

Challenges in Fragment Based Drug Discovery for Protein Kinases

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Outline

- Presentation Goals and Background Information
- Useful Concepts/Tools for Drug Discovery Teams
- Introduction to Fragment-Based Drug Discovery
- FBDD/SBDD Case Study: MET Inhibitor

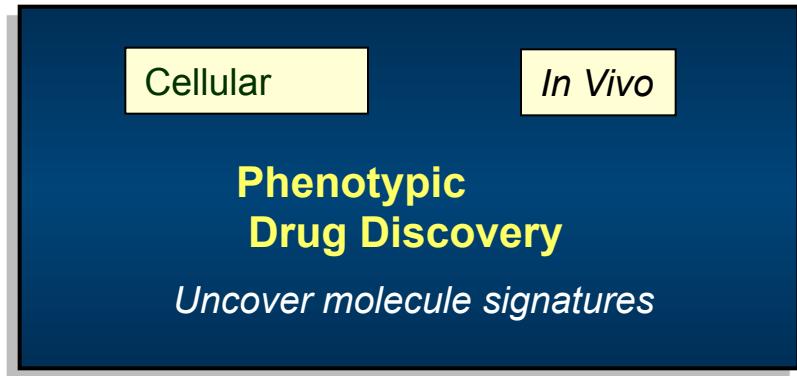
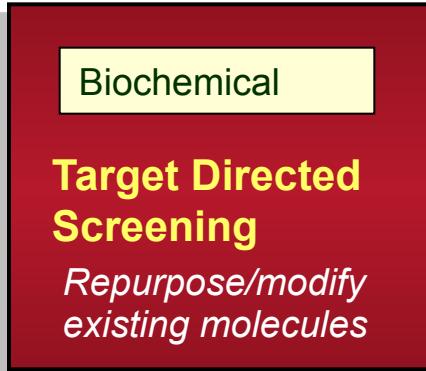
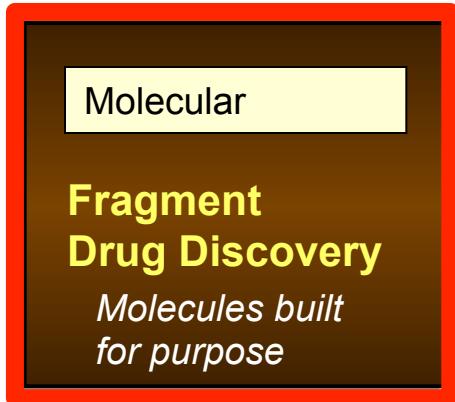
Presentation Goals

- Understand some of the challenges in small-molecule drug discovery
- Understand some of the advantages offered by fragment-based drug discovery
- Appreciate the possibility of unforeseeable pitfalls

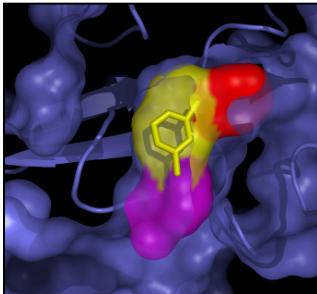
Drug Discovery Strategies

“Distinct Target Hypotheses”

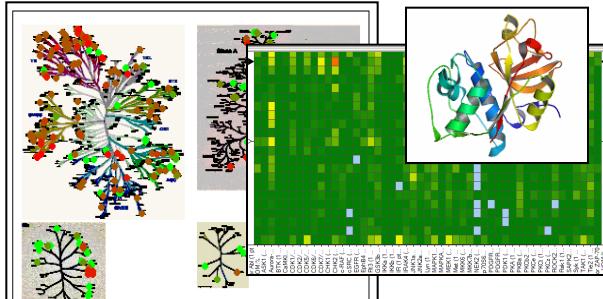
“Biological Systems Hypotheses”



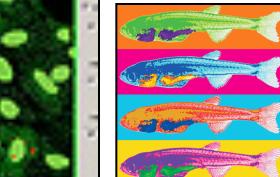
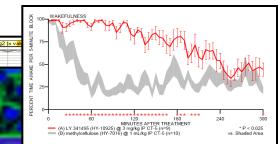
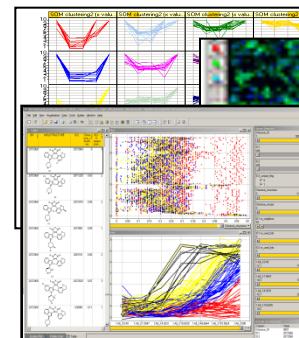
- HT Crystallography
- SPR
- HDX
- Fragment libraries
- High conc. assays



- Gene family platforms
- Diversity/iterative screening (HTS)
- Compound libraries
- Computational models/informatics
- Structural Biology
- Cellular and biochemical assays

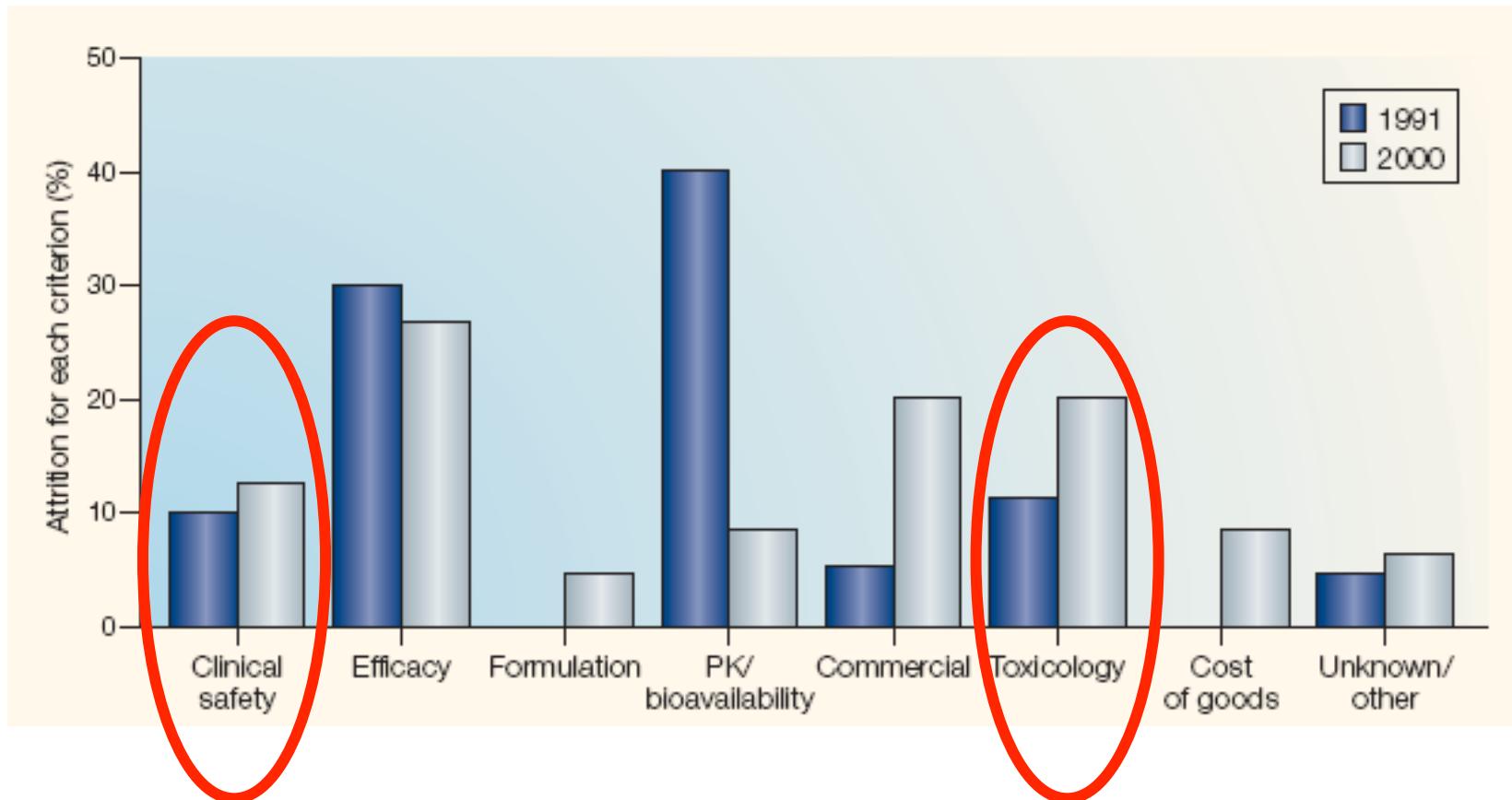


- High Content Imaging
- Advanced informatics
- Alternative diversity
- Advanced cellular assays
- Stem cells



- SCORE
- Zebra fish
- In vivo imaging

Causes of Attrition in Pharma

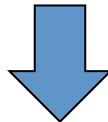


How can MedChemists help address ~1/3 of Pharma Attrition?

