Global Medicinal Chemistry and GPCR Summit

London, 28 November 2017

The systematic use of highly profiled antitumor agents in probing the sensitivity contexts of cancer cells and assessing the validity of target combinations

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Nerviano Medical Sciences S.r.l. (NMS)

• Nerviano Medical Sciences (NMS) is a research-based Italian company dedicated to the discovery and development of new drugs for the treatment of cancer.

• NMS is the Drug Discovery branch of NMS Group.

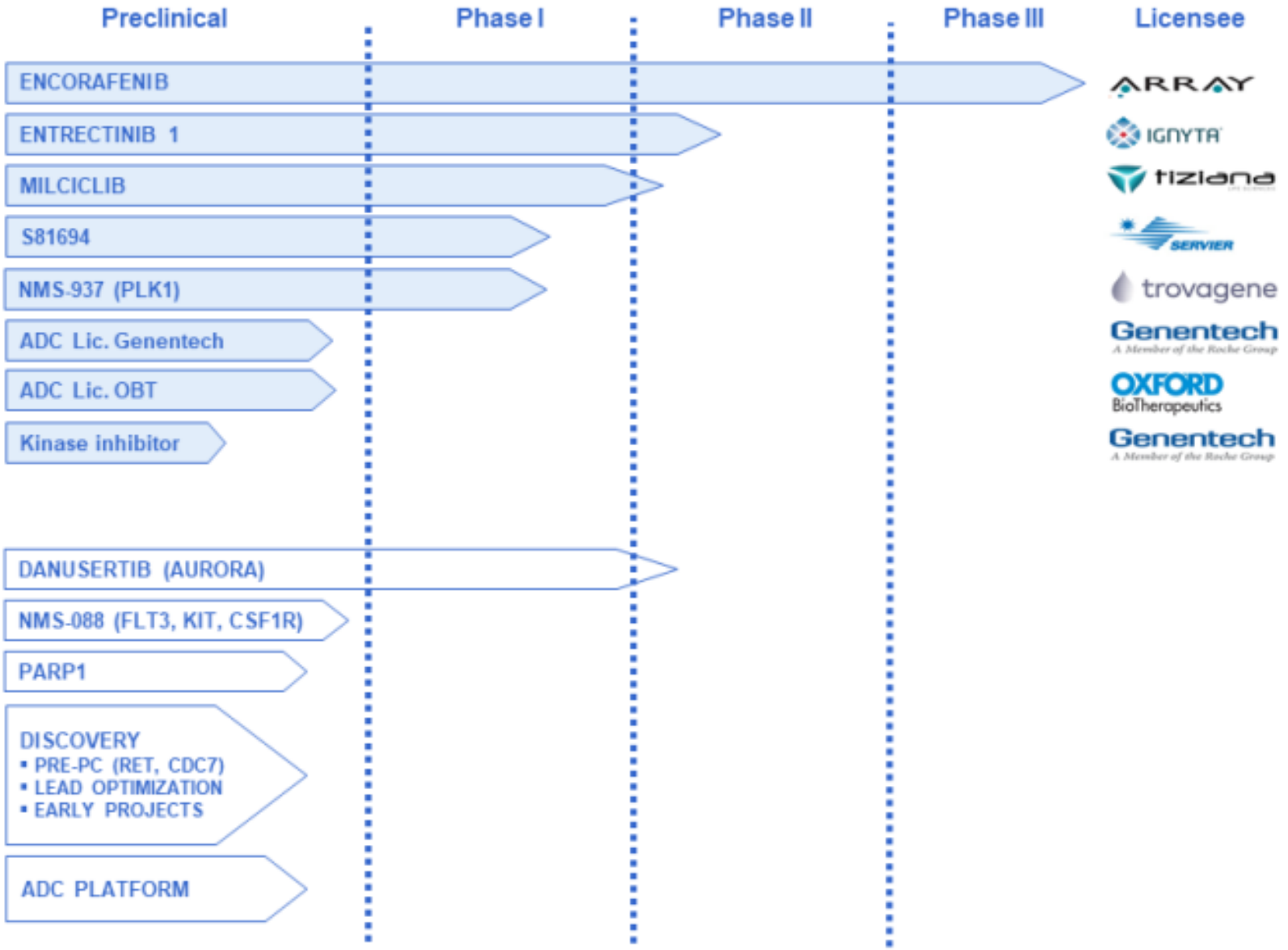
• NMS Group affiliates provide preclinical development, manufacturing and clinical CRO services to Academia, Hospitals, Biotechs and Pharmaceutical Companies wolrdwide.

Preclinical development

Pharmaceutical development
Manufacturing

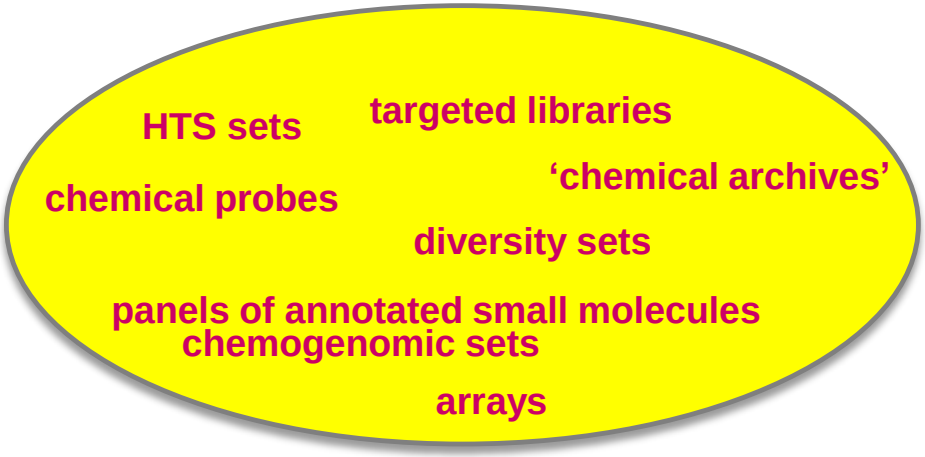
Clinical development

Drug Discovery Pipeline



Outline

- Oncology complexity
- The Purinome Platform *(targets, compounds)*
- Chemical Collections, compound annotations
  - Public
    - commercial
    - shared
    - open innovation
  - Proprietary



- Bioactivity profiling, Dissection of target involvements
  - biochemical
  - cell based

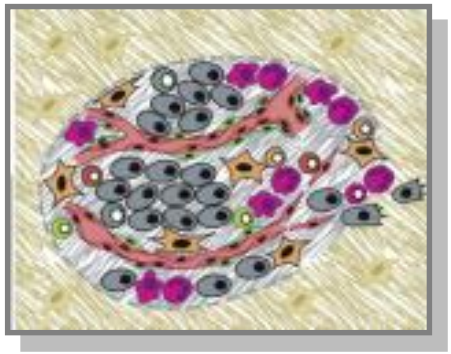
integration of 'tools', from crystal structures to chemical probes to patient derived tissues

- Applications
  - MELK relevance in carcinomas
  - Chordoma targets

Oncology complexity

- Heterogeneity and variability embedded in cancer cells
- The complexity applies also to the tumor microenvironment, *i.e.* the supportive and interactive stroma
- The redundancy in proliferative signaling pathways variably limits the efficacy of targeted therapies in different patient populations
- Emergence of secondary resistance to growth inhibitory drugs variably limits the efficacy as well (genetic drift → limited duration of clinical benefit)

Driving forces in cancer cells



Complementary forces from stromal cell constituents

intra-tumoral metastatic variations

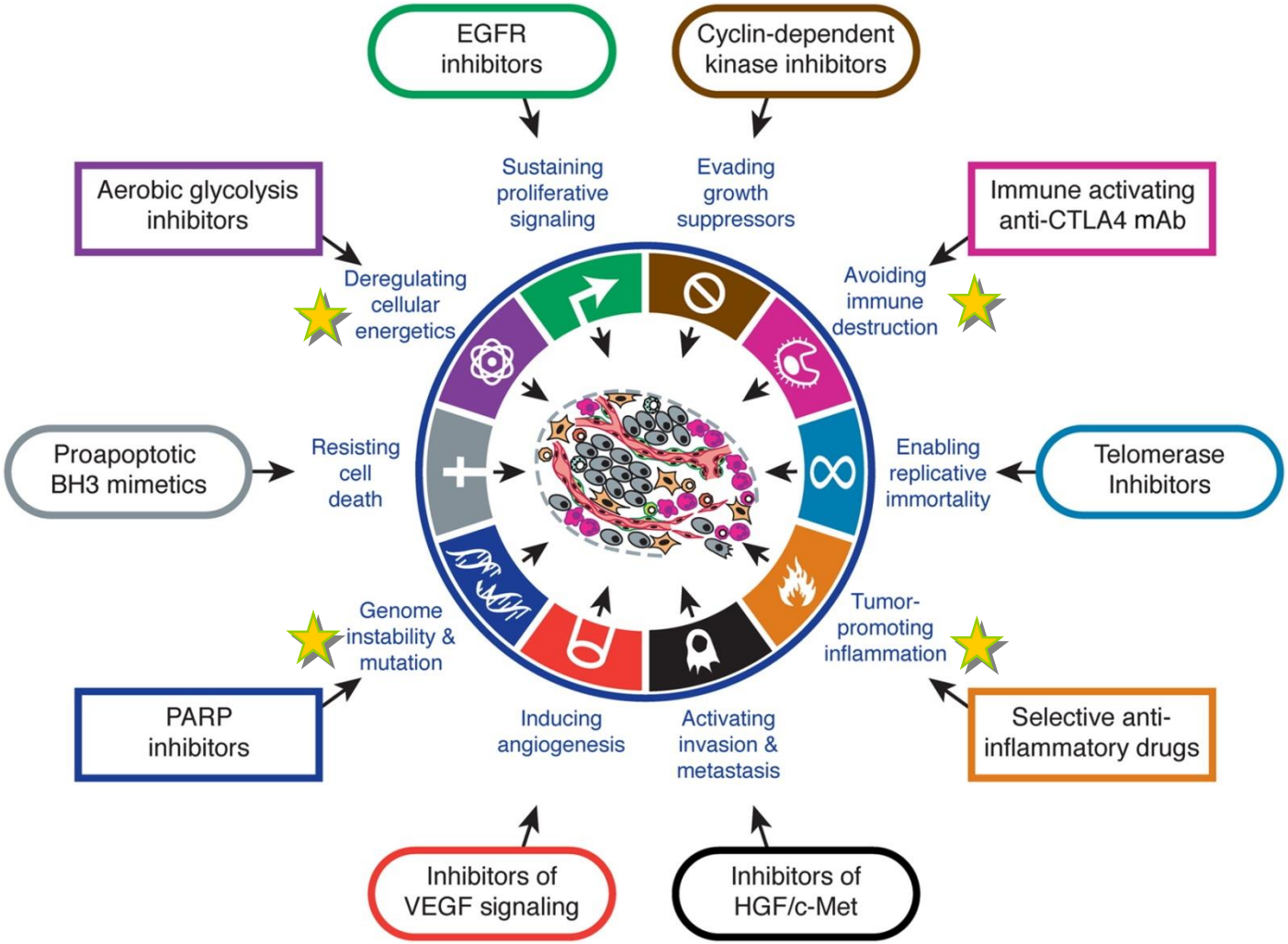
Tumor-antagonizing vs. –promoting cell types

Signaling molecules play a critical role in these processes; several are now recognized as therapeutic targets

Cells usually need to accrete several cancer-promoting, or oncogenic, mutations in separate genes to acquire the hallmark properties of malignancy

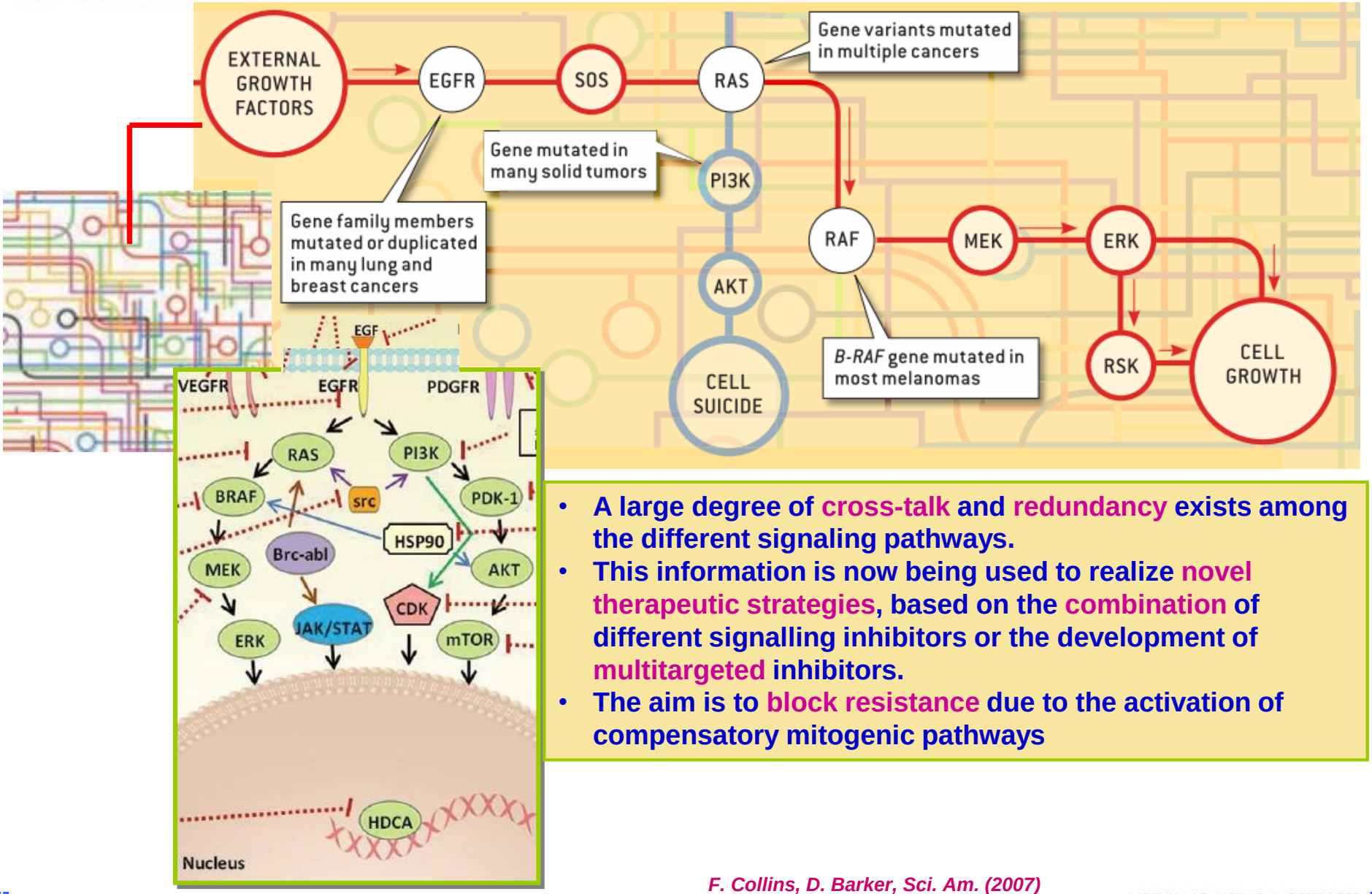
M. De Palma, D. Hanahan, Mol. Oncol. 6, 111 (2012)

