DESIGN OF WORKFLOWS TO IDENTIFY PROBLEM COMPOUNDS FROM SMALL MOLECULE BIOCHEMICAL AND CELL-BASED SCREENS

Agenda

1. Update on drug discovery pipelines
2. Evaluation of biochemical and cell-based assays
3. Workflows to identify problem compounds from small molecule screens

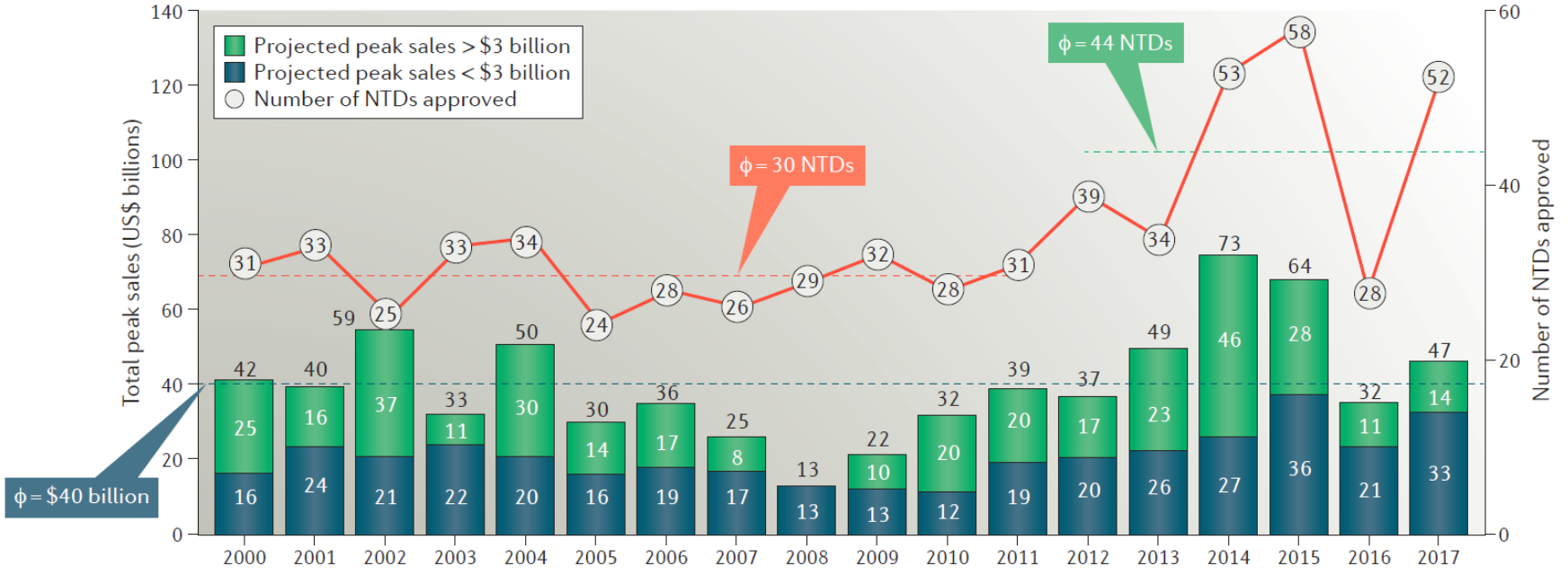
Update on drug discovery pipelines

Drug approvals

Nature Reviews Drug Discovery | Published online 19 Jan 2018; doi:10.1038/nrd.2018.4

2017 FDA drug approvals

The FDA approved 46 new drugs last year, the highest total in more than two decades.



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	
Average peak sales per NTD ($ billions)	1.3	1.2	2.3	1.0	1.5	1.3	1.3	0.9	0.4	0.7	1.1	1.3	0.9	1.4	1.4	1.1	1.1	0.9	ϕ = $1.2 billion
Median peak sales per NTD ($ billions)	0.5	0.7	0.9	0.3	0.7	0.6	0.8	0.5	0.3	0.3	0.4	0.5	0.3	0.6	0.4	0.5	0.7	0.5	ϕ = $0.5 billion

Figure 1 | **FDA drug approvals and projected aggregated peak sales: 2000–2017.** The graph shows the number and aggregate projected peak worldwide annual sales values of new therapeutic drugs (NTDs) by year of FDA approval. All values are inflation-adjusted to 2017. Because of rounding, not all numbers add up to the totals shown. Sources: EvaluatePharma; FDA; Boston Consulting Group analysis.

Drug approvals

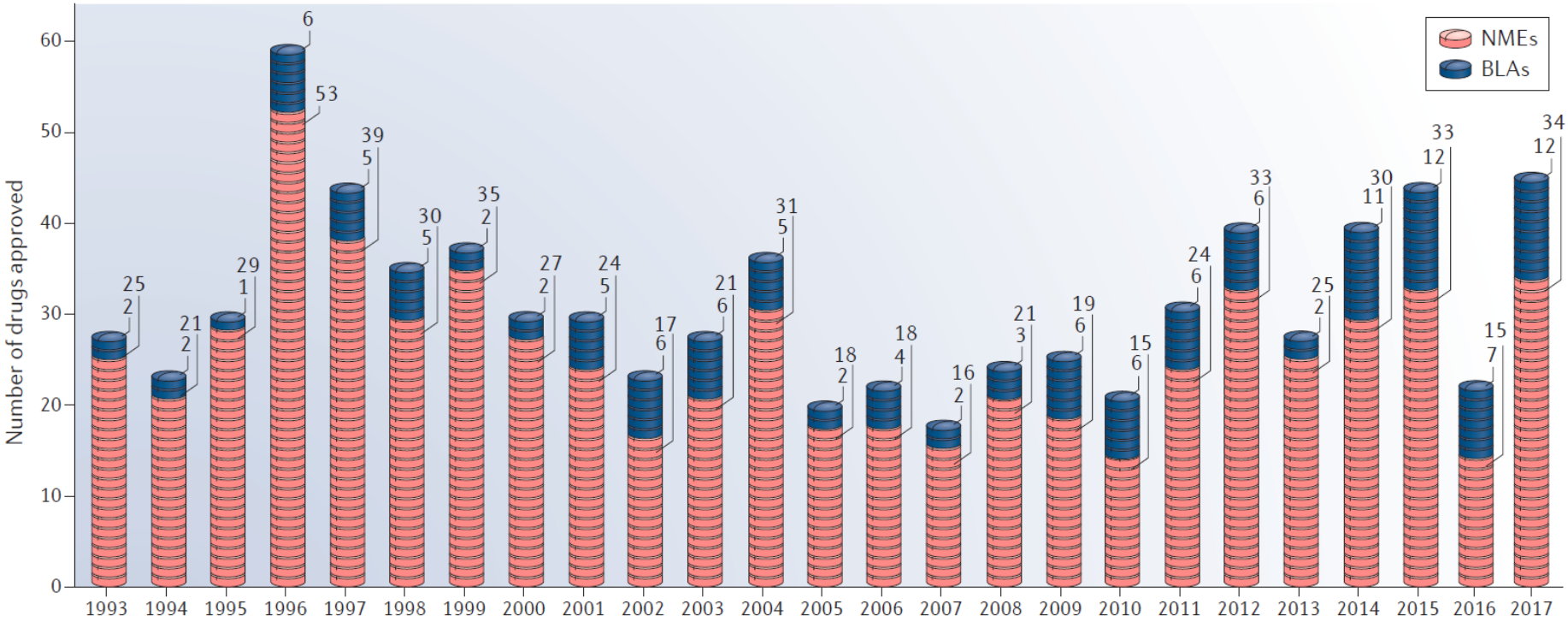


Figure 1 | **Novel FDA approvals since 1993.** New molecular entities (NMEs) and Biologics License Applications (BLAs) approved by the Center for Drug Evaluation and Research since 1993 (see also TABLE 1). Approvals by the Center for Biologics Evaluation and Research are not included in this drug count (see TABLE 2). Data are from Drugs@FDA.

