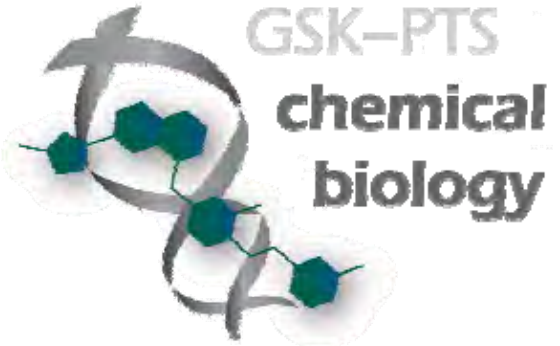


The Published Kinase Inhibitor Set: A resource to develop probes for the untargeted kinome

David Drewry and Bill Zuercher

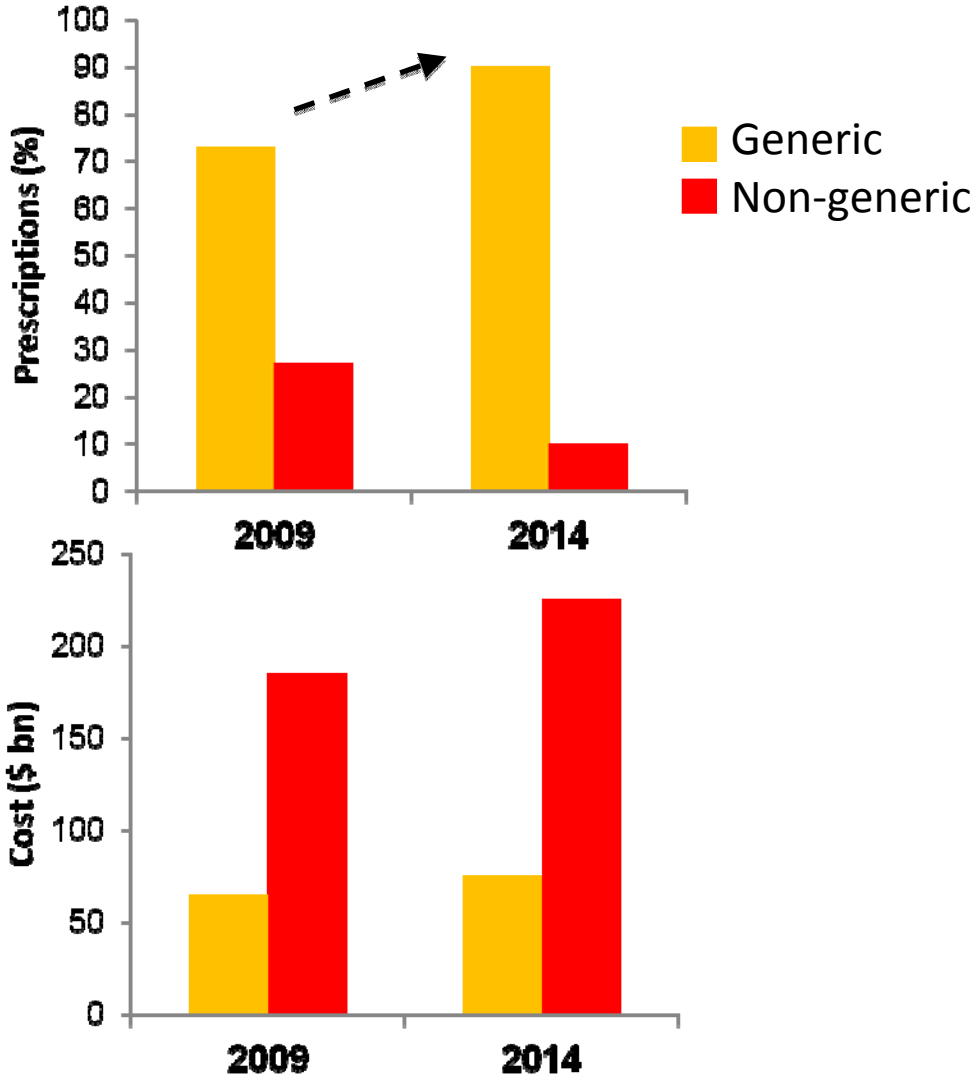
GlaxoSmithKline

2nd RSC Symposium on Chemical Biology for Drug Discovery
March 20, 2012

Why the Pharmaceutical Industry is Changing

Top 25 prescribed drugs in US

	DISPENSED PRESCRIPTIONS MN	2010
	Total US Market	3,995.2
1	hydrocodone/acetaminophen	131.2
2	simvastatin	94.1
3	lisinopril	87.4
4	levothyroxine sodium	70.5
5	amlodipine besylate	57.2
6	omeprazole (RX)	53.4
7	azithromycin	52.6
8	amoxicillin	52.3
9	metformin HCL	48.3
10	hydrochlorothiazide	47.8
11	alprazolam	46.3
12	Lipitor®	45.3
13	furosemide	43.4
14	metoprolol tartrate	38.9
15	zolpidem tartrate	38.0
16	atenolol	36.3
17	sertraline HCL	35.7
18	metoprolol succinate	33.0
19	citalopram HBR	32.1
20	warfarin sodium	32.0
21	oxycodone/acetaminophen	31.9
22	ibuprofen (RX)	31.1
23	Plavix®	29.5
24	gabapentin	29.3
25	Singulair®	28.7

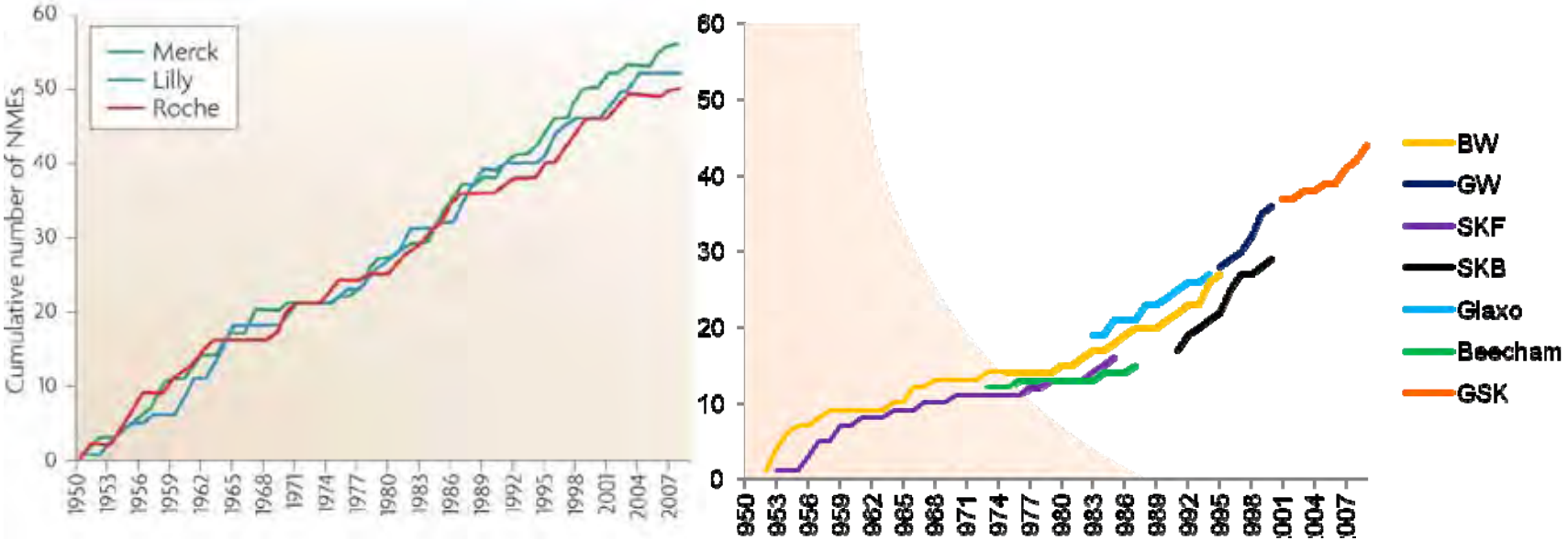


Only 3 non-generics

All 3 lose patent protection in 2011/2

…but the Rate of Drug Discovery is Constant!

- Bernard Munos, Nature Reviews Drug Discovery (2009) 8 959-968



"Nothing that companies have done in the past 60 years has affected their rates of new-drug production"

Still Searching Under the Street Light?

COMMENT

Call for unity in the science of human beings p.168

Reviewed: two primers on personal genomics p.169

Sanitation, not vaccination, is most important in Haiti p.171

Jack Oliver, key player in proof of plate tectonics, remembered p.178

Too many roads not taken

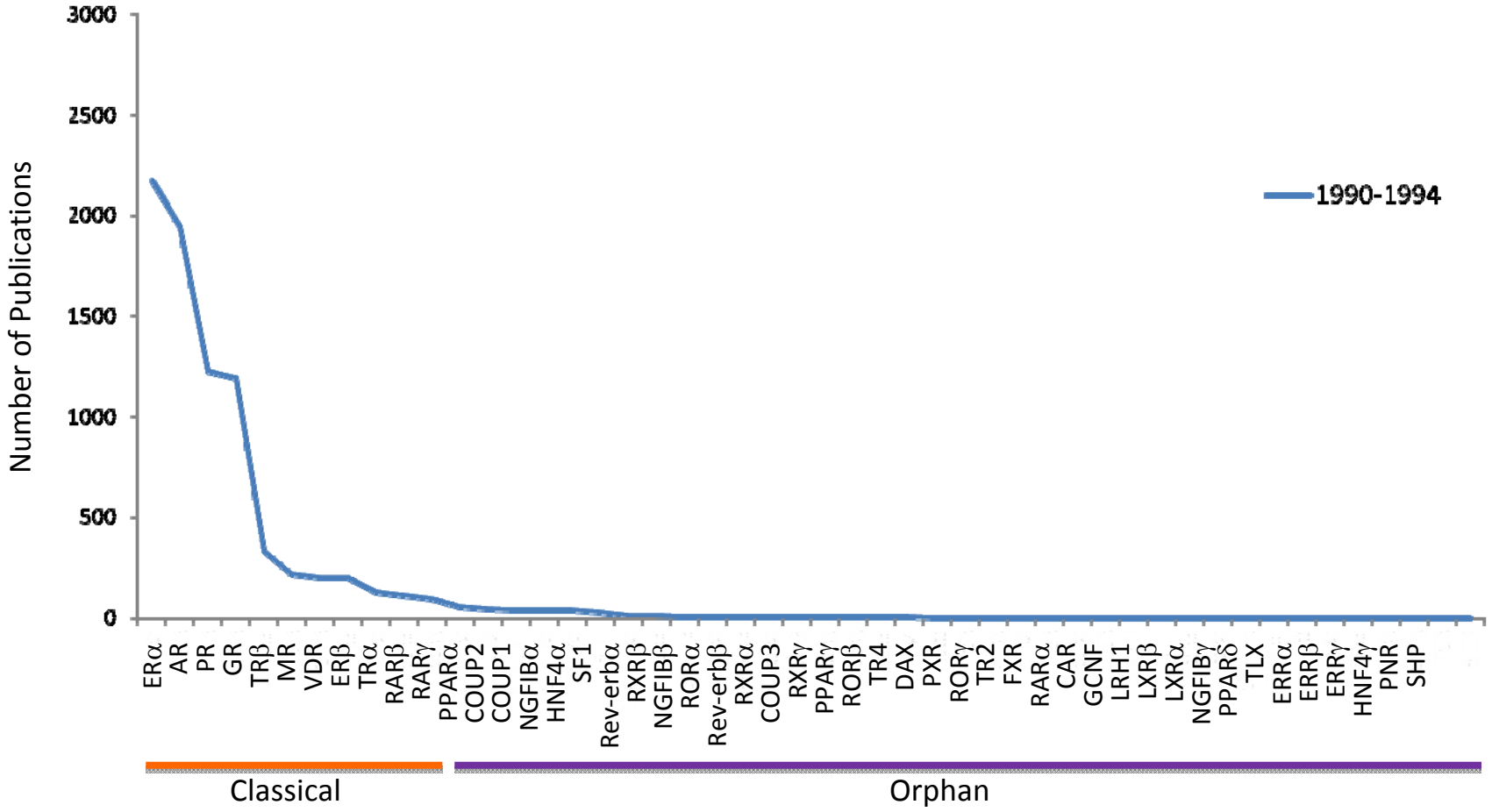
Most protein research focuses on those known before the human genome was mapped. Work on the slew discovered since, urge Aled M. Edwards and his colleagues.