Targeting the expanded set of cancer hallmarks



D. Hanahan, R.A. Weinberg, Cell 2011 144, 646-674

Signaling networks regulate the cancer cell



- A large degree of cross-talk and redundancy exists among the different signaling pathways.
- This information is now being used to realize novel therapeutic strategies, based on the combination of different signalling inhibitors or the development of multitargeted inhibitors.
- The aim is to block resistance due to the activation of compensatory mitogenic pathways

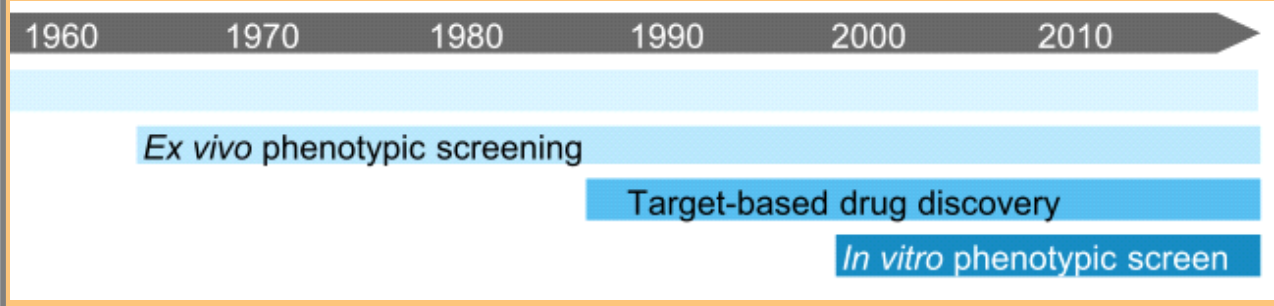
F. Collins, D. Barker, Sci. Am. (2007)

Emphasis on molecular targets in spite of new atypical therapeutic modalities

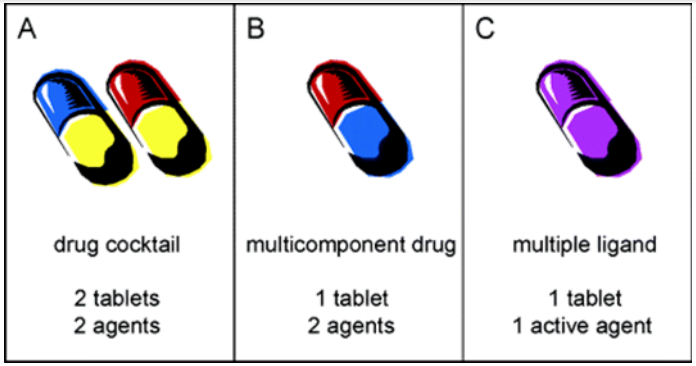
- Immune system manipulation
- Bacteriotherapy
- Stem cell therapy
- RNA therapeutics
- Gene therapy
- Tissue-engineered products
- Electroceuticals
- Oncolytic virotherapy

Lack of validated targets in some disease areas

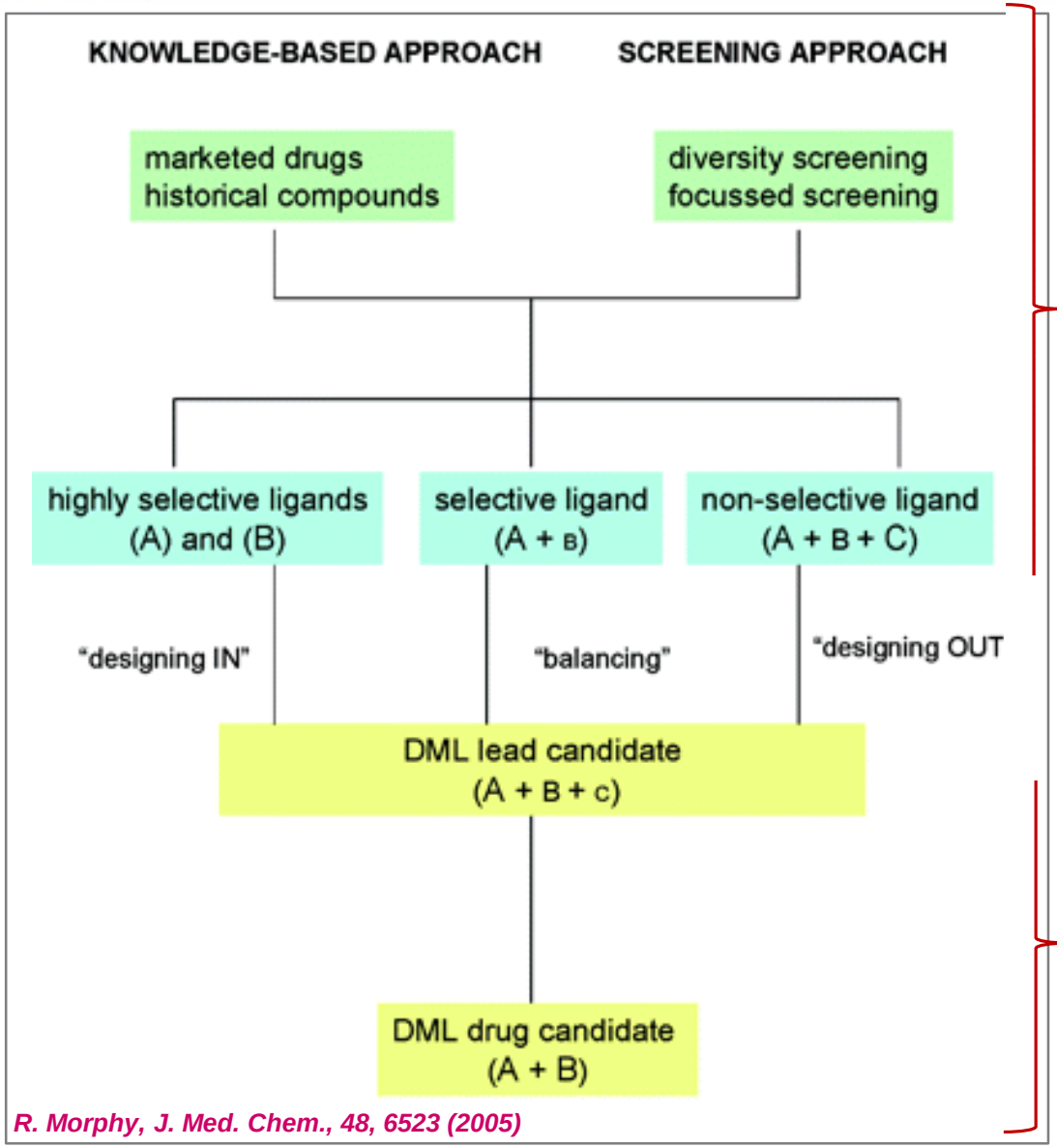
renewed interest in phenotypic screening



- **Include polypharmacology as a drug development option in the early phases**
- **Challenge the reductionist 'one-target, one-disease' approach**



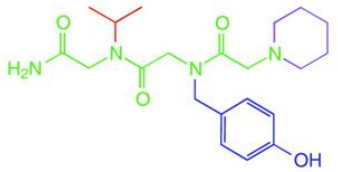
Designed or evolutionary optimization of ligands



R. Morphy, J. Med. Chem., 48, 6523 (2005)

readily approachable

lead generation phase



SMILES: NC(=O)CN(C(C)C)C(=O)CN(Cc1ccc(O)cc1)C(=O)CN2CCCCC2

Decimal: 1 2 1 88 1 101

Binary: 1001 1110 1111

Cyclic: ~~1~~001 1110 1111 01–

challenging

Designed Multiple Ligand (DML) or …
Selected by evolutionary approaches?

lead optimisation phase

Multi-component reactions and evolutionary chemistry encoding molecules from a combinatorial library and applying the genetic algorithm

Purinome Targets

Purinome targets are widely diversified in terms of their function, phylogenetic origins and structural architecture

Kinases

Non-Kinase targets

Is there a similarity of the purine binding site among the different purinome members, sufficient to design a common chemistry?

- ATP- , GTP- , NAD-dependent enzymes
- Bind ligands possessing a purine substructure
- Modes of binding and the sites of interaction may vary considerably
- Interaction with phosphate groups of ATP/ADP is dominant in certain ATPases
- Need for new, purinome-targeted libraries (**PTL**), including diversified ATP-mimicking designs
- Kinase Targeted Libraries (KTL) are viewed as a subset of PTL, without implying a reduction of their important role in drug discovery projects

The Purinome addressed with a Chemical Biology approach

Objectives

- Target (identify) pathway components **that drive a defined set of cancers** and contribute to cancer growth
- Target (identify) mechanisms that support the oncogenic process or represent a vulnerability that can be exploited through synthetic lethality
- Discover bioactive New Chemical Entities with drug development potential

Purinome Assets

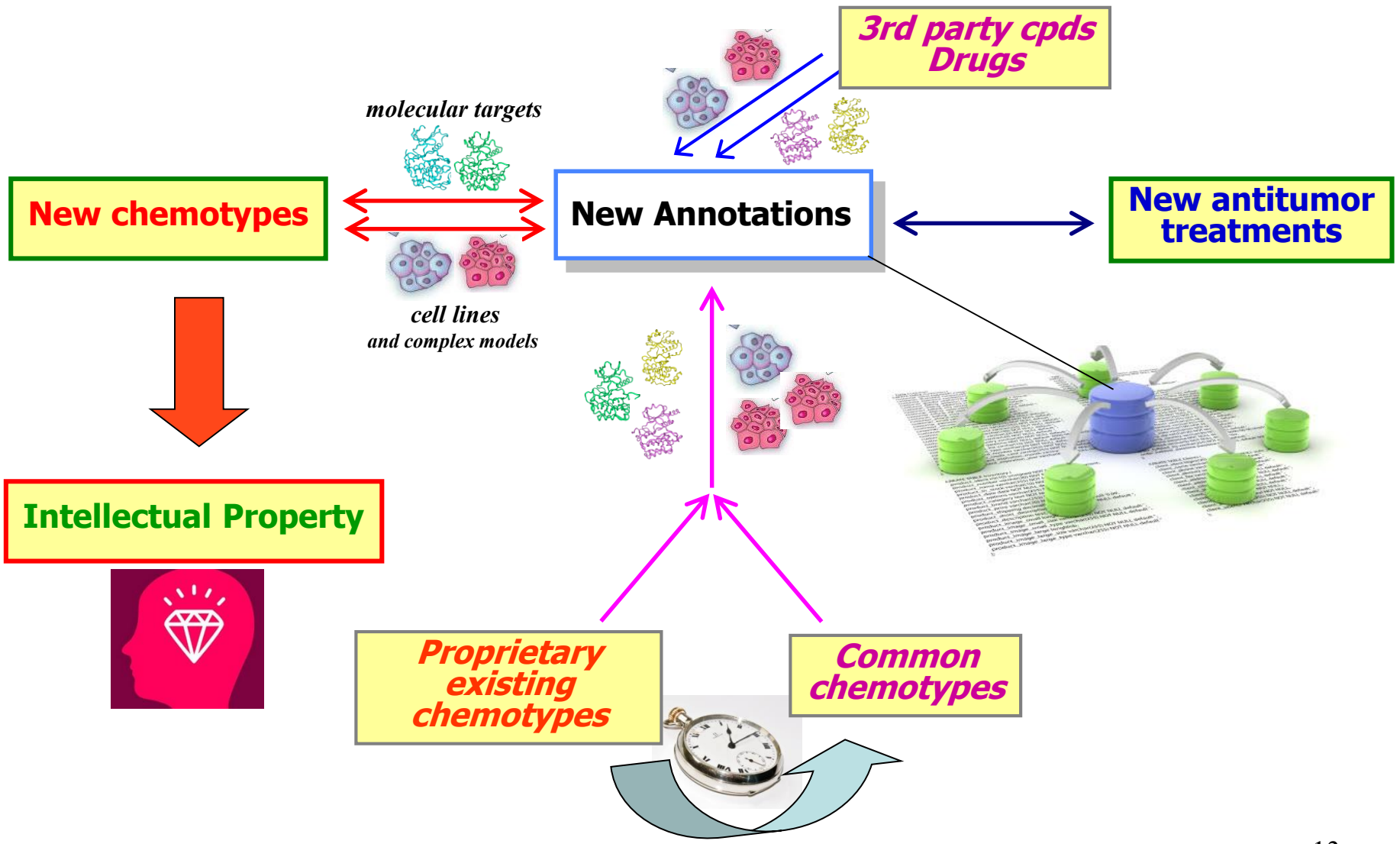
- One of the main mechanisms by which a normal cell appropriately transduces signals is the reversible and dynamic process of protein phosphorylation
- Cross-profiling of inhibitors generated for one particular kinase, has traditionally been a rich source for hits of other kinases. In case, one clinical candidate can be explored as an inhibitor of more than one kinase

Functional Classification *(M. Knapp et al.)*

Protein Functional Class	Total # of Proteins	Substrate/Cofactor
Small G Proteins	750	GTP
Protein Kinases	518	ATP
Dehydrogenases *	456	NAD/NADP
ATPases	453	ATP
Motor proteins (Kinesins, Myosins, Dyneins)	22	ATP
Helicases	217	ATP
Non-conventional purine-utilizing proteins [such as HSP90]	357	ATP, ADP, AMP, GTP
Synthetases	213	ATP
Deaminases	85	ATP
Lipases	78	ATP
Sulfotransferases	40	ATP
CTK	34	ATP
Carboxylases	26	ATP
Puringenic receptors	17	Adenosine, ATP

P-Loop
Structural motif

‘Chemical innovation’ , Chemical matter



Open collections (annotated, curated, pre-competitive)