List of drugs approved in 2017

Table 1 | Center for Drug Evaluation and Research approvals in 2017

Drug (brand name)	Sponsor	Properties	Indication	Review type
Plecanatide (Trulance)	Synergy Pharmaceuticals	Guanylate cyclase C agonist	Chronic idiopathic constipation	S
Etelcalcetide (Parsabiv)	Amgen/Kai Pharmaceuticals	Calcium-sensing receptor agonist	Secondary hyperparathyroidism in patients with chronic kidney disease on haemodialysis	S
Deflazacort (Emflaza)	PTC Therapeutics	Corticosteroid	Duchenne muscular dystrophy	P, O
Brodalumab (Siliq)*	Valeant Pharmaceuticals	IL-17RA antagonist	Plaque psoriasis	S
Telotristat etiprate (Xermelo)	Lexicon Pharmaceuticals	Tryptophan hydroxylase inhibitor	Carcinoid syndrome diarrhoea	P, O
Ribociclib (Kisqali)	Novartis	CDK4/6 inhibitor	HR-positive, HER2-negative breast cancer	P, B
Safinamide (Xadago)	US WorldMeds	MAO-B inhibitor	Parkinson disease	S
Naldemedine (Symproic)	Shionogi	Opioid antagonist	Opioid-induced constipation	S
Avelumab (Bavencio)*	Merck KGaA/Pfizer	PDL1-blocking antibody	Merkel cell carcinoma	P, O, B, A
Niraparib (Zejula)	Tesaro	PARP inhibitor	Epithelial ovarian, fallopian tube or primary peritoneal cancer	P, O, B
Ocrelizumab (Ocrevus)*	Roche/Genentech	CD20-directed cytolytic antibody	Relapsing or primary progressive forms of multiple sclerosis	P, B
Dupilumab (Dupixent)*	Regeneron/Sanofi	IL-4Rα antagonist	Atopic dermatitis	P, B
Deutetrabenazine (Austedo)	Teva	VMAT2 inhibitor	Chorea associated with Huntington disease	S, O
Valbenazine (Ingrezza)	Neurocrine Biosciences	VMAT2 inhibitor	Tardive dyskinesia	P, B
Cerliponase alfa (Brineura)*	BioMarin Pharmaceutical	Tripeptidyl peptidase	Tripeptidyl peptidase 1 deficiency	P, O, B
Midostaurin (Rydapt)	Novartis	FLT3 inhibitor	FLT3-positive AML	P, O, B
Abaloparatide (Tymlos)	Radius Health	Parathyroid hormone-related protein	Osteoporosis	S
Brigatinib (Alunbrig)	Ariad Pharmaceuticals/ Takeda	ALK inhibitor	ALK-positive NSCLC	P, O, B, A
Durvalumab (Imfinzi)*	AstraZeneca	PDL1-blocking antibody	Urothelial carcinoma	P, B, A
Edaravone (Radicava)	Mitsubishi Tanabe	Unknown (radical scavenger)	ALS	S, O
Sarilumab (Kevzara)*	Sanofi/Regeneron	IL-6 receptor antagonist	Rheumatoid arthritis	S
Delafloxacin (Baxdela)	Melinta Therapeutics	Fluoroquinolone antibacterial	Acute bacterial skin and skin structure infections	P
Betrixaban (Bevyxxa)	Portola Pharmaceuticals	FXa inhibitor	Prophylaxis of venous thromboembolism	P
Guselkumab (Tremfya)*	Janssen/Johnson & Johnson	IL-23 blocker	Plaque psoriasis	P
Neratinib (Nerlynx)	Puma Biotechnology	EGFR, HER2 and HER4 irreversible kinase inhibitor	HER2-overexpressed breast cancer	S
Sofosbuvir; velpatasvir; voxilaprevir (Vosevi)	Gilead Sciences	Nucleotide analogue NS5B polymerase inhibitor plus an NS5A inhibitor plus an NS3/4A protease inhibitor	HCV	P, B
Enasidenib mesylate (Idhifa)	Celgene/Agios	IDH2 inhibitor	IDH2-mutated AML	P, O
Glecaprevir; pibrentasvir (Mavyret)	AbbVie	NS3/4A protease inhibitor plus a NS5A inhibitor	HCV	P, B
Inotuzumab ozogamicin (Besponsa)*	Pfizer	CD22-directed antibody–drug conjugate	B cell precursor ALL	P, O, B
Benznidazole (Benznidazole)	Chemo Research	Nitroimidazole antimicrobial	Chagas disease	P, O, A
Meropenem; vaborbactam (Vabomere)	The Medicines Company/ Rempex Pharmaceuticals	Carbapenem antimicrobial plus a β-lactamase inhibitor	Complicated urinary tract infections	P
Copanlisib dihydrochloride (Aliqopa)	Bayer	PI3Kα/δ inhibitor	Follicular lymphoma	P, O, A

Table 1 (cont.) | Center for Drug Evaluation and Research approvals in 2017

Drug (brand name)	Sponsor	Properties	Indication	Review type
Secnidazole (Solosec)	Lupin	Nitroimidazole antimicrobial	Bacterial vaginosis	P
Abemaciclib (Verzenio)	Eli Lilly	CDK4/6 inhibitor	HR-positive, HER2-negative breast cancer	P, B
Acalabrutinib (Calquence)	AstraZeneca/Acerta Pharma	BTK inhibitor	Mantle cell lymphoma	P, O, B, A
Latanoprostene bunod (Vyzulta)	Bausch and Lomb/ Valeant Pharmaceuticals	Prostaglandin analogue	Intraocular pressure	S
Letermovir (Prevymis)	Merck & Co.	CMV DNA terminase complex inhibitor	Prophylaxis of CMV	P, O, B
Benralizumab (Fasenra)*	AstraZeneca	IL-5Rα-directed monoclonal antibody	Severe asthma	S
Vestronidase alfa (Mepsevii)*	Ultragenyx	Recombinant human lysosomal β-glucuronidase	Mucopolysaccharidosis VII	P, O
Emicizumab (Hemlibra)*	Roche/Genentech	Bispecific FIX and FX-directed antibody	Haemophilia A	P, O, B
Semaglutide (Ozempic)	Novo Nordisk	GLP1 receptor agonist	Type 2 diabetes mellitus	S
Ozenoxacin (Xepi)	Ferrer Internacional	Quinolone antimicrobial	Impetigo due to *Staphylococcus aureus* or *Streptococcus pyogenes*	S
Netarsudil (Rhopressa)	Aerie Pharmaceuticals	RHO kinase inhibitor	Open-angle glaucoma or ocular hypertension	P
Ertugliflozin (Steglatro)	Merck & Co./Pfizer	SGLT2 inhibitor	Type 2 diabetes mellitus	S
Macimorelin (Macrilen)	Aeterna Zentaris	Growth hormone secretagogue receptor agonist	Diagnosis of adult growth hormone deficiency	S, O
Angiotensin II (Giapreza)	La Jolla Pharmaceutical Company	Synthetic human angiotensin	Blood pressure in adults with septic or other distributive shock	P