10 FEBRUARY 2011 | VOL 470 | NATURE | 163

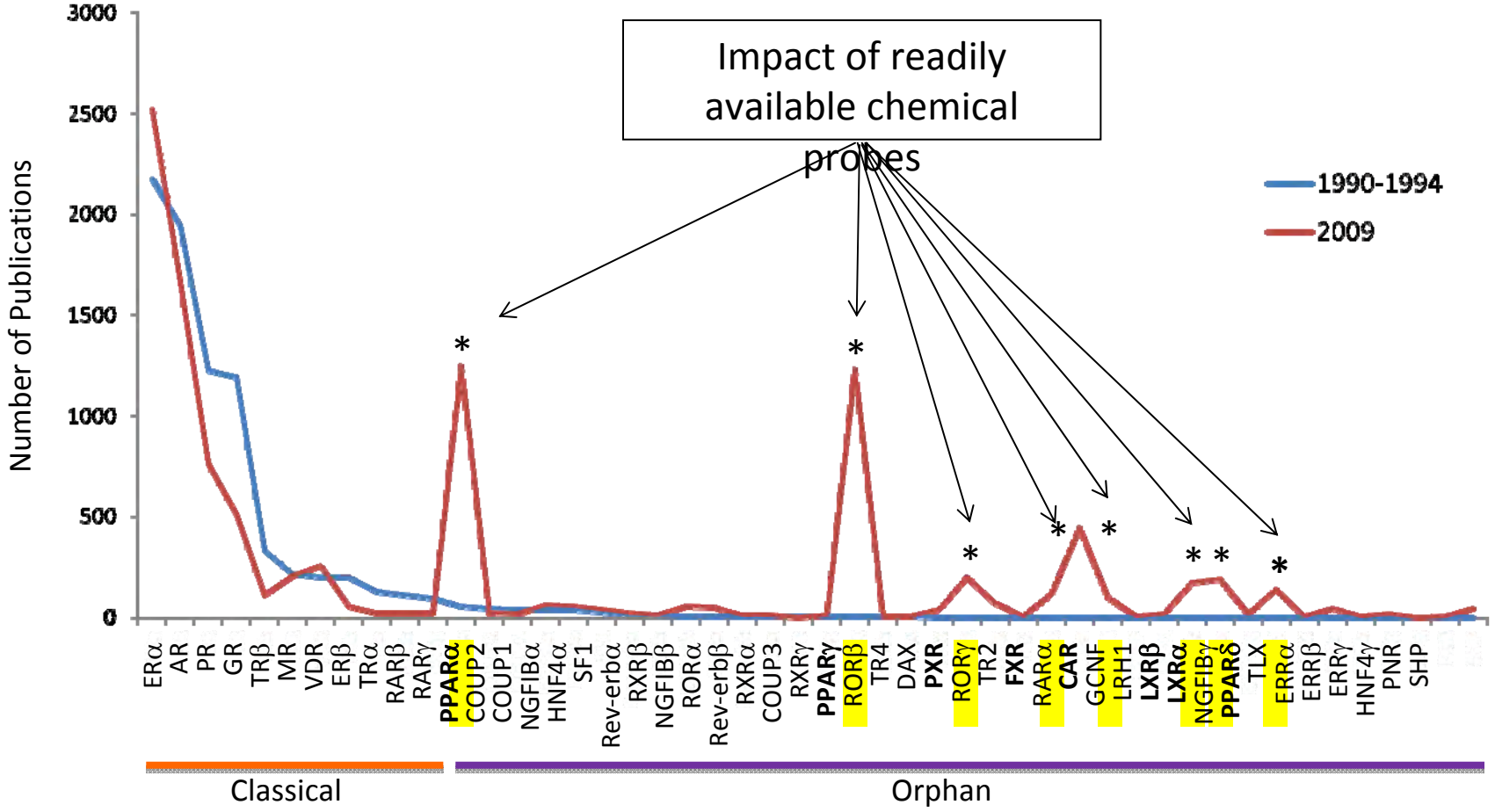
- <10% of the genome has been the focus of pharmaceutical drug discovery
- We work on the same limited set of proteins in industry AND academia

Al Edwards, U. Toronto and The SGC

NR Publications (1990-1994)



NR Publications (1990-1994 and 2009)



Nature (2011) 470 163-165

COMMENTARY

Open access chemical and clinical probes to support drug discovery

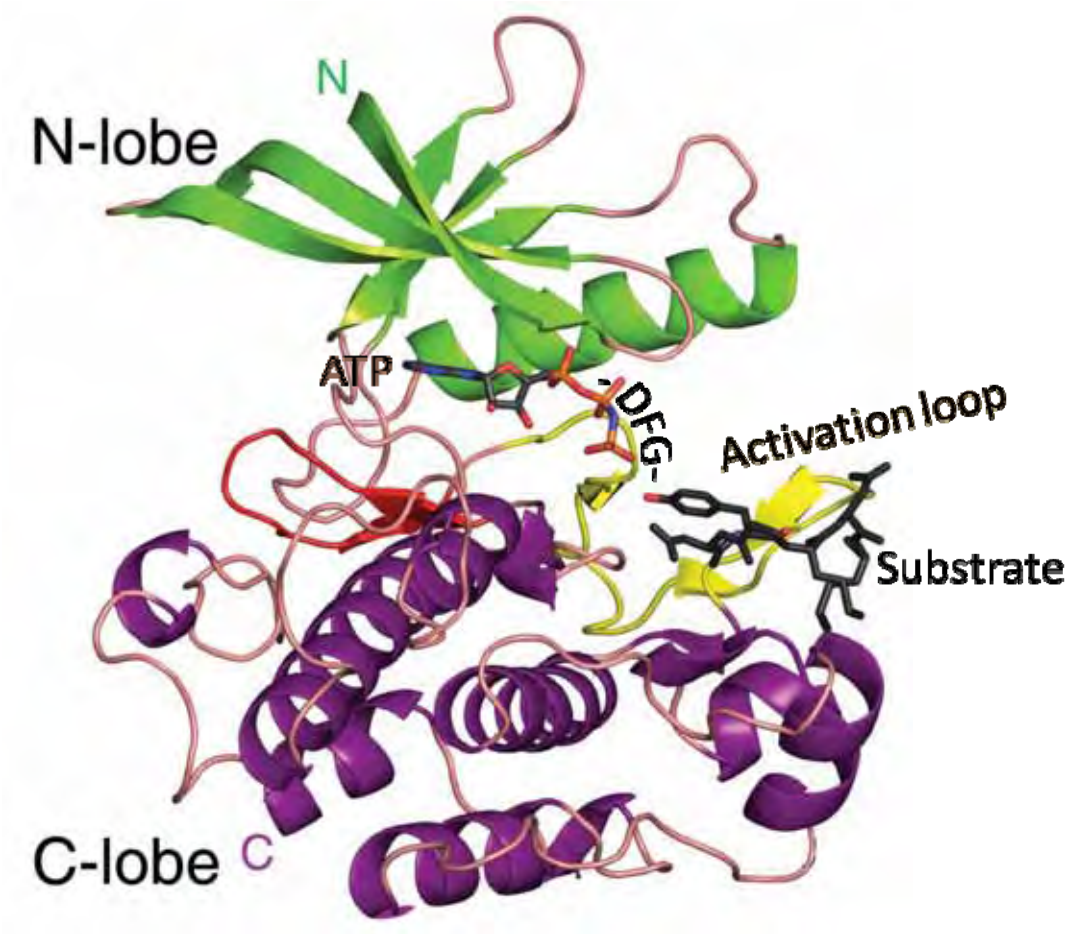
Aled M Edwards, Chas Bountra, David J Kerr & Timothy M Willson

Drug discovery resources in academia and industry are not used efficiently, to the detriment of industry and society. Duplication could be reduced, and productivity could be increased, by performing basic biology and clinical proofs of concept within open access industry-academia partnerships. Chemical biologists could play a central role in this effort.

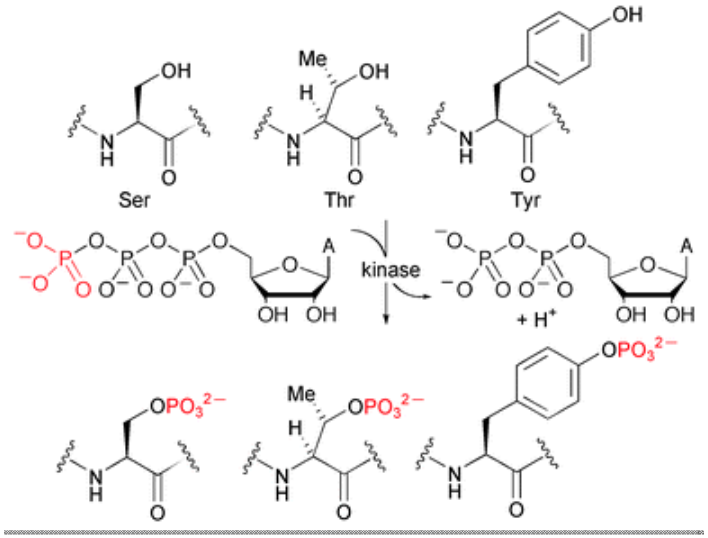
436 VOLUME 5 NUMBER 7 JULY 2009 NATURE CHEMICAL BIOLOGY

- Chemical probes freely available to the scientific community
- Combine the innovation of academia with infrastructure of industry
- Identification of new molecular targets for drug discovery
- Precompetitive publicly-funded endeavor for the benefit of society

Protein Kinases



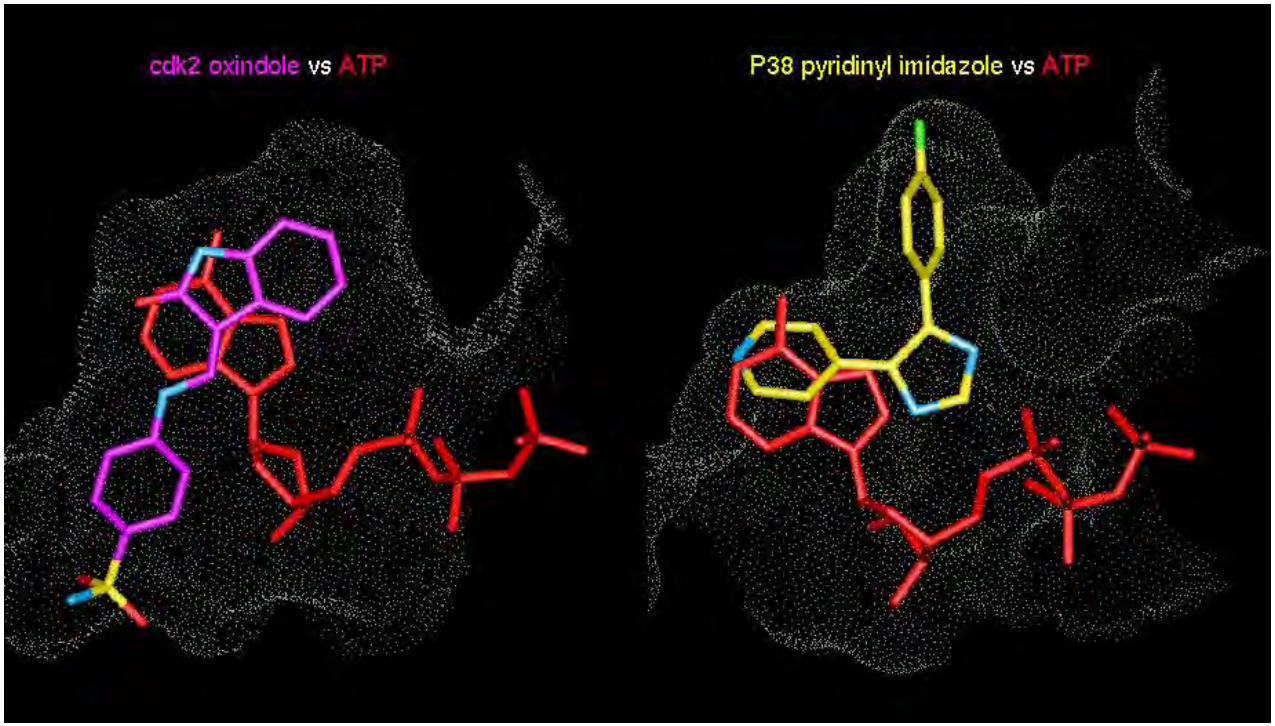
cAMP-dependent protein kinase (PKA)



- 518 kinases in the human genome
- Key regulators of cellular physiology and pathology
- Successful targets for drug discovery using ATP competitive inhibitors

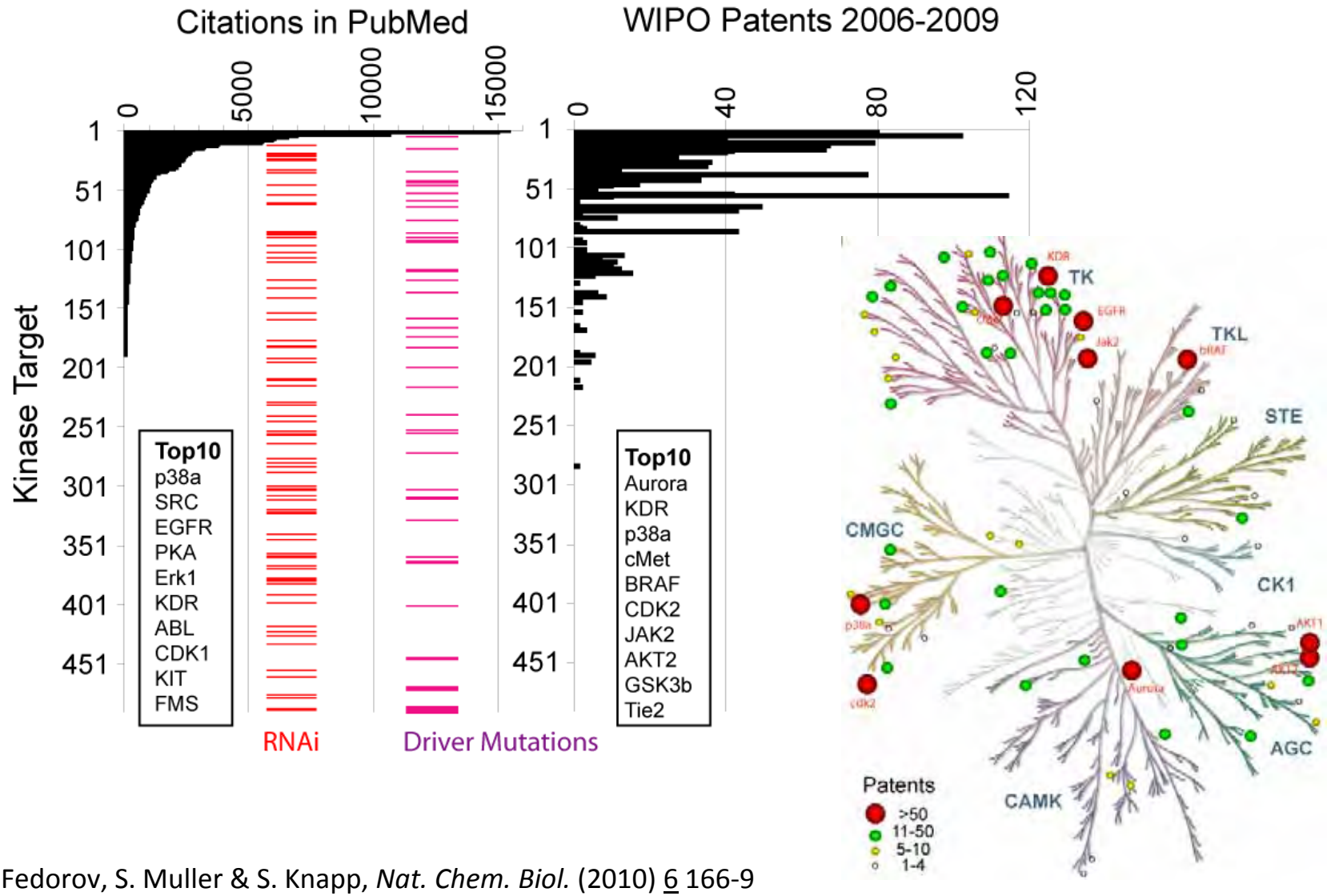
Chemically Connected System: Why it works