- In 2005, **NIH** launched the decade-long **Molecular Libraries Program**
  - to innovate and broaden access to small-molecules
  - enabling the exploration of biological pathways and therapeutic hypotheses

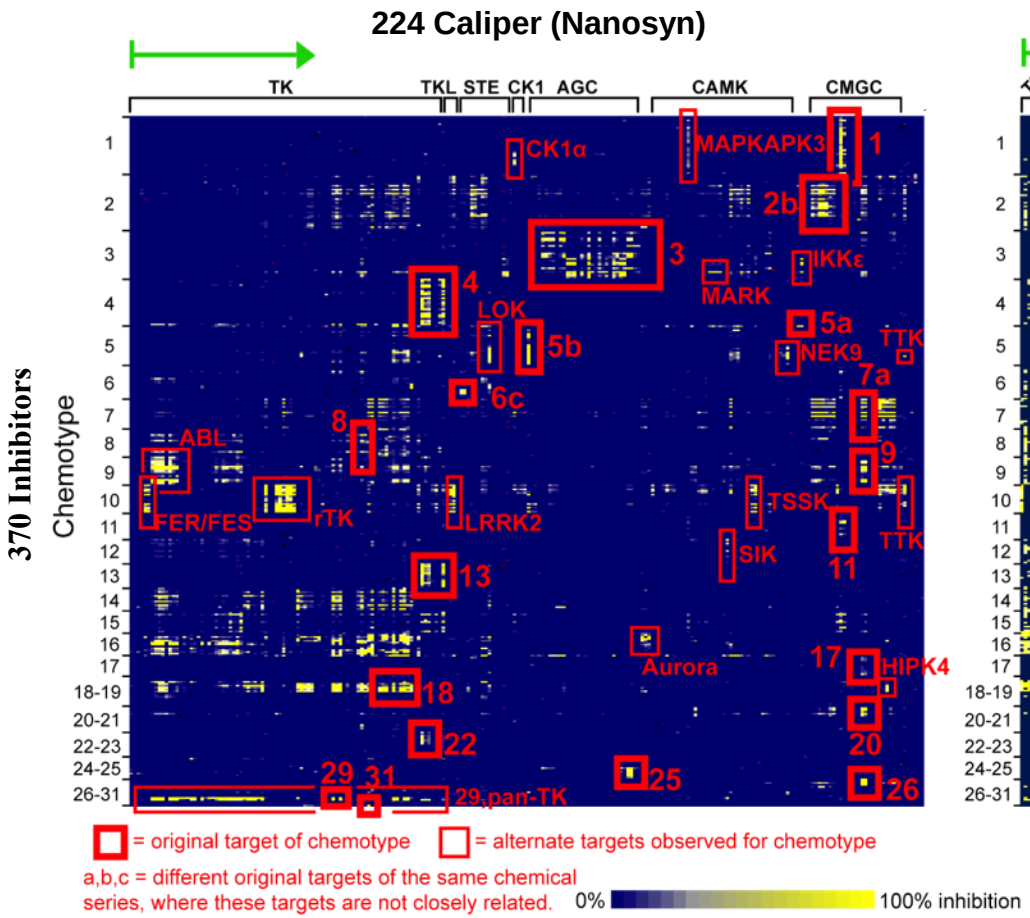
- In 2011, **AstraZeneca** and **Bayer** open mutual access to their libraries, but only on targets that were not relevant to the other company
  Years later **AstraZeneca** and **Sanofi** announced a swap of 210,000 compounds with no restrictions on screening

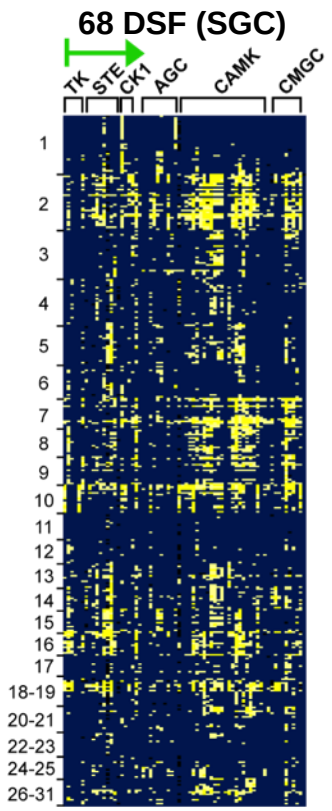
- In 2014, **AstraZeneca** launched a partnership with the **Academic Drug Discovery Consortium**, a network of more than 130 academic drug discovery centers formed in 2012.
  Selected researchers get access to 250,000 AZ compounds for the assays they developed.
  AstraZeneca, typically gets the first chance to license

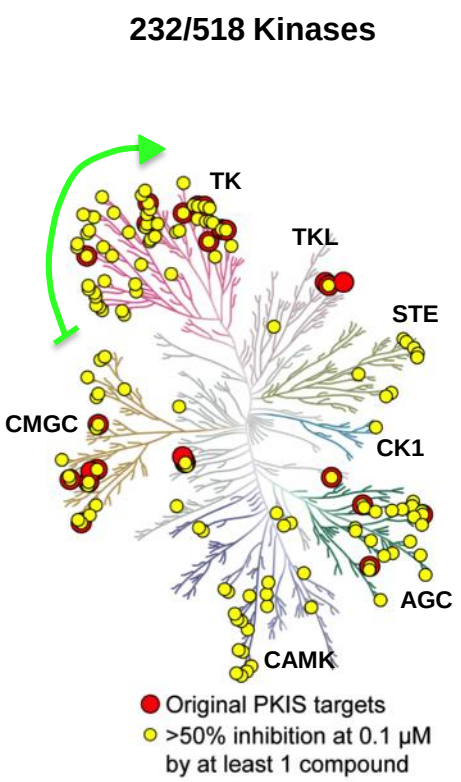
- In 2015 in Europe the Joint European Compound Library (JECL) is formed:
  - with 321,000 compounds that originated in seven pharmaceutical companies
  - with additional 200,000 compounds (PCC) planned by 2019
  - open to academics and biotech companies

- In 2016 comprehensive characterization of GlaxoSmithKline's **PKIS**, a set of 367 kinase inhibitors triaged and selected from 3000 kinase inhibitors previously published in 2014

Published Kinase Inhibitor Set (PKIS)







All structures and data in ChEMBL

J. M. Elkins, Nature Biotechnology (2016) 34, 95-103.

Kinase Chemogenomic Set (KCGS) - expansion in progress

- A set of 1000 kinase inhibitors as a goal
- All kinases in the kinome will be inhibited by at least one inhibitor in the set (ideally several)
- Each compound has a narrow kinase inhibition profile
- The set will be freely distributed for use in disease relevant phenotypic screens, so that kinases involved in that disease model can be identified
- Through broad screening of the set, the community will learn which kinases to pursue (invest drug discovery effort) for which disease
- Some approaches, such as whole-genome short interfering RNA (siRNA) or CRISPR–Cas9 screening, may be carried out in parallel to expedite target identification

D.H. Drewry, PLOS ONE | PLoS ONE 12 (8): e0181585 (2017)

The Nerviano Compound Collection - Definitions

PTL

- *Purinome Targeted Libraries* – widely covering mimicks and surrogates of purine analogs and derivatives, extending into space adjacent to the purine binding site *including:*
- *KTL* Kinase Targeted Libraries

Universal Library UL

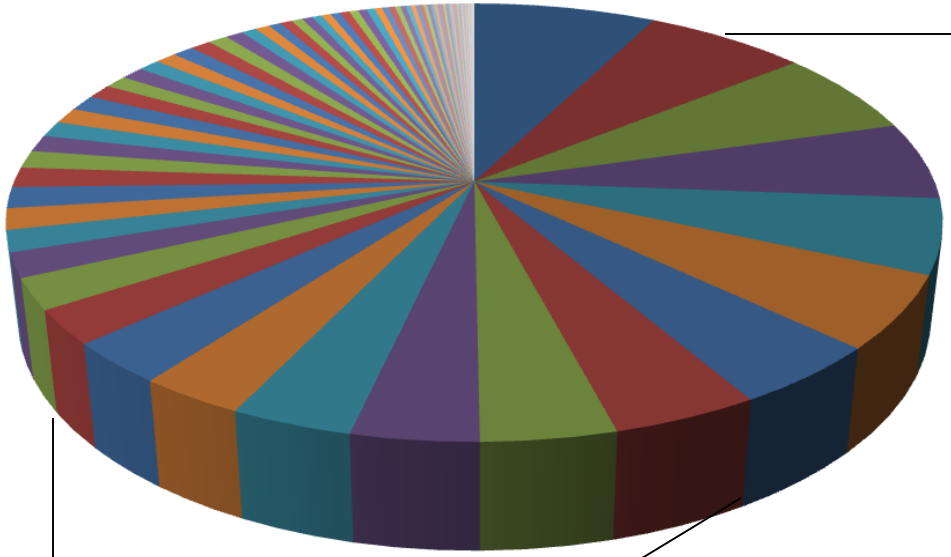
- *Primary libraries* (File enrichment program → Expansion of screening sets)
- *HIS*
  - Historical 'non-kinase' cpds (Legacy compounds Farmitalia, Pharmacia, Pfizer)
- *GEN*
  - ***Generic* cpds (non-kinase)**
  - **Intermediates from 'non-kinase' NMS projects**
- *Commercial compounds*
  - *Selection of 200,000 ChemDiv cpds complementary to KTL*
  - Other commercial sources

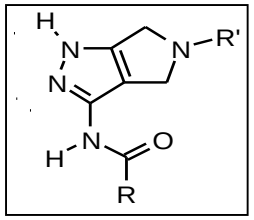
FBA

- *Fragments*
  - *Cpds with MW <300 Da, undecorated scaffolds, 'rule-of-three'*

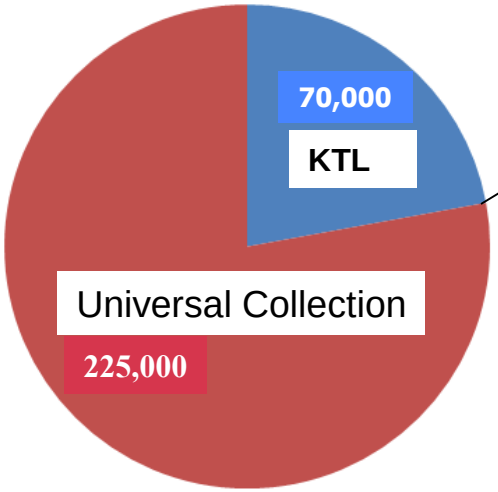
NMS chemical collection (~300,000 compounds)

Purinome Targeted Libraries (KTL): ~70,000 compounds, ~100 chemical classes





Pyrrolopyrazole library:
4300 compounds



Universal Collection (~225,000 compounds)
Explore diverse chemical space for non kinase targets

Scaffolds: Wide scope and high potential for selectivity

Chemical classes

- with wide scope
- 'expressing' selective individual compounds

e.g. 1,4,5,6-Tetrahydro-pyrrolo[3,4-c]pyrazoles for inhibition of kinases

PHA-E544

Cdk2: 30 nM
Aurora-2: 4,100 nM
PAK-4: >10,000 nM
Cdc7: > 10,000 nM

PHA-E363

Cdk2: 1,350 nM
Aurora-2: > 4,000 nM
PAK-4: >10,000 nM
Cdc7: > 10,000 nM
Flk-1: 80 nM

PHA-E468

Cdk2:>10,000 nM
Aurora-2: 50 nM
PAK-4: >10,000 nM
Cdc7: > 10,000 nM

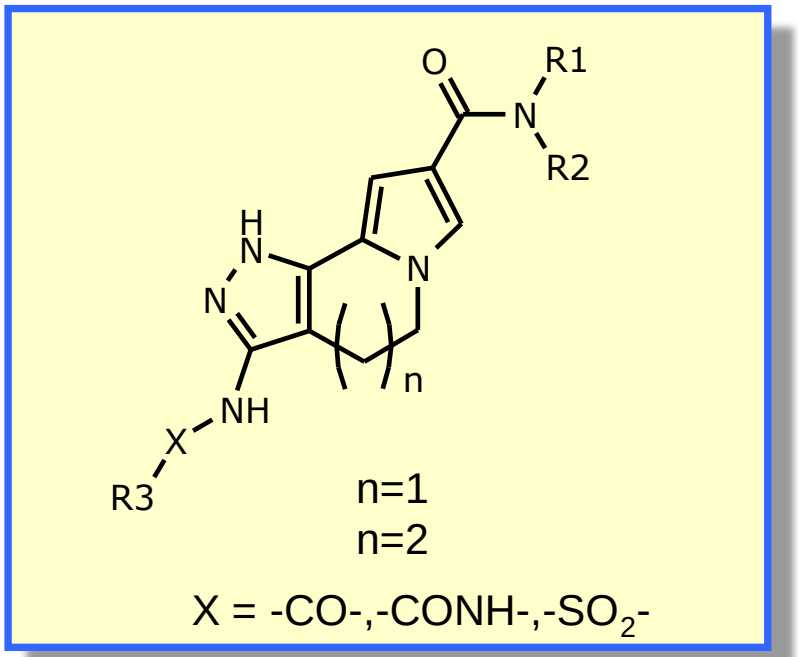
PHA-E779

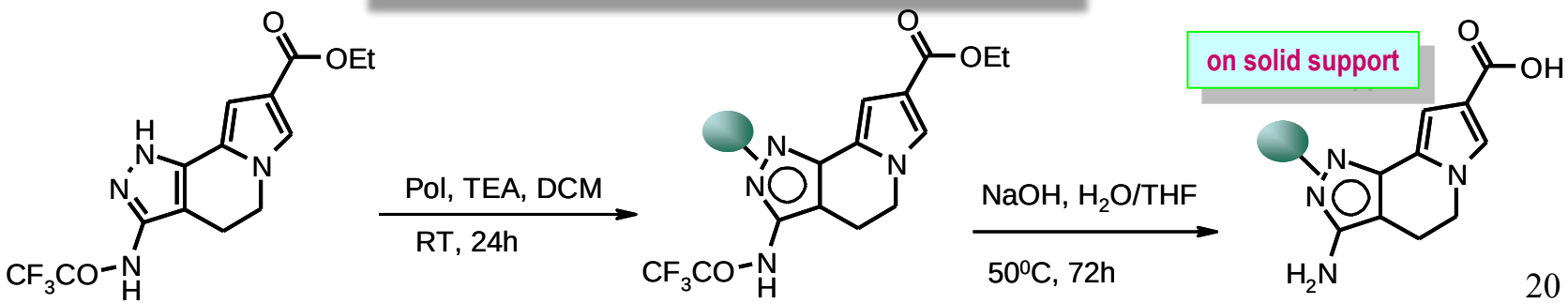
Cdk2: 4200 nM
Aurora-2: 2700 nM
PAK-4: 52 nM
Cdc7: > 10,000 nM

