A CRITICAL EVALUATION OF THE COMMONLY USED BIOCHEMICAL AND CELL-BASED ASSAYS

Agenda

1. Overview of the Fraunhofer
2. The role of assays pre-clinical drug discovery
3. Evaluation of biochemical and cell-based assays

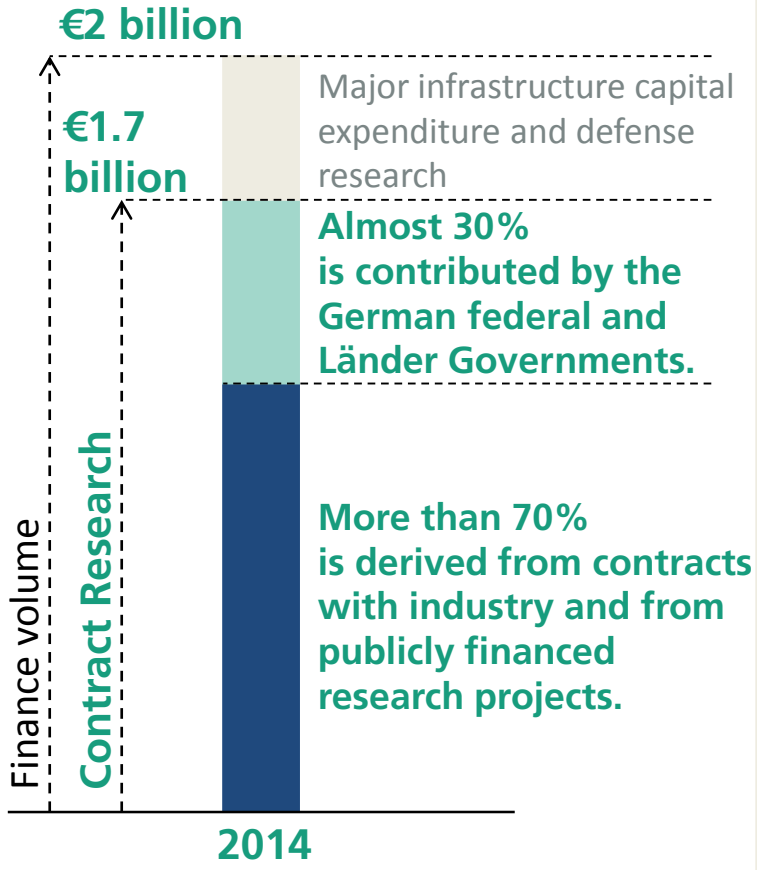
Overview of the Fraunhofer

The Fraunhofer-Gesellschaft

The Fraunhofer-Gesellschaft undertakes applied research of direct utility to private and public enterprise and of wide benefit to society.

Nearly **24,000** staff

66 institutes and research units

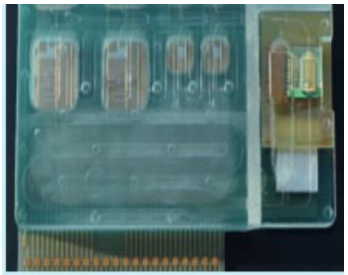


Fraunhofer Health Collaborations

Prevention research
Preventing childhood obesity

Infection research
The green biofactory in the fight against AIDS and malaria

Cancer research
Treating cancer accurately with laser beams



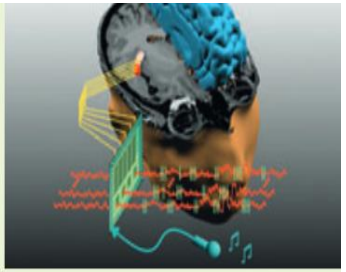
Biomedical engineering
Developing diagnostic microsystems

Prevention research
National cohort to investigate the genesis of common diseases

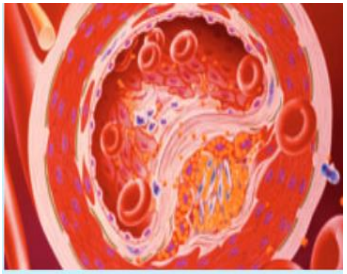
Diabetes research
Customized prevention and treatment

Gerontology
Assistance systems for the home

Biomedical engineering
Factory-made skin

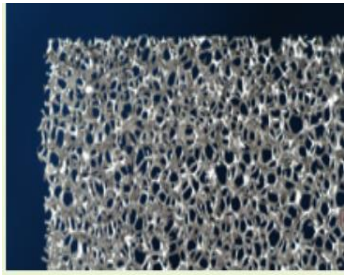


Brain research
Neuroprosthetics and deep-brain stimulation



Cardiovascular research
Gene variant determines cholesterol levels

Prevention research
Identifying risks associated with medication



Medical materials
Metallic foam structures as a bone substitute

Capabilities of the Fraunhofer-IME

National

International

Applied Ecology Division
Schmallenberg (1959)

Molecular Biology Division
Aachen (2000)

Center for Molecular Biotechnology
Newark, USA (2001)

Bioresources
Gießen (2009)

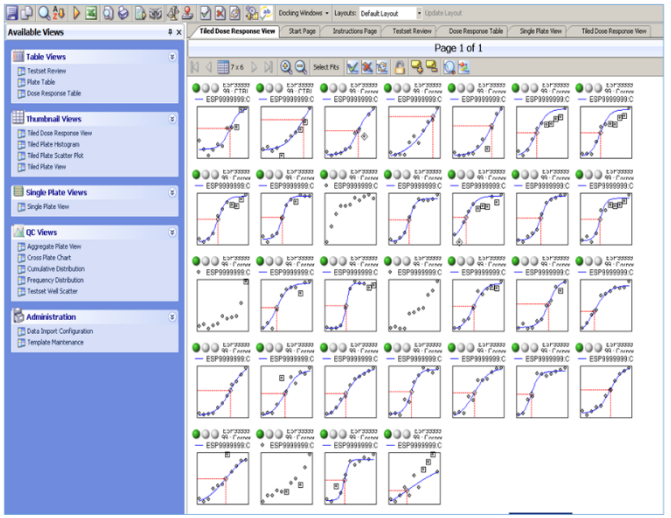
Biopolymers
Münster (2010)

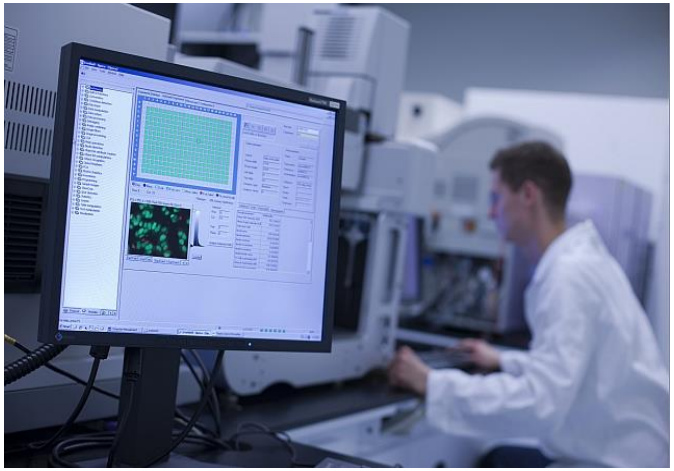
TMP
Frankfurt (2012)

ScreeningPort
Hamburg (2014)

Center for Systems Biotechnology
Santiago, Chile (2010)

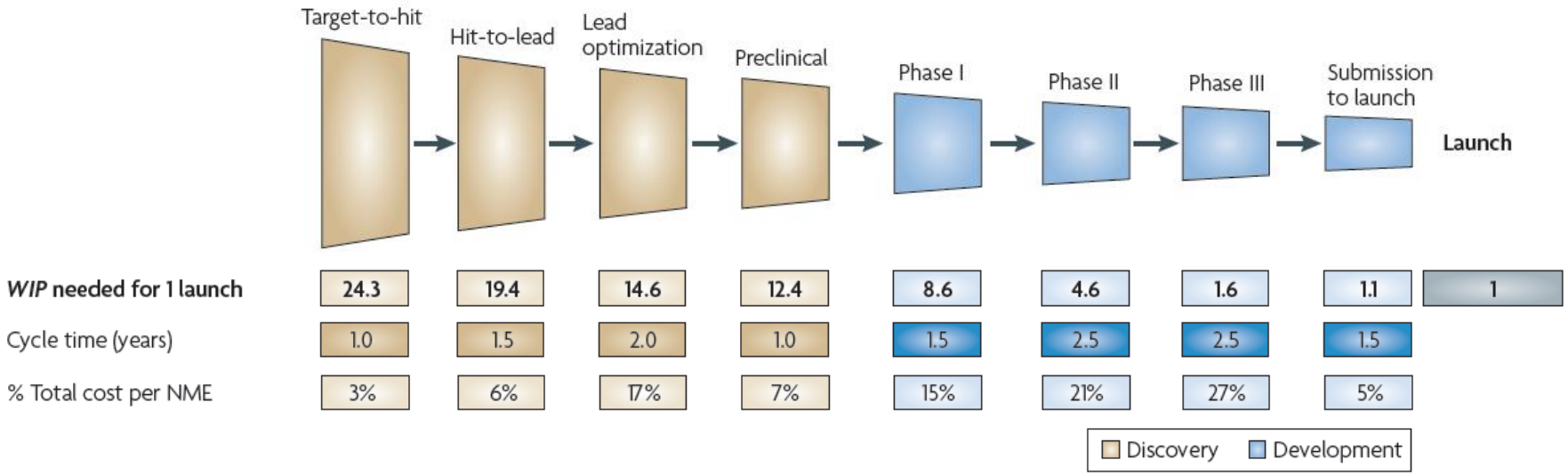
Capabilities of the Fraunhofer-IME SP





The role of assays pre-clinical drug discovery

Drug discovery cascade



Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, USA.