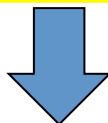
Kola and Landis(2004)

Statement of the Problem

CNOF-containing Cpds with MW<500 ~ 10^{60}



Subset with Pharmacologic Activity ~ $<10^{26}$

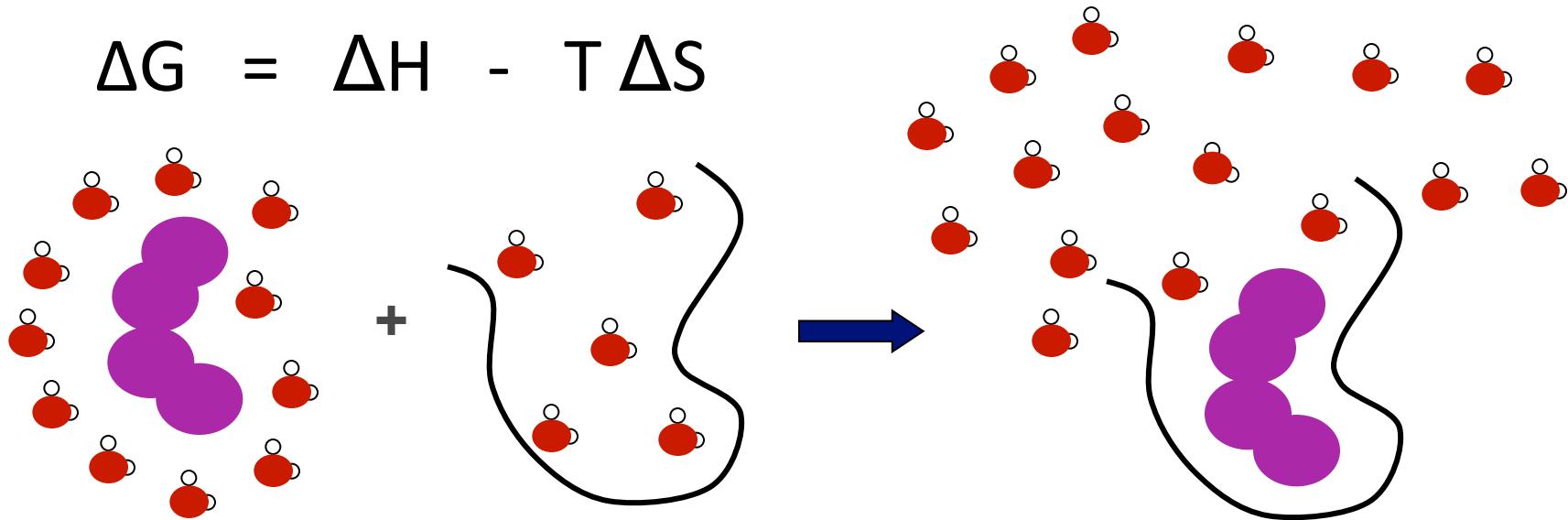


Subset with Good ADMET Properties ~ $10^{??}$

- Lipinski *et al.* (1997) Poor Absorption or Permeability more likely if # H-bond don>5, # H-bond acc>10, MW>500, cLogP>5
→Broad Adoption of the “Rule of 5”
- Medicinal Chemists need to bias design → Green Subset

Hydrophobic Effect

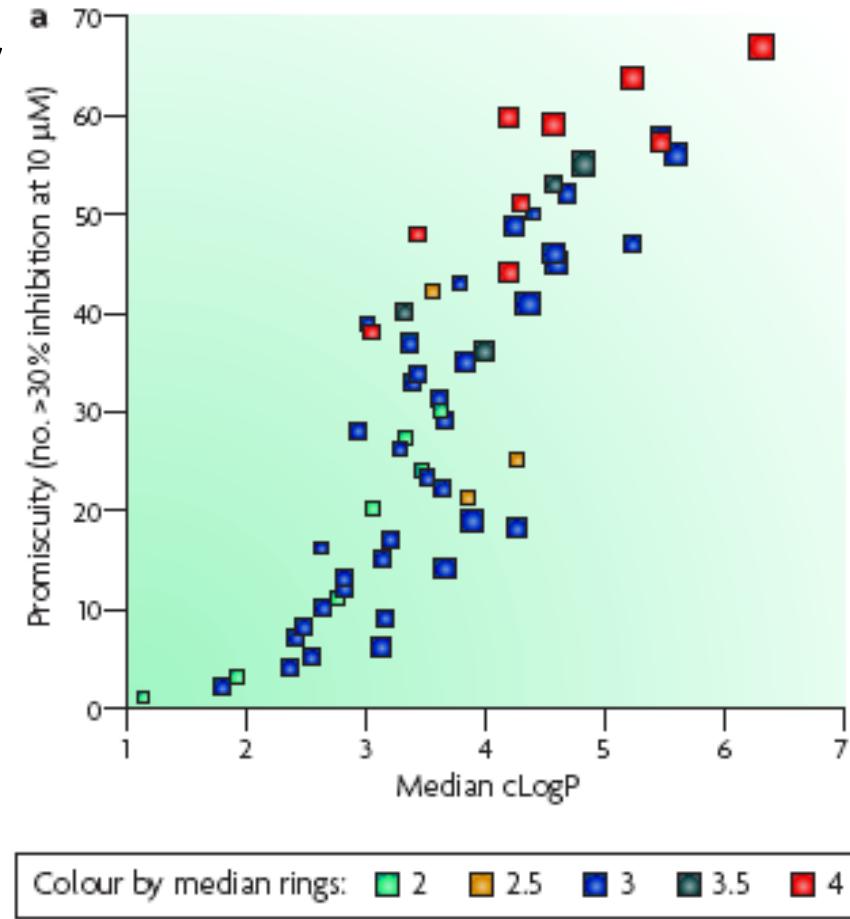
$$\Delta G = \Delta H - T \Delta S$$



- Ligand displacement of partially ordered waters from a hydrophobic surface feature is favorable!
- Lipophilicity does contribute to target binding
- Too much lipophilicity → Unwanted off-target binding

Why Lipophilicity Matters ($c\text{LogP}<3$)!

- Median $c\text{LogP}$ versus Promiscuity assays
- Promiscuity = # Cpd's with >30% inhibition at [10 μM]
- Sigmoidal relationship ($r = 0.84$)
- $c\text{LogP}<3$ more favorable!



Leeson and Springthorpe (2007)

LLE (Lipophilic Ligand Efficiency)

LLE: Potency without too much Grease!

$$\text{LLE} = -\text{Log}(\text{IC}_{50}) - \text{cLogP}$$

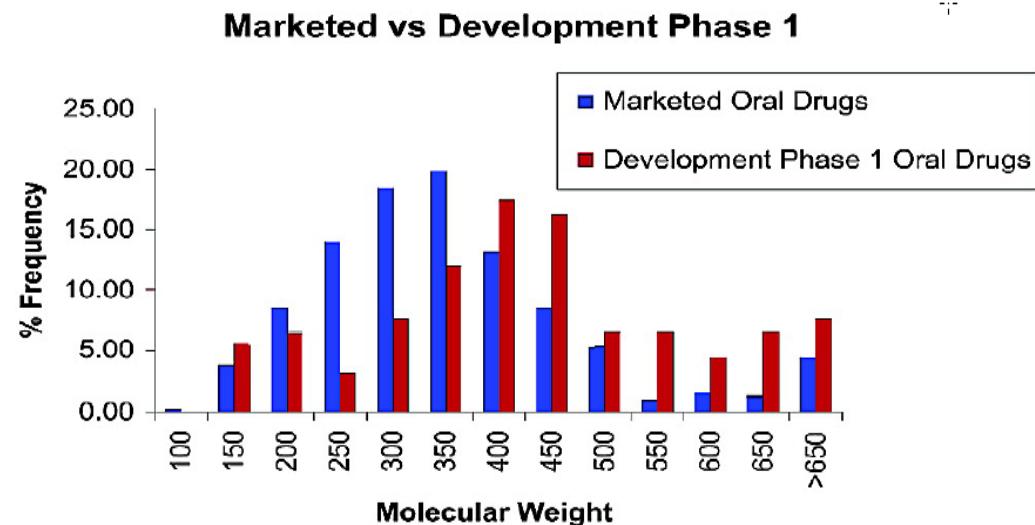
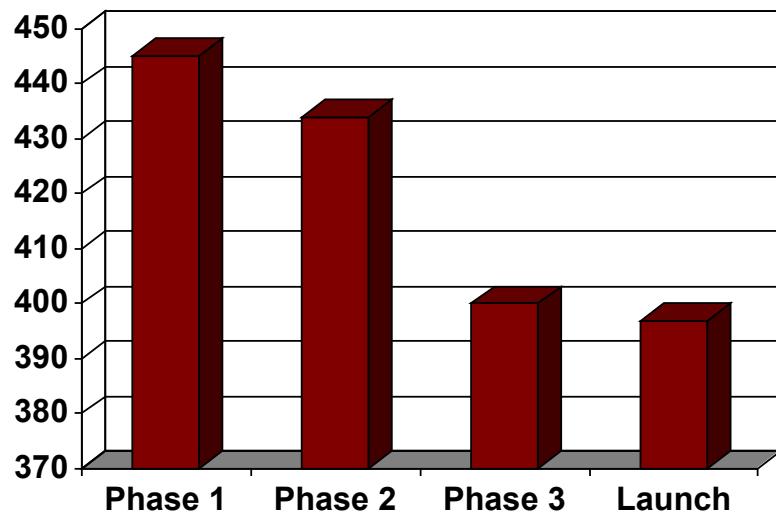
Exemplar Values: LLE>5; IC₅₀<10nM and cLogP<3

- Adding grease is an easy way to accrue “bogus” Potency
- Must consider the efficiency of each lipophilic addition
- LLE allows identification/tracking of “good” Potency
(i.e., not driven by grease → high protein binding)

Leeson and Springthorpe (2007)

Why Size Matters (MW<400)!

- Lower MW Cpds have superior Pr(oral agent approval)!