Purinophosphate mimicking libraries

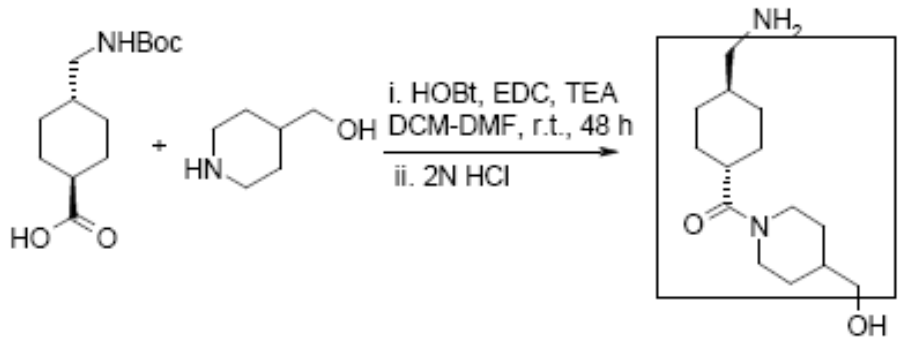
Novel tricyclic Adeninomimetics

3- and 8-substituted Pyrazolo[4,3-g]indolizines
3- and 9-substituted Pyrazolo[3,4-c]pyrrolo[1,2-a]azepines

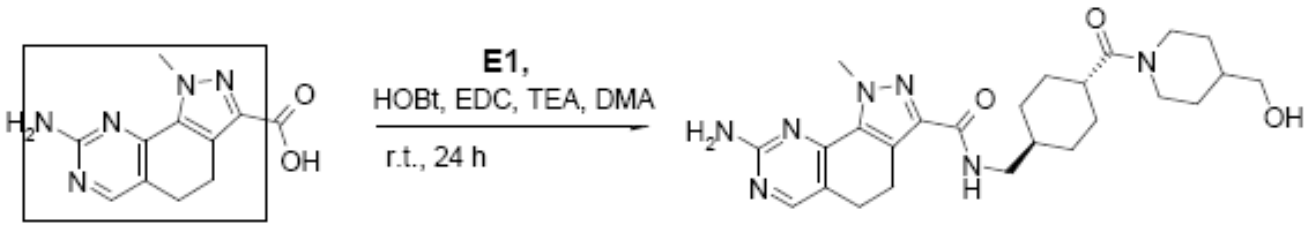




Scaffold → Extension → Phosphorylation

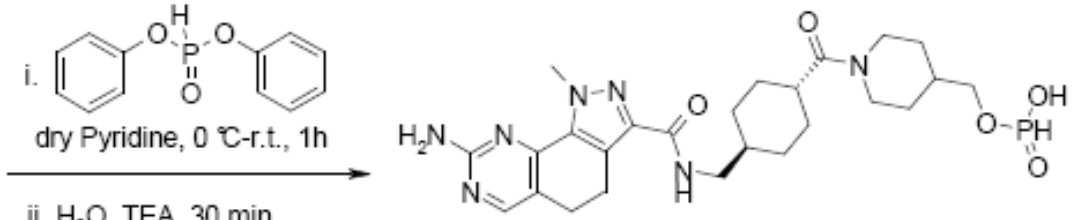


Extension 1



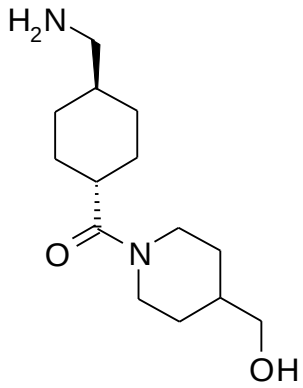
Scaffold 2

S2-E1

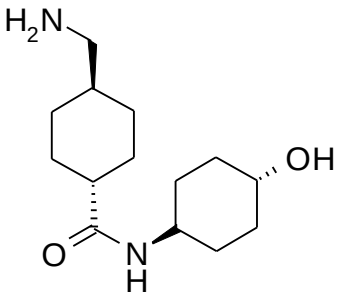


S2-E1-HPO$_3$

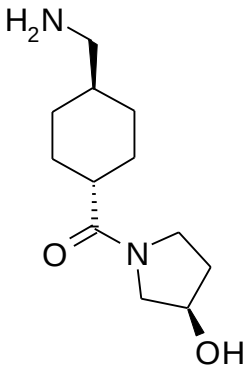
Extensions / Scaffolds



E1



E2



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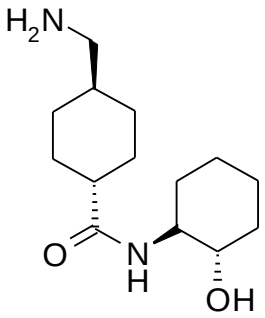

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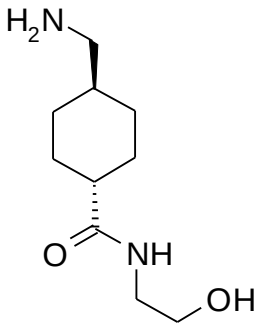
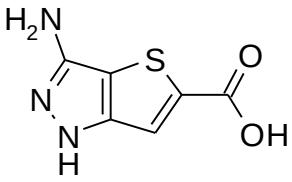
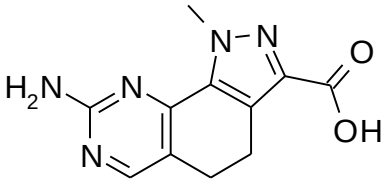


E5



S1



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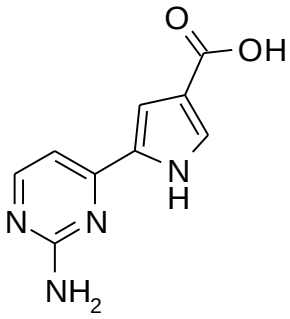
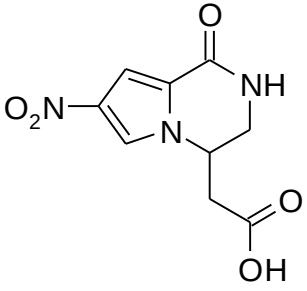
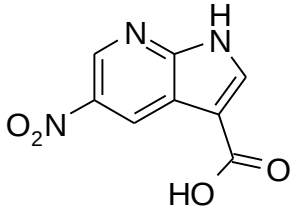


S3

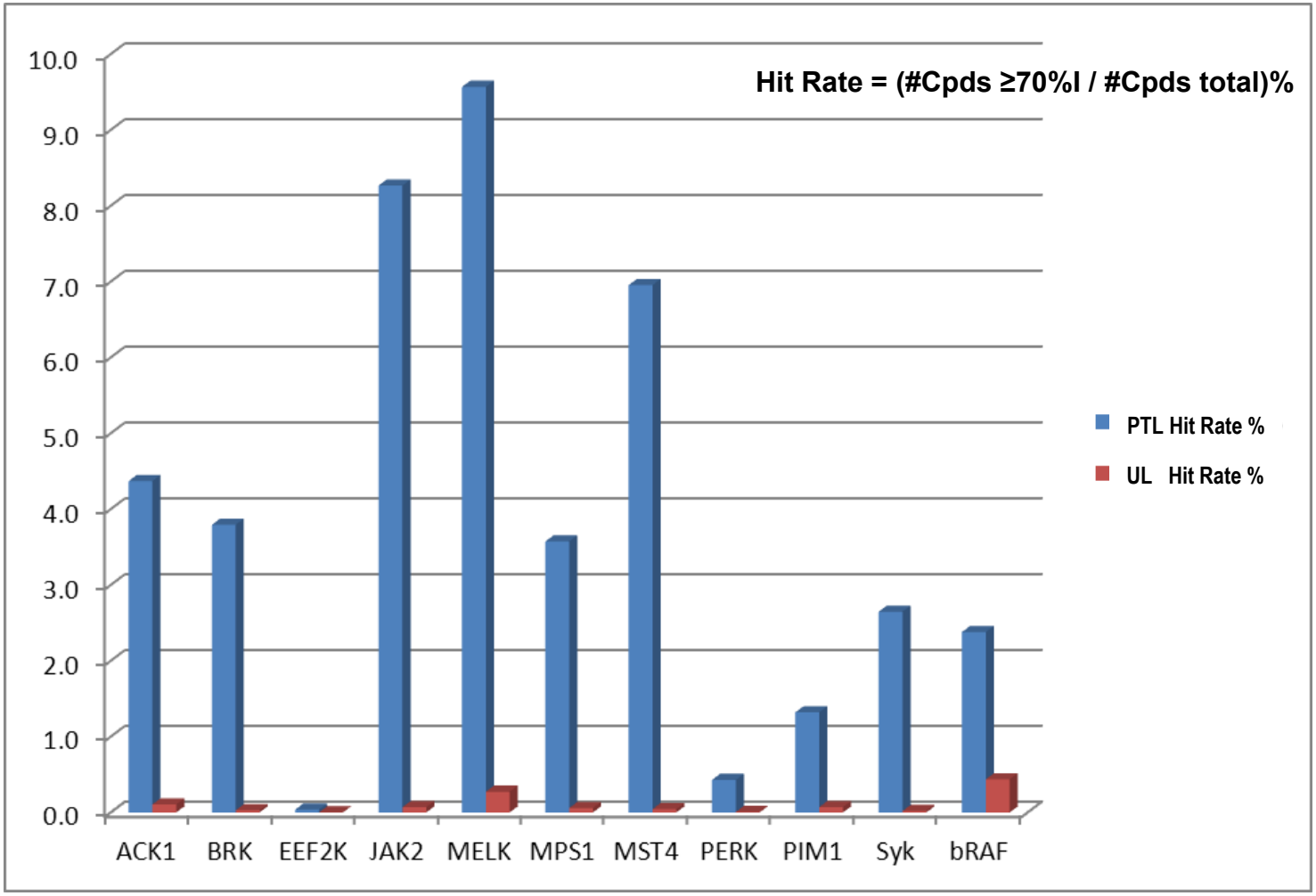


S4

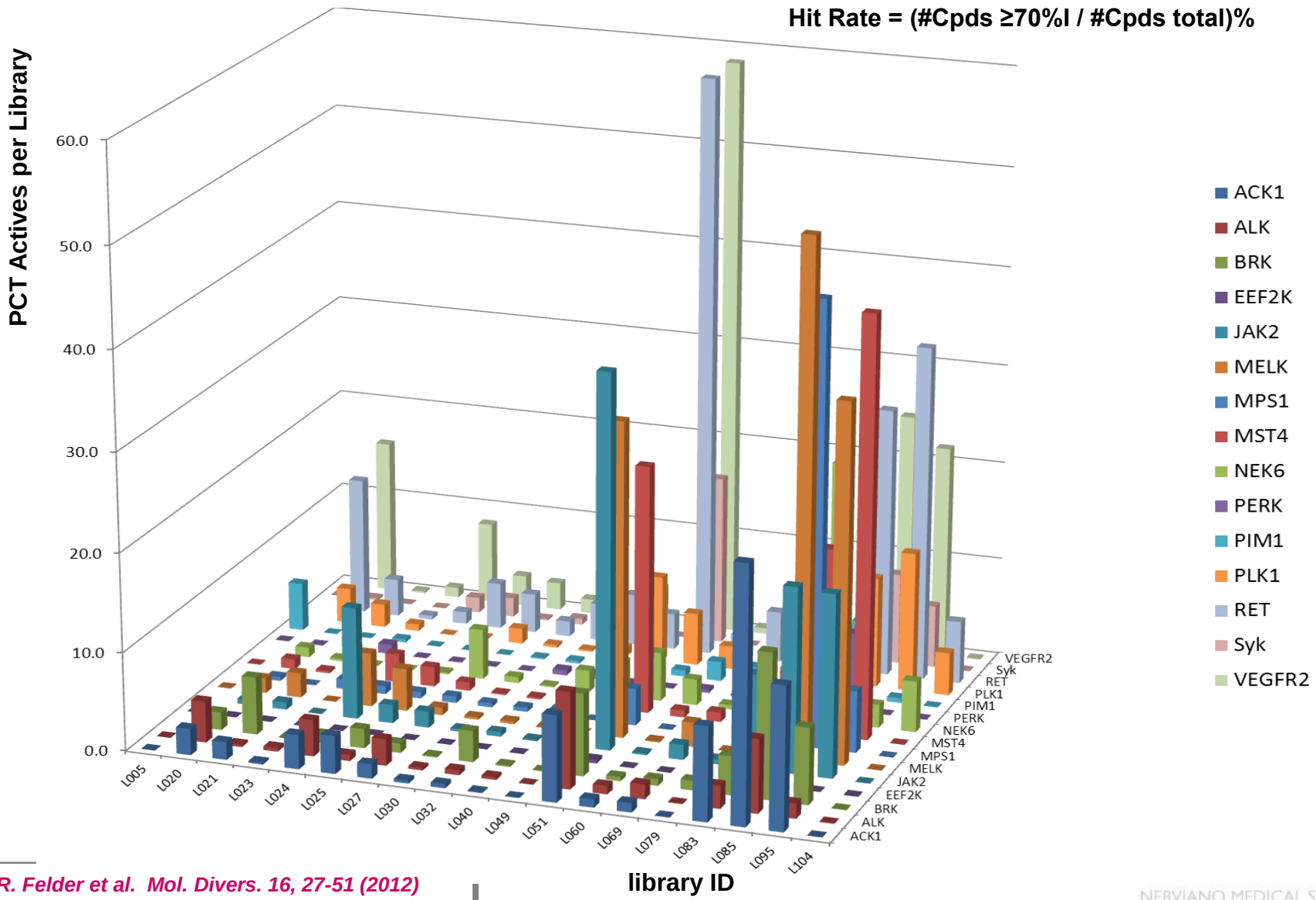


S5

Hit rate of entire PTL collection *vs.* “random” library (UL)