- ATP site - conserved but **not** optimized for ATP
- large database of structures allows for:
  - greater understanding of key pharmacophores and SAR
  - improved homology models
  - novel template design



- Exploit unique features of ATP site to achieve potency and selectivity

The (Orphan) Human Kinome

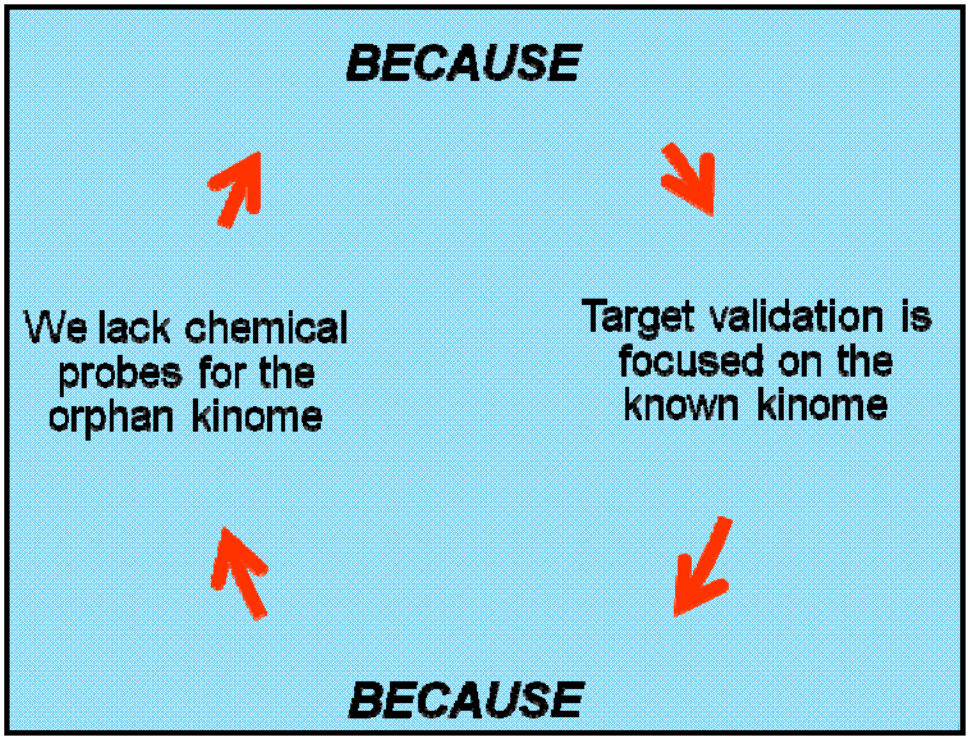


O. Fedorov, S. Muller & S. Knapp, *Nat. Chem. Biol.* (2010) 6 166-9

The orphan kinome: why do we keep focus on the “usual suspects”?

Reasons for this vicious circle

- Kinome size leads to “looking in the light”
- Conservative funding mechanisms and decision making
- Historical lack of methods for broad kinome activity assessment
- Lack of high quality, well-characterized chemical probes



How do we prosecute the orphan kinome? A proposal

- *Situation*: The therapeutic potential of the orphan kinome remains unrealized
- *Task*: Seed kinase research by establishing a loose collaborative network of researchers
- *Proposal*: Define and release an open access set of kinase inhibitors
  - Engage a diverse range of experts
  - ID probes or, more likely, chemical starting points for probe development
  - ID interesting phenotypic profiles and kinases for therapeutic targeting

But wait… we don’t do that!

- Why would we give away compounds!??!?
- Mitigate risk: include only published compounds
- Mitigate cost: include only materially available compounds
- Stipulation of material transfer: all data deposited into public domain
- Move from individual engines of innovation to an ***innovative network of experts***
- Open the door for future collaboration: further dispensing of compounds under the MTA is facilitated

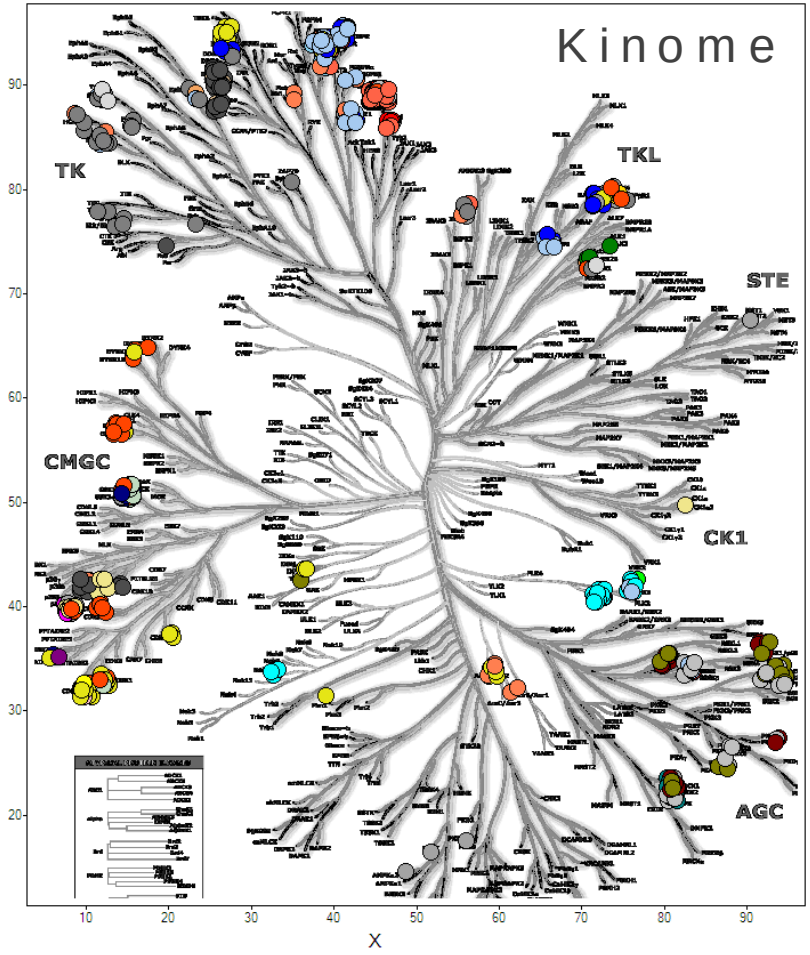
Defining the Set of Kinase Inhibitors

- GSK has long track record with kinases
  - 2 marketed drugs
  - Numerous clinical compounds
  - >100 publications describing 1000s of compounds
- Compound selection
  - Must be published and materially available in house
  - Removed clinical compounds
  - Reduced over-representation of kinases and chemotypes
  - Maximized potential for broad kinome coverage
- End result
  - 367 compounds
  - Not a perfect set but a useful starting point

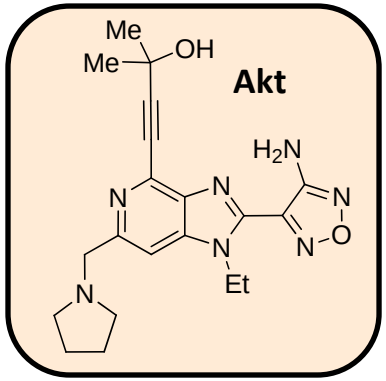
GSK Published Kinase Inhibitor Set (PKIS)

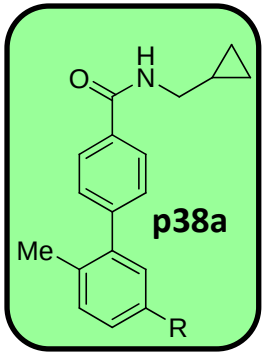
- Set design
  - 367 inhibitors published by GSK
  - >20 chemotypes
  - Limited annotation across <50 kinases
- Availability
  - Available to any academic investigator with structures and selectivity data
  - Investigators required to deposit data in the public domain (www.sarfari.org/kinasesarfari is the suggested site)
- Contacts
  - david.h.drewry@gsk.com
  - william.j.zuercher@gsk.com

GSK annotation colored by chemotype

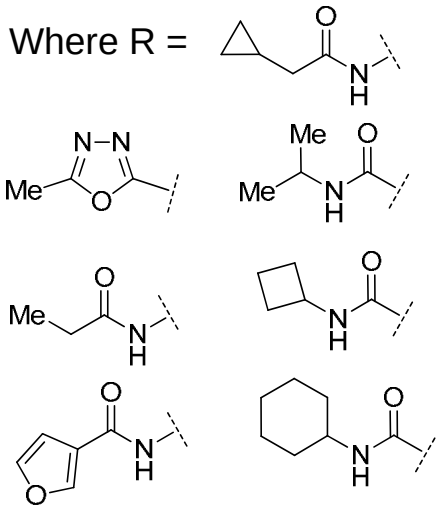


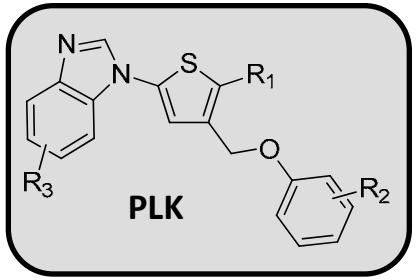
Exemplars from set





Where R =





- 10 PLK inhibitors
- variation at 3 sites
- PLK activity from 10 nM to > 1 μM

Kinase

Akt1: 6 nM

Akt2: 200 nM

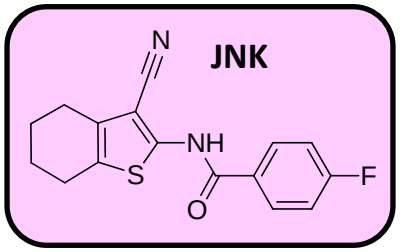
Akt3: 22 nM

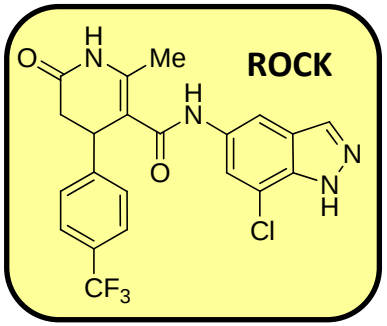
Cellular proliferation

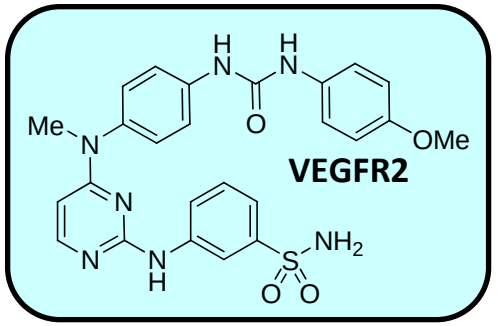
LNCaP: 0.3 μM

HLF: > 30 μM

- p38α IC_{50} values range from 100 nM to 10 μM
- Cellular activity and pharmacokinetic properties described



