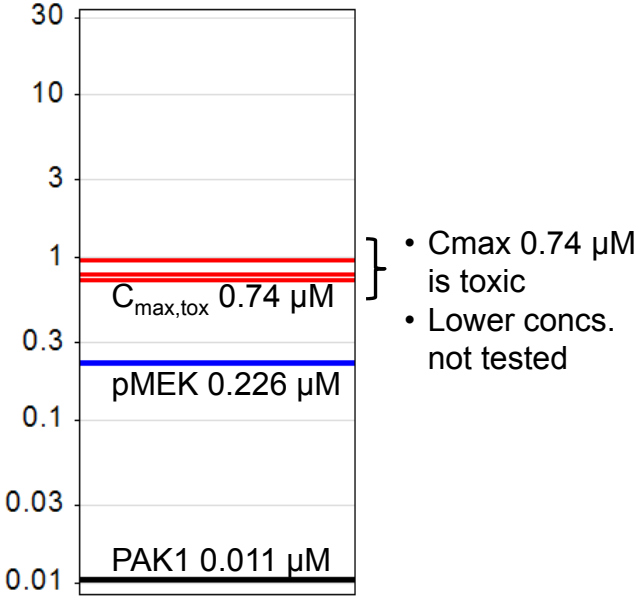
Structurally Closely Related PAK1 Active/Inactive Pair: *Only Active Pair Member Causes Lethality in Mice*



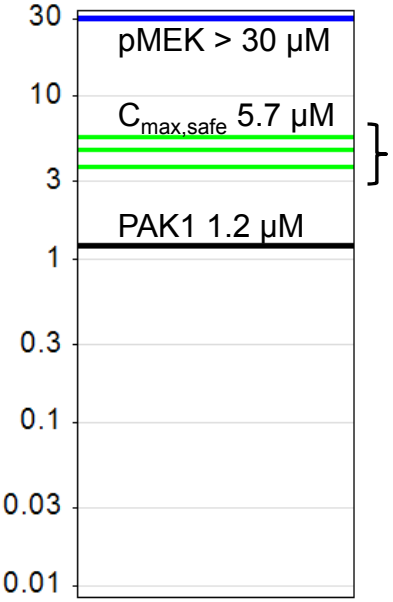
G1608
PAK1 0.011 μM
pMEK 0.226 μM



G7702
PAK1 1.2 μM
pMEK >30 μM



- Cmax 0.74 μM is toxic
- Lower concs. not tested



- Cmax 5.7 μM is safe
- Higher concs. not tested

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Acute CV Tox of PAK1/2 Inhibitors Likely "On-Target"

	Supporting observations	Comments
Toxicity present with chemically-diverse inhibitors	• Three chemotypes: Afraxis, aminopyridine, Pfizer all acutely lethal	No overlapping off-targets (CEREP, Kinases and others)
Toxicity absent with an inactive analog	• Active/inactive pair: only the active analog G1608 was toxic	
+ve Relationship between target potency and toxicity	• Lethality correlated well with pMEK potency with 11 compounds (1-4x pMEK EC_{50})	~300-fold potency range
Target expressed in tissue with toxicity	• PAK1 and 2 are widely expressed, including the cardiovascular system	
Toxicity consistent with phenotype of KO mice?	• PAK1 KO – viable and fertile • **PAK2 KO – embryonic lethal (vasculature development; Chernoff, 2012);** **PAK2 adult cKO – lethal (Chernoff, 2015)**	Neither PAK2 kinase-dead KI nor PAK1/PAK2 double cKO generated to date

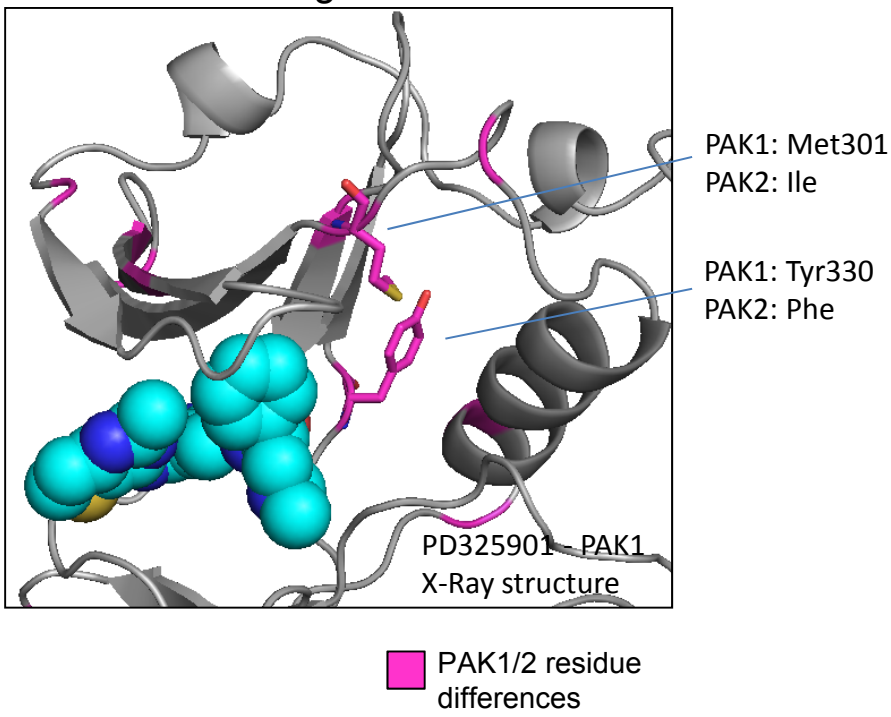
Hypothesis:

