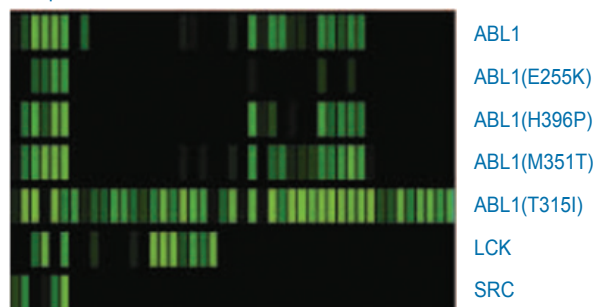


Mutant Kinase Disease Association [GUIDE]

Exploring Compound Selectivity in Mutant Kinases: Opportunities & Strategies

Differentiating Leads Based on Activity Against Related Targets

Compounds



Analysis of a chemical series against multiple related targets. The heatmap reveals that compounds may bind important mutant variants of a kinase with much higher affinity than the wild type version.

Binding Constants (nM) of Reference Kinase Inhibitors for Drug-resistant Kinase Variants

Kinase variant	Imatinib	Dasatinib	PD-180970	BIRB-796	VX-680	SU-11248	MLN-518
ABL1	2*	0.5	1	2,000*	20	1,000*	>10,000
ABL1(Q252H)	20*	1	2	4,000*	10	2,000*	>10,000
ABL1(Y253F)	40*	1	1	2,000*	20	700*	>10,000
ABL1(E255K)	100*	2	4	>10,000	50	>10,000	>10,000
ABL1(M351T)	10*	0.7	0.7	2,000*	8	500*	>10,000
ABL1(F359V)	20	0.3	1	8,000	20	1,000	7,000
ABL1(H396P)	60*	1	1	>10,000	7	900*	>10,000
ABL1(T315I)	6,000*	600	600	40*	5	200*	>10,000
ABL1(T315N)	>10,000	40	300	>10,000	100	400	>10,000
KIT(N822K)	3	0.4	4	200	100	3	5
KIT(V559D)	20	0.7	1	200	300	0.4	4
KIT(V559D,T670I)	3,000	>10,000	3,000	300	600	0.3	1,000

Data reveals VX-680 as a potent *in vitro* inhibitor of ABL1(T315I), commonly associated with imatinib, dasatinib, and nilotinib resistance in CML.

* Previously published binding constants. Table originally published in Carter, T.A. et al. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc Natl Acad Sci USA*. 102, 11011-11016 (2005).

Protein kinases play an integral role as mediators of multiple signal transduction pathways and are responsible for the modulation of essential cellular functions including cell proliferation and differentiation, metabolism, and apoptosis. Although many mechanisms have been elucidated by which kinases become uncoupled from normal cellular regulatory processes, mutations have been implicated as causative agents in a diverse range of diseases, including many forms of cancers. These same mechanisms can also confer resistance to therapeutic agents thereby rendering them ineffective and leading to relapse even after initial positive response is observed. These characteristics represent a significant hurdle to both the development of efficacious therapies, as well as the management of disease progression.

It is important to note that compounds can frequently be identified that bind mutant variants of a kinase with high affinity, but bind much weaker or not at all to the corresponding wild type kinase (e.g. BIRB-786 binds ABL1(T315I) with much higher affinity than wild type ABL1). This observation also suggests a strategy in which existing clinical compounds can be tested for binding and inhibition of important mutant variants to serve as novel therapeutic opportunities where resistance to first line inhibitors is observed as part of a multidrug therapy to delay or eliminate resistant kinase variants, or as starting points for next generation drugs.