Data from Drugs@FDA. A, accelerated; ALS, amyotrophic lateral sclerosis; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; B, breakthrough; BTK, Bruton tyrosine kinase; CDK, cyclin-dependent kinase; CMV, cytomegalovirus; EGFR, epidermal growth factor receptor; FXa, factor Xa; FLT3, FMS-like tyrosine kinase 3; GLP1, glucagon-like peptide 1; HCV, hepatitis C virus; HR, hormone receptor; IDH2, isocitrate dehydrogenase 2; IL-17RA, interleukin-17 receptor A; MAO-B, monoamine oxidase type B; NSCLC, non-small-cell lung cancer; O, orphan; P, priority; PARP, poly(ADP-ribose) polymerase; PDL1, programmed cell death 1 ligand 1; PI3K, phosphoinositide 3-kinase; S, standard; SGLT2, sodium/glucose cotransporter 2; VMAT2, vesicular monoamine transporter 2. *Biologic therapy.

2017 potential blockbusters

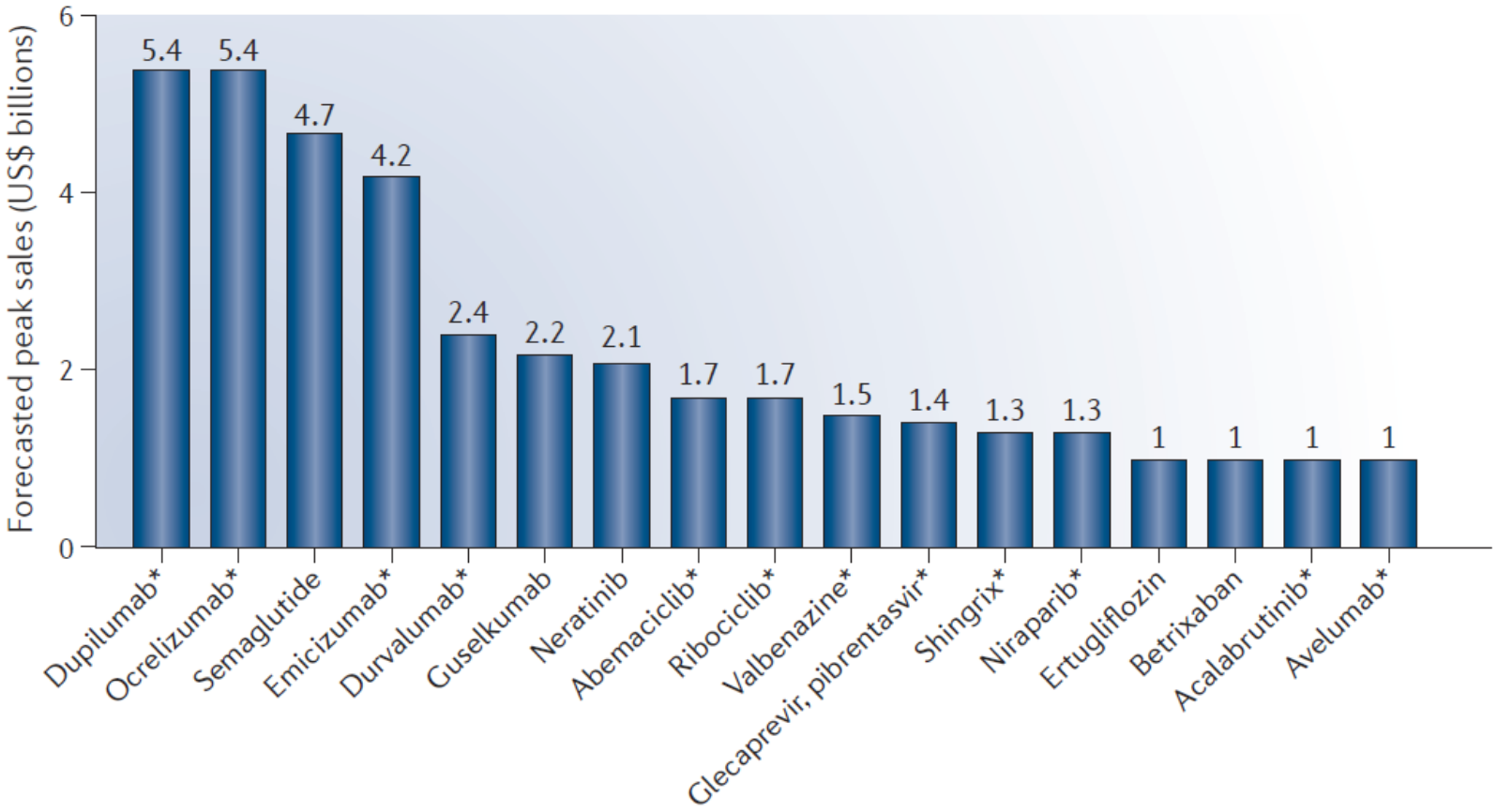


Figure 2 | **2017's potential blockbuster approvals.** Sales forecasts are average, annual, global consensus sales estimates for 2023, as reported by Clarivate Analytics' Cortellis database on 30 December 2016. *Drugs with breakthrough therapy designation.

Therapeutic area and 2018 potential approvals

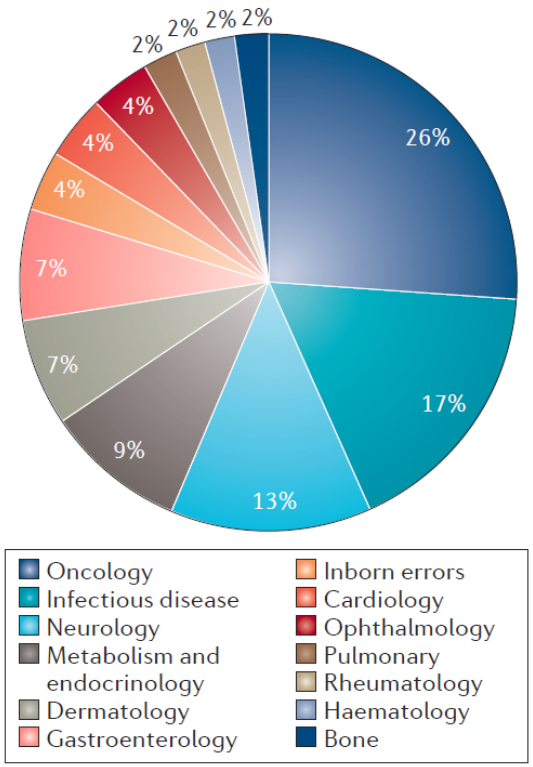


Figure 4 | **CDER approvals by therapeutic area in 2017.** Data from Drugs@FDA.