How to improve R&D productivity: the pharmaceutical industry's grand challenge

Steven M. Paul, Daniel S. Mytelka, Christopher T. Dunwiddie, Charles C. Persinger, Bernard H. Munos, Stacy R. Lindborg and Aaron L. Schacht

NATURE REVIEWS | DRUG DISCOVERY
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HTS in Big Pharma: small molecules

Table 2 | **Examples of recently approved drugs with origins in HTS hits**

Drug (US trade name; company)	Indication	Target class	Year HTS was run	Year of FDA approval
Gefitinib (Iressa; AstraZeneca)	Cancer	Tyrosine kinase	c. 1993	2003
Erlotinib (Tarceva; Roche)	Cancer	Tyrosine kinase	c. 1993	2004
Sorafenib (Nexavar; Bayer/Onyx Pharmaceuticals)	Cancer	Tyrosine kinase	1994	2005
Tipranavir (Aptivus; Boehringer Ingelheim)	HIV	Protease	c. 1993	2005
Sitagliptin (Januvia; Merck & Co)	Diabetes	Protease	c. 2000	2006
Dasatinib (Sprycel; Bristol-Myers Squibb)	Cancer	Tyrosine kinase	1997	2006
Maraviroc (Selzentry; Pfizer)	HIV	GPCR	1997	2007
Lapatinib (Tykerb; GlaxoSmithKline)	Cancer	Tyrosine kinase	c. 1993	2007
Ambrisentan (Letairis; Gilead)	Pulmonary hypertension	GPCR	c. 1995	2007
Etravirine (Intelence; Tibotec Pharmaceuticals)	HIV	Reverse transcriptase	c. 1992	2008
Tolvaptan (Samsca; Otsuka Pharmaceutical)	Hyponatraemia	GPCR	c. 1990	2009
Eltrombopag (Promacta; GlaxoSmithKline)	Thrombocytopaenia	Cytokine receptor	1997	2008

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Dejan Bojanic is at the Lead Finding Platform, Novartis Institutes for Biomedical Research Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts 02139, USA.

David J. Burns is with the Early Pain Discovery Team, Abbott Laboratories, R4DI AP52N 200 Abbott Park Road, Abbott Park, Illinois 60064, USA.

Darren V. S. Green is at the Department of Computational and Structural Chemistry, GlaxoSmithKline, Stevenage, Hertfordshire SG1 2NY, UK.

Robert P. Hertzberg is at the Department of Screening and Compound Profiling, GlaxoSmithKline, Mail Stop UP 12-L05, 1250S Collegeville Rd, Collegeville, Pennsylvania 19426, USA.

William P. Janzen is at the Division of Medicinal Chemistry and Natural Products, Center for Integrative Chemical Biology and Drug Discovery, Eshelman School of Pharmacy, The University of North Carolina, Chapel Hill, North Carolina 27599-7363, USA.

Dragan A. Cirovic is at the Department of Lead Generation and Candidate Realization, Sanofi-Aventis, 1041 Route 202-206, Mail Stop BRJR1-002A, Bridgewater, New Jersey 08807, USA.

Tina Garyantes is at the Department of Lead Generation and Candidate Realization, Sanofi-Aventis, 1041 Route 202-206, P.O. BOX 6800, JR-303D, Bridgewater, New Jersey 08807, USA.

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G. Sitta Sittampalam is at the Department of Pharmacology, Toxicology & Therapeutics, Institute for Advancing Medical Innovations, The University of Kansas Cancer Center, 3901 Rainbow Blvd., Kansas City, Kansas 66160, USA.

OPINION

Impact of high-throughput screening in biomedical research

Ricardo Macarron, Martyn N. Banks, Dejan Bojanic, David J. Burns, Dragan A. Cirovic, Tina Garyantes, Darren V. S. Green, Robert P. Hertzberg, William P. Janzen, Jeff W. Paslay, Ulrich Schopfer and G. Sitta Sittampalam

NATURE REVIEWS | DRUG DISCOVERY

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Phenotypic screening & cancer drug discovery

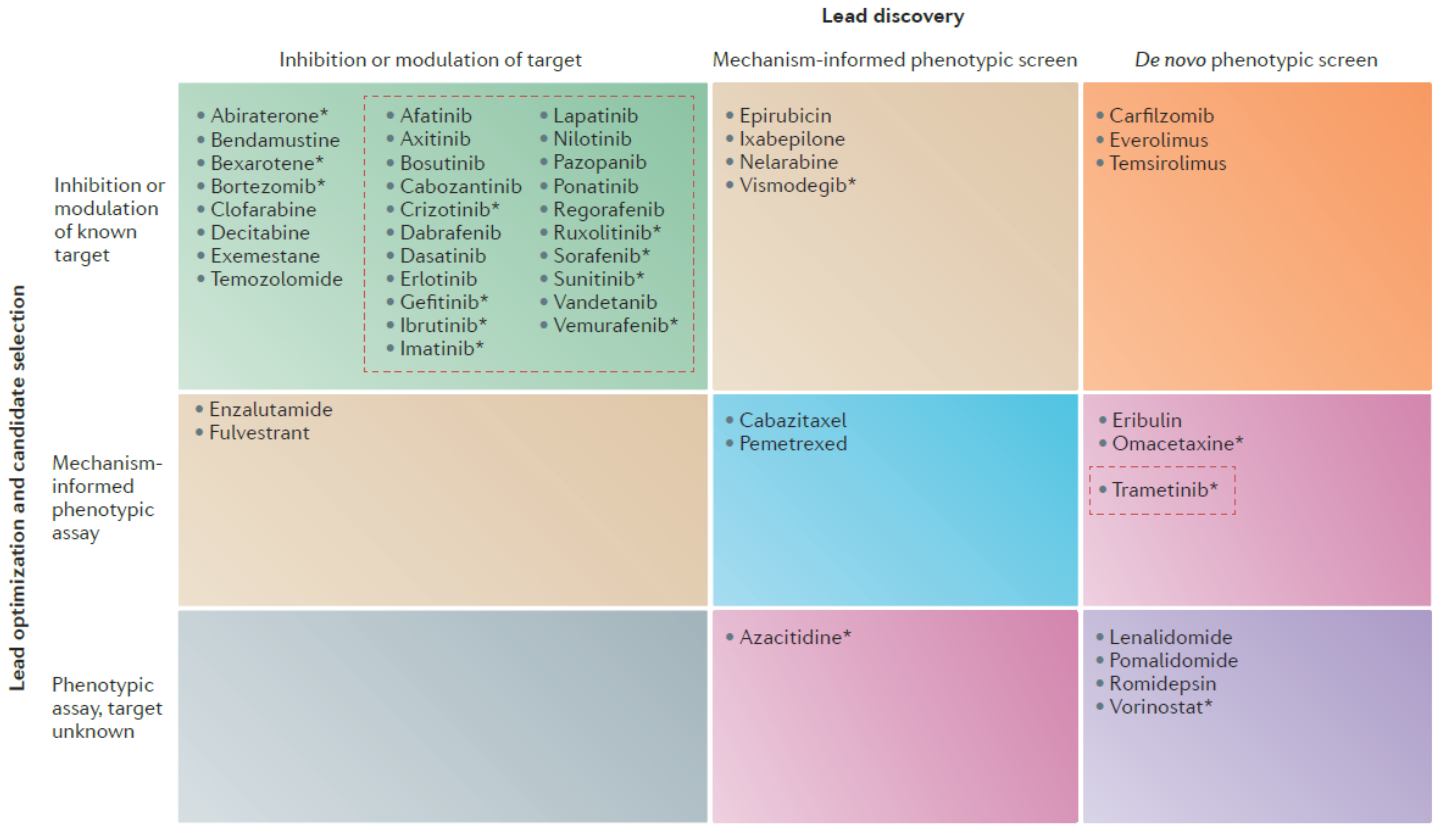


Figure 1 | **Origins of new small-molecule cancer drugs approved by the FDA between 1999 and 2013.** Kinase inhibitors are highlighted within the dotted boxes. Information on the drugs to be analysed was obtained from the US Food and Drug Administration (FDA) website. *First-in-class drug.