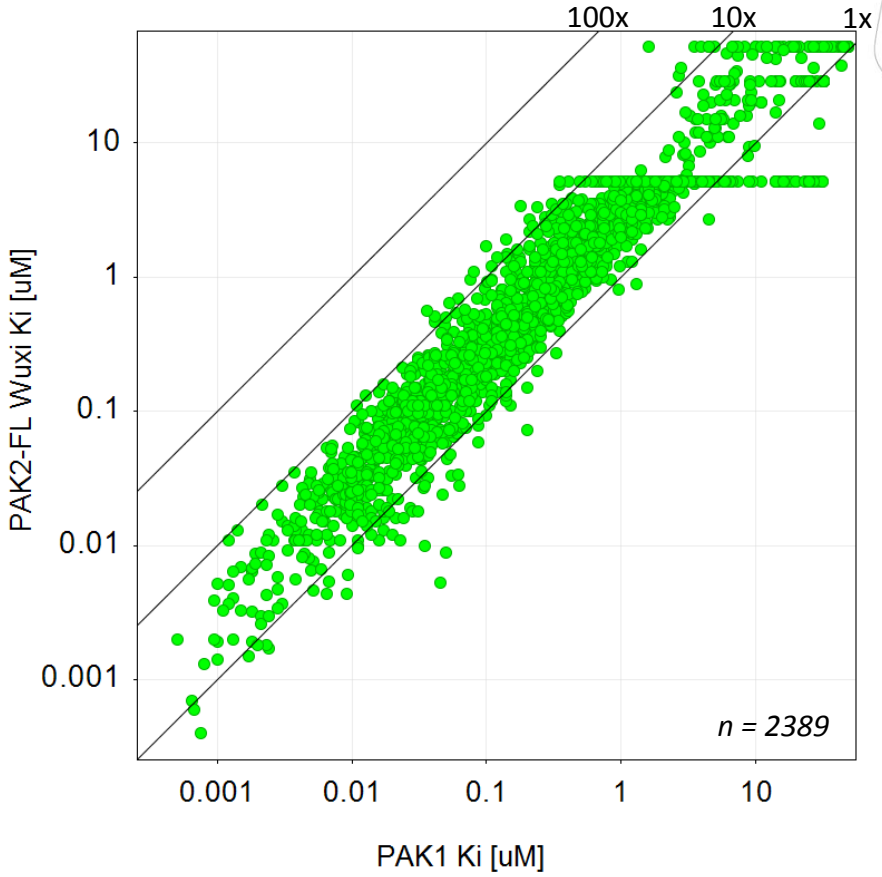
PAK2 is the driver of toxicity, either alone or in combination with PAK1

J Med Chem **2016**, *59*, 5520-5541

Path Forward for a PAK2-Sparing PAK1 Inhibitor?

PAK1 ATP binding site





- PAK1/PAK2 kinase domain homology: 93%
- 2 residue differences in ATP binding site; similar residues and difficult to target

- For ~2400 ATP-competitive inhibitors made, PAK1 vs PAK2 selectivity never obtained