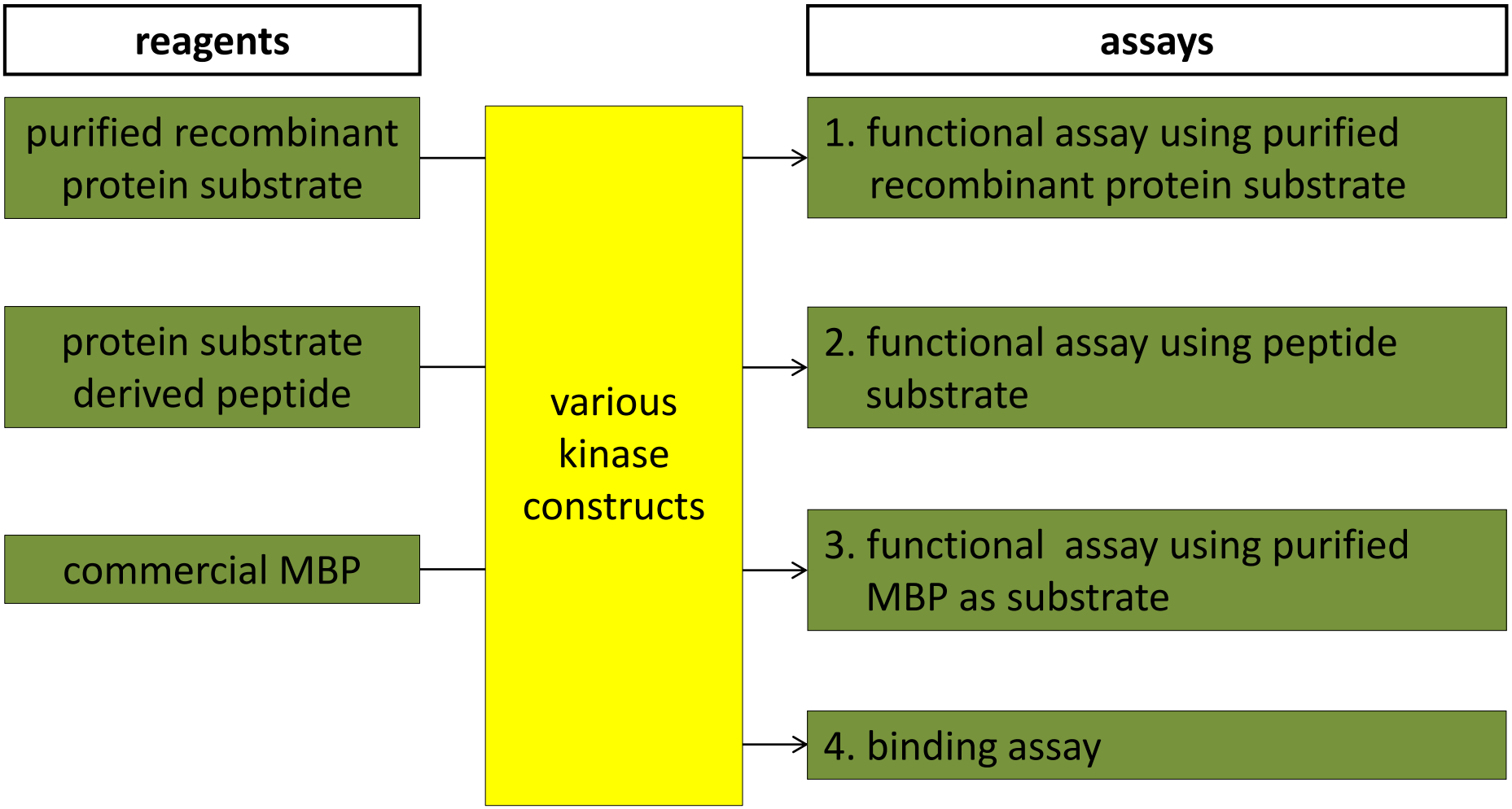
Table 4 | **Potential first approvals in 2018**

Drug name	Sponsor	Properties	Indication	Expected PDUFA date
Tezacaftor[a]	Vertex	CFTR corrector	Cystic fibrosis	February
Ibalizumab[a]	Theratechnologies	Anti-CD4 mAb	HIV	April
Fostamatinib	Rigel Pharmaceuticals	SYK inhibitor	Immune thrombocytopenic purpura	April
Andexanet alfa[a]	Portola Pharmaceuticals	Universal factor Xa inhibitor	Anticoagulant reversal	May
Erenumab	Amgen	Anti-CGRP receptor mAb	Migraine	May
Plazomicin[a]	Achaogen	Novel aminoglycoside antimicrobial	Antibacterial indications	June
Fremanezumab	Teva	Anti-CGRP mAb	Migraine	June
Tafenoquine[a]	GlaxoSmithKline	Antiparasitic DNA synthesis inhibitor	Malaria	July
Patisiran[a]	Alnylam Pharmaceticals	First RNAi drug	TTR-mediated amyloidosis	August
Galcanezumab	Eli Lilly	Anti-CGRP mAb	Migraine	September
Oliceridine[a]	Trevena	Biased μ-opioid receptor agonist	Acute pain	November
Larotrectinib[a]	Loxo Oncology	TRK inhibitor	NTRK-fusion solid tumours	NA (NDA completion in early 2018)

Source: BioMedTracker. CFTR, cystic fibrosis transmembrane conductance regulator; CGRP, calcitonin gene-related peptide; mAb, monoclonal antibody; NA, not applicable; NDA, new drug application; RNAi, RNA interference; TRK, tropomyosin receptor kinase; TTR, transthyretin. [a]Drug has breakthrough therapy designation.

Example cell-based assay screen output

Strategy for biochemical kinase assays



NIK phosphorylates IKK-α

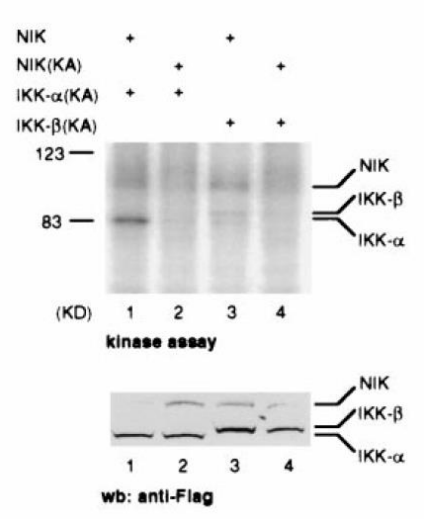
Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 3792–3797, March 1998
Immunology