[1]Department of Biochemical and Cellular Pharmacology, Genentech, South San Francisco, California 94080, USA.
[2]Department of Discovery Chemistry, Genentech, South San Francisco, California 94080, USA.
[3]IOTA Pharmaceuticals, St Johns Innovation Centre, Cowley Road, Cambridge CB4 0WS, UK.

Phenotypic screening in cancer drug discovery — past, present and future
John G. Moffat[1], Joachim Rudolph[2] and David Bailey[3]
NATURE REVIEWS | DRUG DISCOVERY
588 | AUGUST 2014 | VOLUME 13

How may drug targets are there?

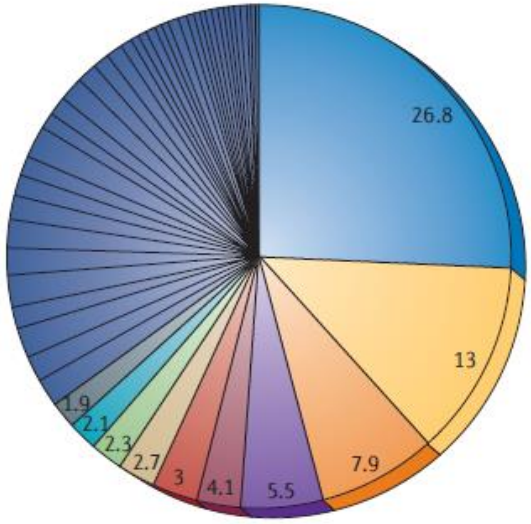


Figure 1 | **Gene-family distribution of current drugs per drug substance.**

Table 1 | **Molecular targets of FDA-approved drugs**

Class of drug target	Species	Number of molecular targets
Targets of approved drugs	Pathogen and human	324
Human genome targets of approved drugs	Human	266
Targets of approved small-molecule drugs	Pathogen and human	248
Targets of approved small-molecule drugs	Human	207
Targets of approved oral small-molecule drugs	Pathogen and human	227
Targets of approved oral small-molecule drugs	Human	186
Targets of approved therapeutic antibodies	Human	15
Targets of approved biologicals	Pathogen and human	76

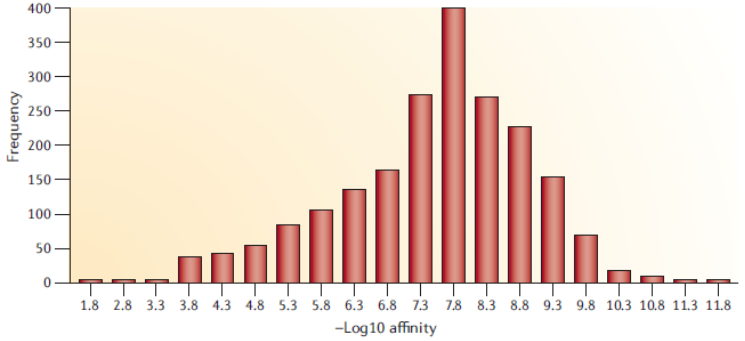


Figure 2 | **Frequency distribution for small-molecule drug potencies.**

John P. Overington and Bissan Al-Lazikani are at Inpharmatica Ltd., 1 New Oxford Street, London, WC1A 1NU, UK.

Andrew Hopkins is at Pfizer Global Research and Development, Sandwich, Kent, CT13 9NJ, UK.

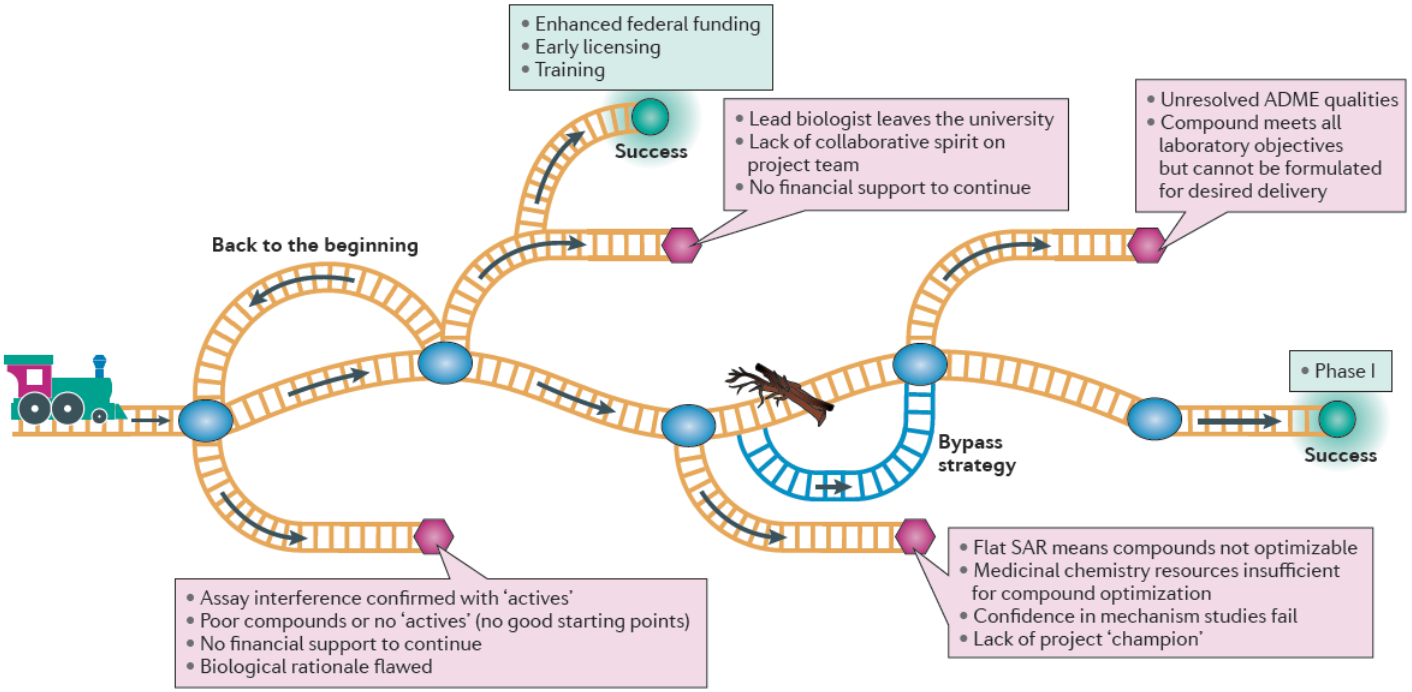
OPINION

How many drug targets are there?

John P. Overington, Bissan Al-Lazikani and Andrew L. Hopkins

NATURE REVIEWS | DRUG DISCOVERY
VOLUME 5 | DECEMBER 2006 | 993

Realistic drug discovery cascade



Targets

- Select validated, novel targets
- Gauge druggability and biomarkers
- Engage structural biology
- Consider phenotypic targets

Assays

- Ensure the HTS assay is on target
- Require orthogonal/ cross-validating assays

Medicinal chemistry

- Build a better HTS library
- Avoid the lure of nuisance compounds
- Demand an effective screening tree that includes ADMET
- Make sure compounds are well characterized

Preclinical pharmacology

- Establish redundant lines of evidence
- Ensure target engagement
- Demand an interpretable SAR
- Check for mechanistic soundness
- Assess promiscuity and off-target effects