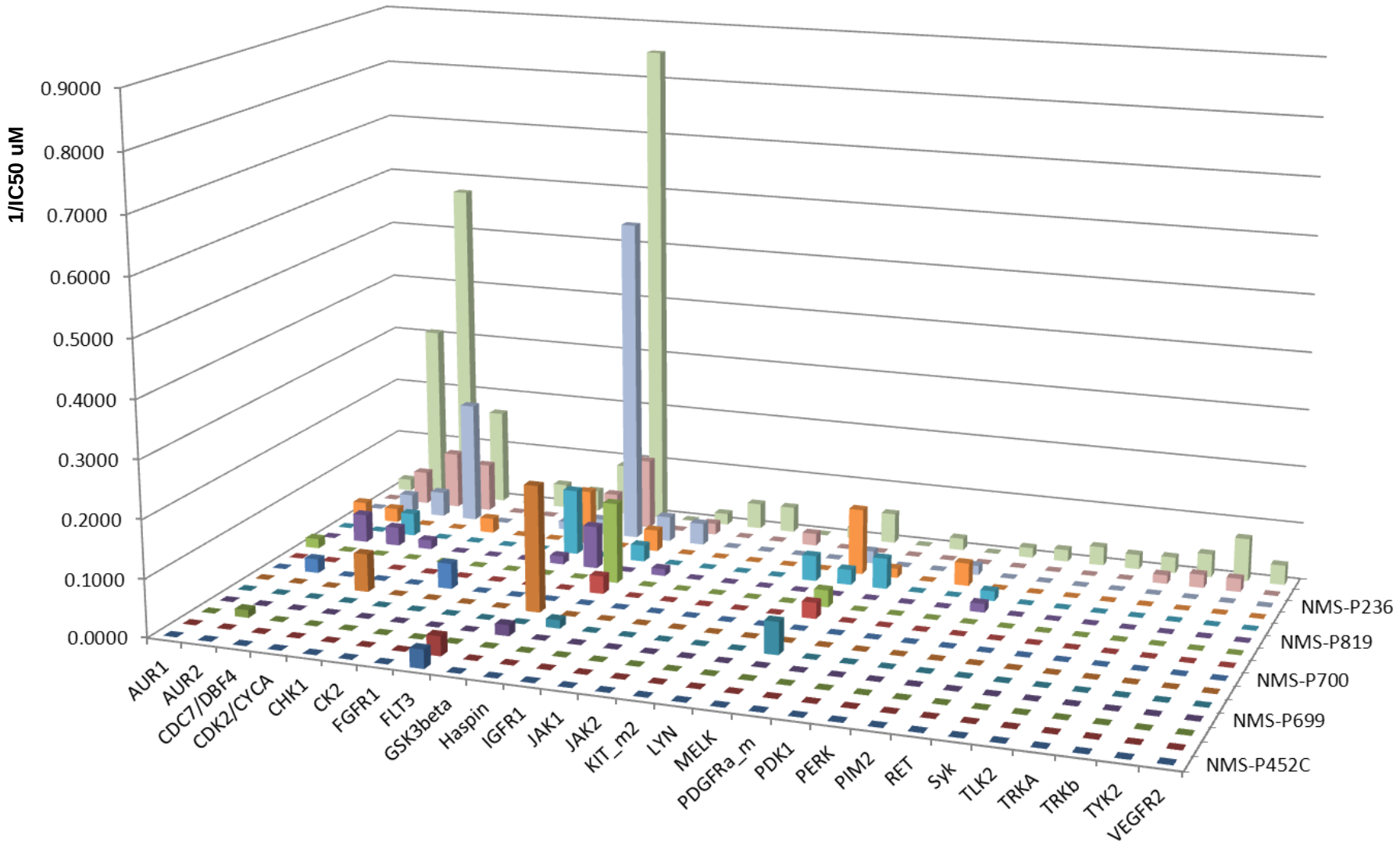
Hit Rates of PTL main chemical classes (>1000 cpds each)



E.R. Felder et al. Mol. Divers. 16, 27-51 (2012)

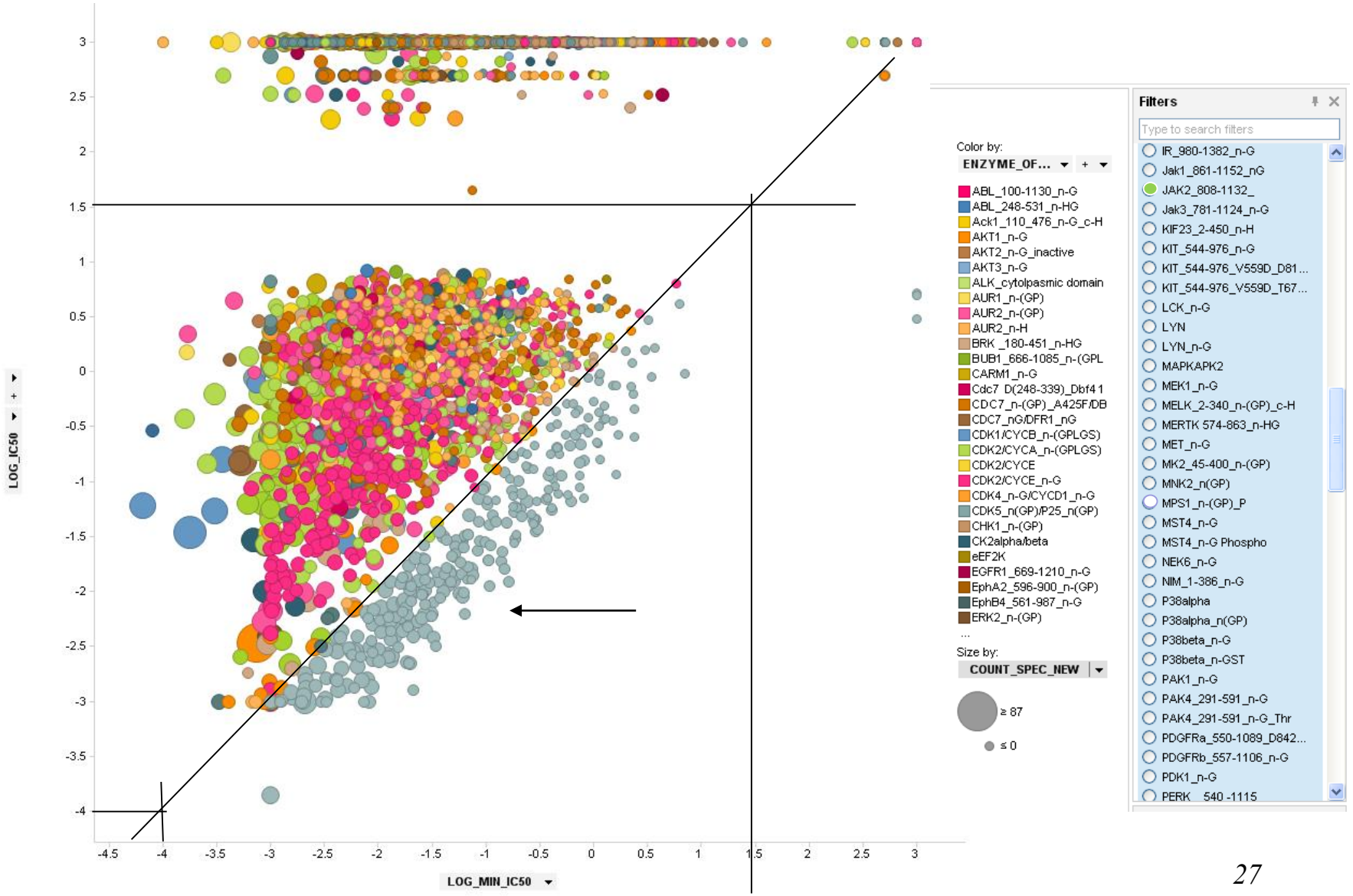
NERVIANO MEDICAL SCIENCES

Kinase selectivity profile of active compounds from a prototype ADP mimics library

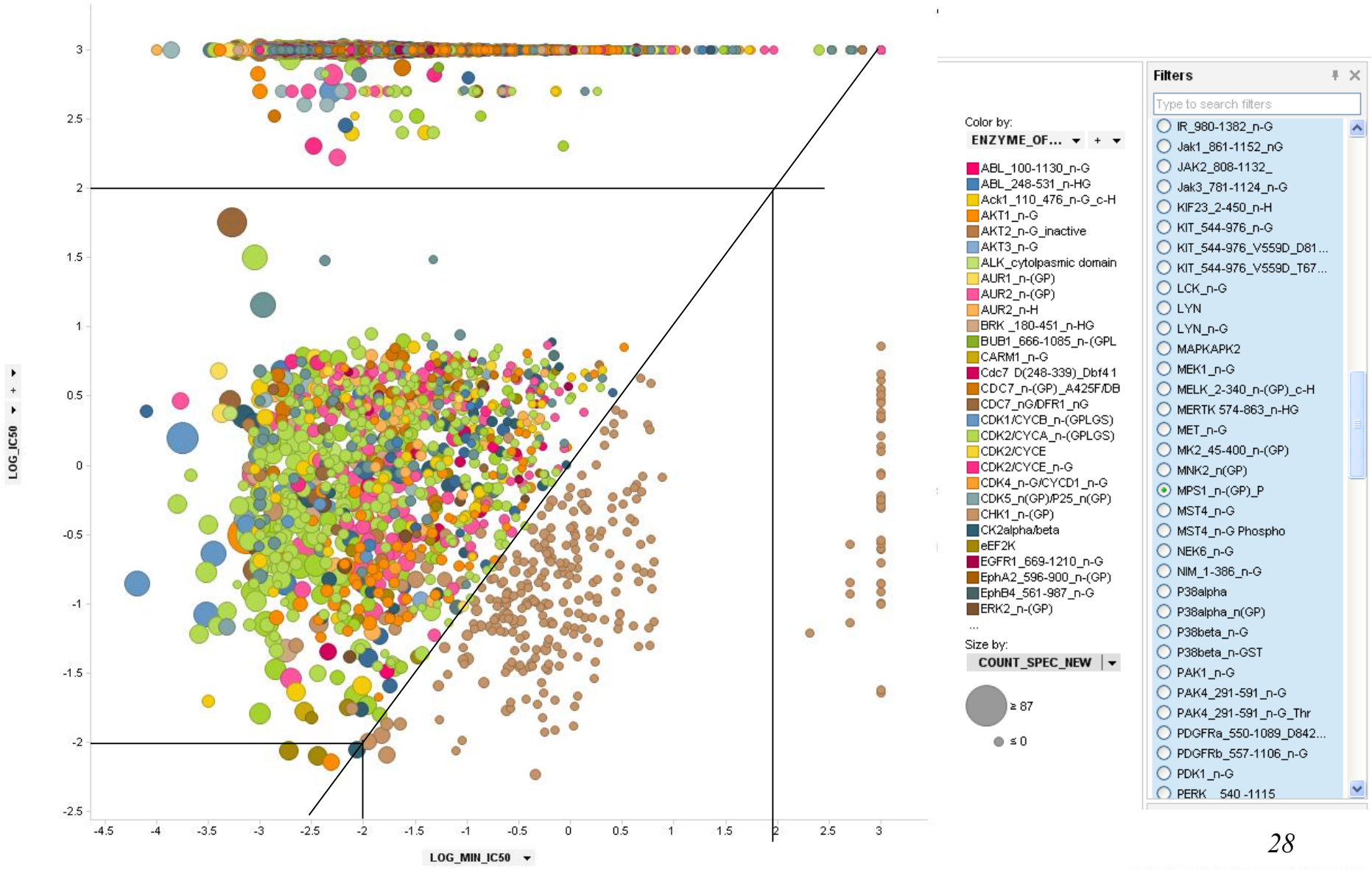


Activities on selected kinases for two chemotypes

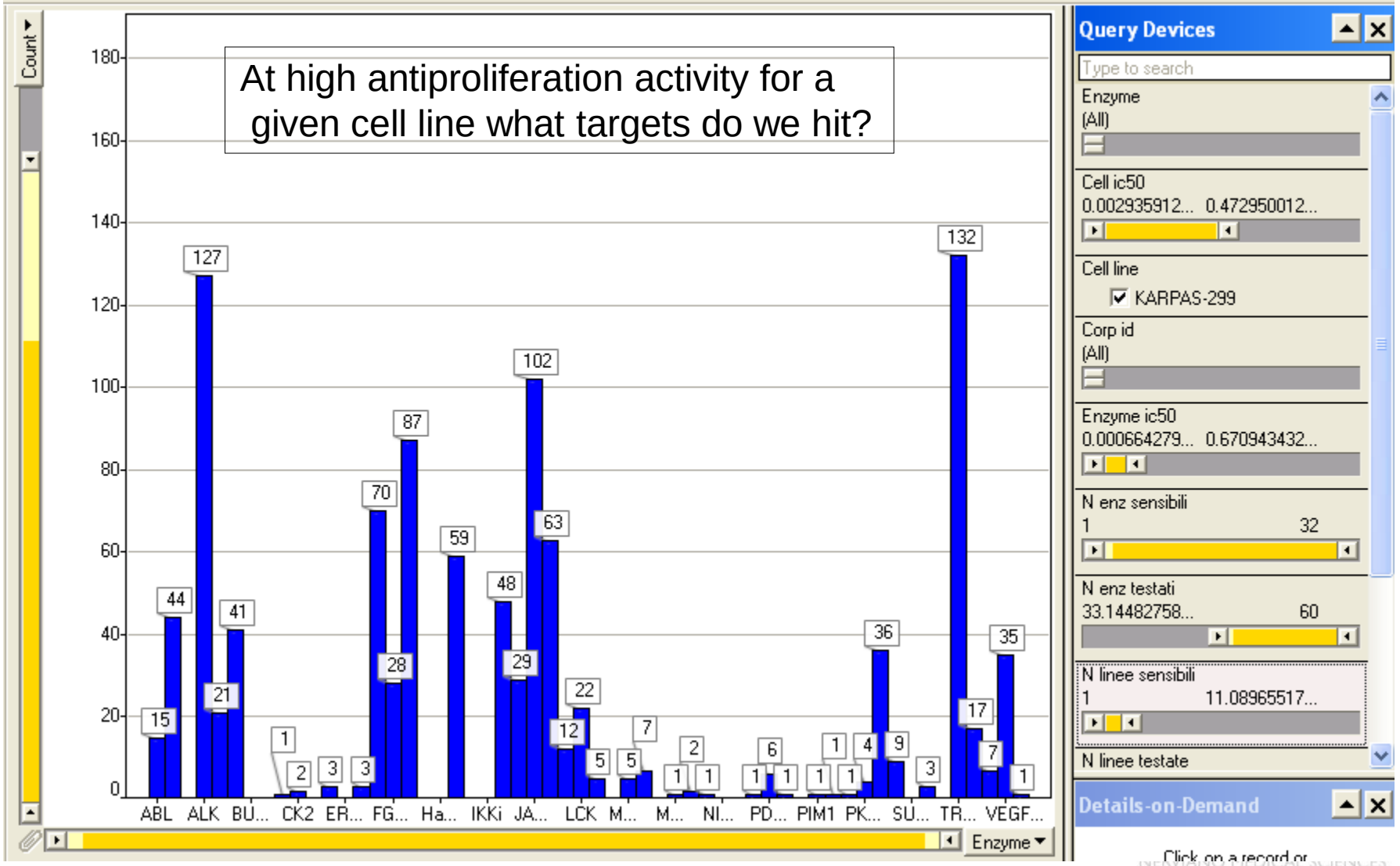
Specificity challenge: JAK2



Specificity success: MPS1

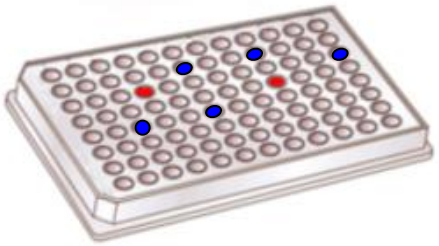


Antiproliferation vs. Kinase Panel Activity

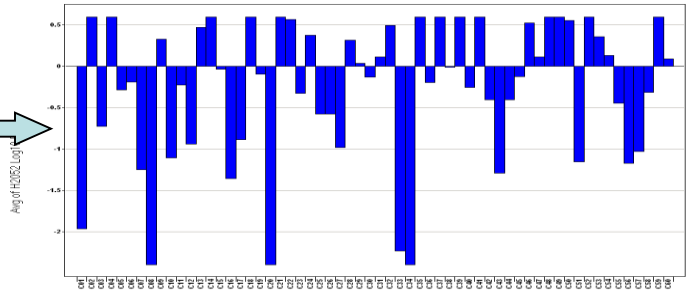


Searching growth inhibitory targets of outstanding relevance in particular cell lines