LEAN: Drive for Potency at the Right Price!

$$\text{LEAN} = -\log(\text{IC}_{50})/n \quad [n = \# \text{ of non H atoms}]$$

Exemplar Values: LEAN>0.27; IC₅₀<10nM and MW<400

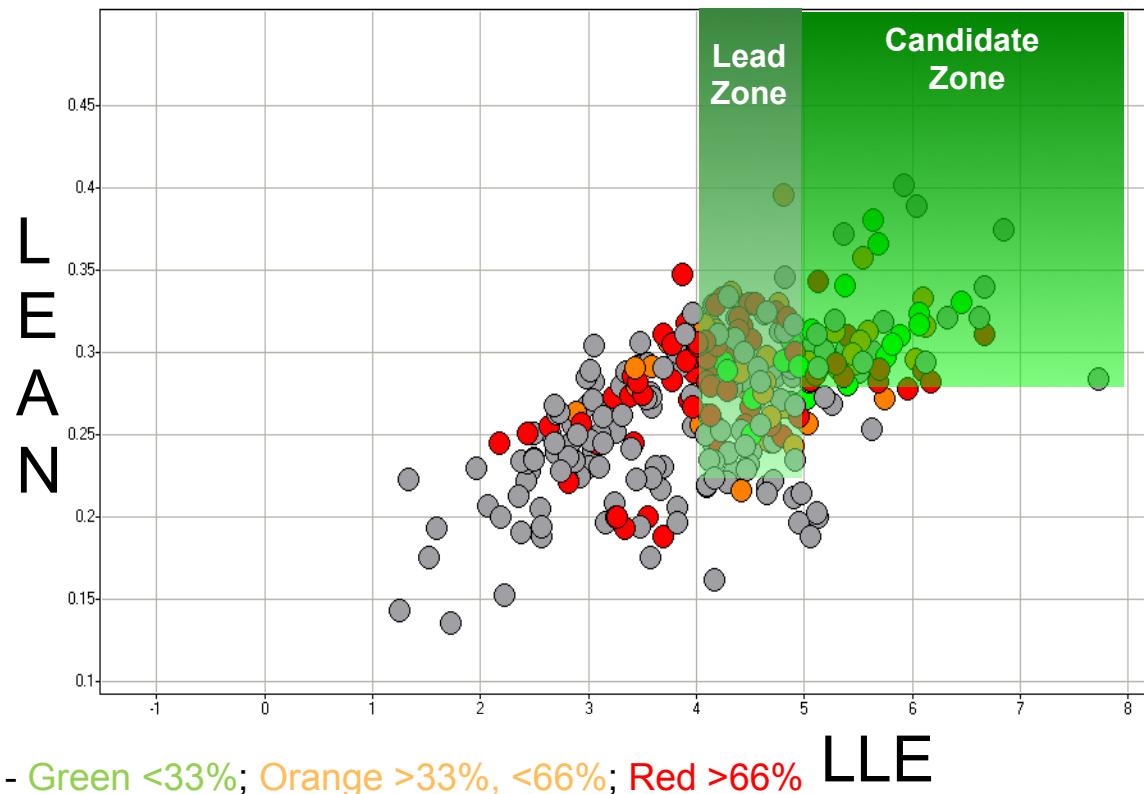
Wenlock *et al.* (2003); Hopkins *et al.* (2004)

LEAN versus LLE Trends

- Optimization of both LEAN and LLE in drive from LEAD→CS
- Example: Kinase Project

Lean vs. LLE

- Target Values:
- Lead:
 - MW \leq 400/ IC_{50} \leq 100nM
→ LEAN \geq ~0.23
 - cLogP \leq 3/ IC_{50} \leq 100nM
→ LLE \geq ~4-5
- Candidate:
 - MW \leq 400/ IC_{50} \leq 10nM
→ LEAN \geq ~0.27
 - cLogP \leq 3/ IC_{50} \leq 10nM
→ LLE \geq ~5-8



Guidelines for Oral Drug Candidates

MW < 400

cLogP < 3

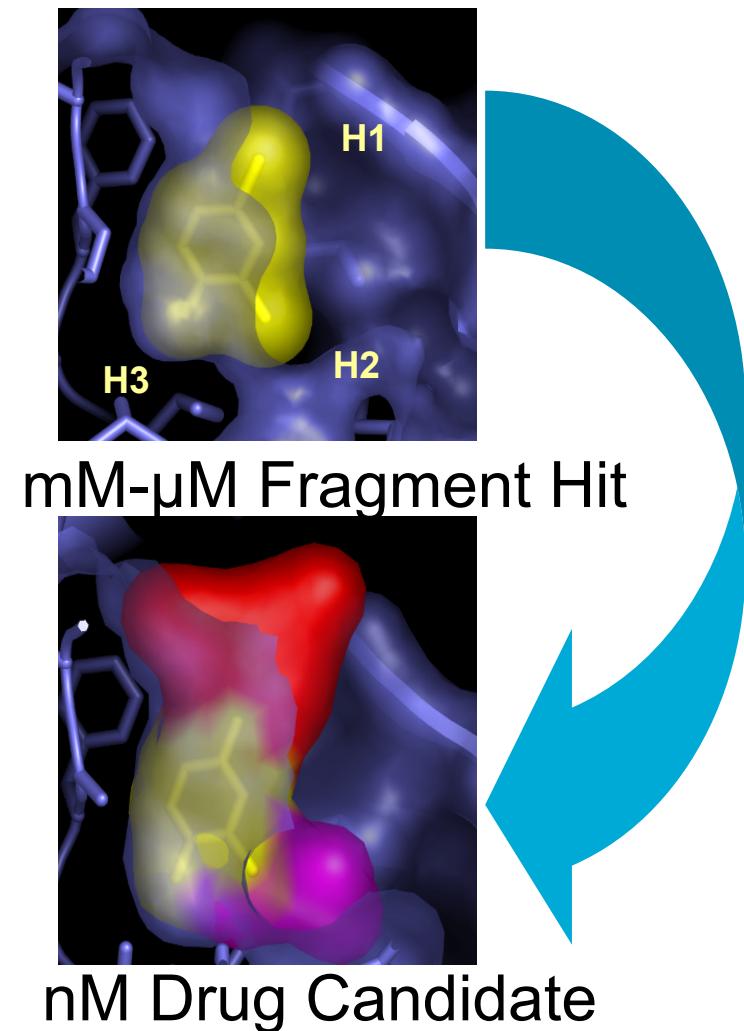
Lean > 0.27

LLE > 5



- These are the ‘gold zone’ targets
- There is a ‘gray zone’ but be mindful that operating there may come with more risk
- “Make Drugs looks like Natural Products”

Fragment-Based Drug Discovery

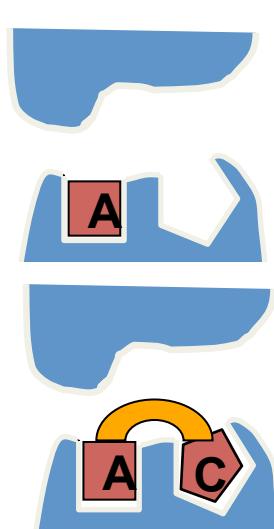


- Fragment library screening Hits
- Exploit literature precedents
- Structure-guided triage process
- Pick “promising” opportunities
- Drive Potency/Selectivity → Candidate
- Perceived/Real Challenges
 - Lower affinity starting points
 - Modest fragment libraries (1000s)
 - Hydrophobic Effect: Friend or Foe?
 - Lipophilicity Matters!
 - Size does Matter!
 - Structure is essential
 - Are fragments selective *per se*?

mM - μM Affinity Starting Points

$$\Delta G_{\text{total}} = \Delta G_{\text{intrinsic}} + \Delta G_{\text{rigid}} \quad (\Delta G_{\text{rigid}} \sim 3.5\text{-}5 \text{ kcal/mol})$$

ΔG_{rigid} penalty due to loss of entropy



	# Heavy Atoms/MW	K_d	ΔG_{total} (kcal/mol)	$\Delta G_{\text{intrinsic}}$ (kcal/mol)
Scaffold A	11/140Da	100 μM	-5.4	-9 to -10
CandidateAC	30/400Da	3nM	-11.4	-15 to -16

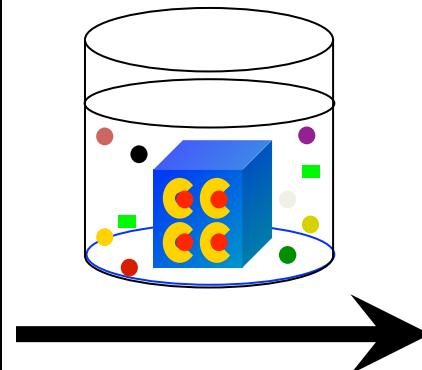
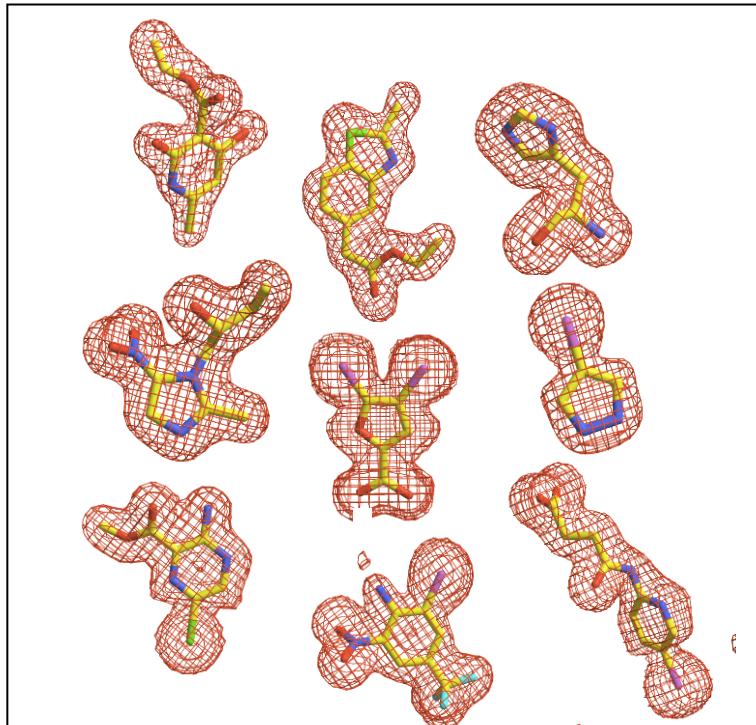
MW \uparrow ~3-fold \rightarrow Affinity \uparrow ~33000-fold

Scaffold A contributes significantly to $\Delta G_{\text{intrinsic}}$!