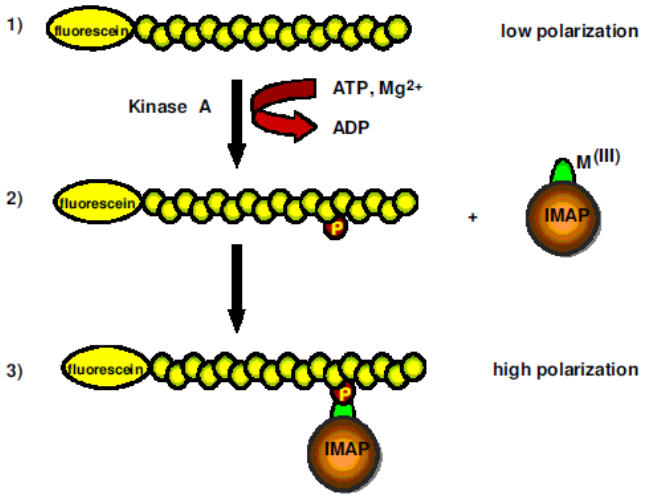
Organization

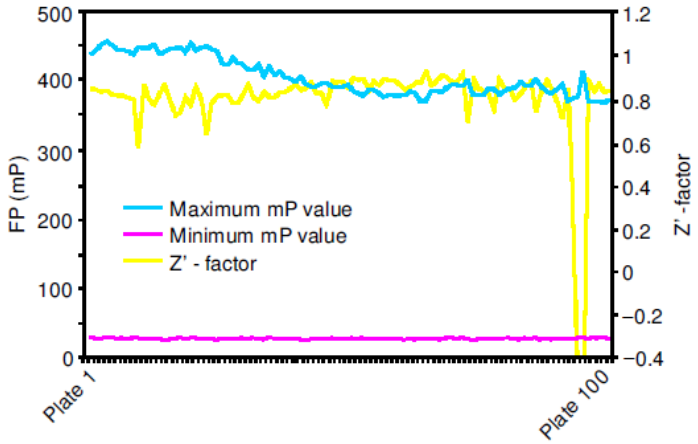
- Finance collaborations
- Play to academic strengths
- Forage for best projects
- Fund for initial results
- Consult experts
- Reward translational research

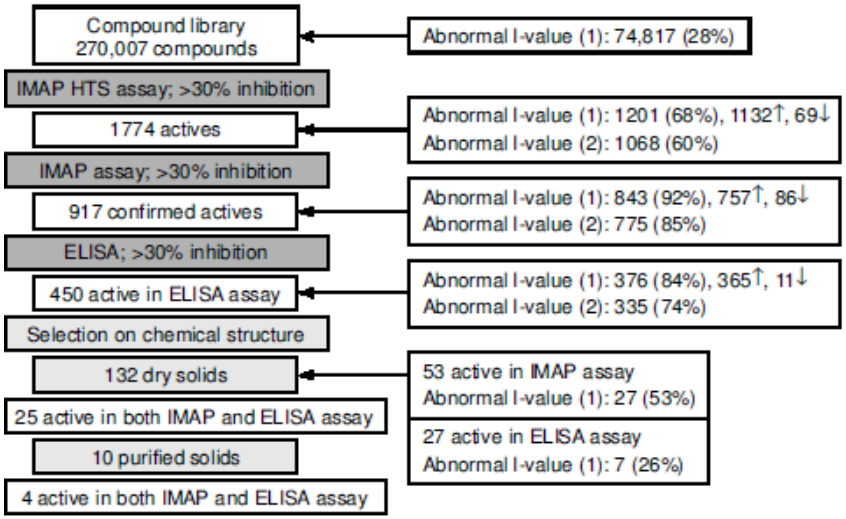
Mitigating risk in academic preclinical drug discovery
Jayme L. Dahlin[1], James Inglese[2,3] and Michael A. Walters[4]
NATURE REVIEWS | DRUG DISCOVERY
VOLUME 14 | APRIL 2015 | 279

Assay systems and High Throughput Screening

Example biochemical assay HTS output





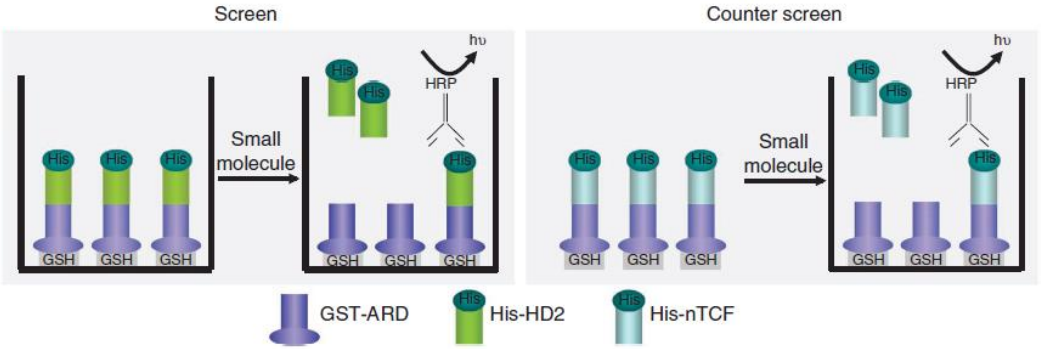


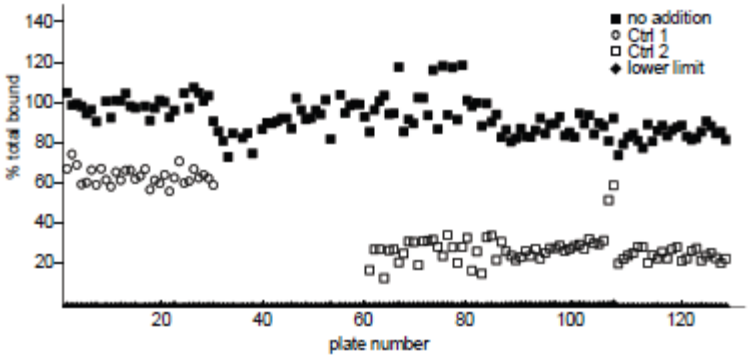
ASSAY and Drug Development Technologies
Volume 1, Number 3, 2003

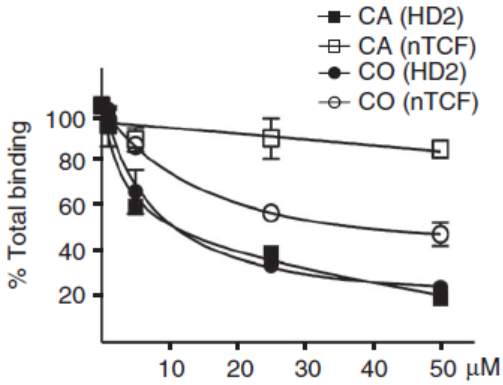
High-Throughput Screening with Immobilized Metal Ion Affinity-Based Fluorescence Polarization Detection, A Homogeneous Assay for Protein Kinases

Elma E.M.G. Loomans, Antoon M. van Doornmalen, Jesse W.Y. Wat, and Guido J.R. Zaman

Example ELISA HTS output







statistics	LOPAC screen	Phytopure screen	MRCT Screen
average Z	0.35	0.32	0.70
# of compounds	1280	1280	45'000
percent hit rate	2.5% (32)	3.8% (49)	0.024% (12)
# of discarded hits	32	46	12
% confirmed hits (#)	0% (0)	0.15% (3)	0% (0)

nature COMMUNICATIONS OPEN

ARTICLE

Received 27 Sep 2011 | Accepted 11 Jan 2012 | Published 21 Feb 2012 DOI: 10.1038/ncomms1680

An intrinsically labile α-helix abutting the BCL9-binding site of β-catenin is required for its inhibition by carnosic acid

Marc de la Roche[1], Trevor J. Rutherford[1], Deepti Gupta[1], Dmitry B. Veprintsev[1,†], Barbara Saxty[2], Stefan M. Freund[1] & Mariann Bienz[1]

Example *in silico* output

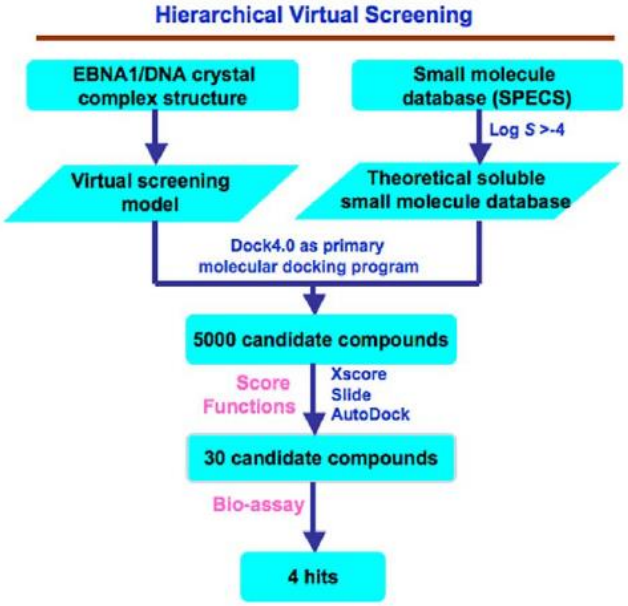


Figure 1. Flow chart of virtual and experimental screening strategy for discovering EBNA1 inhibitors. The EBNA1/DNA crystal structure was computationally fitted into a 6 Å grid containing every residue of the EBNA1 DNA-binding pocket was used to dock a library of compounds from the SPECS database. Compounds were preselected for solubility in an aqueous solution using a log *S* value of greater than −4. A database of ~90,000 small-molecule compounds were then analyzed by one primary docking programs and three score functions to calculate the free energy of binding. 5000 candidates were then re-examined using Xscore, Slide, and AutoDock programs to select 30 top candidates. The top 30 compounds from 15 manually classified groups were selected for experimental DNA binding and cell-based bioassays.