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Mutant Kinase	Entrez Gene Symbol	Kinase Family	Sugen Gene Family	Significance	References
ABL1(E255K)	ABL1	TK	ABL1	Mutation confers imatinib-resistance in chronic myeloid leukemia (CML).	Lancet. 359, 487-491 (2002).
ABL1(F317I)	ABL1	TK	ABL1	Mutation confers dasatinib-resistance in cell culture.	Proc Natl Acad Sci USA. 102, 3395-3400 (2005); Blood. 108, 2332-2338 (2006).
ABL1(F317L)	ABL1	TK	ABL1	Mutation confers imatinib- and dasatinib-resistance in chronic myeloid leukemia (CML).	Blood. 100, 1014-1018 (2002); J Clin Oncol. 24, e51-52 (2006).
ABL1(H396P)	ABL1	TK	ABL1	Mutation confers imatinib-resistance in chronic myeloid leukemia (CML).	Lancet. 359, 487-491 (2002).
ABL1(M351T)	ABL1	TK	ABL1	Mutation confers imatinib-resistance in chronic myeloid leukemia (CML).	Blood. 100, 1014-1018 (2002)
ABL1(Q252H)	ABL1	TK	ABL1	Mutation confers imatinib-resistance in chronic myeloid leukemia (CML).	Cancer Cell. 2, 117-125 (2002).
ABL1(T315I)	ABL1	TK	ABL1	Mutation confers imatinib-, dasatinib- and nilotinib-resistance in chronic myeloid leukemia (CML).	Science. 293, 876-880 (2001); Lancet. 359, 487-491 (2002).
ABL1(Y253F)	ABL1	TK	ABL1	Mutation confers imatinib-resistance in chronic myeloid leukemia (CML).	Proc Natl Acad Sci USA. 99, 10700-10705 (2002).
BRAF(V600E)	BRAF	TKL	BRAF	Activating mutation found in malignant melanoma.	Nature. 417, 949-954 (2002).
EGFR(E746-A750del)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	N Engl J Med. 350, 2129-2139 (2004); Science. 304, 1497-1500 (2004); Proc Natl Acad Sci USA. 101, 13306-13311 (2004).
EGFR(G719C)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	N Engl J Med. 350, 2129-2139 (2004).
EGFR(G719S)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	Science. 304, 1497-1500 (2004).
EGFR(L747-E749del, A750P)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	Science. 304, 1497-1500 (2004).
EGFR(L747-S752del, P753S)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	Science. 304, 1497-1500 (2004).
EGFR(L747-T751del,Sins)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	N Engl J Med. 350, 2129-2139 (2004); Science. 304, 1497-1500 (2004).
EGFR(L858R)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	N Engl J Med. 350, 2129-2139 (2004); Science. 304, 1497-1500 (2004); Proc Natl Acad Sci USA. 101, 13306-13311 (2004).
EGFR(L858R,T790M)	EGFR	TK	EGFR	T790M mutation confers gefitinib- and erlotinib-resistance in non-small-cell lung cancer (NSCLC).	N Engl J Med. 352, 2136 (2005); PLoS Med. 2, e73 (2005).

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EGFR(L861Q)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	N Engl J Med. 350, 2129-2139 (2004).
EGFR(S752-I759del)	EGFR	TK	EGFR	EGFR inhibitor sensitizing mutation in non-small-cell lung cancer (NSCLC).	Science. 304, 1497-1500 (2004).
EGFR(T790M)	EGFR	TK	EGFR	T790M mutation confers gefitinib- and erlotinib-resistance in non-small-cell lung cancer (NSCLC).	Cancer Res. 66, 7854-7858 (2006).
FGFR3(G697C)	FGFR3	TK	FGFR3	Activating mutation found in oral squamous cell carcinoma.	Int J Cancer. 117, 166-168 (2005).
FLT3(D835H)	FLT3	TK	FLT3	Activating mutation found in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).	Blood. 97, 2434-2439 (2001); Br J Haematol. 113, 983-988 (2001).
FLT3(D835Y)	FLT3	TK	FLT3	Activating mutation found in acute myeloid leukemia (AML).	Blood. 97, 2434-2439 (2001); Br J Haematol. 113, 983-988 (2001).
FLT3(ITD)	FLT3	TK	FLT3	Activating mutation found in acute myeloid leukemia (AML). ITD is an activating Internal Tandem Duplication in the juxtamembrane domain.	Leukemia. 10, 1911-1918 (1996).
FLT3(K663Q)	FLT3	TK	FLT3	Activating mutation found in acute myeloid leukemia (AML).	Leukemia. 20, 2008-2014 (2006).
FLT3(N841I)	FLT3	TK	FLT3	Activating mutation found in acute myeloid leukemia (AML).	Blood. 104, 1855-1858 (2004).
FLT3(R834Q)	FLT3	TK	FLT3	Activating mutation found in acute myeloid leukemia (AML).	Cancer Cell. 12, 501-513 (2007).
KIT(A829P)	KIT	TK	KIT	A829P mutation confers imatinib-resistance in gastrointestinal stromal tumors (GIST).	J. Clin. Oncol. 24, 4764-2774 (2006).
KIT(D816H)	KIT	TK	KIT	Activating mutation found in systemic mastocytosis, D816H confers imatinib-resistance in gastrointestinal 1542-stromal tumors (GIST).	Oncogene 20, 4528-4536 (2001); Am J Pathol 163, 305-313 (2004); Proc Natl Acad Sci USA 106, 1547 (2009).
KIT(D816V)	KIT	TK	KIT	Activating mutation found in systemic mastocytosis and acute myeloid leukemia. Imatinib resistant	Nature Gen. 12, 312-314 (1996); Blood 107, 3463-3468 (2006).
KIT(L576P)	KIT	TK	KIT	Mutation found in malignant melanoma.	Clin Cancer Res. 14 6821-6828 (2008).
KIT(V559D)	KIT	TK	KIT	Activating mutation found in gastrointestinal stromal tumors (GIST).	Clin. Cancer Res. 11, 3668-3677 (2005).
KIT(V559D,T670I)	KIT	TK	KIT	T670I mutation confers imatinib-resistance in gastrointestinal stromal tumors (GIST).	Clin. Cancer Res. 11, 4182-4190 (2005).
KIT(V559D,V654A)	KIT	TK	KIT	V654A mutation confers imatinib-resistance in gastrointestinal stromal tumors (GIST).	Clin. Cancer Res. 11, 4182-4190 (2005).