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

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

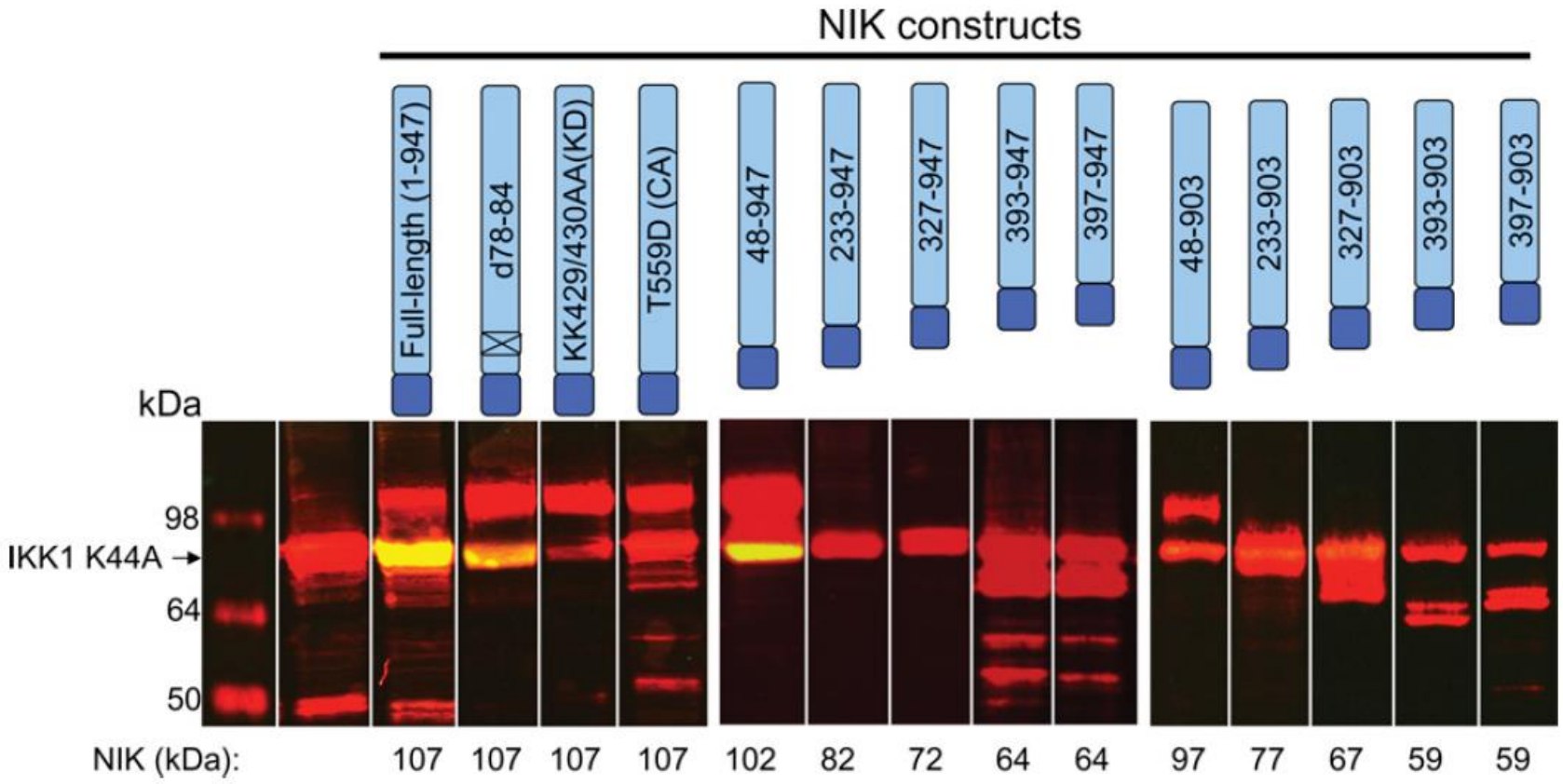
Tularik, Inc., Two Corporate Drive, South San Francisco, CA 94080

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

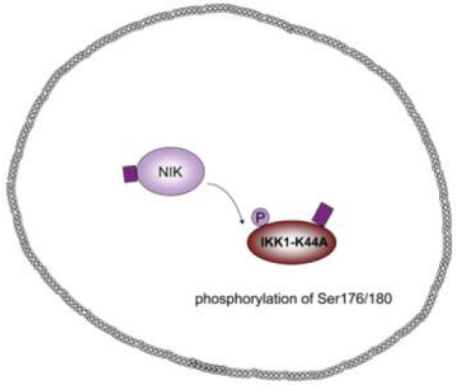


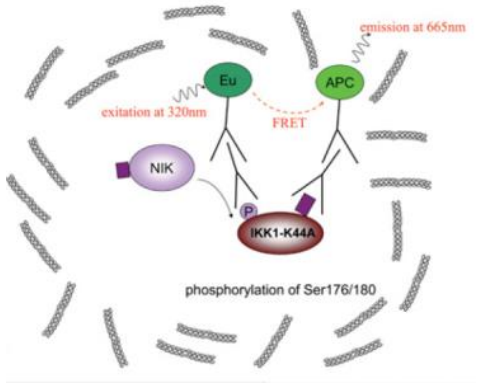
BJ www.biochemj.org
BJ ChemBio
Biochem. J. (2009) 419, 65–73 (Printed in Great Britain) doi:10.1042/BJ20081646
65

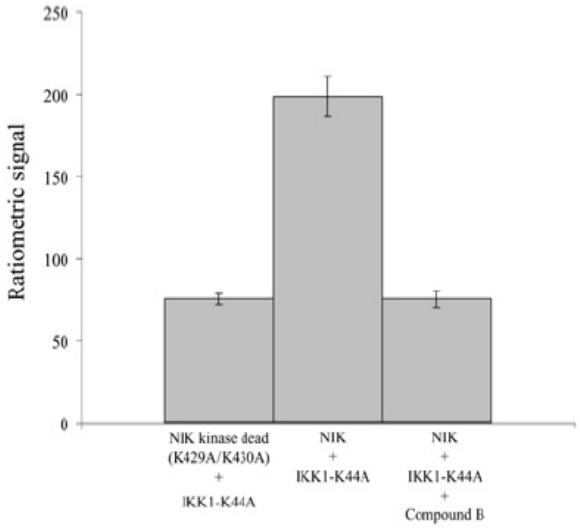
Development of an insect-cell-based assay for detection of kinase inhibition using NF-κB-inducing kinase as a paradigm

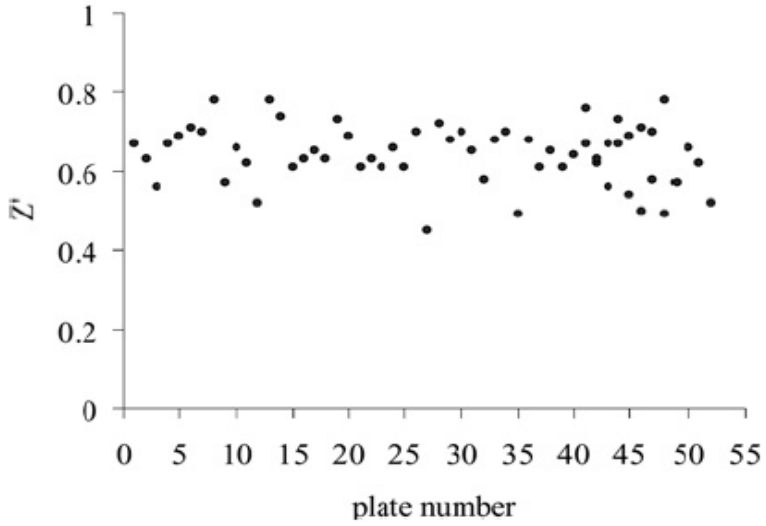
Namir J. HASSAN*[1,2], Sheraz GUL*[1], Fiona FLETT*, Edward HOLLINGSWORTH*, Angela A. DUNNE†, Amanda J. EMMONS†, Jonathan P. HUTCHINSON*, Martin J. HIBBS*, Susan DYOS‡, Jeremy D. KITSON§, Emma HILEY*, Martin RÜDIGER*, David G. TEW*, David J. POWELL* and Mary A. MORSE‖