Only well anchored scaffolds show up in fragment screens

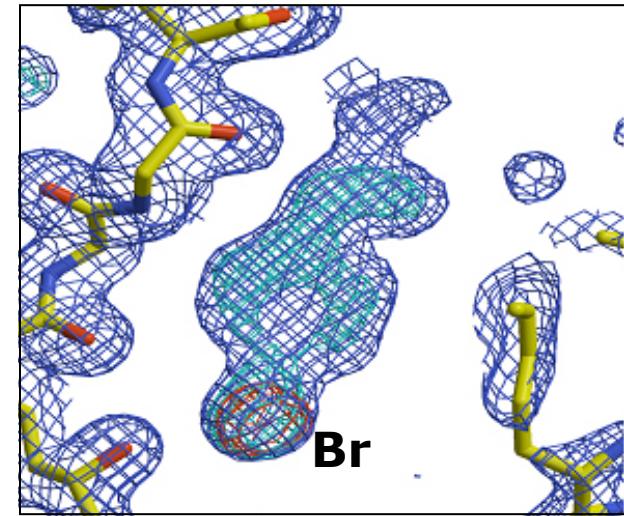
Murray and Verdonk (2002)

Crystallographic Fragment Screening



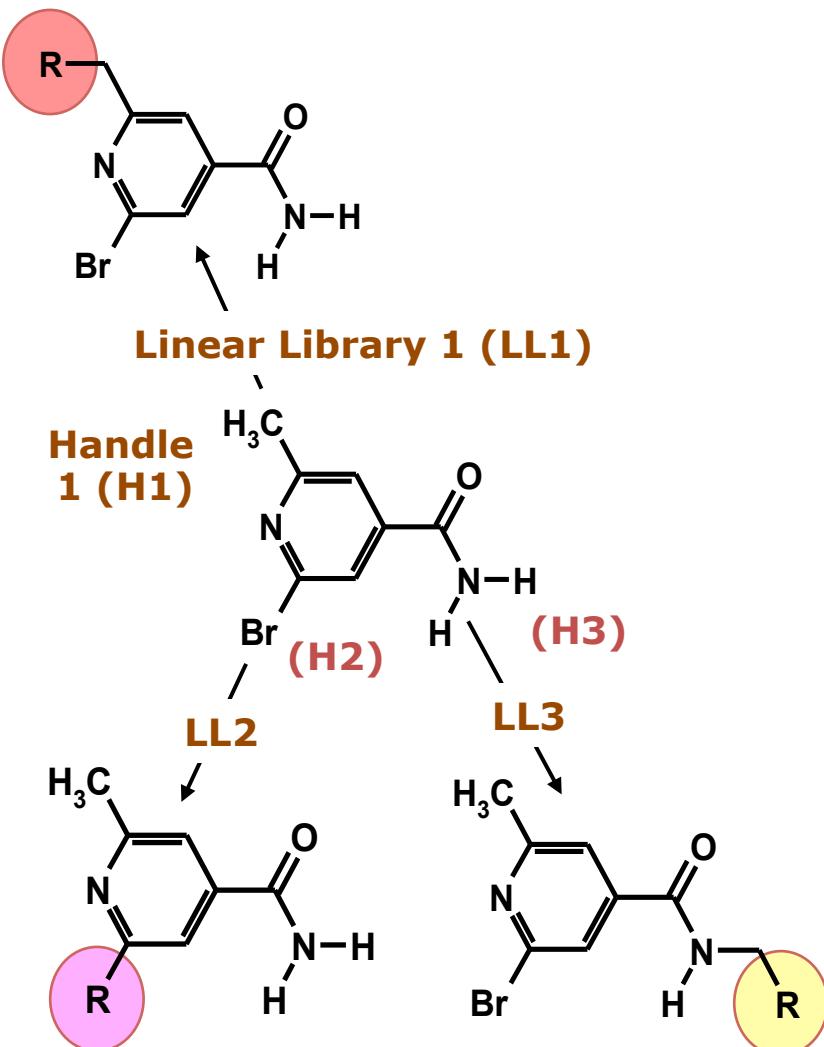
Crystal soaking and data collection

Hit rate~1-5%



- Fragment library (1500-2000 cpds) is divided into pools of 10 shape diverse compounds → soaked into preformed crystals
- Bound fragment is identified from shape of electron density features
- Entire library can be screened within a few days at a synchrotron

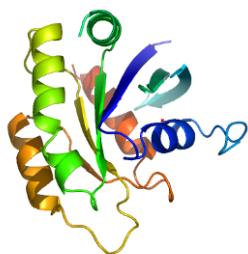
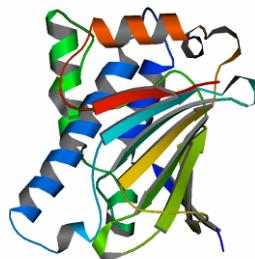
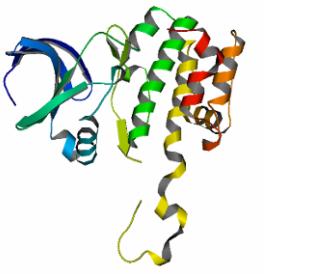
Fragment Library Design/Diversity/Utility



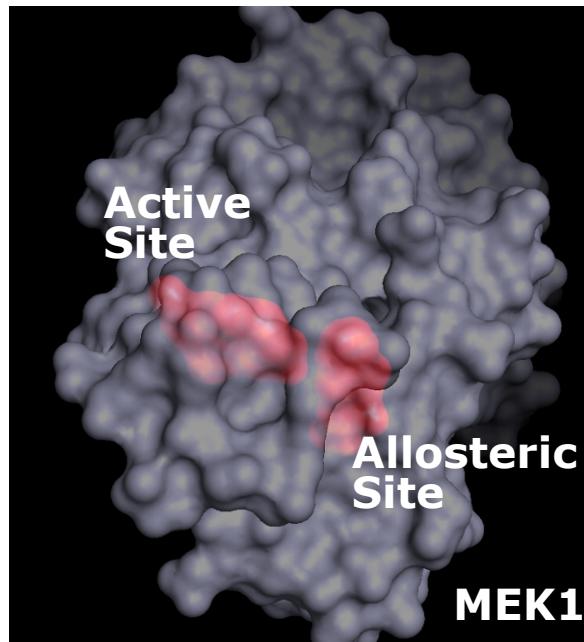
- ~2000 fragments (scaffolds)
- Scaffolds have ~3 Handles amenable to modification
- 1000s-10000s of commercial R groups/Handle
- Potential Diversity Enormous
 - $2 \times 10^3 * 1000^3 \rightarrow 2 \times 10^{12}$
 - $2 \times 10^3 * 10000^3 \rightarrow 2 \times 10^{15}$
 - CHFNOcpds(<400Da)~ 10^{30}
- Fragment Libraries support rapid hit SAR exploration with automated synthesis