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Mutant Kinase	Entrez Gene Symbol	Kinase Family	Sugen Gene Family	Significance	References
LRRK2(G2019S)	LRRK2	TKL	TKL	Activating mutation found in Parkinson's disease.	Proc Natl Acad Sci USA. 102, 16842-16847 (2005).
MET(M1250T)	MET	TK	MET	Mutation found drug-resistant papillary renal carcinomas.	Proc Natl Acad Sci USA. 95, 14379-14383 (1998); Oncogene. 20, 5493-5502 (2001).
MET(Y1235D)	MET	TK	MET	Activating mutation found in lymph node metastases of head and neck squamous-cell carcinomas.	Oncogene. 19, 1547-1555 (2000); Biochemistry. 44, 14110-14119 (2005).
PIK3CA(C420R)	PIK3CA	LIPID		Activating mutation found in diverse cancers.	Cancer Res. 65, 4562-4567 (2005); Proc Natl Acad Sci USA. 104, 5569-5574 (2007).
PIK3CA(E542K)	PIK3CA	LIPID		Activating mutation found in diverse cancers.	Proc Natl Acad Sci USA. 103, 1475-1479 (2006); Proc Natl Acad Sci USA. 104, 5569-5574 (2007).
PIK3CA(E545A)	PIK3CA	LIPID		Activating mutation found in diverse cancers.	Proc Natl Acad Sci USA. 104, 5569-5574 (2007).
PIK3CA(E545K)	PIK3CA	LIPID		Activating mutation found in diverse cancers.	Proc Natl Acad Sci USA. 104, 5569-5574 (2007).
PIK3CA(I800L)	PIK3CA	LIPID		A potential hotspot for resistance mutations.	Cancer Cell. 14, 180-192 (2008). Resistant to PI-103 but sensitized to NVP-BEZ-235.
PIK3CA(H1047L)	PIK3CA	LIPID		Activating mutation found in diverse cancers.	Proc Natl Acad Sci USA. 104, 5569-5574 (2007).
PIK3CA(H1047Y)	PIK3CA	LIPID		Activating mutation found in diverse cancers.	Proc Natl Acad Sci USA. 104, 5569-5574 (2007).
PIK3CA(M1043I)	PIK3CA	LIPID		Activating mutation found in diverse cancers.	Cancer Res. 65, 4562-4567 (2005).
PIK3CA(Q546K)	PIK3CA	LIPID		Activating mutation found in diverse cancers.	Proc Natl Acad Sci USA. 104, 5569-5574 (2007).
RET(M918T)	RET	TK	RET	Activating mutation found in multiple endocrine neoplasias and familial medullary thyroid carcinomas.	Cancer Res. 63, 5559-5563 (2003); Cancer Res. 66, 10741-10749 (2006).
RET(V804L)	RET	TK	RET	Activating mutation found in multiple endocrine neoplasias and familial medullary thyroid carcinomas.	Oncogene. 23, 6056-6063 (2004).
RET(V804M)	RET	TK	RET	Activating mutation found in multiple endocrine neoplasias and familial medullary thyroid carcinomas.	Oncogene. 23, 6056-6063 (2004).

Kinase Family
LIPID Lipid kinases
TK Tyrosine kinases
TKL Tyrosine kinase-like



Kinases: conformationally dynamic proteins

Kinase conformational equilibria are often governed by the phosphorylation of key residues in regulatory elements, including the activation loop. Activation loop phosphorylation can shift the equilibrium to favor a “catalytically active-like” state characterized by a “DFG-in” structure at the loop’s N-terminus. Importantly, inhibitor binding can be conformation-specific and thus affected by the activation/phosphorylation state. For example, the ABL inhibitor Imatinib, which recognizes an “inactive DFG-out” kinase conformation, binds with high affinity to nonphosphorylated ABL but with reduced affinity to activated ABL phosphorylated on the activation loop.