Screening on tumor cell lines



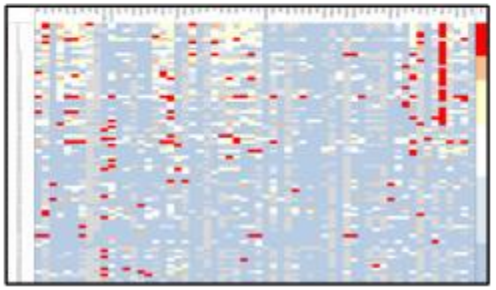
IC_{50} calculation for each cell line



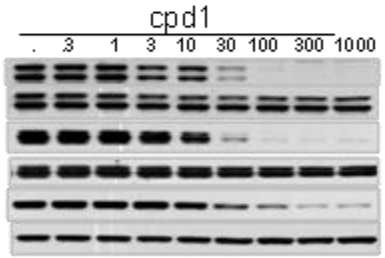
Kinase profiling

Kinases hit by the most active cpd(s)

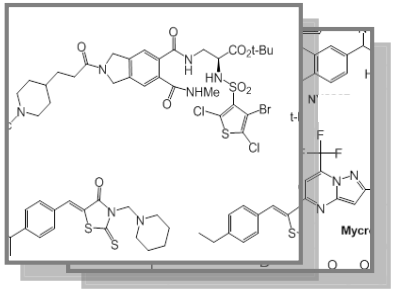
Heatmaps (kinases hit by cell active cpds)



Mechanism of action studies



Cpds selected for additional characterization



Compound selection → Assay Set Up, Phenotypic Screen → Hit-to-Lead → Target and Pathway Deconvolution → Lead Optimization → Preclinical development

Biologically diverse compounds

Hits with target and pathway annotations

Hit expansion by target

Additional assays

Disease model *in vivo* efficacy

Knockout

Target-specific assay

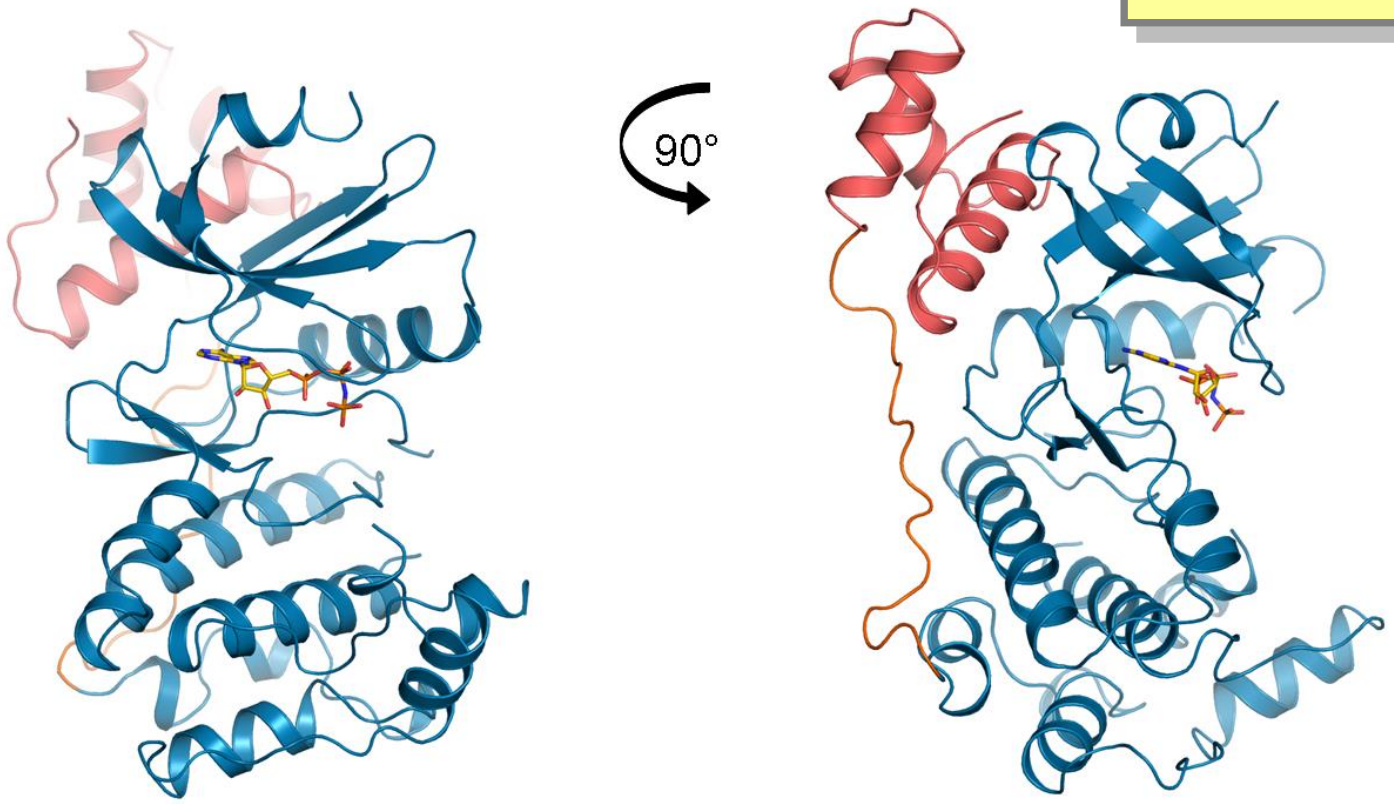
MELK , a controversial target

- Maternal embryonic leucine zipper kinase (MELK) is an AMPK-related serine/threonine kinase
- MELK has active roles in a number of cancer cell lines, and in the physiological cell cycle and embryogenesis
- Enhanced MELK activity was found in aggressive breast cancer cell lines, whose proliferation can be modulated by siRNA knockdown
- Implications in glioblastoma, colon cancer, ovarian cancer have been reported
- OTSSP167, from OncoTherapy Science is in clinical studies
- Overexpression of MELK is observed in cancer stem cells
- Claims that MELK dependence is specific to basal-like breast cancer (BBC)
- BBC largely overlaps with triple-negative breast cancer (TNBC)
- Little is known about whether MELK plays a causal role in fueling these cancer phenotypes
- J. Sheltzer et al. at Cold Spring Harbor found that knocking MELK out via CRISPR treatment in a whole list of different cancer cell lines has no effect on their growth

MELK Crystal Structure

The N-terminal kinase domain is flanked by a smaller ubiquitin-associated (UBA) domain, a TP dipeptide-rich domain and a C-terminal kinase associated domain (KA1)

First reported structure Enables Drug Design



G. Canevari et al. , Biochemistry 52, 6380–6387 (2013)

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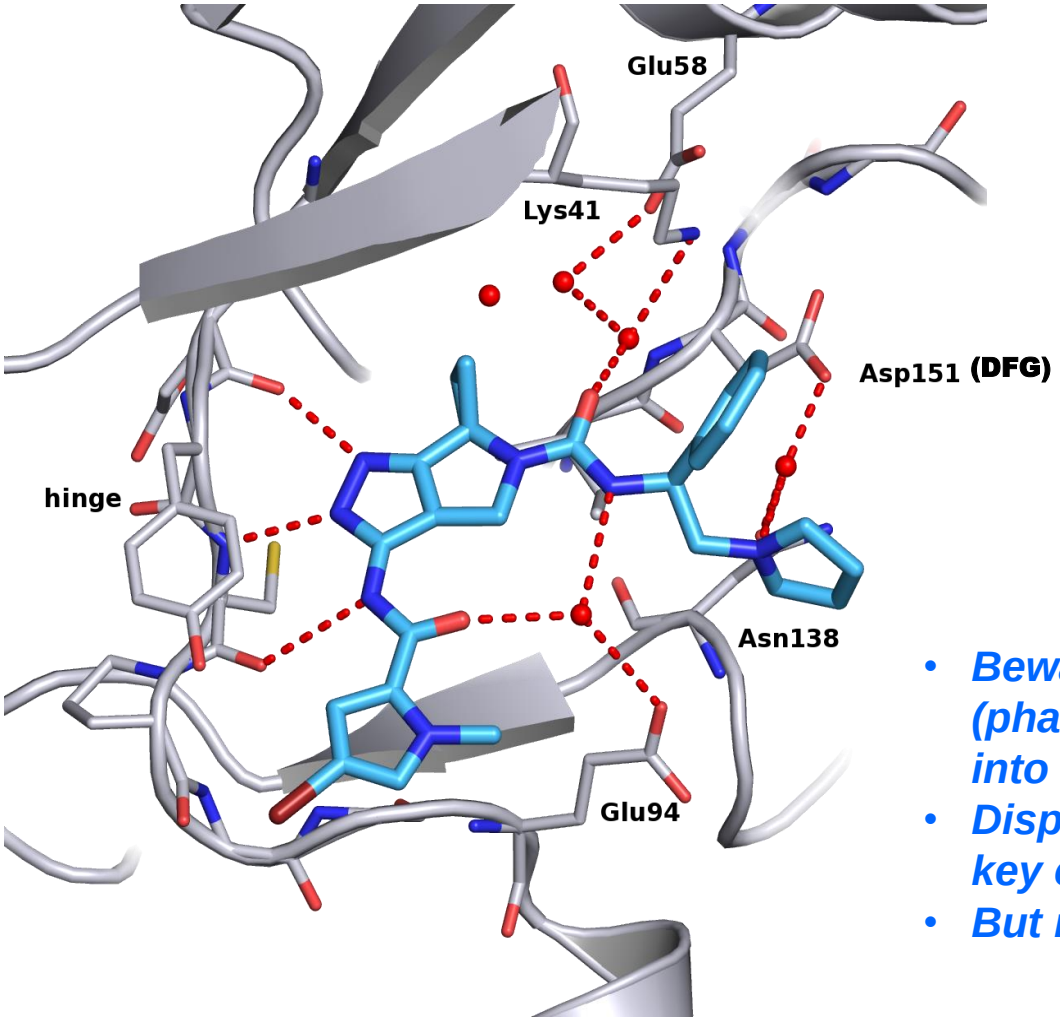
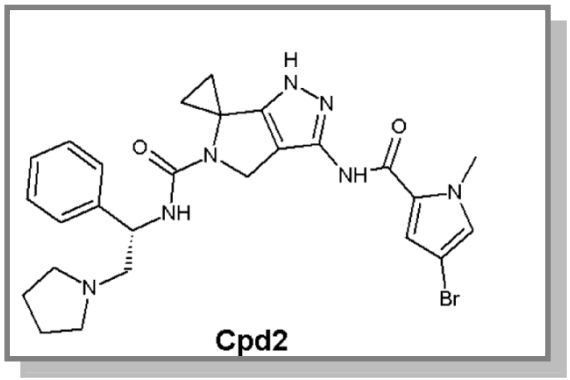
MELK Crystal Structure w. Inhibitors