FBDD Generates Multiple Opportunities

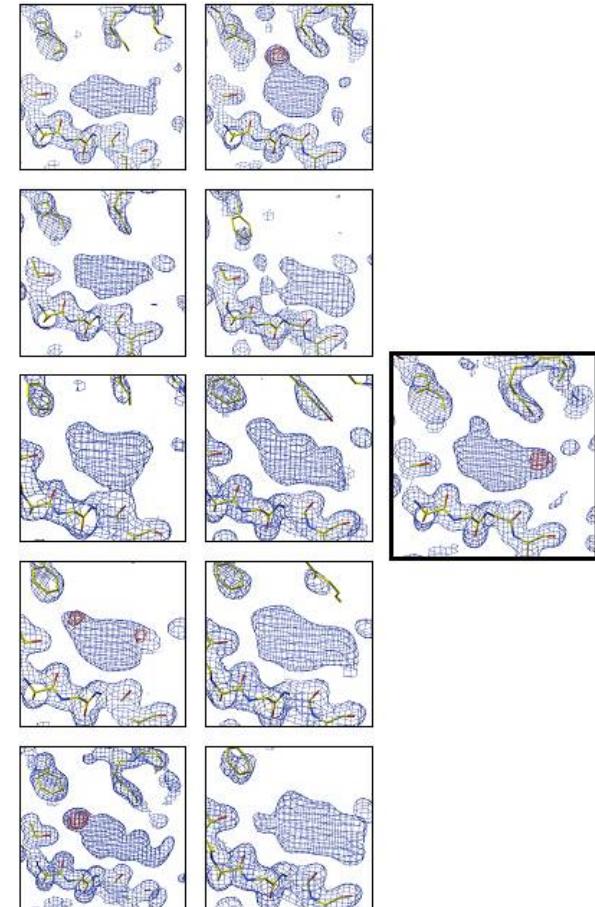
Multiple Targets



Multiple Binding Sites



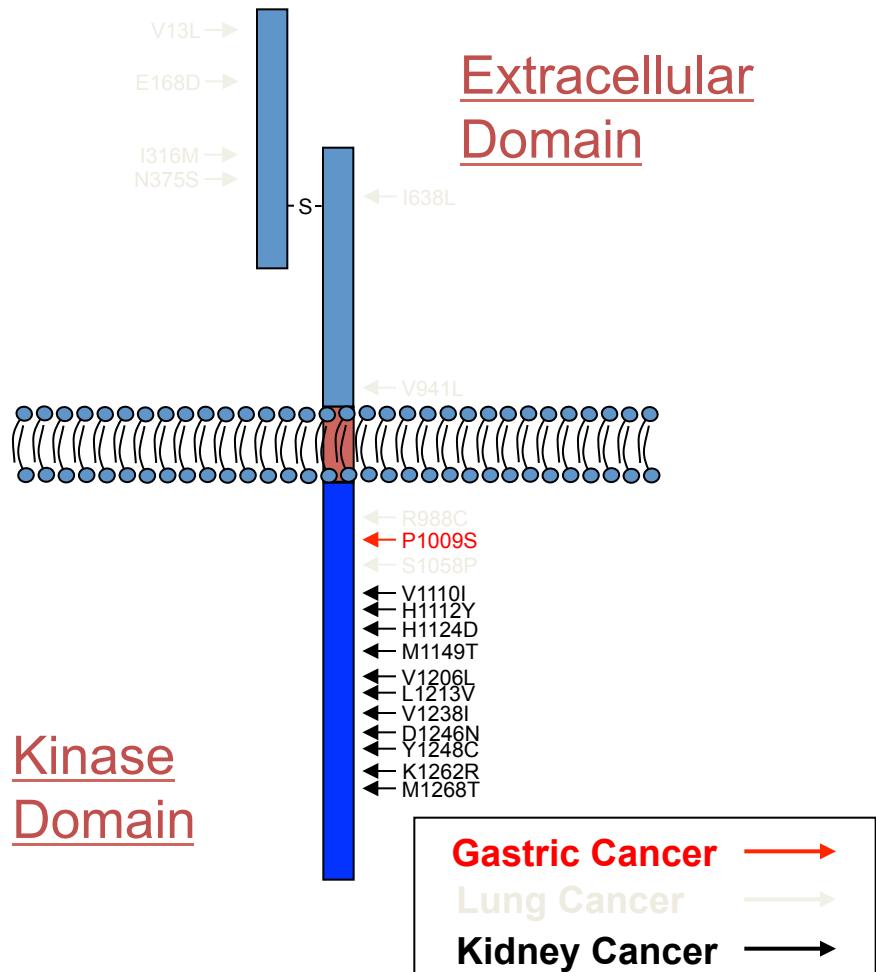
Multiple Chemotypes



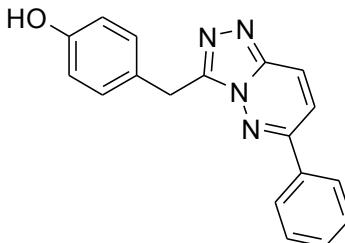
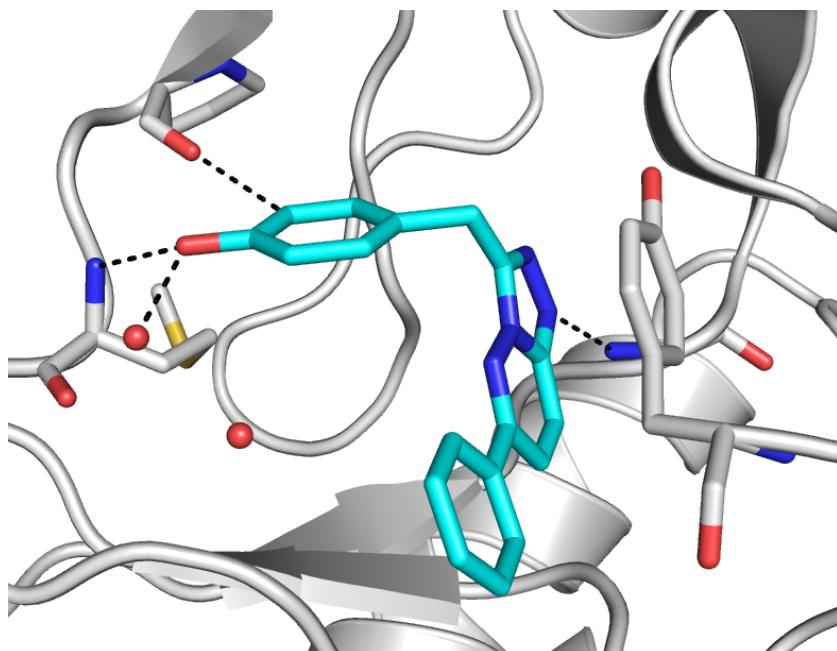
Wealth of Opportunities!

MET Receptor Tyrosine Kinase Inhibitor

- MET (HGFR) is the receptor for hepatocyte growth factor/scatter factor
- Activating mutations occur in various human cancers
 - Hereditary Papillary Renal Cell Carcinoma (HPRCC)
 - Sporadic, e.g., PRCC, non-small cell lung cancer (NSCLC)
 - Activating *MET* mutations have been observed in metastases
- MET gene amplification seen in other tumors
 - Gastric cancer
 - EGFR inhibitor-resistant lung cancer



Fragment Hit Evaluation/Lead Optimization

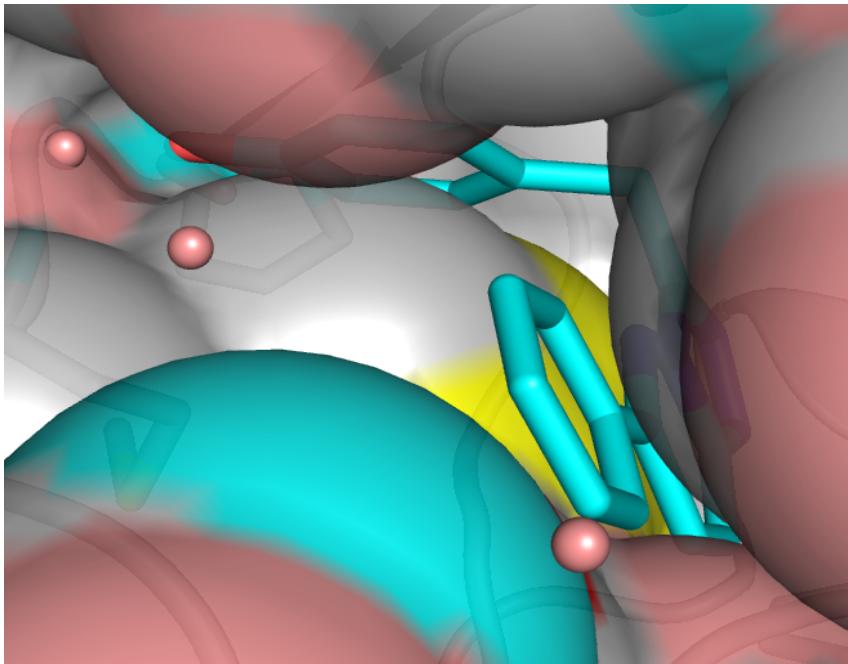


MET IC₅₀ = 712 nM
GTL16 IC₅₀ = 1.37 μM
MW = 302; LEAN = 0.28
cLogP 3.0; LLE = 3.1

**Good ligand efficiency AND
Novel binding mode**

R		
<chem>RCc1nc2ccccc2n1</chem>	<chem>c1ccsc1</chem>	<chem>c1ccncccc1</chem>
MET IC ₅₀ (nM)	156	114
GTL16 IC ₅₀ (nM)	558	179
MW	343	337
cLogP	3.5	3.6
LEAN	0.27	0.27
LLE	3.3	3.3

Achieving LEAN>0.27/LLE>5



Exploring the Cleft

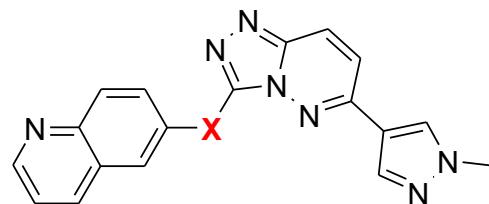
Goal: Reduce cLogP

**Design: Phenyl variants
and Heterocycles**

	R	
<chem>CN1C=NC2=C1C(=O)N(C)c3cc(F)ccc3C2</chem>	<chem>CC1=NC=NC2=C1Cc3cc(F)ccc3C2</chem>	
MET IC ₅₀ (nM)	25	61
GTL16 IC ₅₀ (nM)	14	151
MW	412	341
cLogP	2.5	1.5
LEAN	0.25	0.28
LLE	5.1	5.7

Reducing Clearance (<10mL/min/kg)