Inhibitor classification and binding mode

The majority of ATP-competitive kinase inhibitors are classified as either Type I or Type II. Although both Type I and II inhibitors generally contact the ATP binding site, only Type II inhibitors access an “allosteric site” unmasked in the inactive-DFG-out conformation. Consequently, Type II inhibitor binding can be significantly more activation state-dependent than Type I inhibitor binding. Examples of Type I and Type II inhibitors are listed in Table 1.

Characterization of compound binding mode

Activation state-sensitive (e.g. Type II) and insensitive inhibitors (e.g. Type I) are embodiments of related but distinct paradigms for ATP-competitive kinase inhibition. The binding mode can impact several key parameters in drug discovery, including enzyme inhibition kinetics, offsets between *in vitro* and cellular potency, nearest neighbor & kinome-wide selectivity, on target residence time & pharmacodynamics, interactions with upstream and downstream signaling molecules, and intellectual property position. Since the optimal inhibition paradigm is likely to be target-specific, it is essential to understand the binding mode of multiple leads at program outset and during optimization. Furthermore, if the optimal binding mode is unknown *a priori*, a strategy to pursue two lead series with distinct binding modes can de-risk early lead selection decision making. However, binding mode determination can be difficult, time consuming, and expensive, often requiring the use of x-ray crystallography or *in silico* modeling.

KINOMEscan now offers *scanMODE*, a novel biochemical tool that can simplify inhibitor binding mode elucidation. Comprised of a panel of phosphorylated/nonphosphorylated ABL assay pairs,

Table 1. Select Type I and Type II kinase inhibitors

Drug	Inhibitor Type	Primary Kinase Target	Status
Imatinib	II	ABL1	FDA-Approved
Nilotinib	II	ABL1	FDA-Approved
Dasatinib	I	ABL1	FDA-Approved
Sorafenib	II	VEGFR2	FDA-Approved
Gefitinib	I	EGFR	FDA-Approved
Erlotinib	I	EGFR	FDA-Approved

scanMODE

a novel biochemical tool employing phosphorylated/nonphosphorylated ABL assay pairs to elucidate compound binding mode. An ideal tool for understanding how kinase phosphorylation state affects inhibitor affinity.

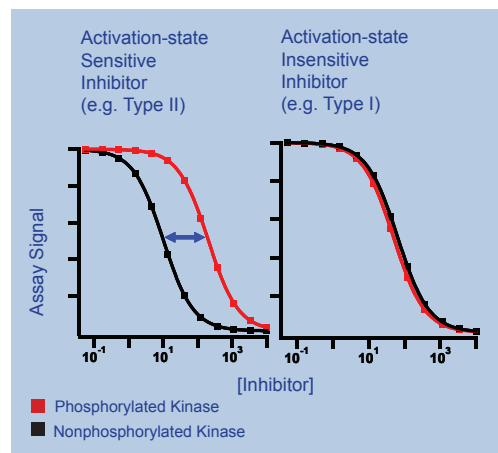


Figure 1. *scanMODE* explores inhibitor binding mode by measuring phosphorylation state-dependent affinity changes.

Key reference: Liu, Y and Gray, N.S. (2006) Rational design of inhibitors that bind to inactive kinase conformations. *Nat. Chem. Biol.* 2, 358-364.