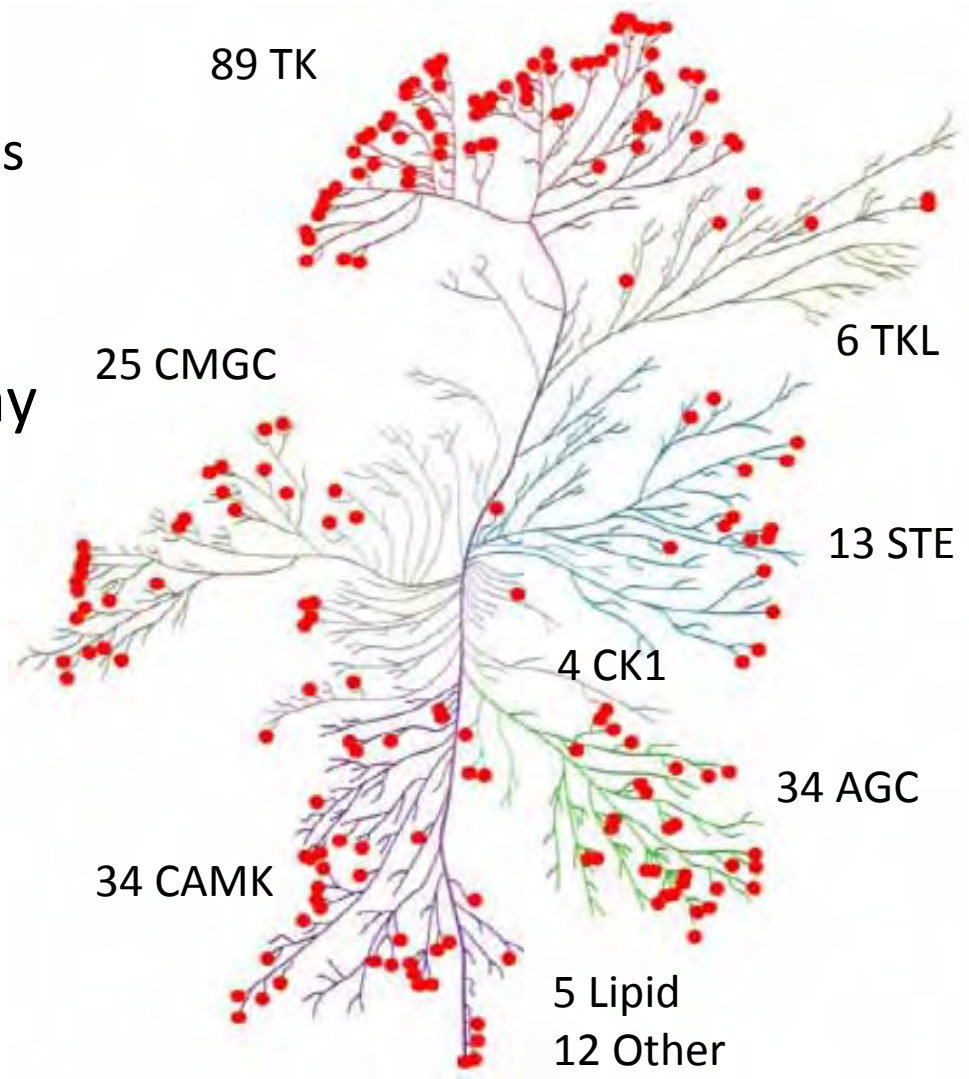




Akt: *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1508.
p38α: *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4428.
PLK: *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1018.
JNK: *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1296.
ROCK: *J. Med. Chem.* **2007**, *50*, 6.
VEGFR2: *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3519.

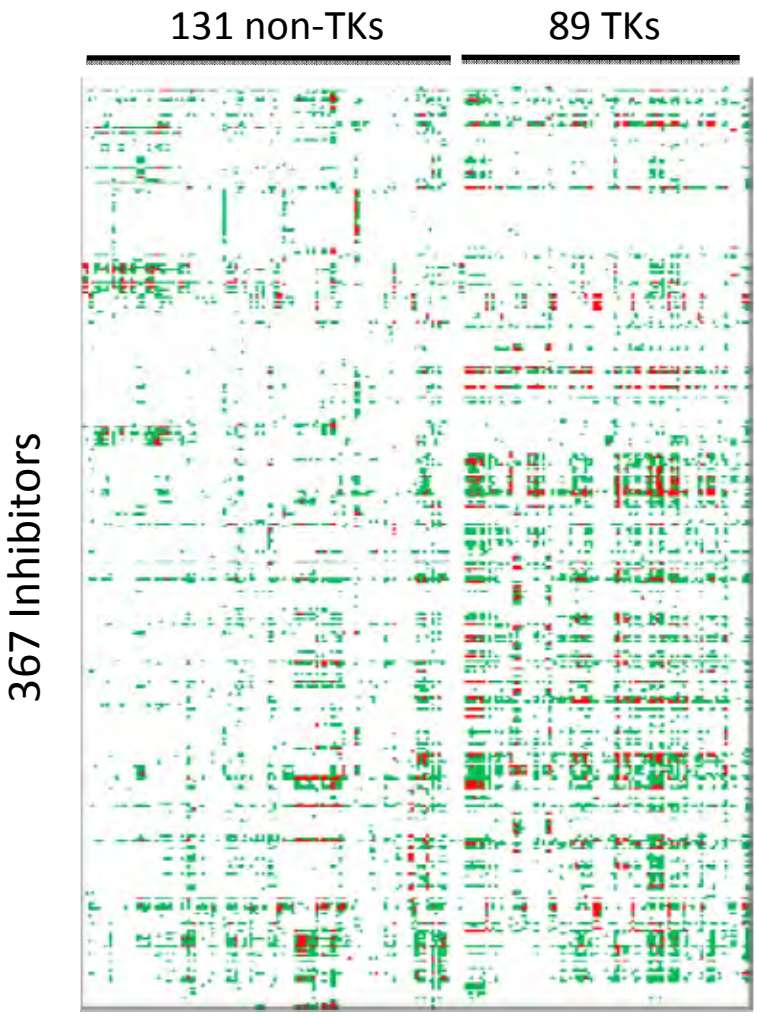
How Broad is the Kinome Coverage?

- PKIS vs. 220 kinases
  - ID of starting points for probes
  - a map to guide phenotypic results
- NANOSYN Microfluidics Assay
  - Activity-based assay
  - Ratiometric detection of product and substrate = increased precision
  - Performed at K_m of ATP for each kinase
  - Dual assay at 1.0 and 0.1 μM



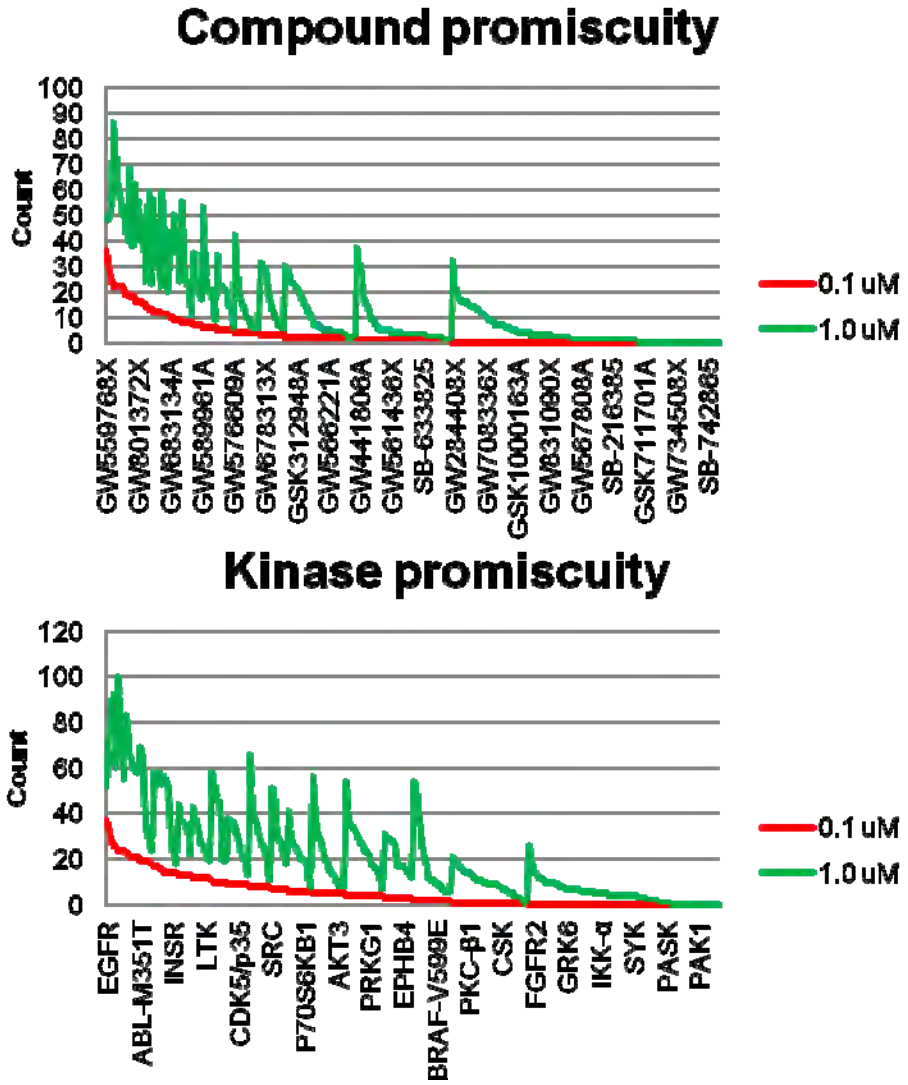
NANOSYN

Kinome Coverage (Nanosyn)

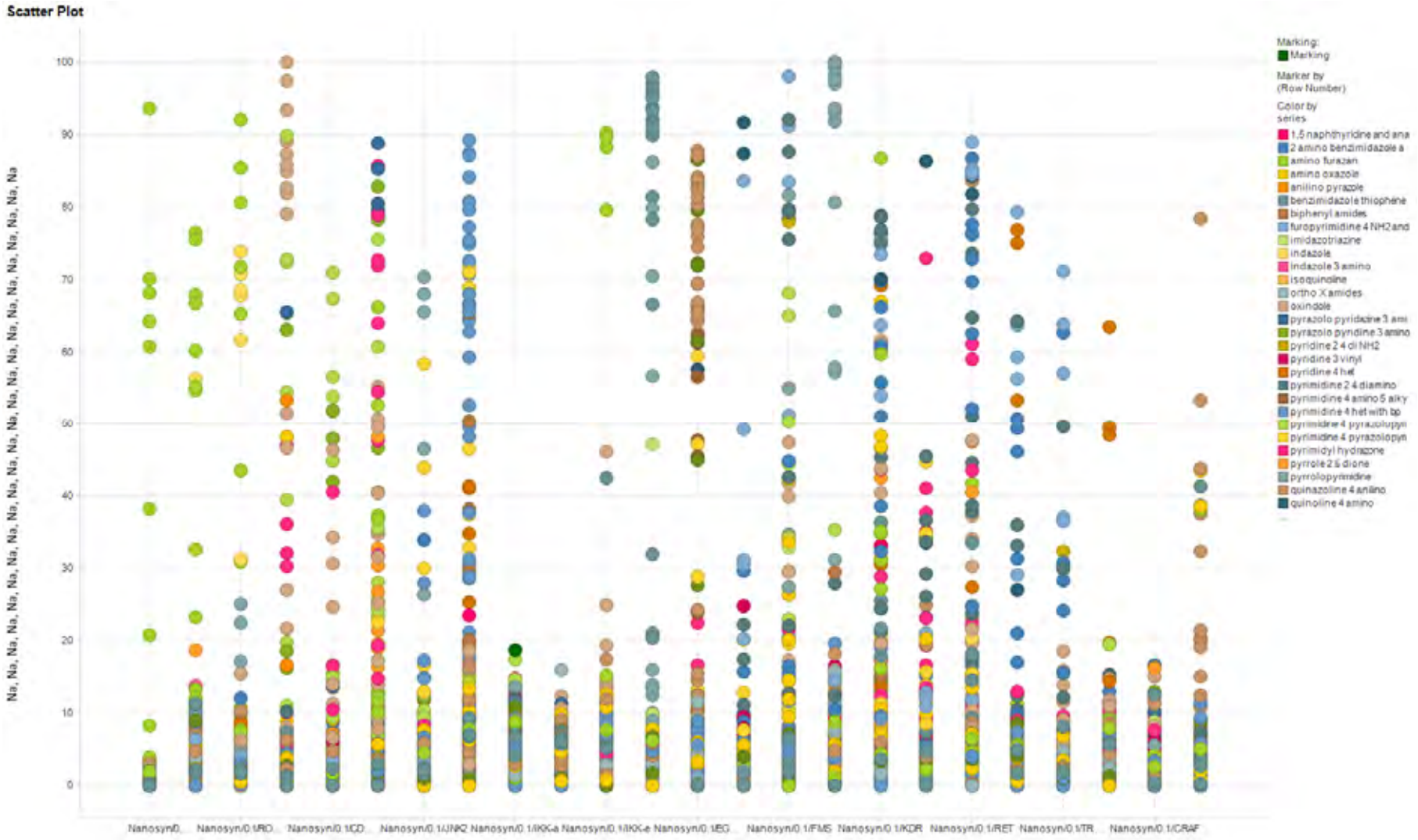


- PKIS had activity across the TKs and non-TKs
- Potent inhibitors were found more often against the TKs
- PKIS had activity on 127/130 non-TKs

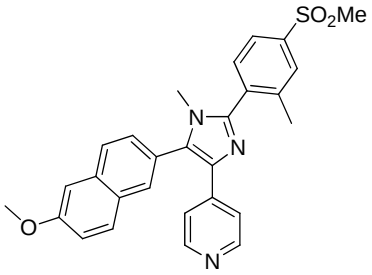
Selectivity results



PKIS %I at 100 nM vs. original targets



Potential LOK (STK10) starting point

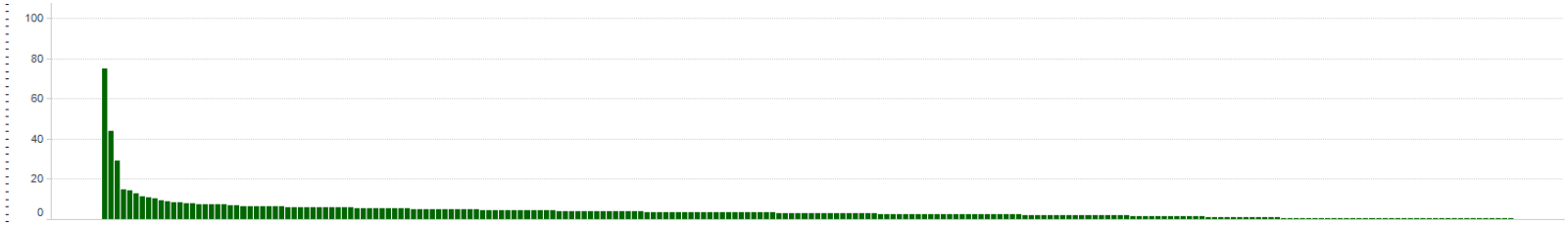


SB-633825
NJ44157-082B1

0.1 uM
LOK = 44%
TIE2 = 75%
BRK = 29%

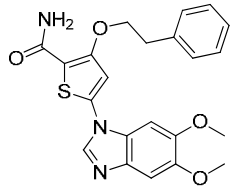
1 uM
LOK = 95%
TIE2 = 79%
BRK = 85%

SB-633825
@ 100 nM



- LOK (STK10) associates with PLK1 and phosphorylates it in vitro
- Crystal structure 2J7T by SGC of different scaffold (Met kinase oxindole SU11274 from Sugen)
- A chemical starting point for a LOK probe?