Insect cell-based assay for NIK









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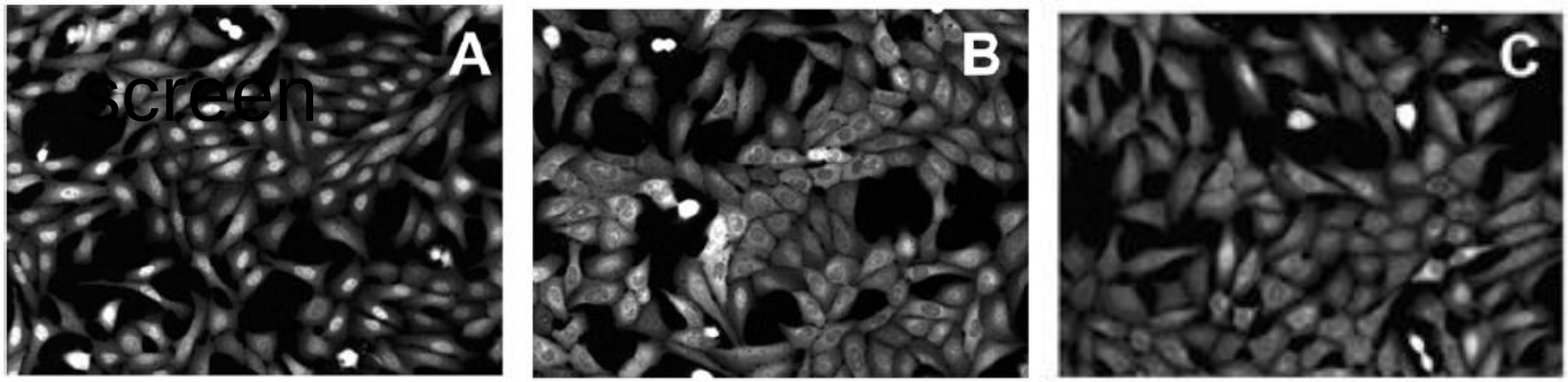
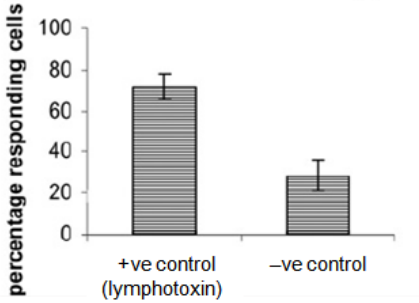
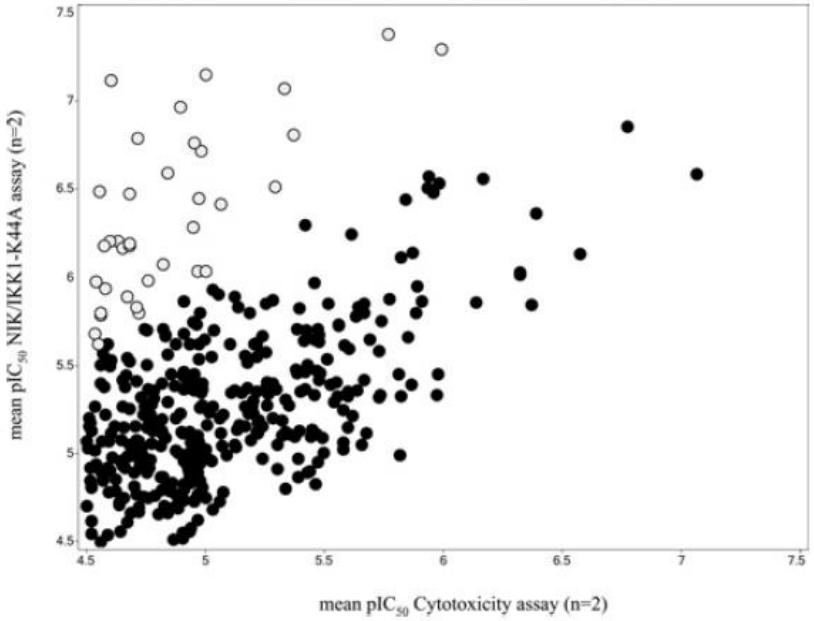
Use of HCS after cell-based NIK inhibitor