Type I binding mode with key involvement of water molecules

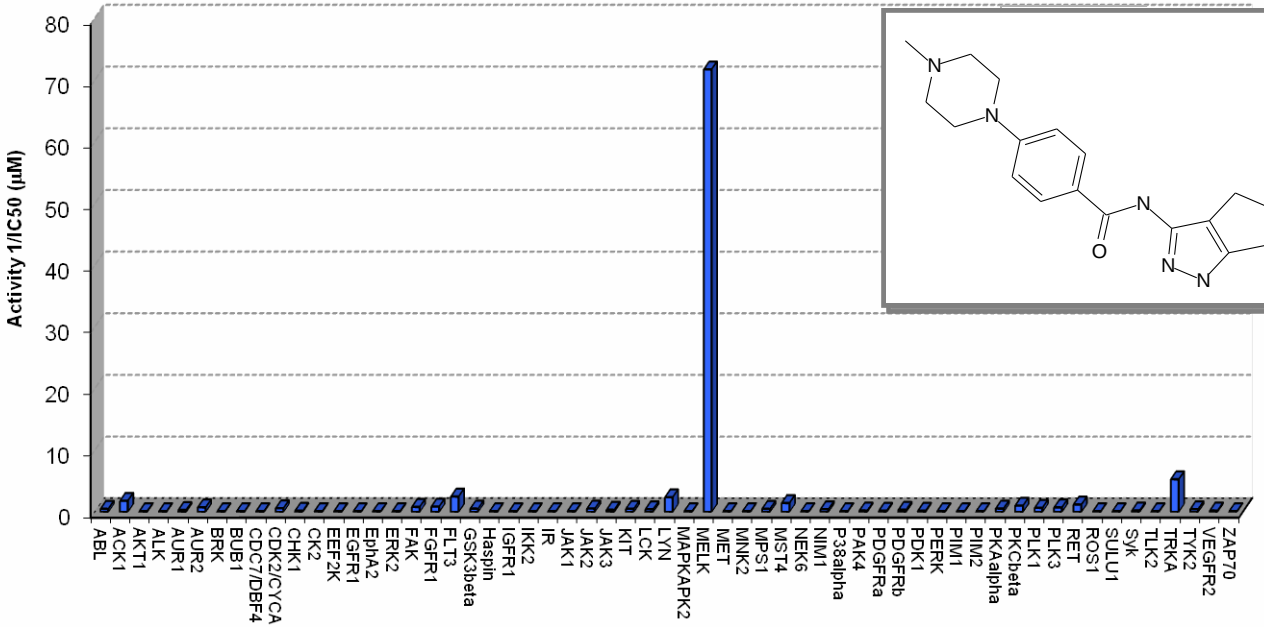




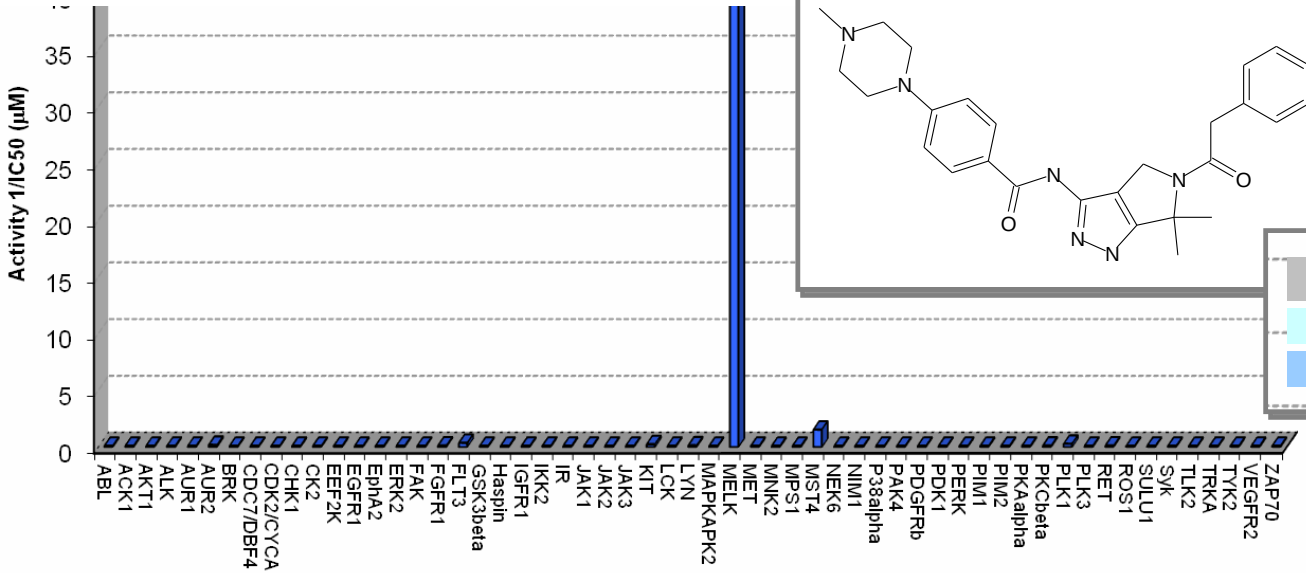
- *Beware of force fitting ligands (pharmacophores) without taking water into account*
- *Displacing water from a binding site is a key component of ligand binding*
- *But not all waters are equal …*

G. Canevari et al. . Biochemistry 52, 6380–6387 (2013)

Potent and selective MELK inhibitors

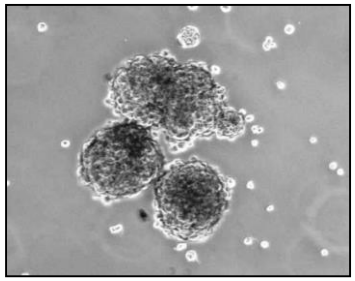


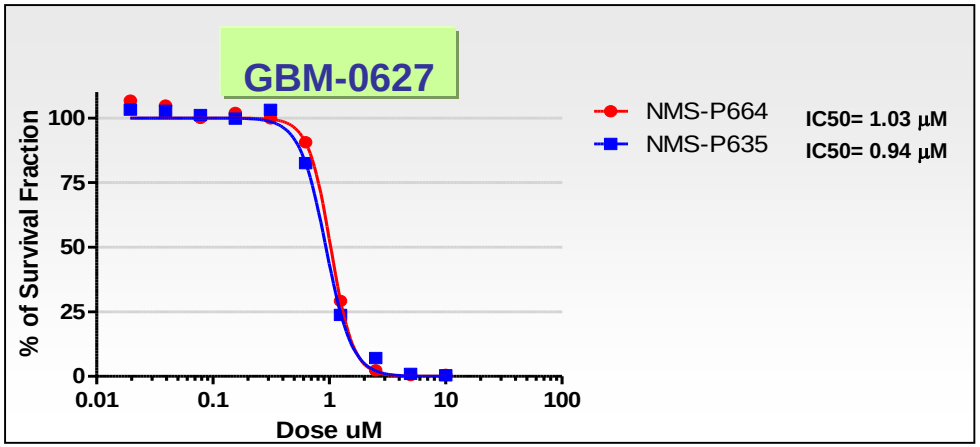
NMS-P0635	IC50 (nM)
MELK	14
TRKA	190
FLT3	406

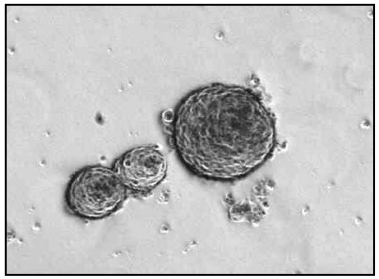


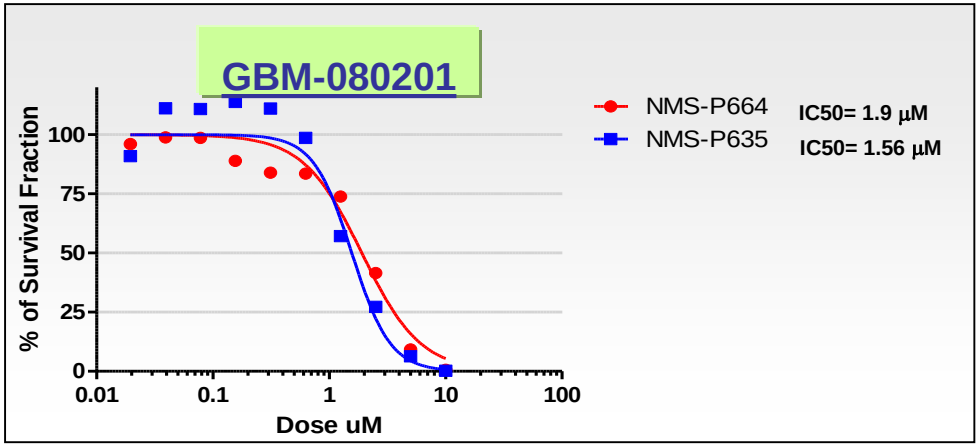
NMS-P0664	IC50 (nM)
MELK	23
MST4	654

MELK inhibitors effective on Glioblastoma cancer stem cells









- **Melk inhibitors are effective in inhibiting the growth of Glioblastoma cancer stem cells.** GBM-0627 and GBM-080201 cancer stem cell lines were seeded and then treated with different concentrations of the Melk inhibitors. After 7 days, the cell numbers were estimated using CellTiter-Glo assay.

P. Carpinelli et al. 26th EORTC-NCI-AACR (2014)
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MELK as a marker for poor prognosis

Chemical proteomics reveals the target landscape of clinical kinase drugs

Bernhard Kuster & coworkers, Chair of Proteomics and Bioanalytics, Technical University of Munich, Germany

Data revealing numerous novel targets for existing drugs
Offering a view on the druggable kinome, highlighting non-kinase off-targets and suggesting potential applications in immune or cancer therapy

In this study, we elucidated the target space, selectivity and full dose response characteristic of clinical kinase inhibitors in lysates of cancer cell lines using Kinobeads and quantitative mass spectrometry
Other than protein kinases, Kinobeads also bind other nucleotide binding proteins owing to the fact that the compounds immobilized on beads are ATP mimetics

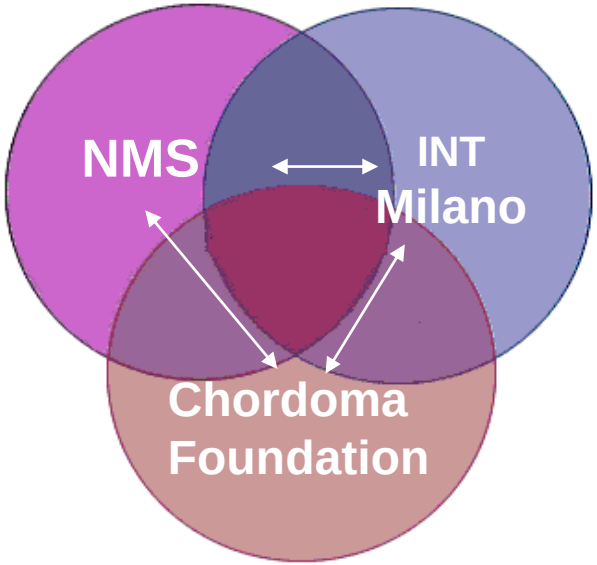
Integration with phosphoproteomic data refined drug affected pathways, identified response markers and provided rationale for combination treatment

The full proteomic data and multiple visualizations will be made publically available in ProteomicsDB and proteomeXchange

Working towards better chordoma treatments

Chordomas form when notochord cells left over in the skull or spine change over time and become cancerous

- ✓ Drug discovery know-how
- ✓ Drug collection



- ✓ Medical need
- ✓ Clinical knowledge
- ✓ Biological reagents

- ✓ Biological reagents
- ✓ Support for efficacy experiments

New pharmacological approaches for the treatment of chordoma:

- ➢ **New targets**
- ➢ **New drugs**

Chordoma cell screening workflow

Anti-proliferative screening of NMS chemical collection on U-CH1 and U-CH2 chordoma cell lines

1400 compounds including 200 reference drugs

Active compounds characterized on a broader panel of chordoma cell lines

6 cell lines, including new Chor-IN-1

New targets

Ongoing, based on:

- Screening results
- Cell lines genomic characterization

Active drugs

- Most drugs inactive on U-CH1 and U-CH2
- Focus on PDGFR, MET, EGFR inhibitors
- **Afatinib** EGFR inhibitor most active drug

P. Magnaghi et al. 5th Int. Chordoma Research Workshop (2016)

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Met and PDGFR inhibitors do not impair proliferation of chordoma cell lines

Compound IC50 (uM)	Cell line							MET amplif.	
	U-CH1	UM-Chor1	MUG-Chor1	U-CH2	U-CH2 (ATCC)	Chor-IN-1	JHC7	MKN-45 Ctrl +	A2780 Ctrl -
Crizotinib	3.972	>10	2.611	3.318	6.421	4.856	8.061	0.098	1.086
Cabozantinib	7.047	4.755	8.651	2.919	2.398	7.508	8.444	0.129	1.269
PHA-665752	4.926	4.681	2.332	3.439	4.073	5.480	3.560	0.119	3.289
Doxorubicin	0.166	0.067	0.455	0.152	0.348	0.340	0.881	0.659	0.010

MET inhibitors

Compound IC50 (uM)	Cell line							PDGFR amplif.	
	U-CH1	UM-Chor1	MUG-Chor1	U-CH2	U-CH2 (ATCC)	Chor-IN-1	JHC7	NCI-H1703 Ctrl +	A2780 Ctrl -
Imatinib	>10	>10	>10	>10	>10	>10	>10	1.936	10.000
Sunitinib	5.739	>10	3.897	3.636	4.441	3.764	9.349	0.088	1.576
Crenolanib	8.635	>10	>10	5.786	>10	8.117	>10	0.505	1.702
Doxorubicin	0.166	0.067	0.455	0.152	0.348	0.340	0.881	0.171	0.010

PDGFR inhibitors

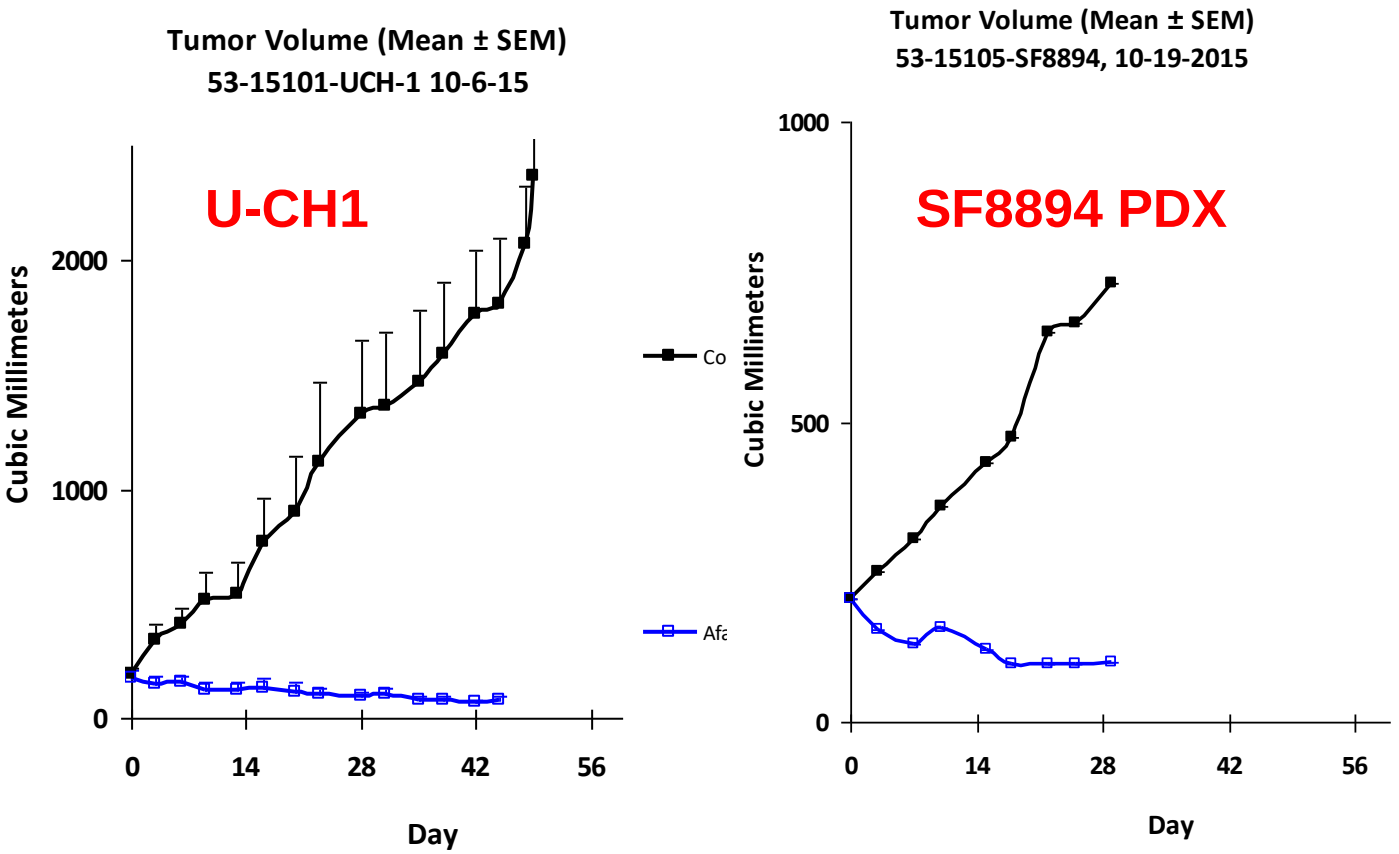
IC50= drug concentration that inhibits 50% cell proliferation
(Red: active drug= IC50< 1 uM)