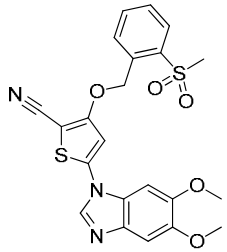
Potential BRSK2 starting point



GSK204925A
U19479/153/8

0.1 uM
BRSK2 = 58%
BRSK1= 42%
PLK1= 32%
LOK = 25%

1 uM
BRSK2 = 80%
BRSK1= 77%
PLK1= 80%
LOK = 67%



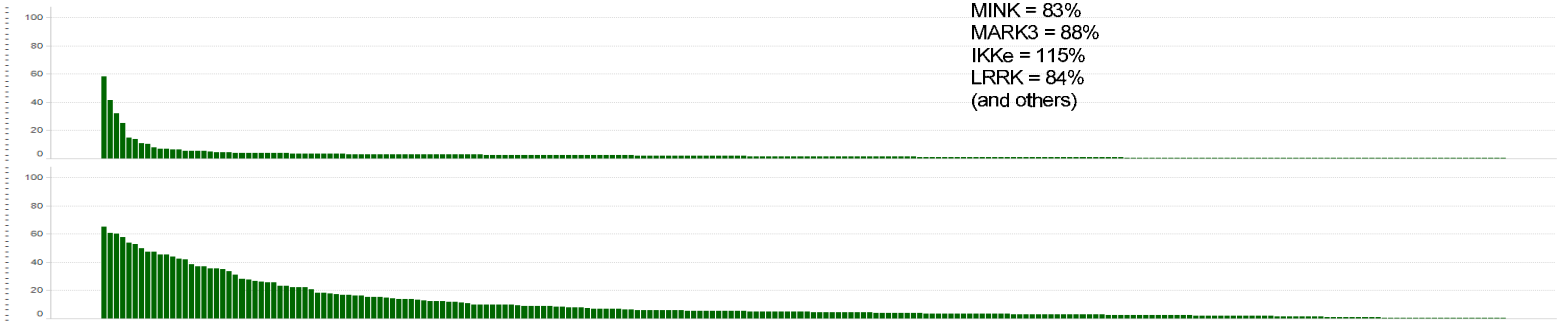
GSK319347A
U24235/171/40

0.1 uM
BRSK2 = 54%
BRSK1= 37%
PLK1= 10 %
TBK1 = 65%
MAP4K4 = 58%

1 uM
BRSK2 = 82%
BRSK1= 76%
PLK1= 47%
AurC = 93%
MAP4K4 = 90%
MINK = 83%
MARK3 = 88%
IKKe = 115%
LRRK = 84%
(and others)

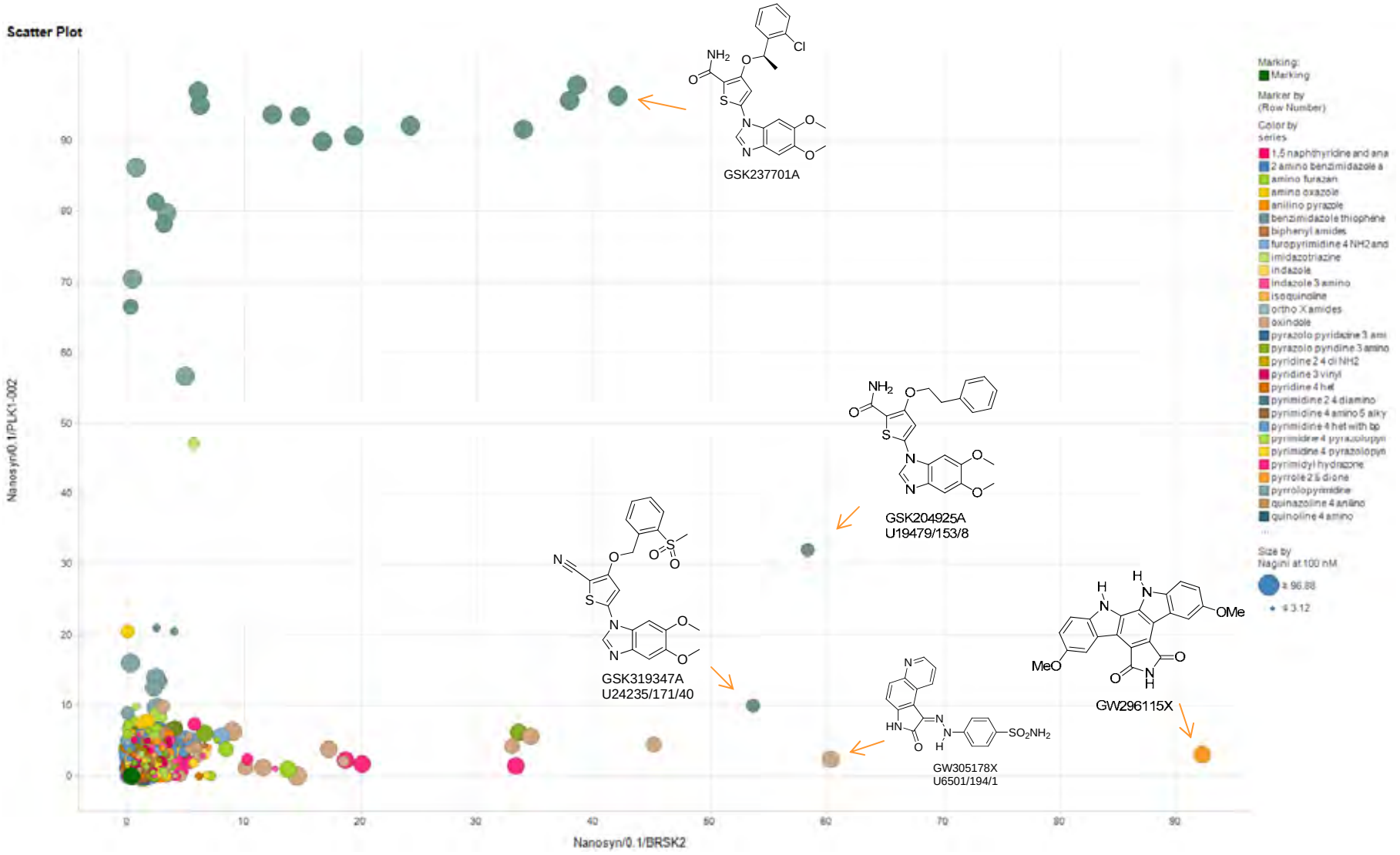
GSK204925A
@ 100 nM

GSK319347A
@ 100 nM



- BRSK2 expressed in brain and required for neuronal polarization; regulation of neurotransmitter release
- SAR between BRSK2 and PLK1 appears divergent (at least some differences)

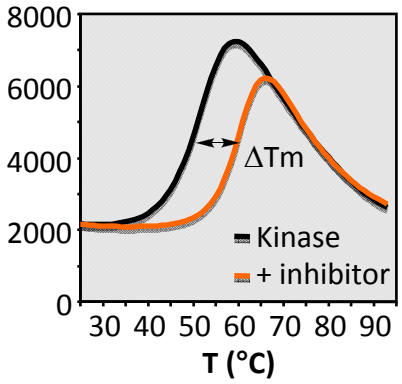
BRSK2 Hits: SAR diverges from PLK1

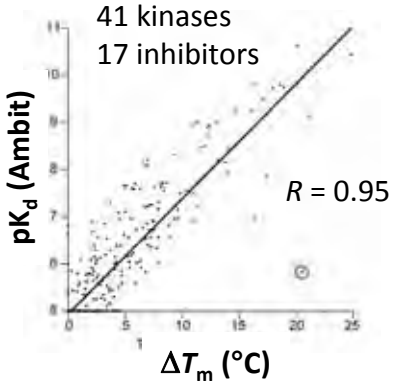


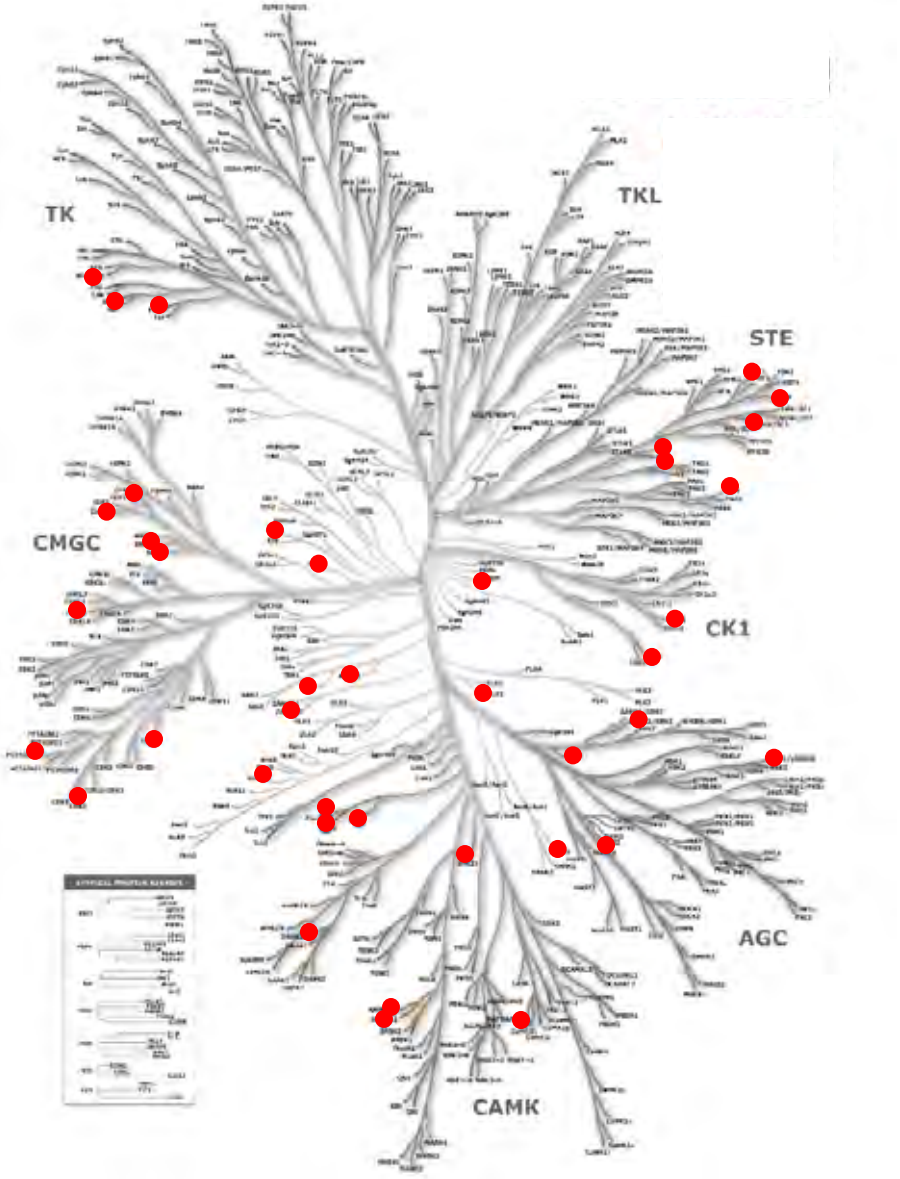
Orphan Kinase Activity

- 40 orphan kinases
- Screened by thermal melt
- Stefan Knapp (SGC-Oxford)