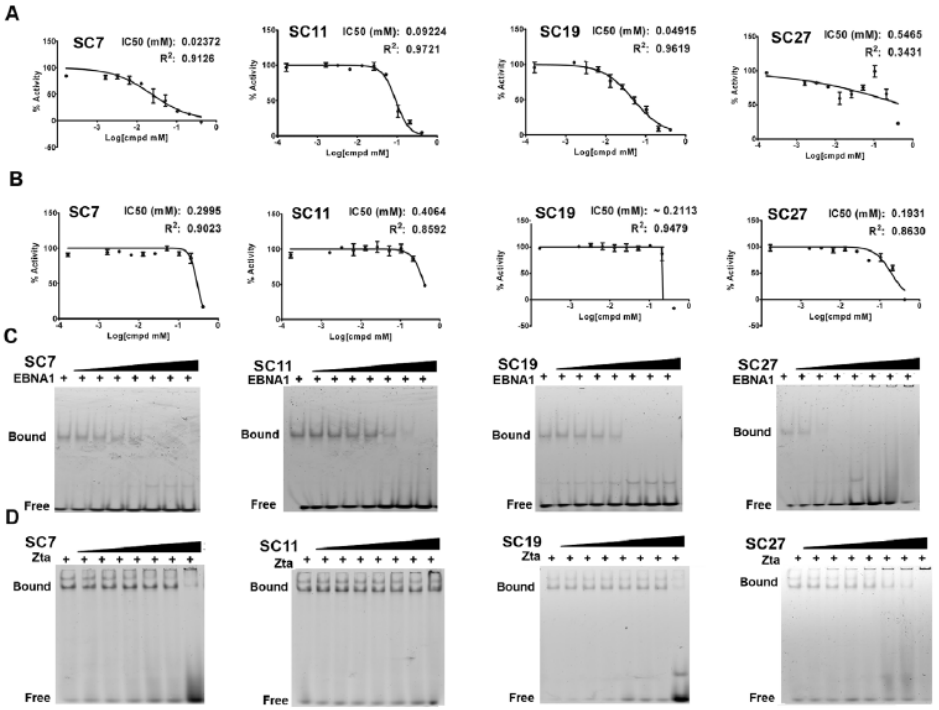


Figure 3. Physical inhibition of EBNA1-DNA binding assays. Candidate inhibitors SC7, SC11, SC19, and SC27 were assayed by fluorescence polarization (FP) for inhibition of EBNA1-DNA binding (panel A) and for inhibition of Zta-DNA binding (panel B). IC50 values were calculated for each isotherm. Inhibitor concentrations were diluted 2-fold from 833 to 7 μM for each compound. Inhibitors were also assayed using a secondary EMSA assay to monitor EBNA1-DNA binding (panel C) or Zta-DNA binding (panel D) using the same concentrations of inhibitor compounds (two fold dilutions from 833 to 7 μM) as that shown for FP assays in panels A and B, above.

OPEN ACCESS Freely available online

PLoS one

Discovery of Selective Inhibitors Against EBNA1 via High Throughput *In Silico* Virtual Screening

Ning Li[1☯], Scott Thompson[2☯], David C. Schultz[2], Weiliang Zhu[1], Hualiang Jiang[1], Cheng Luo[1*], Paul M. Lieberman[2*]

1 Drug Discovery and Design Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China, 2 The Wistar Institute, Philadelphia, Pennsylvania, United States of America

Example stem-cell based screen

Cell Stem Cell
Resource

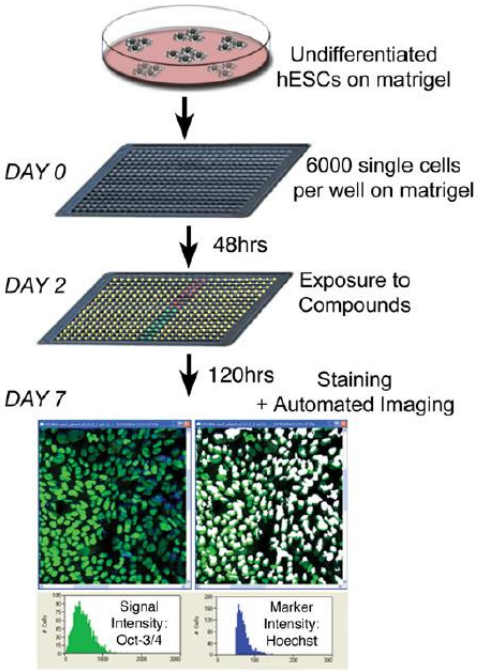
High-Throughput Screening Assay for the Identification of Compounds Regulating Self-Renewal and Differentiation in Human Embryonic Stem Cells

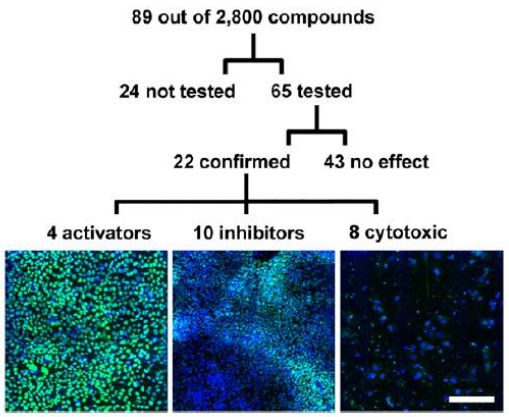
Sabrina C. Desbordes,[1,2,6,*] Dimitris G. Placantonakis,[2,5] Anthony Ciro,[3] Nicholas D. Socci,[4] Gabsang Lee,[1,2] Hakim Djaballah,[3] and Lorenz Studer[1,2,*]
[1]Developmental Biology Program
[2]Department of Neurosurgery
[3]High-Throughput Screening Core Facility
[4]Computational Biology Center
Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA
[5]Department of Neurological Surgery, Weill Cornell Medical College, 525 East 68th Street, New York, NY 10021, USA
[6]Present address: Differentiation and Cancer Program, Centre de Regulació Genòmica, C/Dr. Aiguader 88, 08003 Barcelona, Spain

Cell Stem Cell *2*, 602–612, June 2008

SUMMARY

High-throughput screening (HTS) of chemical libraries has become a critical tool in basic biology and drug discovery. However, its implementation and the adaptation of high-content assays to human embryonic stem cells (hESCs) have been hampered by multiple technical challenges. Here we present a strategy to adapt hESCs to HTS conditions, resulting in an assay suitable for the discovery of small molecules that drive hESC self-renewal or differentiation. Use of this new assay has led to the identification of several marketed drugs and natural compounds promoting short-term hESC maintenance and compounds directing early lineage choice during differentiation. Global gene expression analysis upon drug treatment defines known and novel pathways correlated to hESC self-renewal and differentiation. Our results demonstrate feasibility of hESC-based HTS and enhance the repertoire of chemical compounds for manipulating hESC fate. The availability of high-content assays should accelerate progress in basic and translational hESC biology.





PCSK9 (protease) biochemical assay

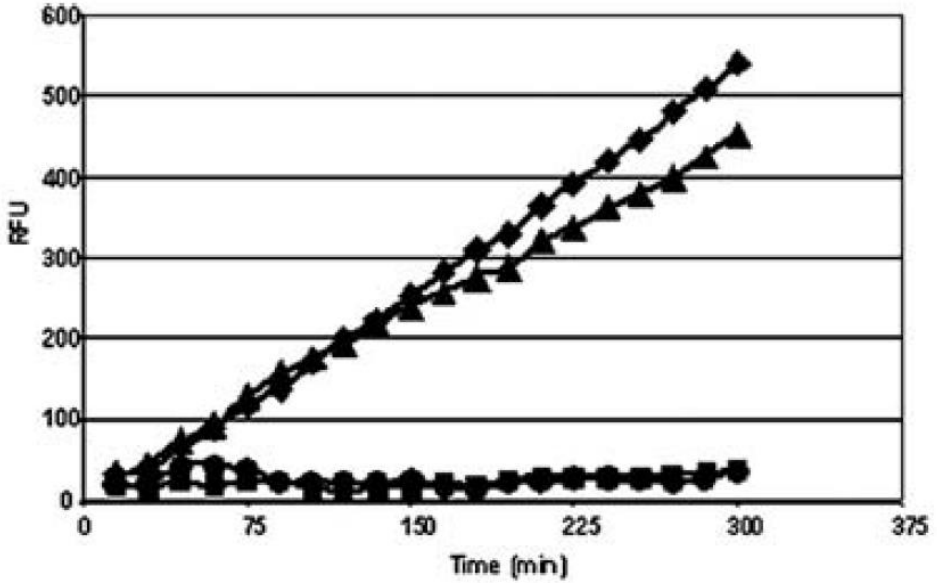
Functional characterization of Narc 1, a novel proteinase related to proteinase K