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





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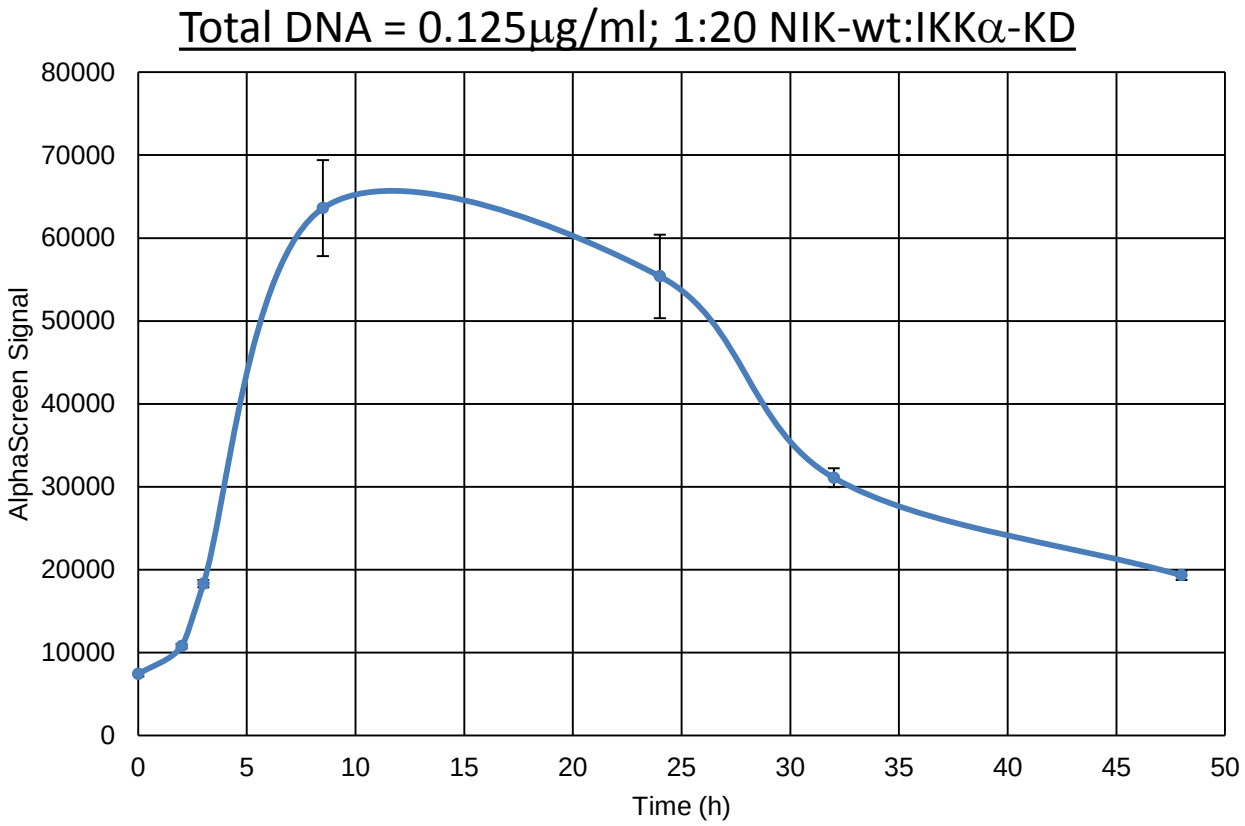
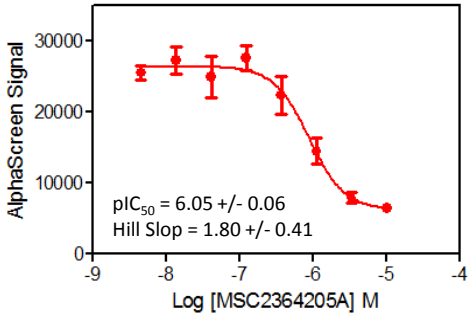
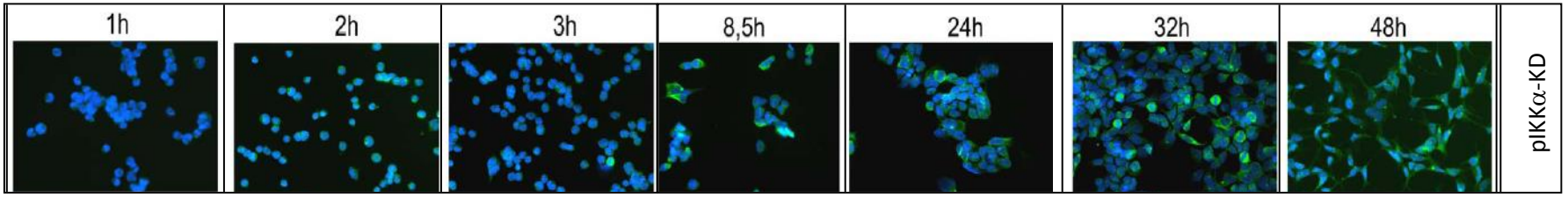
Time-course for IKKα phosphorylation

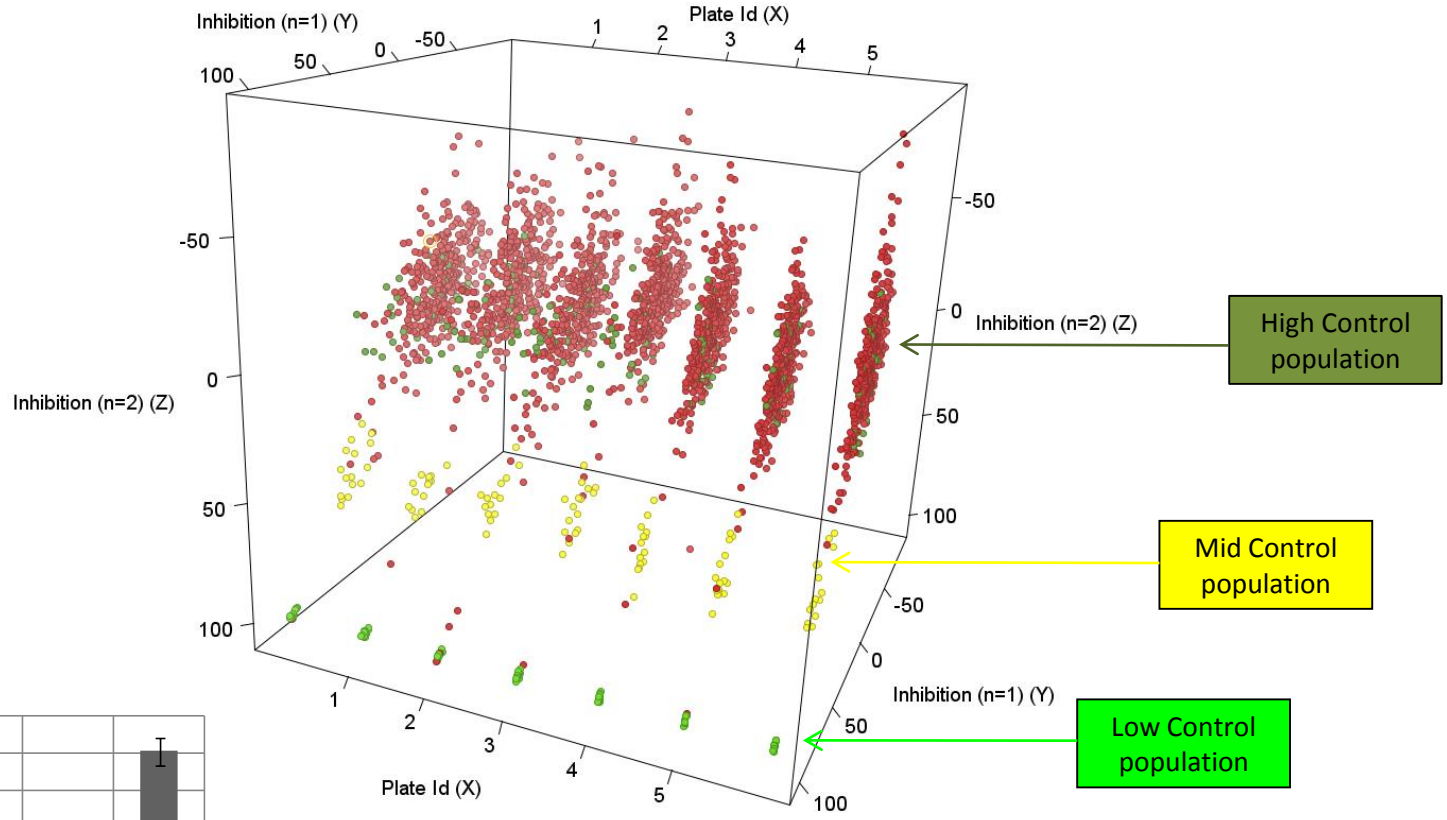






PoC screen (with test compound populations)