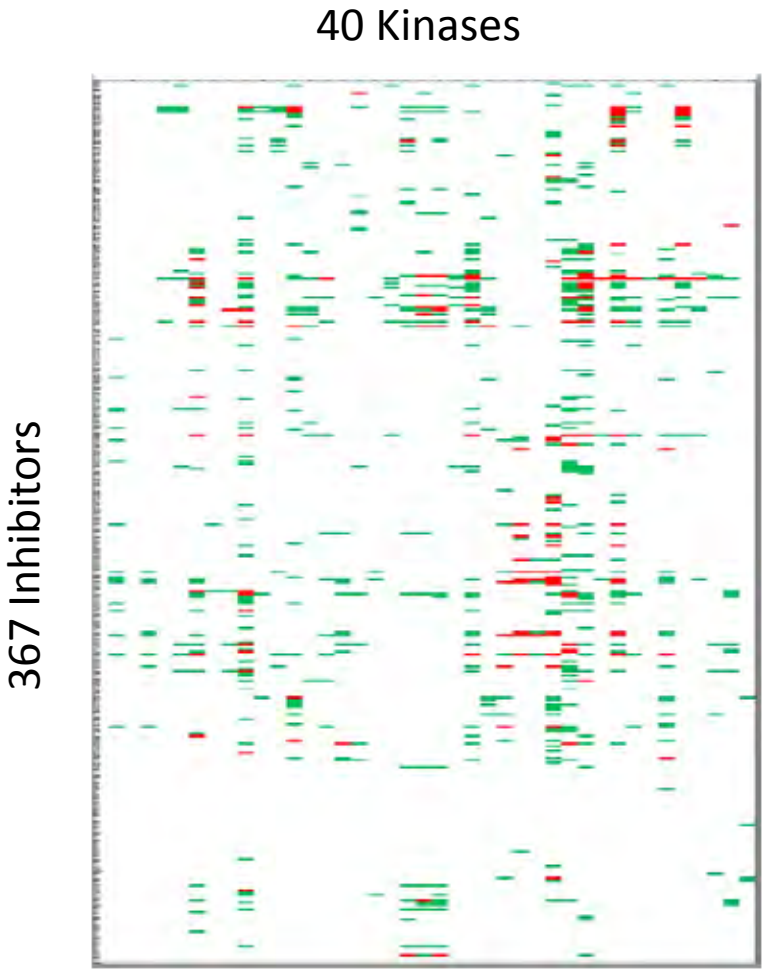
Thermal stability (DSF)



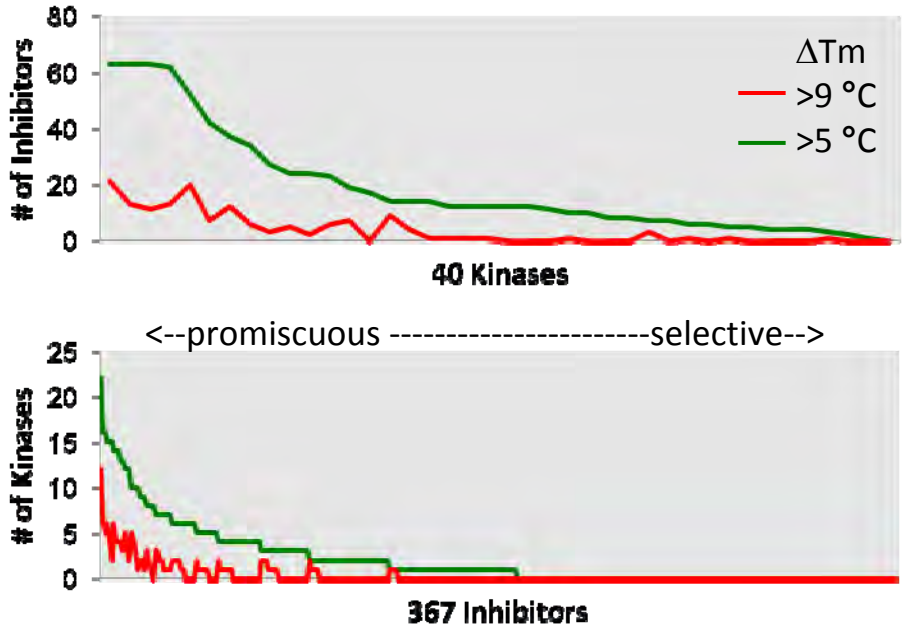




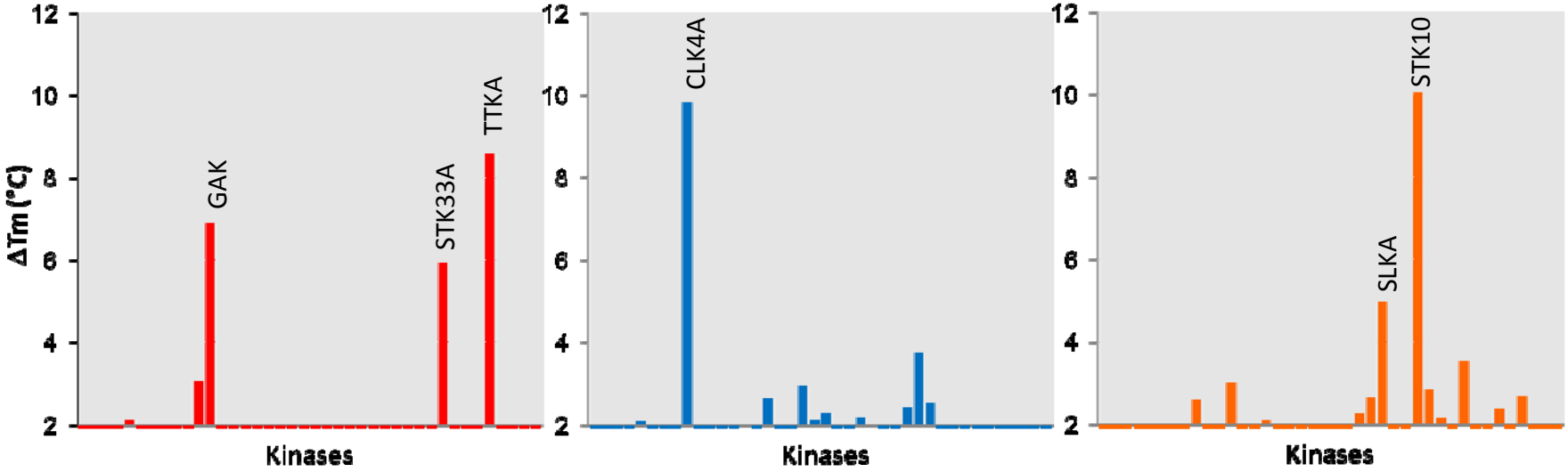
PKIS vs SGC Orphan Kinase Panel

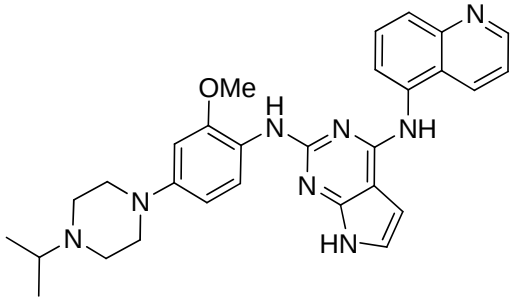


- Sub-μM hits for 39/40 kinases
- Multiple analogs with structure-activity
- Identification of promiscuous kinases
- Identification of selective inhibitors
- *Vice versa*



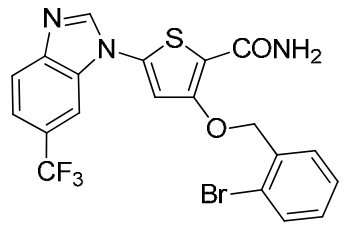
Orphan Kinase Inhibitors





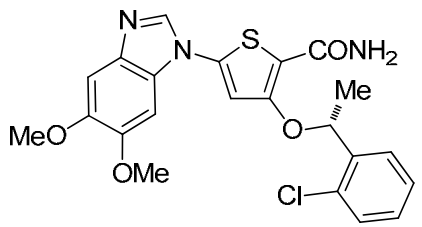
GSK1511931
IGF-1R inhibitor

GAK: involved in centrosome maturation
STK33A: interacts with oncogenic KRAS
TTKA: associated with breast cancer



GW853606
PLK1 inhibitor

CLK4A: CDC-like kinase 4

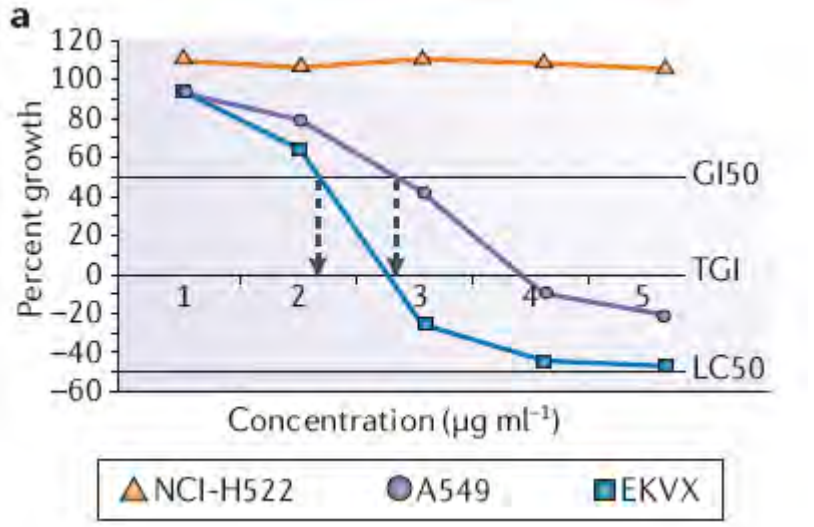


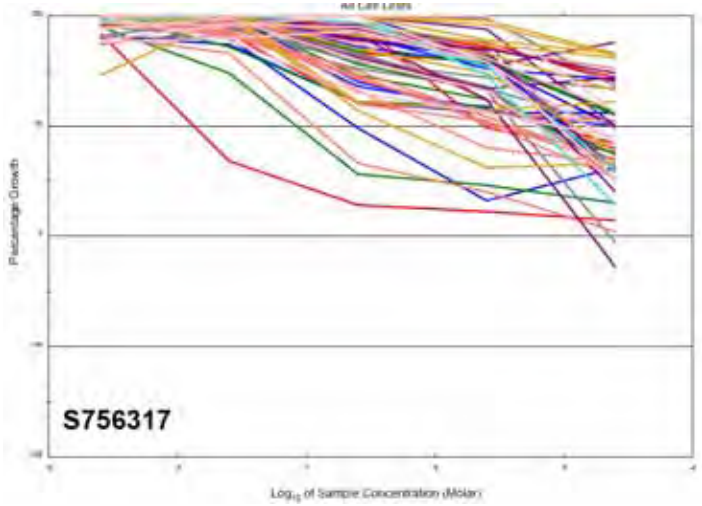
GSK312948
PLK1 inhibitor

SLKA: STE 20-like kinase
STK10: mutated in testicular cancer

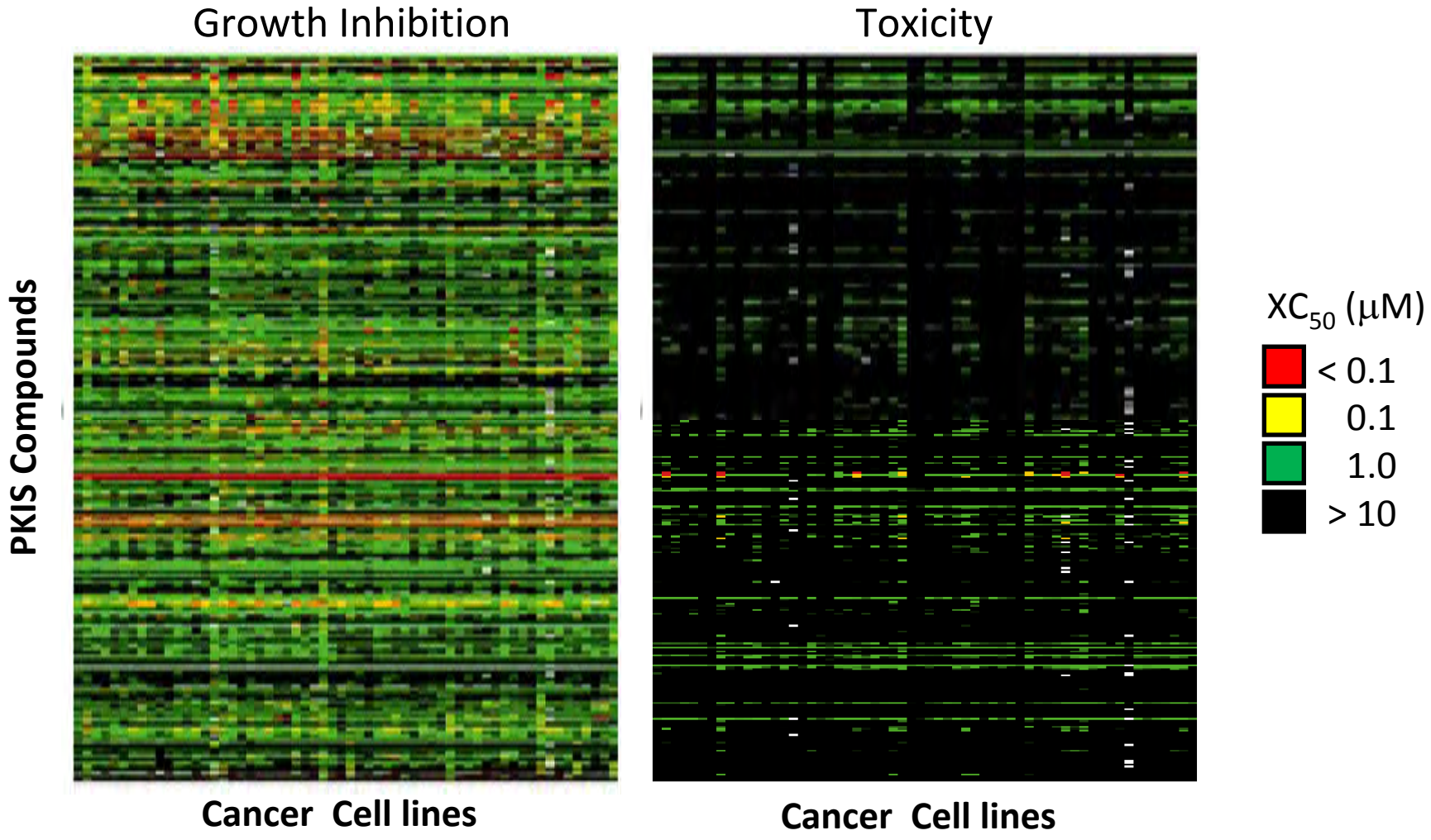
Phenotypic screening: NCI60

- 60 different cell lines spanning 9 cancer types
- ***Extensively*** characterized biologically and pharmacologically
- Dose response curves for PKIS obtained
- Results for cmpds with known MOA (eg, EGFR inhibitors) as expected