Benzylidic center known site of oxidation

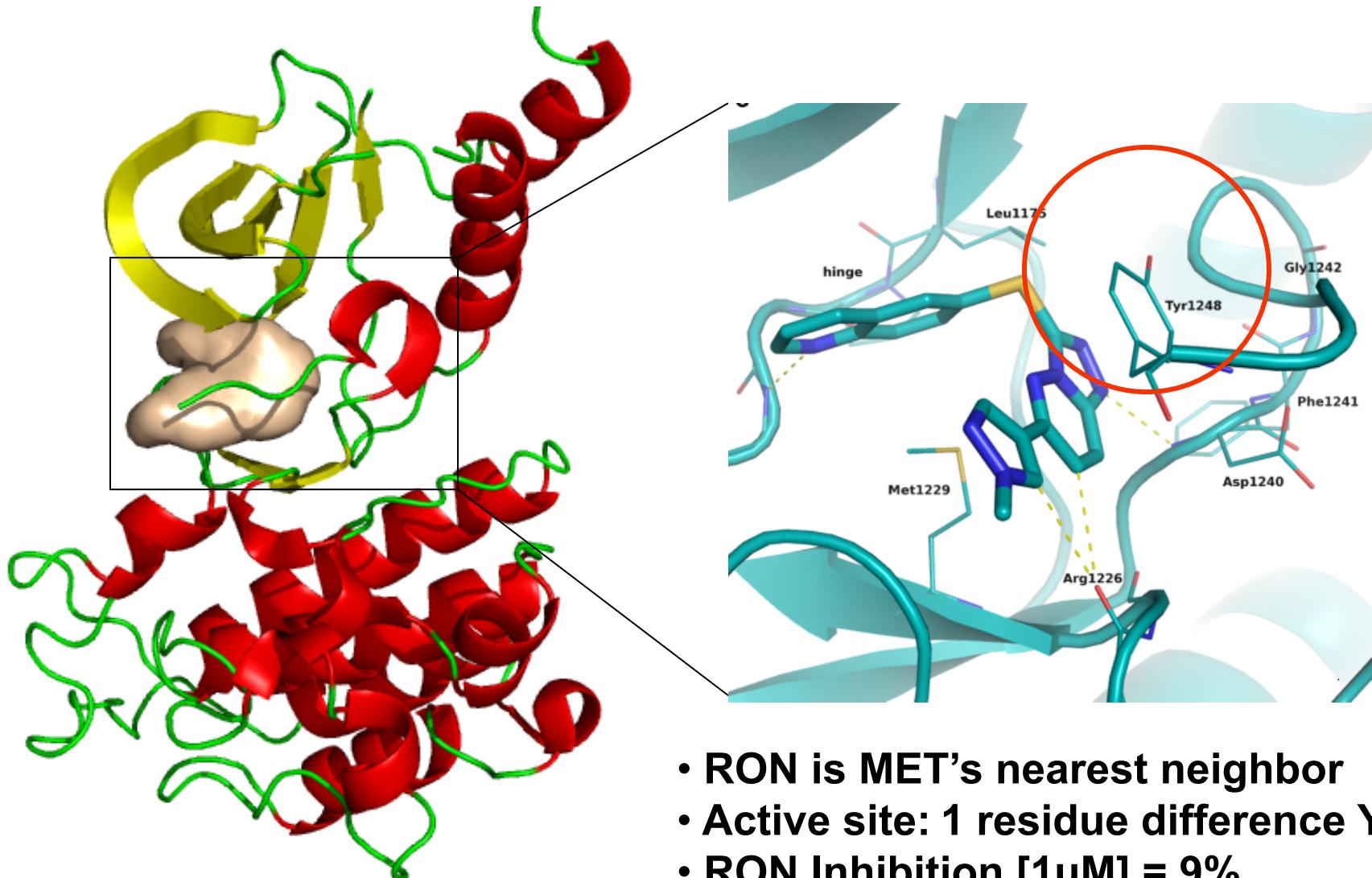


Compound	X	GTL16 IC ₅₀ (nM)	In vitro Cl (mL/min/kg)	T _{1/2} (hr)
	CH ₂	151	High	No Data
	CF ₂	20	5.1	2.2
	CH(CH ₃)	38	11	1.7
SGX523	S	23	7.2	2.7

SGX523: *In-vitro* Profiling

Enzyme Assay: IC ₅₀	SGX523
MET Cytoplasmic portion	4 nM
Cell Assay: IC ₅₀	
GTL16 cell proliferation (XTT) BaF3/TPR-MET (pMET ELISA) Cell assay specificity control	24 nM 17 nM > 10,000 nM
Liver Microsome Metabolism: T _{1/2}	
Rat Human	41 min 279 min
CYP Inhibition (IC ₅₀)	
1A2 2C9 2C19 2D6 3A4 hPXR 3A4 induction	> 10 µM 10 µM > 10 µM > 10 µM > 10 µM > 10 µM
Safety Pharmacology	
hERG AMES/Micronucleus	> 10 µM Neg./Neg.

SGX523: Unique Binding Mode



SGX523: Human Kinome Profiling I

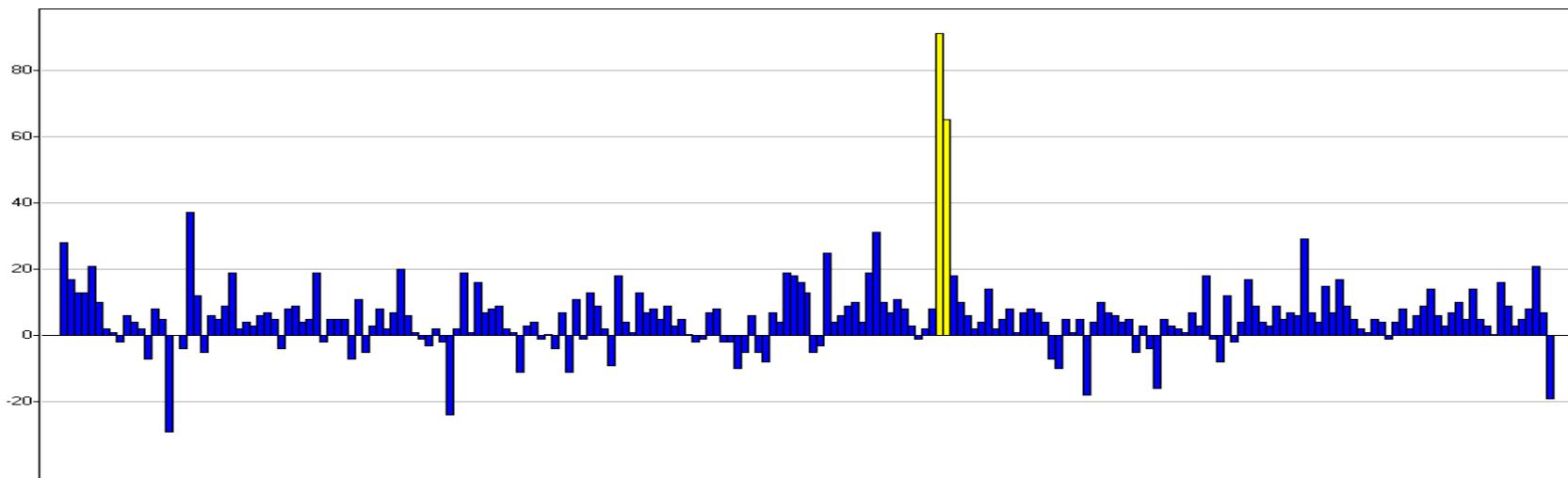
%Inhibition @ 1 μ M for 213 kinases

█ <50% Inhibition

█ >50% Inhibition

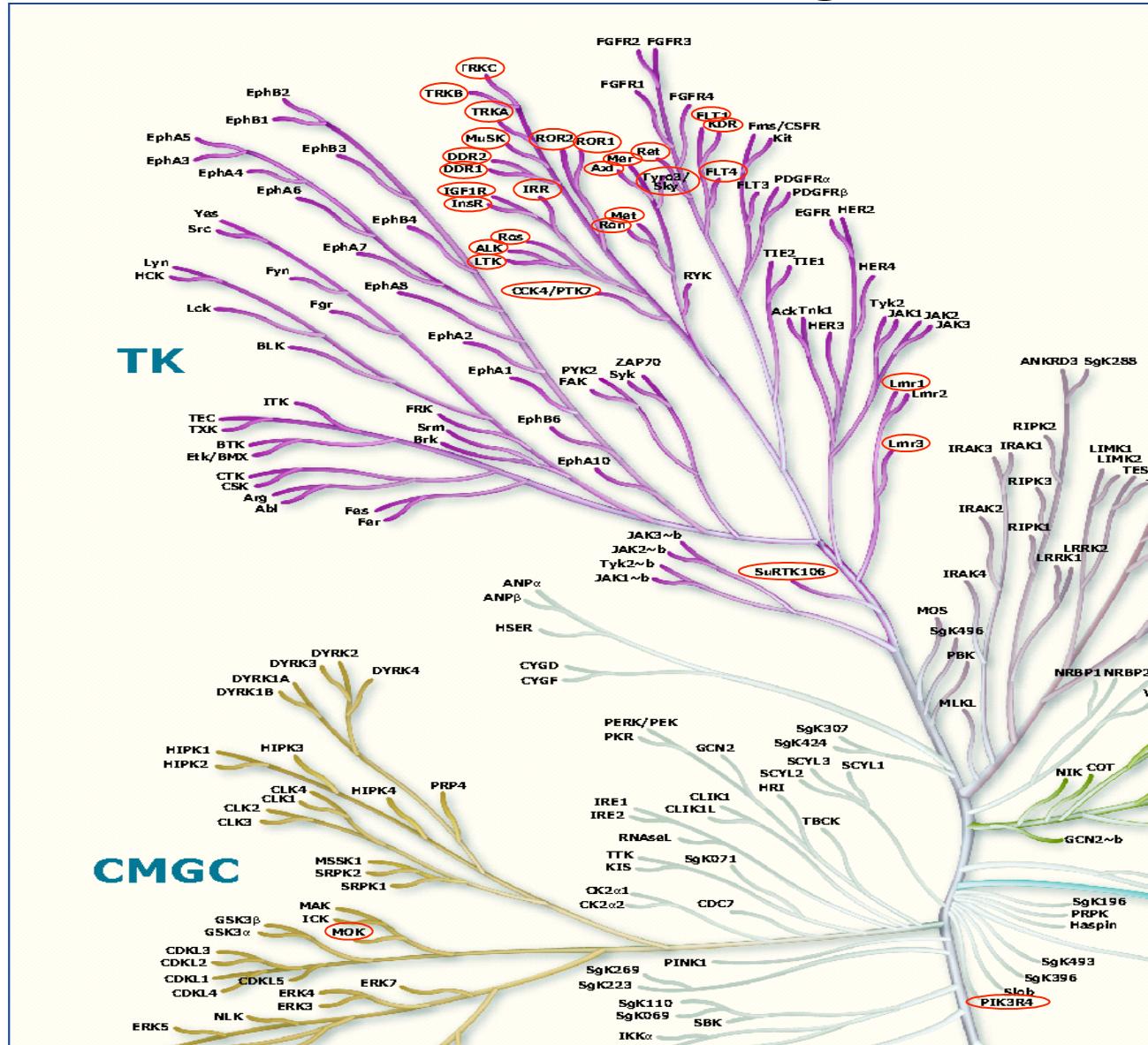
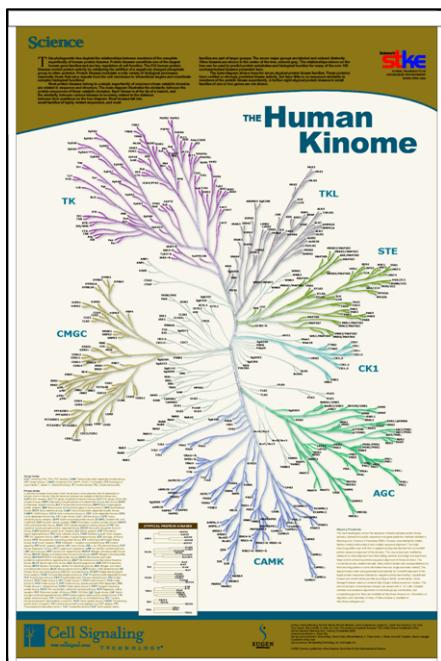
- MET and MET M1250T