Saule Naureckiene,[a] Linh Ma,[a] Kodangattil Sreekumar,[b] Urmila Purandare,[a,1] C. Frederick Lo,[a] Ying Huang,[c] Lillian W. Chiang,[d,2] Jill M. Grenier,[d,3] Bradley A. Ozenberger,[a] J. Steven Jacobsen,[a] Jeffrey D. Kennedy,[a] Peter S. DiStefano,[d,4] Andrew Wood,[a] and Brendan Bingham[a,*]

Archives of Biochemistry and Biophysics 420 (2003) 55–67

Narc 1 activity on fluorogenic substrates[a]

Substrate	Sequence	Relative activity (%)
Subtilisin substrate	Z-GGL-AMC	4.5
Furin substrate	Boc-RVRR-AMC	20
TPP II substrate	H-AAF-AMC	1.4
Processing site substrates		
SN-1	Dnp-FAQSIPK-AMC	100
SN-2	Dnp-DSLVFAK-AMC	1.2
SN-3	Dnp-FANAIPK-AMC	82



However, PCSK9 is bound to its pro-domain and catalytically inactive

The Crystal Structure of PCSK9: A Regulator of Plasma LDL-Cholesterol

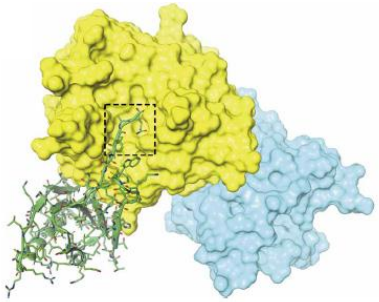
Derek E. Piper,[1] Simon Jackson,[2] Qiang Liu,[3] William G. Romanow,[1] Susan Shetterly,[2] Stephen T. Thibault,[1] Bei Shan,[2] and Nigel P.C. Walker[1,*]

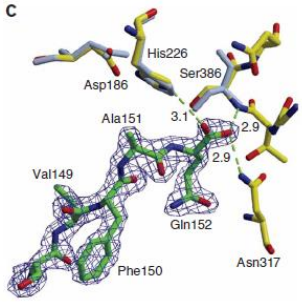
Structure *15*, 545–552, May 2007

Structural and biophysical studies of PCSK9 and its mutants linked to familial hypercholesterolemia

David Cunningham[1], Dennis E Danley[1], Kieran F Geoghegan[1], Matthew C Griffor[1], Julie L Hawkins[1], Timothy A Subashi[1], Alison H Varghese[1], Mark J Ammirati[1], Jeffrey S Culp[1], Lise R Hoth[1], Mahmoud N Mansour[1], Katherine M McGrath[1], Andrew P Seddon[1], Shirish Shenolikar[2], Kim J Stutzman-Engwall[1], Laurie C Warren[1], Donghui Xia[1] & Xiayang Qiu[1]

NATURE STRUCTURAL & MOLECULAR BIOLOGY
VOLUME 14 NUMBER 5 MAY 2007 413





PCSK9: 12 years from target ID to drug

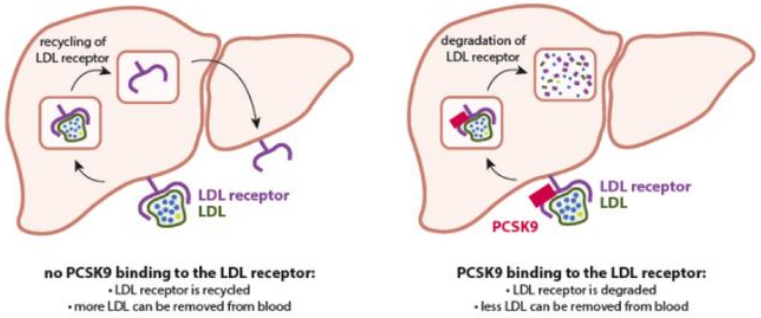


Figure 2. PCSK9 can degrade the LDL receptor. *If the LDL receptor is not bound to PCSK9, it can be recycled back to the cell surface to continue removing LDL from the blood. If PCSK9 binds, it leads to the degradation of the LDL receptor inside the liver cell.*

PRALUENT® (alirocumab) Injection

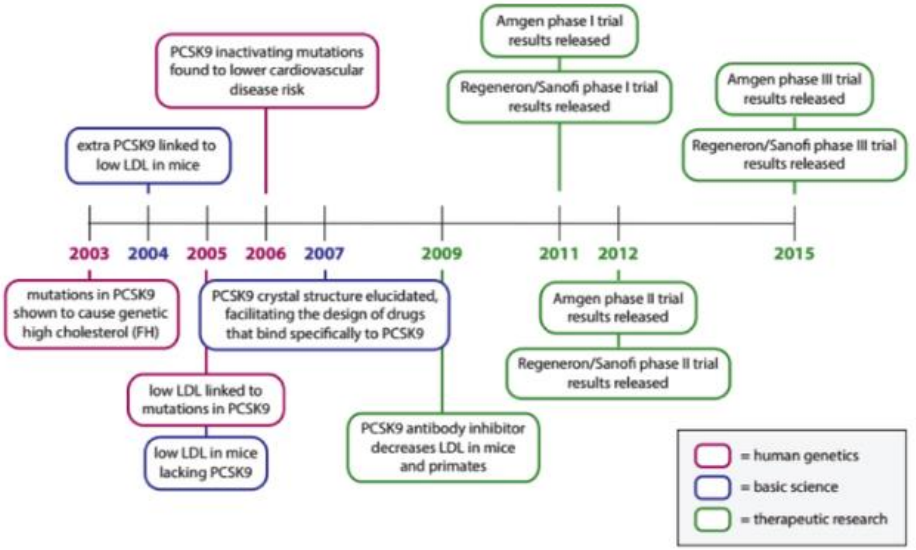
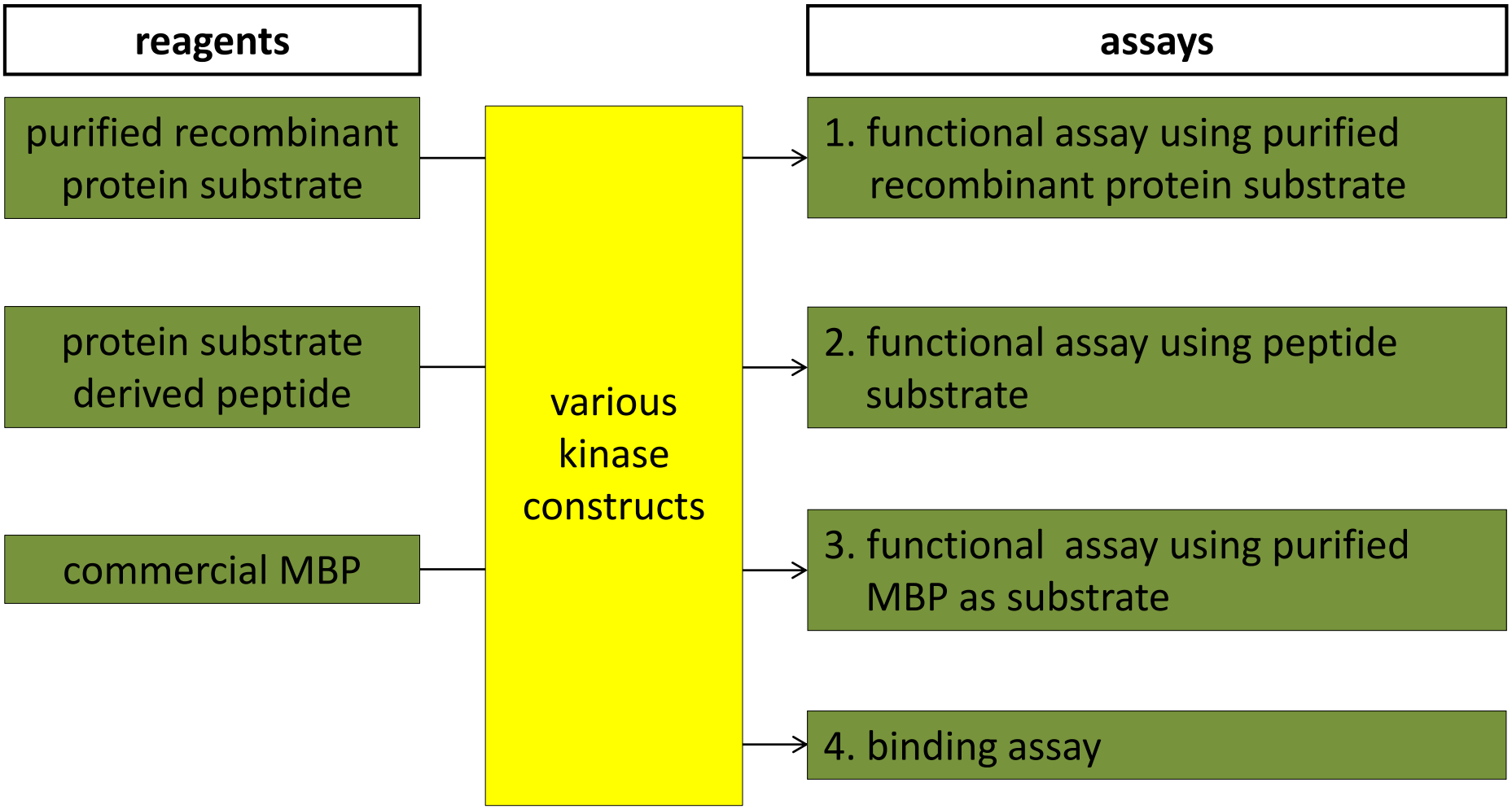