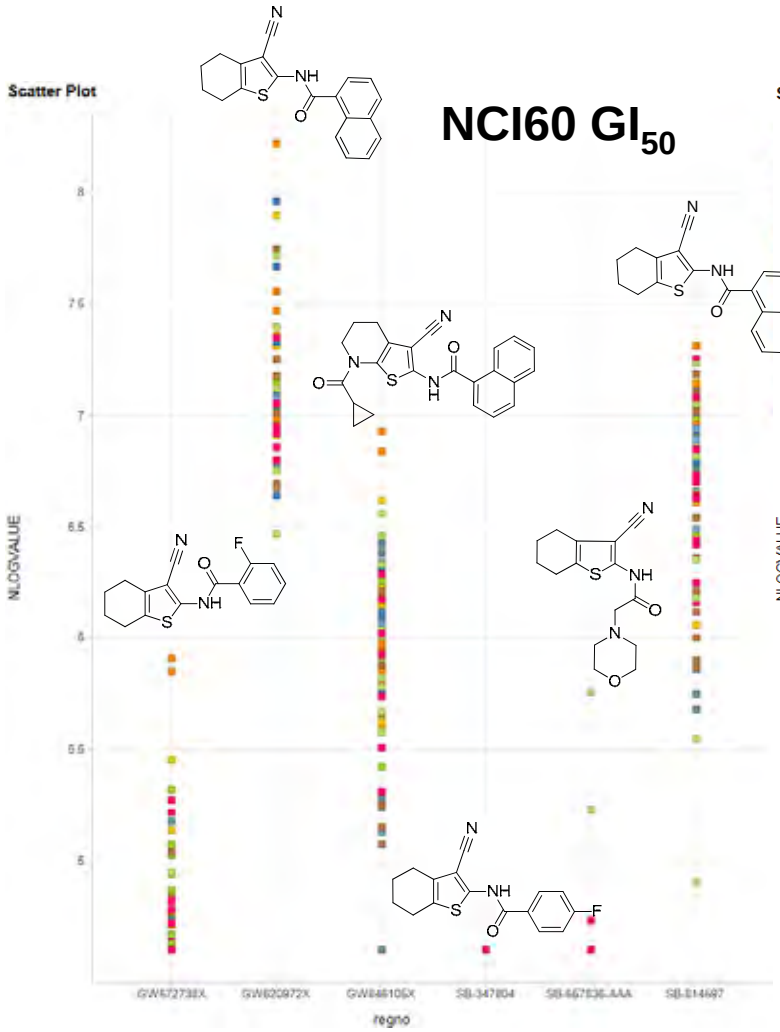




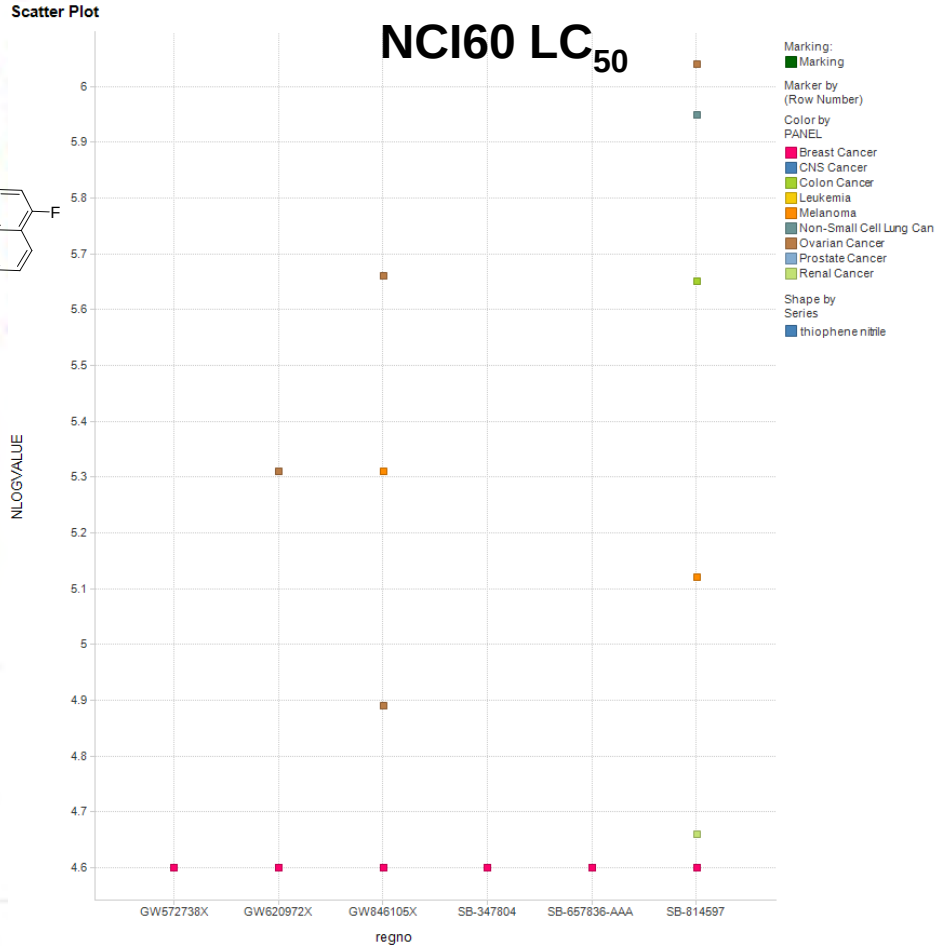
NCI-60 Cancer Cell Lines: high level view



JNK3 compounds

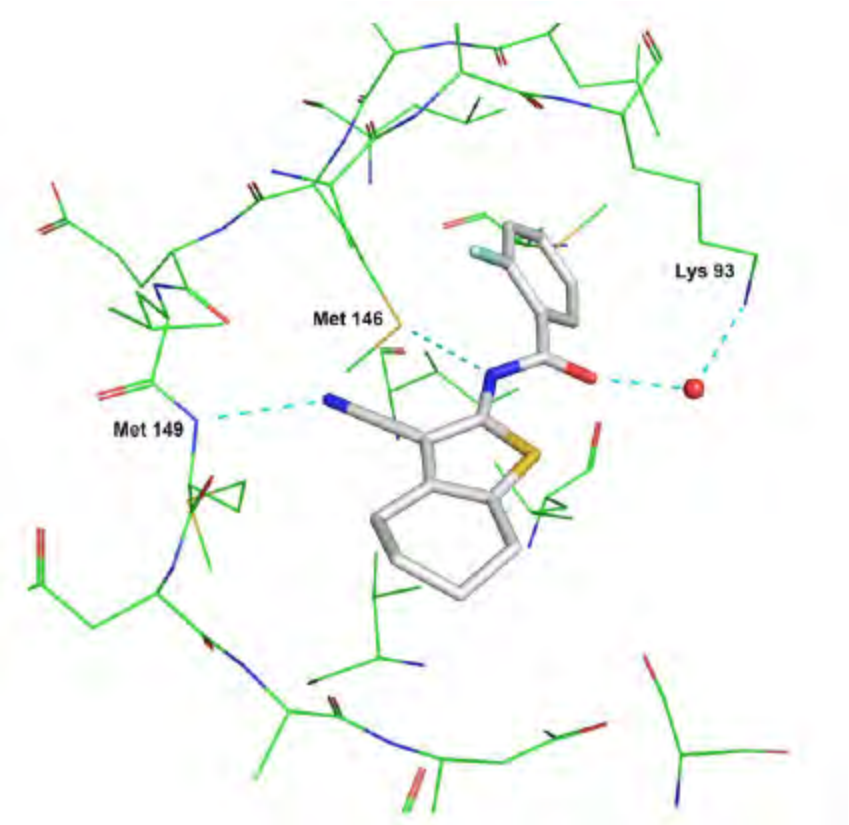


NCI60 GI_{50}



NCI60 LC_{50}

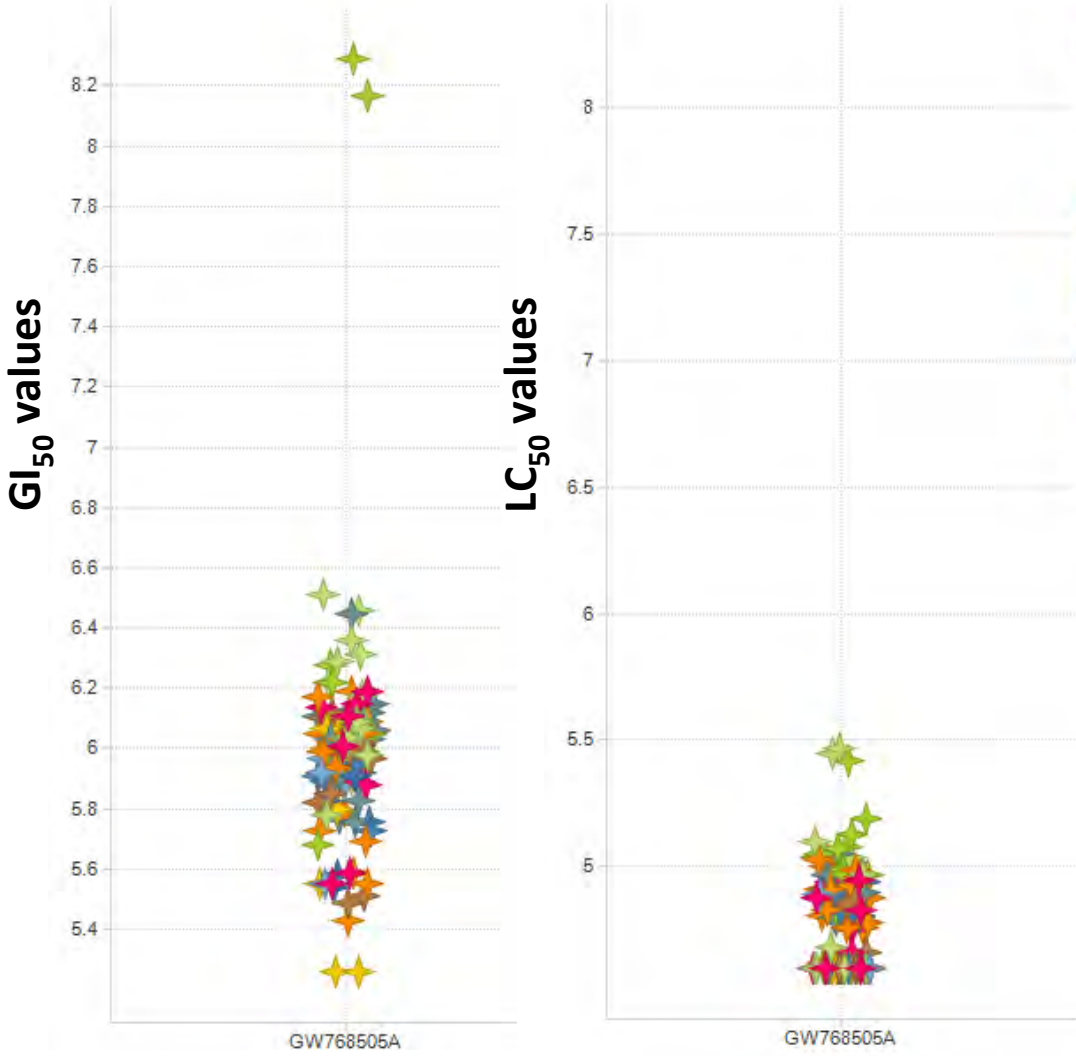
Crystal structure

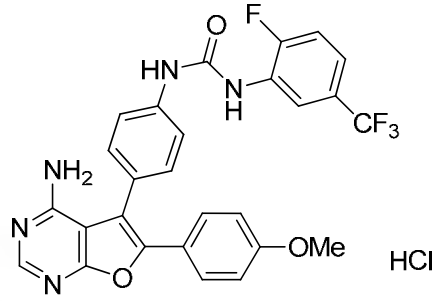


- 2.45 Å crystal structure of GW572738X/JNK3 (PDB code 2O2U)
- Unusual hinge binding: Met149 backbone NH with ligand CN
- H-bond donation from ligand amide NH to Met146 S
- Water-mediated interation of ligand CO with Lys93

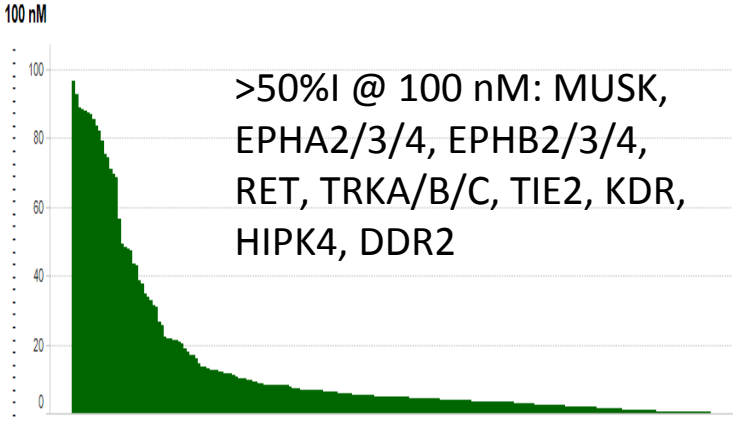
Bioorg. Med. Chem. Lett. **2007**, *17*, 1296