Activity of **EGFR** approved drugs on chordoma cell lines

Compound IC50 (uM) (StdDev)	Chordoma cell lines							EGFR amplif.	
	U-CH1	UM-Chor1	MUG-Chor1	U-CH2	U-CH2 (ATCC)	Chor-IN-1	JHC7	A-431 Ctrl+	A2780 Ctrl-
Afatinib	**0.014** (0.005)	**0.023** (0.007)	**0.258** (0.072)	**0.494** (0.409)	**0.531** (0.203)	**0.668** (0.351)	**1.346** (0.394)	**0.026** (0.009)	1.915 (0.594)
Erlotinib	**0.144** (0.049)	**0.617** (0.069)	3.006 (0.977)	8.042 (1.714)	7.776 (1.953)	2.329 (0.774)	2.281 (0.848)	**0.346** (0.033)	3.919 (0.898)
Lapatinib	**0.656** (0.257)	**0.516** (0.080)	>10 (-)	>10 (-)	>10 (-)	>10 (-)	>10 (-)	**0.562** (0.061)	3.578 (0.771)
Gefitinib	**0.791** (0.446)	**0.751** (0.055)	6.241 (1.390)	6.259 (2.502)	5.936 (2.115)	9.040 (1.389)	7.010 (0.856)	**0.333** (0.069)	4.762 (0.911)
Dacomitinib	**< 0.019** (-)	**<0.019** (-)	2.075 (0.191)	2.400 (0.042)	2.145 (0.276)	0.418 (0.054)	1.230 (0.156)	0.043 (0.014)	2.715 (0.106)

- U-CH1 and UM-Chor-1 are sensitive to all EGFR inhibitors, with different sensitivity
- Sensitivity is comparable to EGFR-dependent cell lines
- Afatinib is the only EGFR inhibitor active across the chordoma cell line panel
- The JHC7 cell line is resistant to Afatinib (note: JHC7 line is not driven by EGFR signaling)

In vivo efficacy of Afatinib in U-CH1 Xenograft and SF8894 PDX models



20 mg/kg; po; qdx42

20 mg/kg; po; qdx28

- **Daily oral treatment with afatinib induces tumor regression in both chordoma models**

P. Magnaghi et al. 5th Int. Chordoma Research Workshop (2016)

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A rationale for clinical trials with Afatinib in chordoma patients

- A subset of chordoma cell lines are highly sensitive to EGFR inhibitors
- Afatinib is the only EGFR inhibitor with activity across the chordoma panel
- This activity appears to be strongly contributed by its unique ability to down-modulate the total level of EGFR and the transcription factor Brachyury *, a feature not shared by the other inhibitors
- The most sensitive cell lines display stronger EGFR activation and lower expression of Axl, a putative resistance pathway
- Afatinib has high efficacy against chordoma tumors *in vivo*
- These data support the use of afatinib in clinical trials and provide the rationale for the upcoming European phase II study on afatinib in advanced chordoma.

***Brachyury* is highly expressed in all cells in nearly every chordoma tumor**

P. Magnaghi et al. 5th Int. Chordoma Research Workshop (2016)
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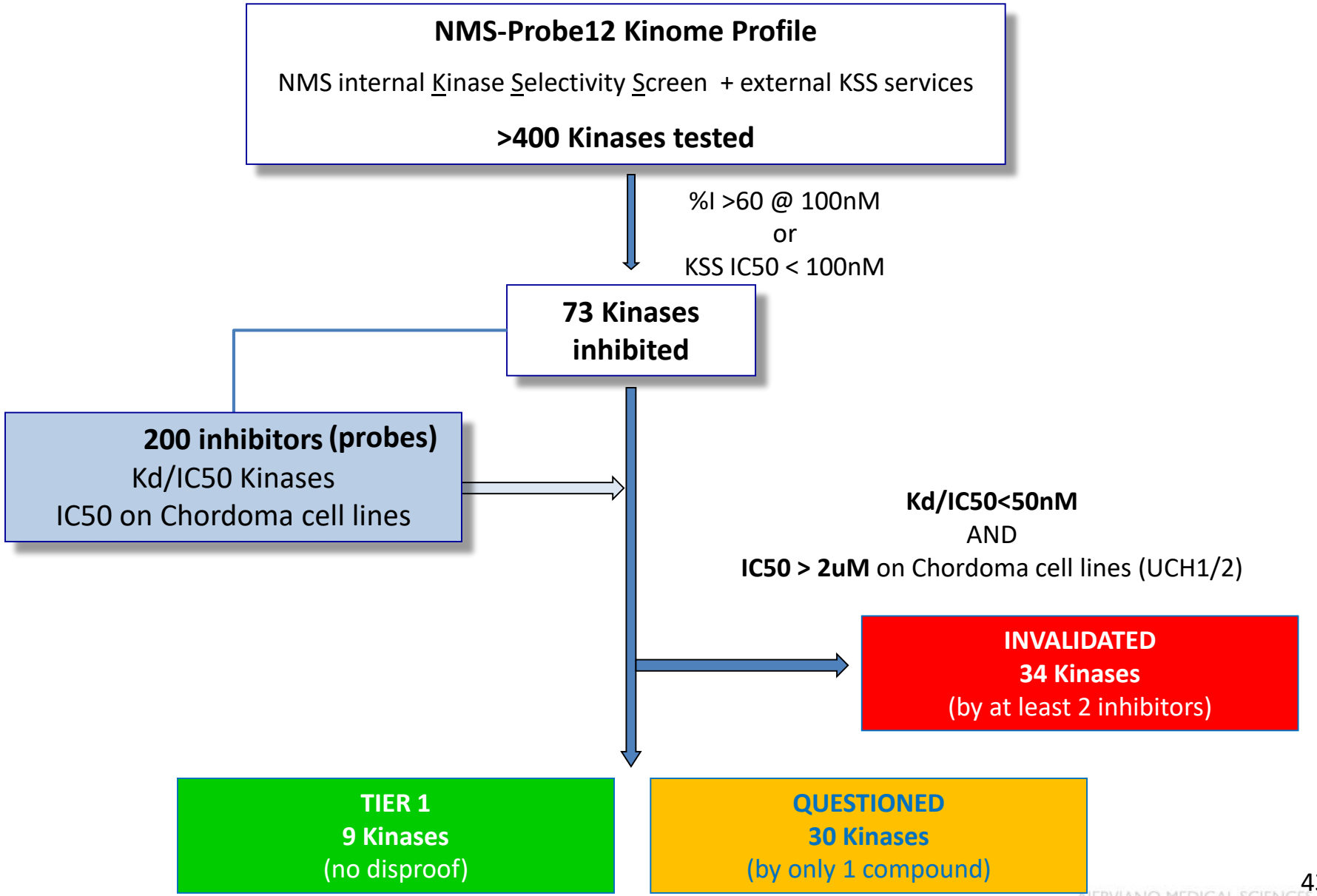
Narrowing the circle of relevant targets



Compound groups of closer interest

Four groups of compounds are under investigation based on potency, cellular selectivity and perspectives for therapeutic applications in Chordomas

- **EGFR inhibitors**: Afatinib has been identified as the most advanced high profile compound suitable for clinical studies

- **HSP90 inhibitors**: very potent cellular activity, correlated to Brachyury and EGFR degradation in multiple cell lines. Good in vivo efficacy in UCH-1 SCID mice

- **CDKs**

- **NMS-Probe12 and analogs:** multi-kinase inhibitors with cellular selectivity.

  Tool for the identification of new potential kinase targets involved in chordoma cell line proliferation

Chordoma cell line inactive kinase inhibitors (probe cpds)

Compounds with IC50 > 2uM on UCH-1/2	UCH-1 (IC50 uM)	UCH-2 (IC50 uM)	Excluded Target	Number of Excluded Targets with compound
AC-220/Quizartinib	>10	>10	CSF1R/FMS; FLT1/VEGFR1; FLT3; FLT4/VEGFR3; KIT; PDGFRA; PDGFRB; RET;	8
Barasertib; AZD1152-HQPA	>10	>10	AURKC; FLT3; KIT; MEK5/MAP2K5; PDGFRA; PDGFRB;	6
Cabozantinib	6.561	5.806	RET; VEGFR2/KDR;	2
Crizotinib	2.943	3.830	LCK; LOK/STK10; SLK/STK2; TRKB;	4
Dabrafenib	37% @ 0.3uM	46% @ 0.3uM	BRAF; RAF1/CRAF;	2
Dasatinib	2.093	3.911	ABL; ABL2/ARG; BLK; CSF1R/FMS; DDR1; DDR2; EPHA2; EPHA8; FGR; FYN; KIT; LCK; LYN; MEK5/MAP2K5; p38-alpha; PDGFRA; PDGFRB; ZAK/MLTK;	18
Doramapimod, BIRB-796 (p38-alpha)	>10	>10	DDR1; DDR2; JNK2; LOK/STK10; p38-alpha; p38-beta;	6
Imatinib	>10	>10	ABL; ABL2/ARG; CSF1R/FMS; DDR1; DDR2; KIT; LCK; PDGFRA; PDGFRB;	9
MLN-518/Tandutinib	>10	>10	CSF1R/FMS; FLT3; KIT; PDGFRA; PDGFRB;	5
Nilotinib	2.407	2.975	ABL; ABL2/ARG; CSF1R/FMS; DDR1; DDR2; EPHA8; KIT; LCK; p38-beta; ZAK/MLTK;	10
NMS-probe1	21.1% @ 0.3uM	6.1% @ 0.3uM	AKT3;	1
NMS-probe2	-16% @ 0.3uM	-14% @ 0.3uM	AKT3;	1
NMS-probe69A	14% @ 0.3uM	0% @ 0.3uM	SULU1;	1
NMS-probe3	11.4% @ 0.3uM	11.8% @ 0.3uM	AKT3;	1
NMS-probe9	>10	>10	BRAF; RAF1/CRAF;	2
NMS-probe76	>10	>10	BRAF; RAF1/CRAF;	2
NMS-probe83	6.790	6.430	BRAF;	1
NMS-probe16 (KIT)	8.519	9.465	CSF1R/FMS; FLT1/VEGFR1; FLT3; KIT; (LOK/STK10; ZAK/MLTK;)	6
NMS-probes etc.				
etc.	↓	↓		
etc.	↓	↓		
	↓	↓		
VX-745 (Vertex p38)	>10	>10	p38-alpha;	1

Invalidated Chordoma driver targets : 34 kinases

Target	NMS-Probe12 A Chordoma cell line active multikinase inhibitor			Chordoma cell line inactive kinase inhibitors
	KSS 1 (% I)	KSS2 (% I)	KSS (IC50 uM)	Compounds with Kd or IC50 <50nM on target and inactive on UCH1/2
ABL1	98	94	0.056	Dasatinib; Imatinib; NMS-probe8; Nilotinib;
ABL2/ARG	98	84		Dasatinib; Imatinib; Nilotinib
AKT3	100	-2		NMS-probe1; NMS-probe2; NMS-probe3
AURKC	81	3		R406 (Fostamatinib active metab); AZD-1152HQPA
BLK	99	74		R406; Dasatinib; NMS-probe8
BRAF	77	37	2.939	Dabrafenib, SB-590885, Plexxikon (PLX-4720), and other NMS-probes.....
CSF1R/FMS	95	61		MLN-518/Tandutinib; Pazopanib; PTK-787; R406; Sorafenib; Sunitinib; AC220; Dasatinib; Imatinib; NMS-probe16 (Kd exper 0.88nM); Nilotinib
DDR1	100	93		R406; Sorafenib; BIRB-796; Dasatinib; Imatinib; Nilotinib
DDR2	88	100		R406; Sorafenib; BIRB-796; Dasatinib; Imatinib; Nilotinib
EPHA2	87	95	0.050	Dasatinib; NMS-probe8 (KSS 58nM); more NMS cpds available
EPHA8	99	92		Dasatinib; Nilotinib
FGR	60	64		R406; Dasatinib;
FLT1/VEGFR1	95	68		Pazopanib; PTK-787; R406; Sorafenib; Sunitinib; AC220; NMS-probe16;
FLT3	100	89	0.071	MLN-518/Tandutinib; R406; Sorafenib; Sunitinib; AC220; AZD-1152HQPA; NMS-probe16; NMS-probe15; NMS-probe97
FLT4/VEGFR3	94	86		Pazopanib; R406; Sunitinib; AC220;
etc.				Cpds X, Y , Z etc.
etc.				etc.
34 targets overall				

Conclusions

- The increasing relevance of context dependent poly-pharmacology in treating complex diseases, such as cancer, has a significant impact on the strategic configuration of compound collections, screening and optimization methods
- Multiparametric optimizations from the onset of *hit-to-lead* phases and leverage on the combination of effects are bound to have a consolidated role in drug discovery
- Chemogenomic screening helps to convert phenotypic screening endeavors into target-based drug discovery, at times involving multiple targets simultaneously
- Multiple, structurally distinct chemotypes with affinity against a particular target provide confidence in a target.
- Developing and applying selective chemical probes against novel (unannotated) targets is an area for collaborative partnerships between academic institutions and pharmaceutical companies
- Opportunities to repurpose existing drugs into new applications are created