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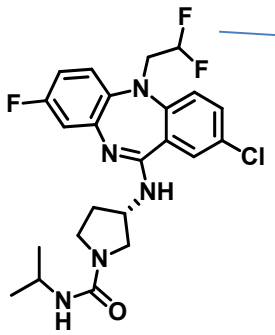
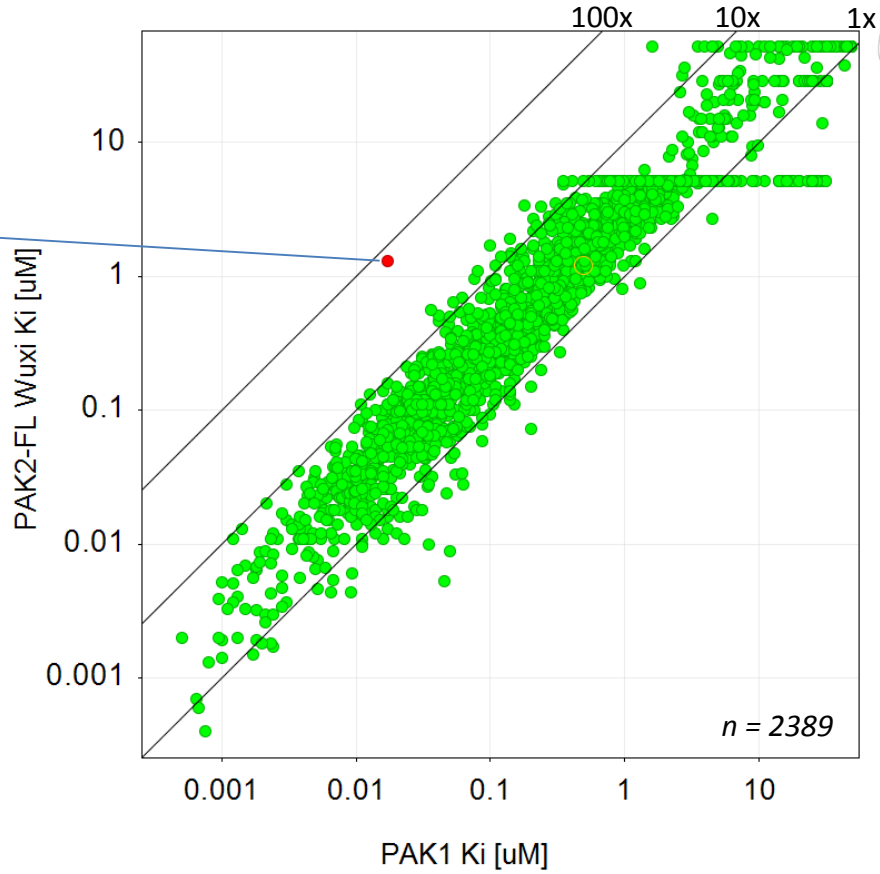
Path Forward for a PAK2-Sparing PAK1 Inhibitor?

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

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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:

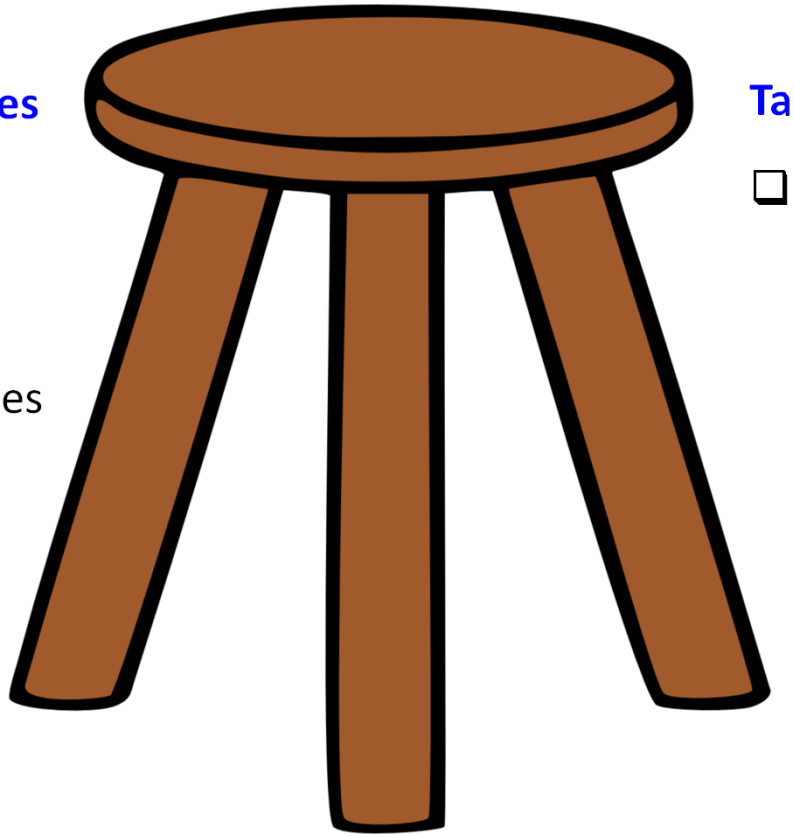
- Certain kinases have been implicated in cardiovascular functions but kinase related CV toxicity of the acuteness and severity observed here is uncommon; watch your preoccupation “Acute cardiac toxicity = ion channel”

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*

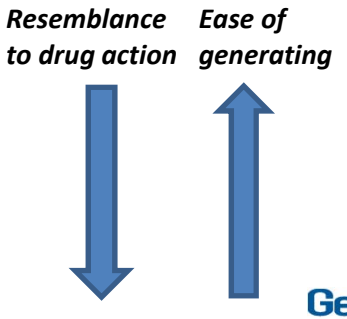


Target expression knowledge

- ❑ 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



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Anneleen Daemen
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Christopher Heise
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Klaus Hoeflich
Tom O'Brien
Beth Blackwood
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Christy Ong
Diana Wadley-Jakubiak
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Amy Young
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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
Angela Oh
Lionel Rouge
Chistine Tam
Jiansheng Wu
Shanghai ChemPartner

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

Diagnostics, Biomarkers
Marcin Kowanetz
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Tim Wilson
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Brian Buckwalter
Alex Andrus
Jori Mandelman

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

Other
David Campbell (Afraxis)
Sergio Duron (Afraxis)
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