Kinase Tested	% Inhibition (1 μ M SGX523)	IC ₅₀ (nM)
MET	92	4
BRAF V599E	36	>66,667
RAF1 (cRAF)	27	>66,667
ABL	26	>7,407
MAPK14 (p38 α)	25	>200,000
ABL1 Y253F	20	>7,407



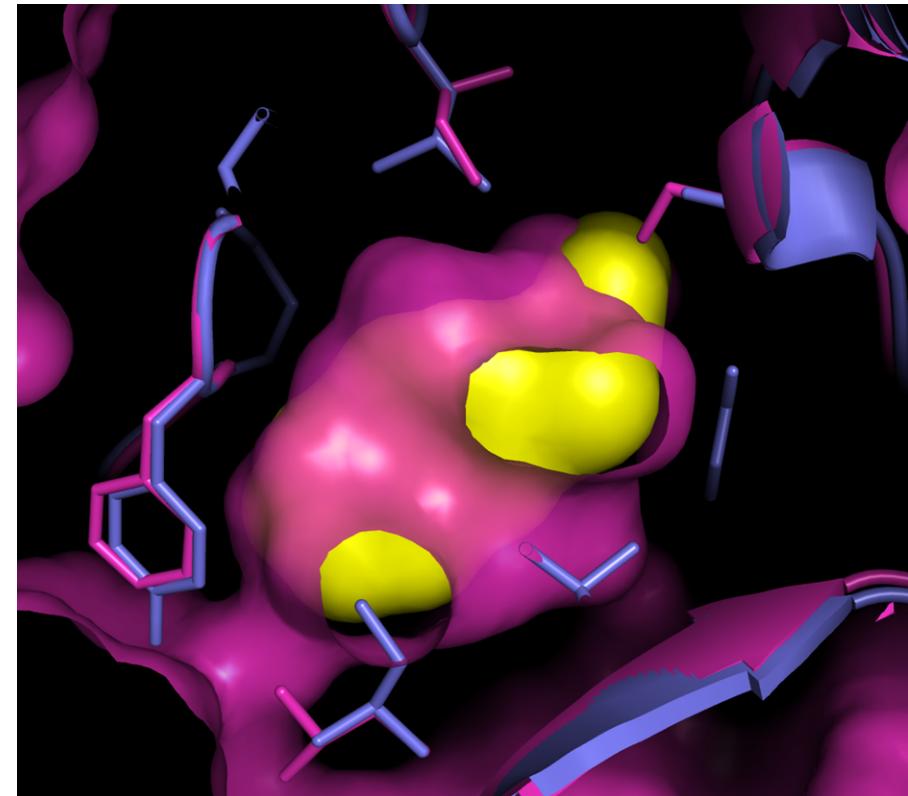
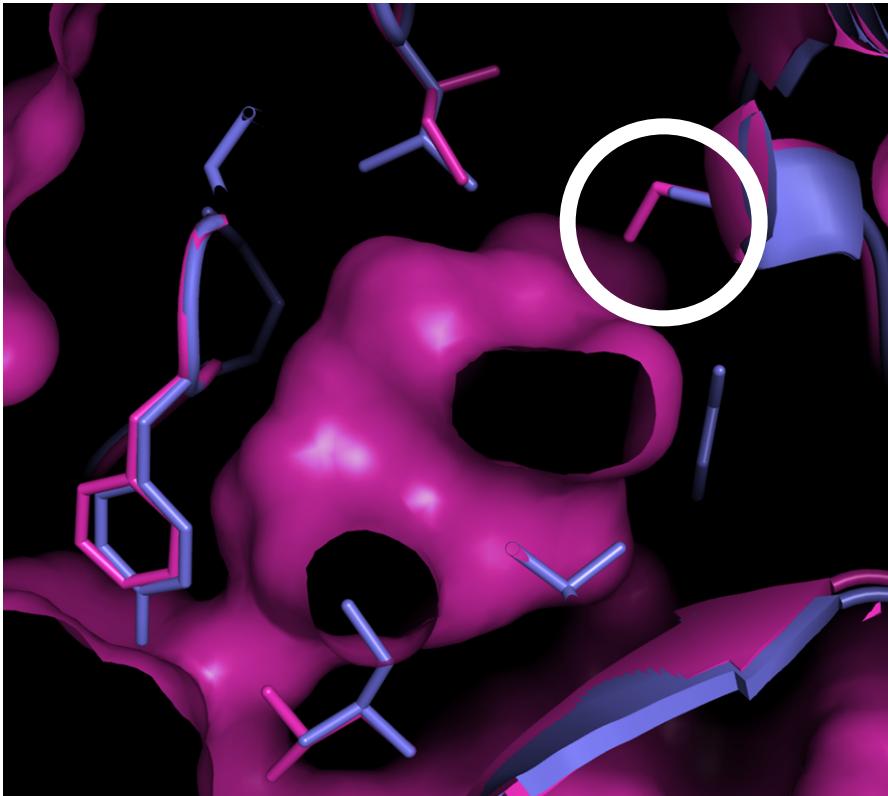
SGX523: Human Kinome Profiling II

- Active site residues in common



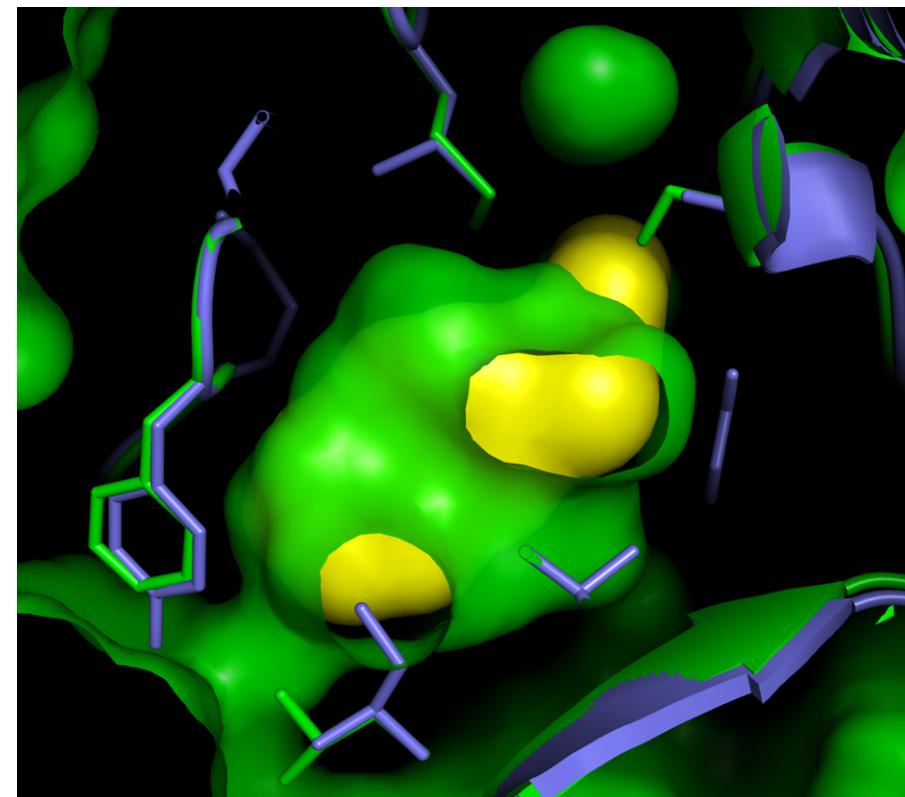
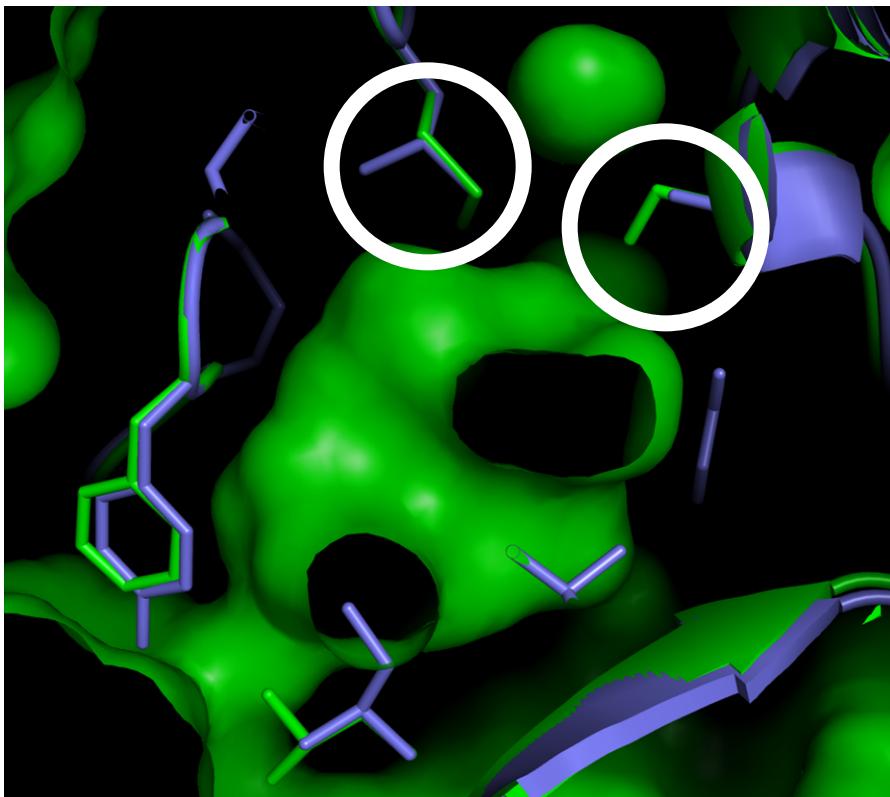
SGX523: MET versus MER