Figure 4. A timeline of PCSK9 biology. *20 years ago, PCSK9 was not known to influence cholesterol levels. Today, it's a major drug target that could change the way we treat heart disease. PCSK9 could be a true biomedical science success story, with important contributions from human genetics and other basic science fields paving the way for rational drug development.*

http://sitn.hms.harvard.edu/flash/2015/a-potential-new-weapon-against-heart-disease-pcsk9-inhibitors/

Example cell-based assay screen output

Strategy for biochemical kinase assays



NIK phosphorylates IKK-α

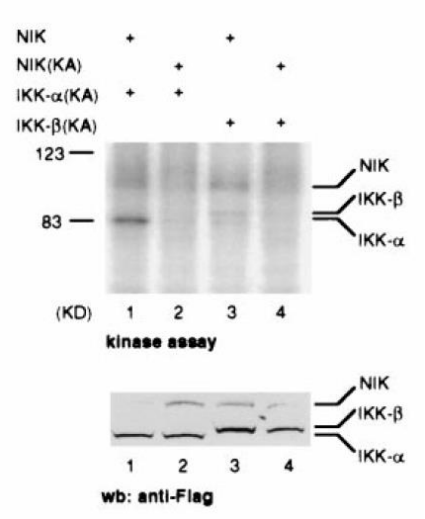
Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 3792–3797, March 1998
Immunology

NF-κB-inducing kinase activates IKK-α by phosphorylation of Ser-176

LEI LING, ZHAODAN CAO, AND DAVID V. GOEDDEL*

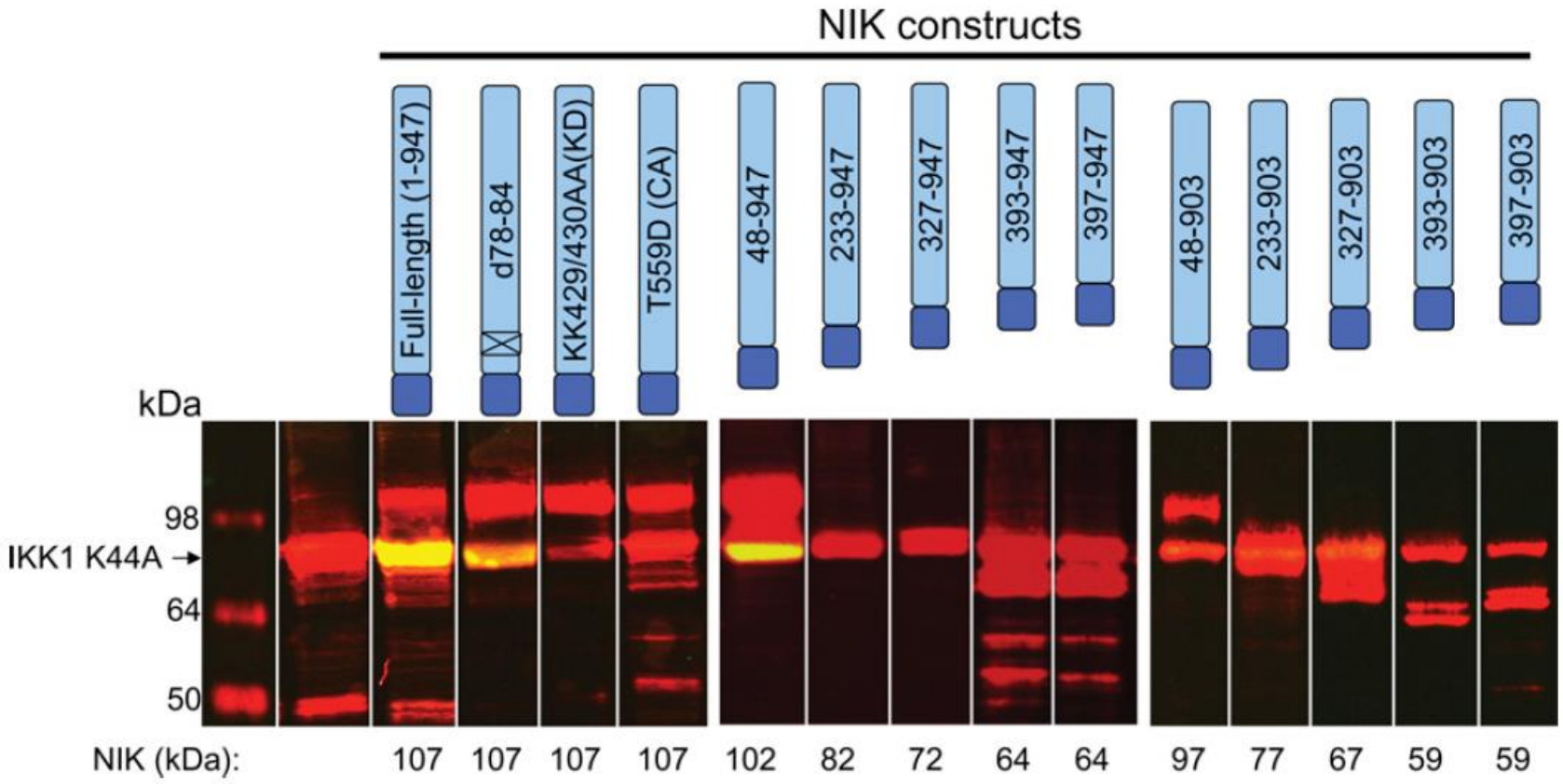
Tularik, Inc., Two Corporate Drive, South San Francisco, CA 94080

Contributed by David V. Goeddel, January 29, 1998



Phosphorylation of IKK-α(KA) and IKK-β(KA) by NIK. 293 cells were transiently transfected with expression plasmids encoding FLAG epitope-tagged wild-type NIK, IKK-α(KA), or IKK-β(KA). Purified proteins were incubated with [γ-^{32}P]ATP, resolved by SDS/PAGE, and analyzed by autoradiography. The amounts of proteins used in the reactions were determined by immunoblotting (wb) with anti-FLAG polyclonal antibodies (*Lower*). The positions of IKK-α, IKK-β, and NIK are indicated.

Cell-based NIK assay



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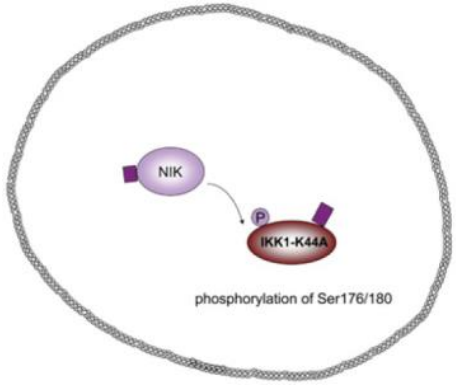
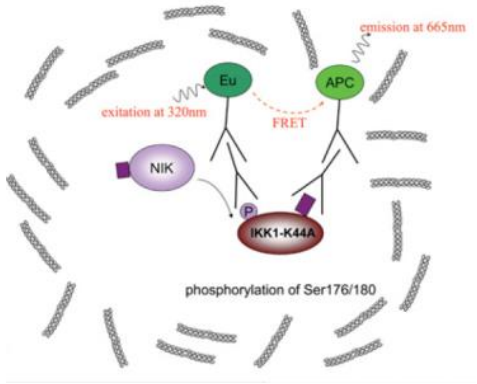
Biochem. J. (2009) 419, 65–73 (Printed in Great Britain) doi:10.1042/BJ20081646
65