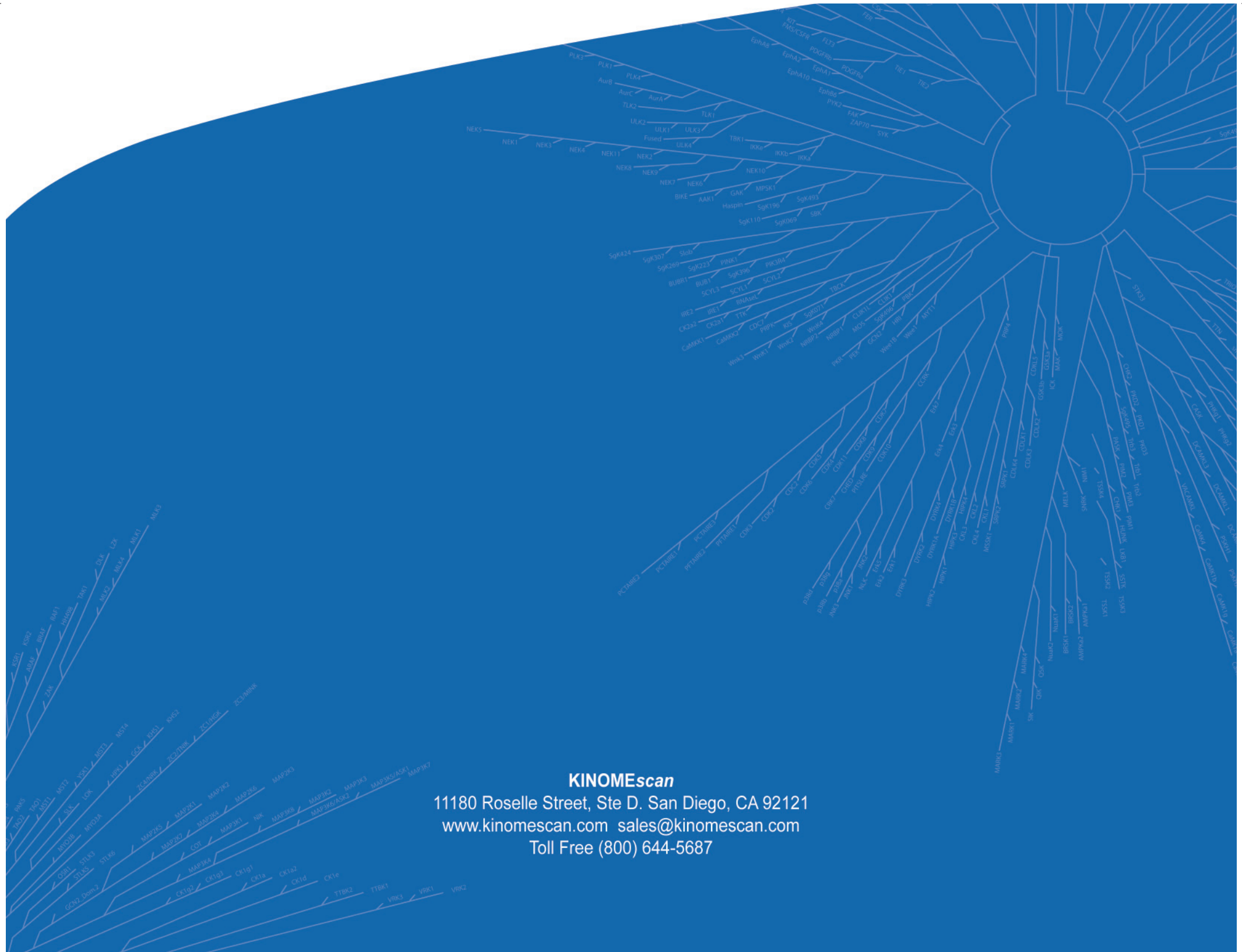
scanMODE capitalizes on two key observations: a) an inhibitor's binding mode is maintained across kinases (e.g. Imatinib is a Type II ABL inhibitor and a Type II LCK inhibitor) and b) a significant fraction of kinase inhibitors have off-target affinity for ABL and/or clinically relevant ABL mutants. Taken together, these observations enable the use of ABL assay pairs to serve as surrogates to study inhibitor binding mode.

Model data are presented in Figure 1. Binding curves for an activation state-sensitive inhibitor measured for phosphorylated and nonphosphorylated ABL show a significant affinity preference for the nonphosphorylated version (left panel), whereas an activation state-insensitive inhibitor does not distinguish between the phosphorylated and nonphosphorylated versions (right panel).

scanMODE Panel

12 Kinases (released May 2009)

Ambit Gene Symbol	Entrez Gene Symbol	Accession Number
ABL1-nonphosphorylated	ABL1	NP_005148.2
ABL1-phosphorylated	ABL1	NP_005148.2
ABL1(F317I)-nonphosphorylated	ABL1	NP_005148.2
ABL1(F317I)-phosphorylated	ABL1	NP_005148.2
ABL1(F317L)-nonphosphorylated	ABL1	NP_005148.2
ABL1(F317L)-phosphorylated	ABL1	NP_005148.2
ABL1(H396P)-nonphosphorylated	ABL1	NP_005148.2
ABL1(H396P)-phosphorylated	ABL1	NP_005148.2
ABL1(Q252H)-nonphosphorylated	ABL1	NP_005148.2
ABL1(Q252H)-phosphorylated	ABL1	NP_005148.2
ABL1(T315I)-nonphosphorylated	ABL1	NP_005148.2
ABL1(T315I)-phosphorylated	ABL1	NP_005148.2



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