- MET (blue) inhibited by SGX523 (yellow)
- Homology model of MER (magenta)
- MET-Ala \rightarrow MER-Ser blocks binding



SGX523: MET versus AXL

- MET (blue) inhibited by SGX523 (yellow)
- Homology model of AXL (green)
- MET-Ala \rightarrow MER-Ser and MET-Leu \rightarrow AXL-Met block binding

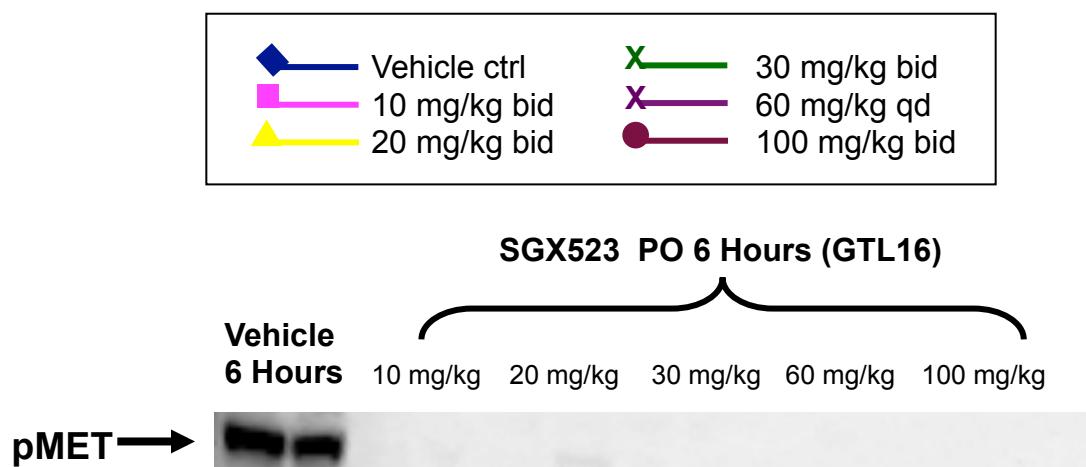
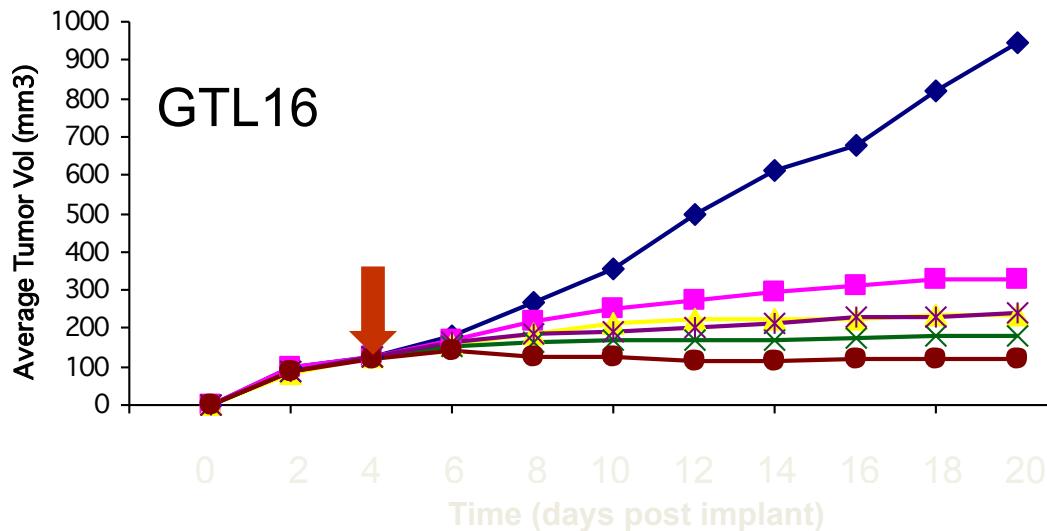
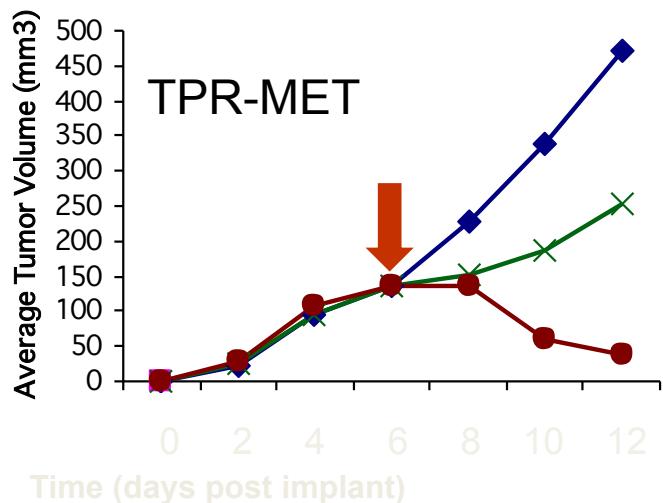


SGX523: *In vivo* Efficacy

Active in two mouse models

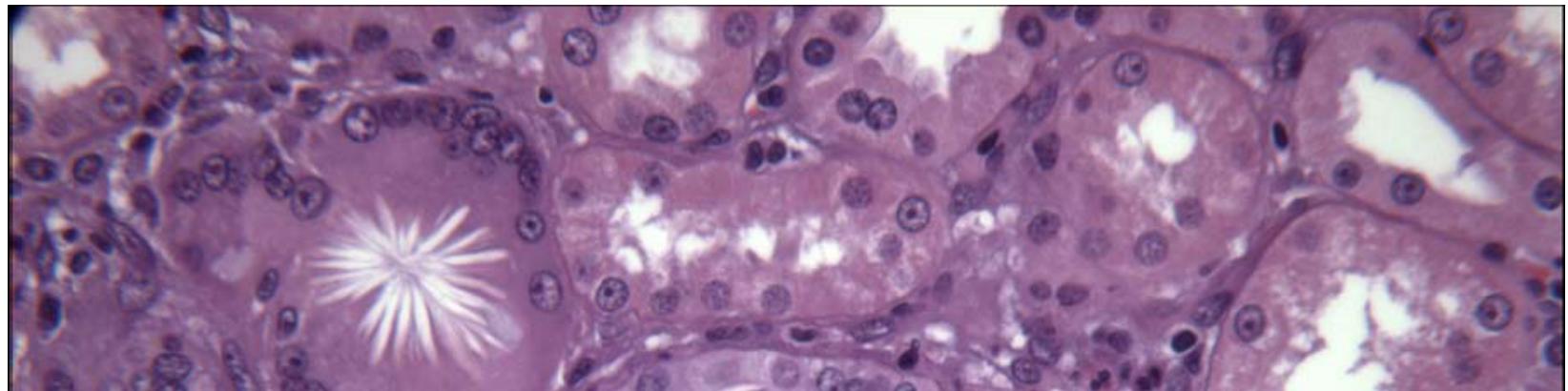
- Human gastric carcinoma cell xenograft (GTL16 cell implant)
- Activated MET xenograft (TPR-MET BaF3 cell implant)

Inhibition of pharmacodynamic marker (pMET)



SGX523: Phase I Clinical Outcome

- Lowest dose group tolerated 40 mg per day
- >80 mg dosing
 - Rapid onset of renal failure
 - Dosing suspended
 - Kidney function in affected patients restored
- Obstructive crystal nephropathy observed in monkeys



Representative photomicrograph of renal histology: Crystals observed

SGX MET Inhibitor Summary

- TZP Leads → LEAN>0.27, LLE>5
- SGX523
 - MET IC₅₀ = 4 nM
 - > 1000-fold selectivity for MET *versus* 211 human protein kinases
 - Human/primate metabolite → crystals in distal tubule → obstructive nephropathy
- Other SGX TZPs → Lilly
- TZP variant → Drug Candidate



Useful References

- Cavalli *et al.* (2002) *J. Med. Chem.* 45, 3844
- Fink *et al.* (2005) *Angew. Chem. Int. Ed.* 44, 1504
- Gleeson (2008) *J. Med. Chem.* 51, 817
- Henkel *et al.* (1999) *Angew. Chem. Int. Ed.* 38, 643
- Hopkins *et al.* (2004) *Drug Disc. Today* 9, 430
- Hughes *et al.* (2008) *BMCL* 18, 4872
- Johnson *et al.* (2009) *BMCL* 19, 5560
- Kelder *et al.* (1999) *Pharm. Res.* 16, 1514
- Kola and Landis (2004) *Nature Rev./Drug Disc.* 3, 711
- Leeson and Springthorp (2007) *Nature Rev./Drug Disc.* 6, 881
- Leeson and St-Gallay (2011) *Nature Rev./Drug Disc.* 10, 749
- Lipinski *et al.* (1997) *Adv. Drug Del. Rev.* 23, 3-25
- Lovering *et al.* (2009) *J. Med. Chem.* 52, 6752
- Paolini *et al.* (2006) *Nature Biotech.* 24, 805
- Ploemen *et al.* (2004) *Exp. Toxic Pathol.* 55, 347
- Price *et al.* (2009) *Expert Opin. Drug Metabol. Toxicol.* 5, 921
- Varma *et al.* (2010) *J. Med. Chem.* 53, 1098
- Waring (2009) *BMCL* 19, 2844
- Waring (2010) *Expert Opin. Drug Discov.* 5, 235
- Waring *et al.* (2006) *BMCL* 17, 1759
- Wenlock *et al.* (2003) *J. Med. Chem.* 46, 1250