Selective growth inhibition





Selective growth inhbition of KM12 colon cancer cell line by GW768505A



>50%I @ 100 nM: MUSK, EPHA2/3/4, EPHB2/3/4, RET, TRKA/B/C, TIE2, KDR, HIPK4, DDR2

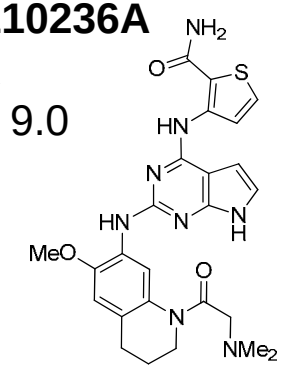
The PKIS Collaboration Network

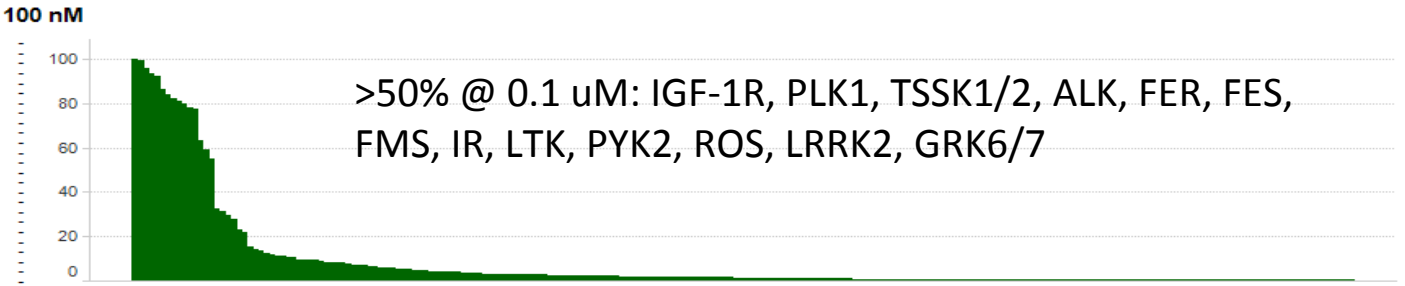
PKIS dispensed to over 60 laboratories across 35 institutions

Ependymoma

- Background
  - 3rd most common brain tumor in children
  - Survival: 24-75% at 5 years; Incurable in up to 40% of cases
- Screening paradigm
  - Proliferation of mEP^{Ephb2} vs. parental NSCs
- IGF-1R as ependymoma target?
  - IGF-1R upregulated in mEP^{Ephb2} NSCs relative to parental
  - PKIS screening IDed GSK2110236A as hit

GSK2110236A
IGF-1R
pIC_{50} > 9.0



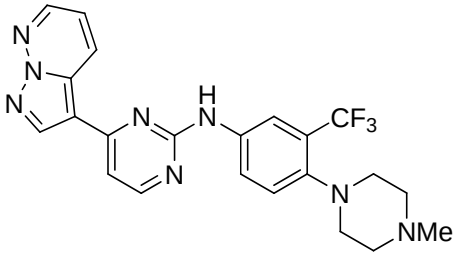


>50% @ 0.1 uM: IGF-1R, PLK1, TSSK1/2, ALK, FER, FES, FMS, IR, LTK, PYK2, ROS, LRRK2, GRK6/7

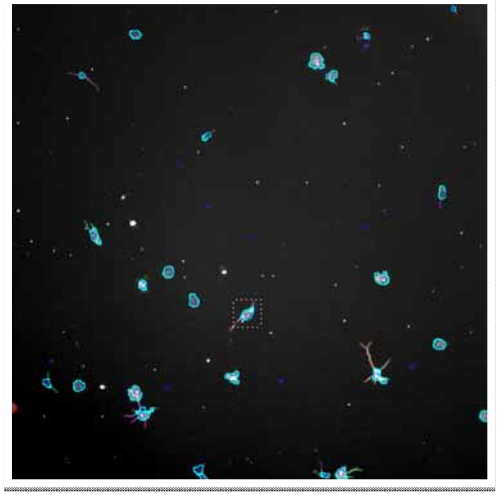
R. Gilbertson, K. Guy et al.
St. Jude Childrens Research Hospital

High-Content Neuronal Imaging

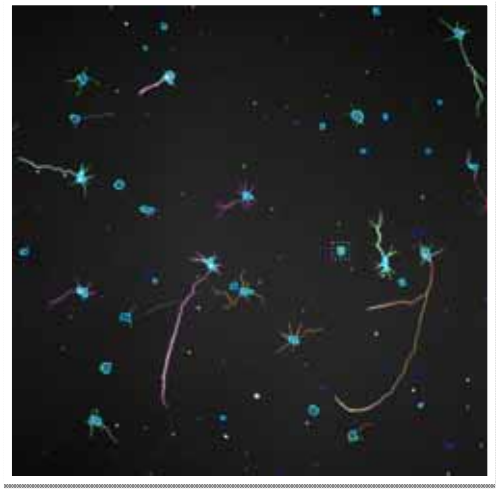
- *Real time* measurement of multiple morphologic parameters
- Previous molecular genetic studies have identified kinases and phosphatases



GW779439X



DMSO Control

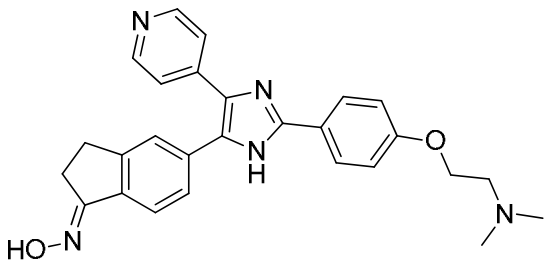


GW779439X (6 nM)

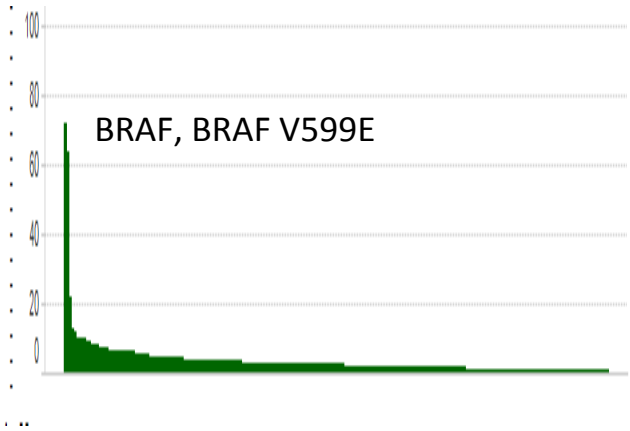
Vance Lemmon, John Bixby (U. Miami)

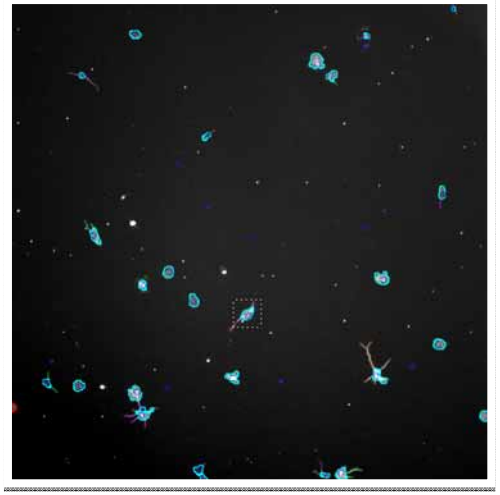
High-Content Neuronal Imaging

- *Real time* measurement of multiple morphologic parameters
- Previous molecular genetic studies have identified kinases and phosphatases

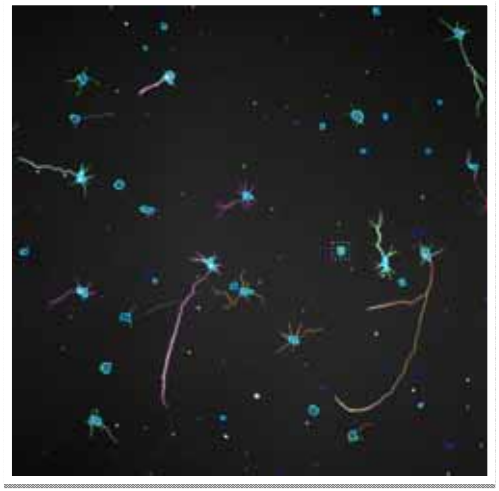


SB-590885-AAD





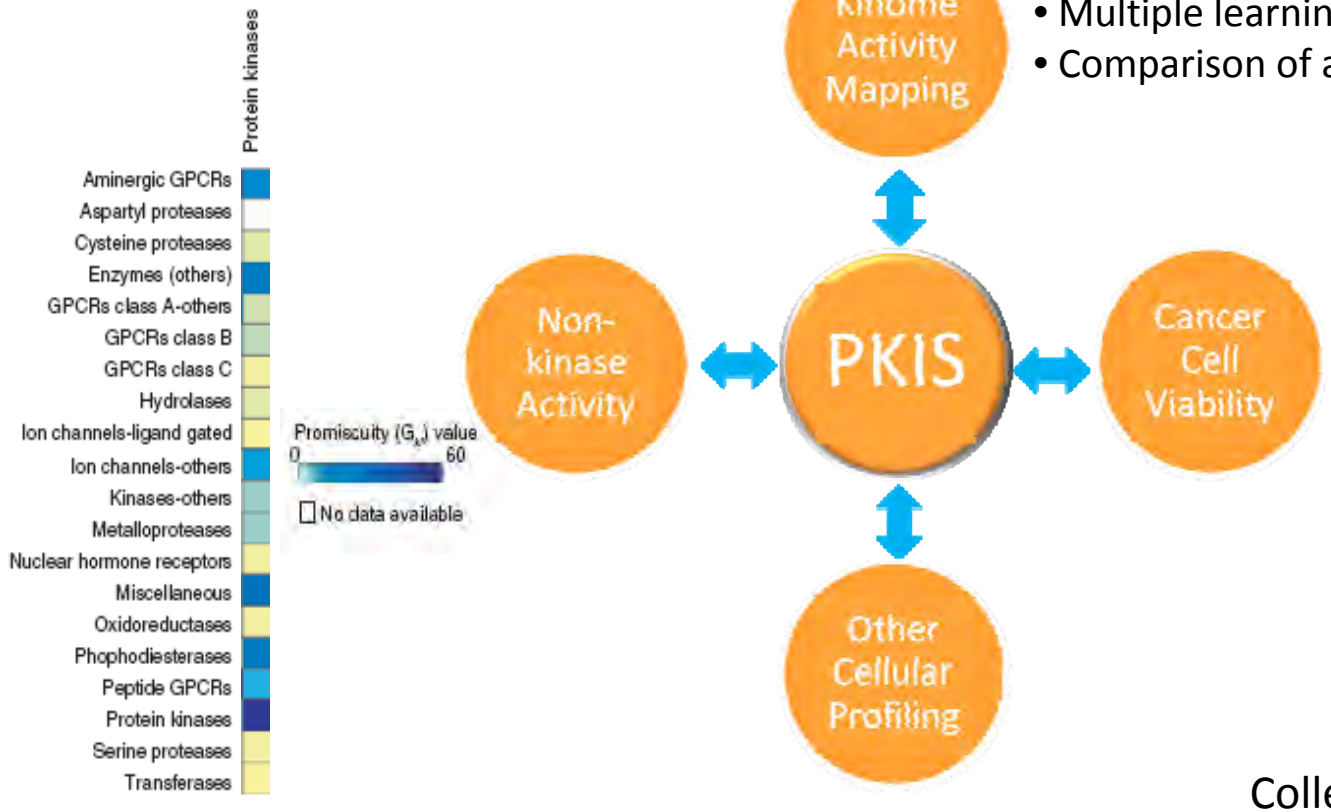
DMSO Control



GW779439X (6 nM)

Vance Lemmon, John Bixby
The Miami Project to Cure Paralysis, University of Miami

Applications of PKIS



- ID of selective probes or chemical starting points
- Multiple learnings from SAR
- Comparison of assay types and conditions

- Annotation around cell lines (e.g., NCI60) combined with activity map may ID kinases or combinations of kinases for targeting
- Mechanistic insight
- Synthetic lethal/sensitization screens

- Screens for phenotypes of interest
- Human, pathogen, etc.

Collective data will enable an improved PKIS

"Global Mapping of Pharmacological Space" Hopkins et al. *Nature Biotech.* **2006**, *24*, 805

Unlocking the Orphan Kinome

1) Dispense inhibitor set. Screen broadly across the kinome and release all data into public domain
2) Refine PKIS by addition of more compounds from GSK and other Pharma + academics.
3) Create open network to enable optimization of new kinase chemical probes

Closing Thoughts

- The challenges of drug discovery demand new ways of doing things
- An experiment in open preclinical target validation:
  - Created PKIS, a set of 367 kinase inhibitors
  - Obtained activity map vs. 220 kinases
  - Engaged several dozen collaborators (and growing)
- Annotation of the orphan kinome creates opportunities for new drug discovery

Acknowledgements

Previous contributors to PKIS compounds

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Olga Issakova, Nikolai Sepetov

Trinity College Dublin: David Lloyd

NCI: Joel Morris, Raj Misra

University of Dundee: Andrew Hopkins

European Bioinformatics Institute: John Overington, Francis Atkinson

A growing network of collaborators!