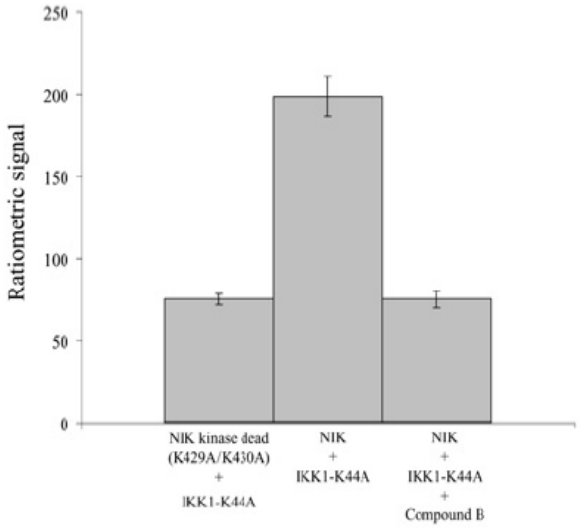
Development of an insect-cell-based assay for detection of kinase inhibition using NF-κB-inducing kinase as a paradigm

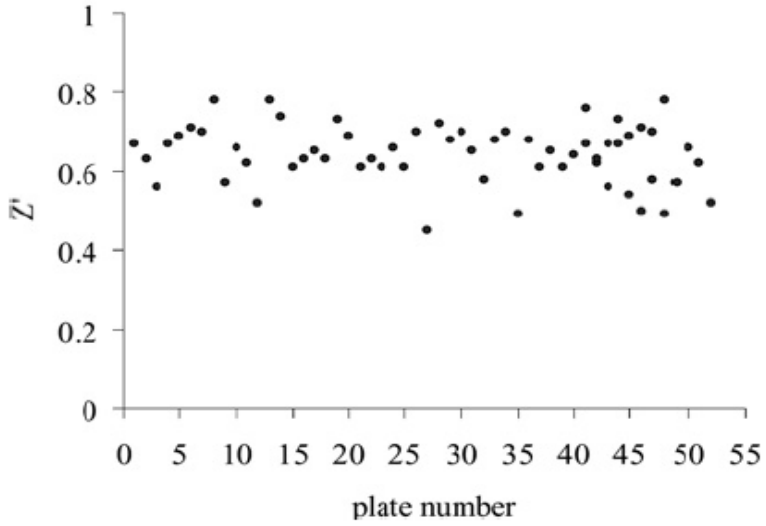
Namir J. HASSAN*[1,2], Sheraz GUL*[1], Fiona FLETT*, Edward HOLLINGSWORTH*, Angela A. DUNNE†, Amanda J. EMMONS†, Jonathan P. HUTCHINSON*, Martin J. HIBBS*, Susan DYOS‡, Jeremy D. KITSON§, Emma HILEY*, Martin RÜDIGER*, David G. TEW*, David J. POWELL* and Mary A. MORSE‖

Insect cell-based assay for NIK









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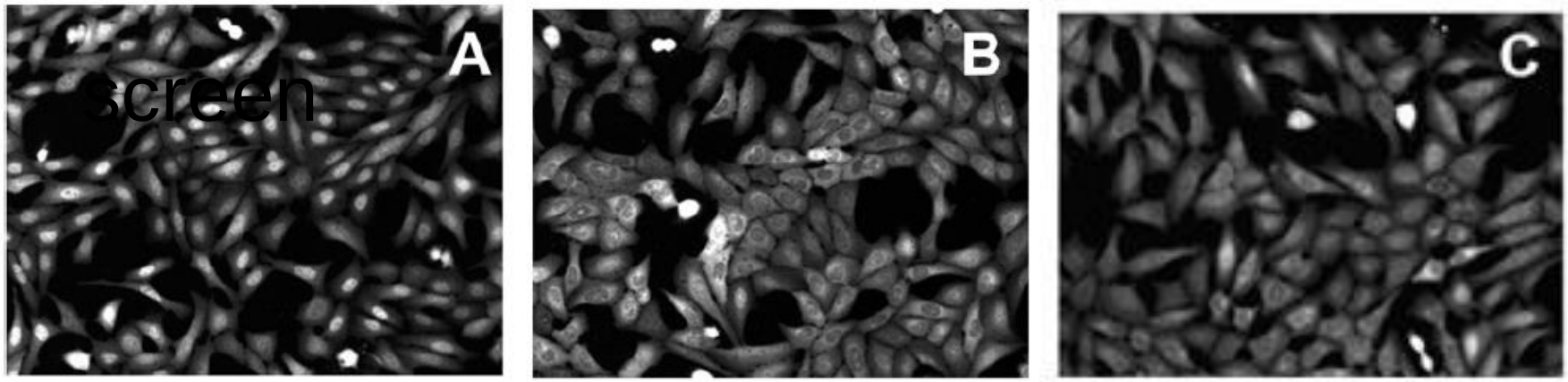
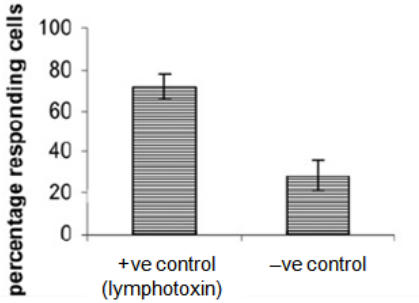
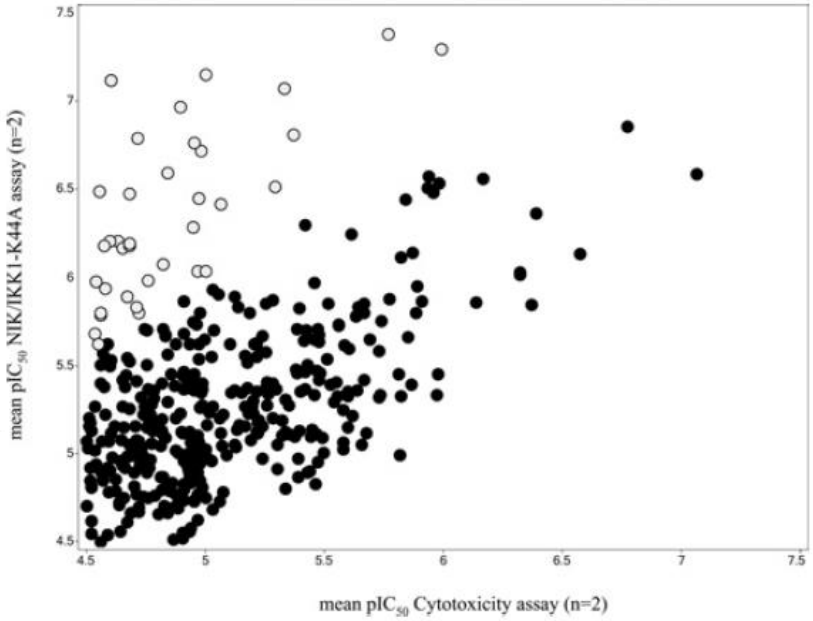
Use of HCS after cell-based NIK inhibitor



+ve control (lymphotoxin) –ve control Hit from cell-based screen





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Further progression of compounds

Assays	Target value	Comments
Aqueous solubility	>100 μM	Important for running *in vitro* assays and for *in vivo* delivery of drug
Log $D_{7.4}$	0–3 (for BBB penetration ca 2)	A measure of lipophilicity hence movement across membranes
Microsomal stability Cl_{int}	<30 $\mu L \cdot min^{-1} \cdot mg^{-1}$ protein	Liver microsomes contain membrane bound drug metabolizing enzymes. This assay measures compound clearance and can give an idea of how fast it will be cleared out *in vivo*
CYP450 inhibition	>10 μM	Main enzymes in body which metabolize drugs and their inhibition can cause toxicity
Caco-2 permeability P_{app}	>1 × 10^{-6} cm^{-1} (asymmetry <2)	Caco-2 colon carcinoma cell line used to estimate permeability across intestinal epithelium, important for drug absorption from gut
MDR1-MDCK permeability P_{app}	>10 × 10^{-6} cm^{-1} (asymmetry <2)	MDCK cells transfected with the MDR1 gene, which encodes the efflux protein P glycoprotein (P-gp). An important efflux transporter in many tissues including intestine, kidney and brain, P-gp can be used to predict intestinal and brain permeability
Hep G2 hepatotoxicity	No effect at 50 × IC_{50} or EC_{50}	Human HepG2 cells can act as a surrogate for effects of toxicity on human liver, an important cause of drug failure in the clinic
Cytotoxicity in suitable cell line	No effect at 50 × IC_{50} or EC_{50}	Reduce the likelyhood of cellular toxicity *in vivo*

CONCLUSIONS

- Conformance with industry stand criteria in drug discovery is essential
- Hurdles for achieving Lead and Candidate compound profiles are high
- Develop a panel of assays for each drug discovery screening project
- Use physiologically assay systems if possible
- Develop both biochemical and cell-based assays for any given target
- Develop a panel of assays and pre-screen all against a training compound library
- Ensure post-screening cascade is in place with suitable Secondary assays