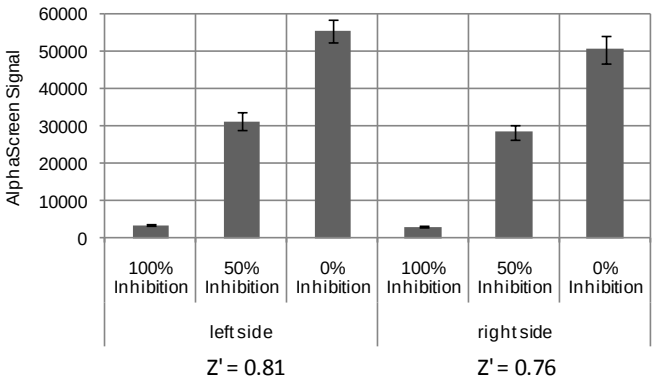
Test Compound population



High Control population

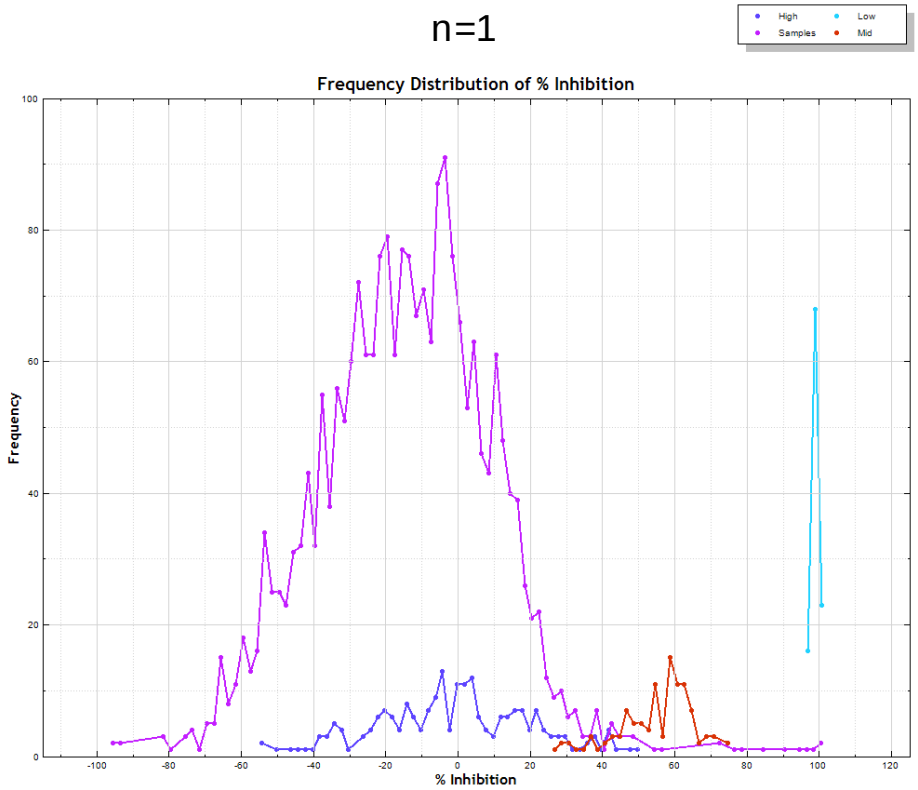
Mid Control population

Low Control population

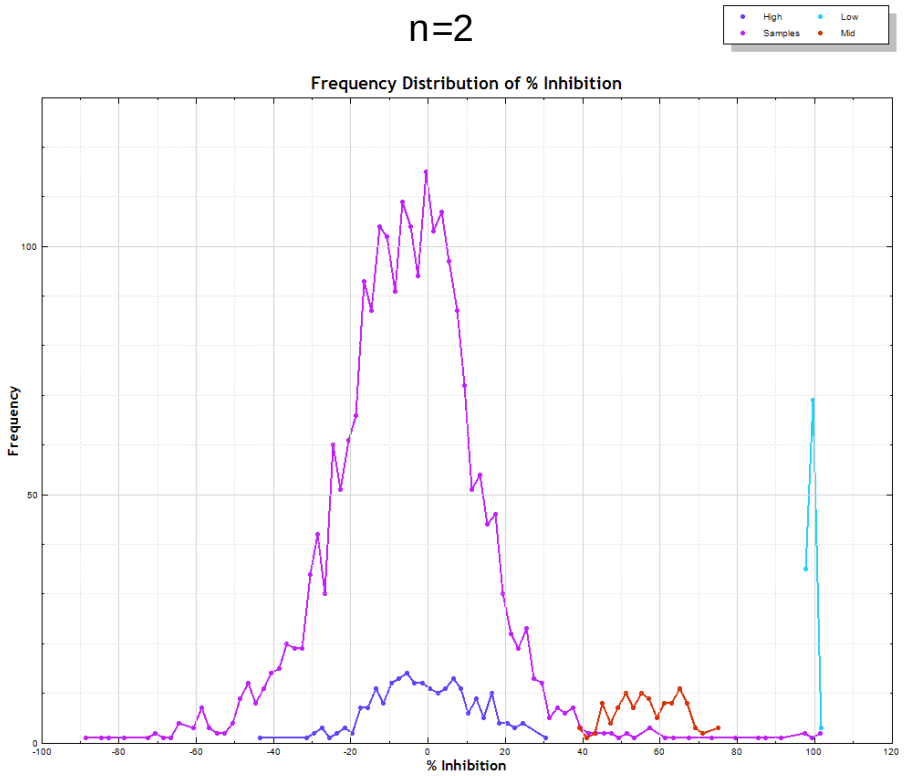


PoC screen output (distributions and hit rates)

n=1



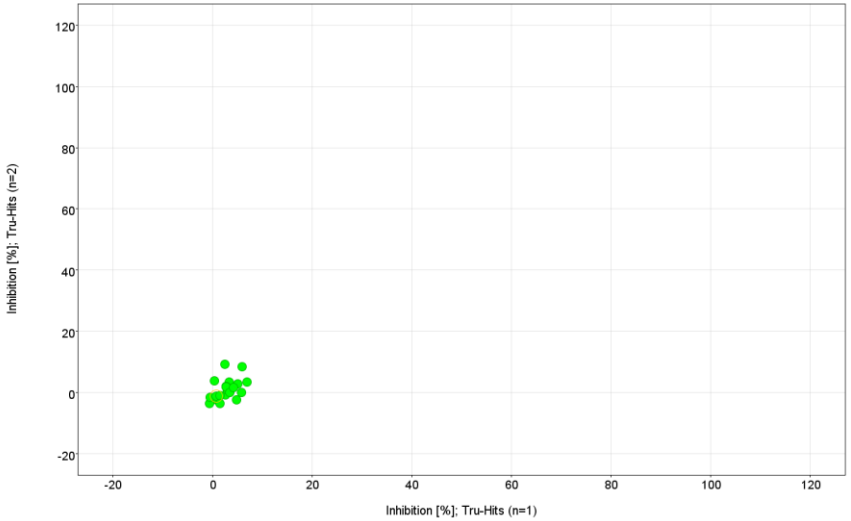
n=2



Inhibition threshold for Hits	Number of Hits (n=1)	Number of Hits (n=2)	Number of Reproducible Hits	Hit Rate (%)
40%	27	31	20	0.89
50%	14	20	13	0.58
60%	11	13	10	0.45
70%	11	10	9	0.40
80%	8	9	8	0.36
90%	6	6	6	0.27

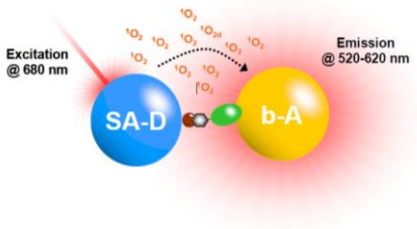
PoC screen output (false-positives)

Tru-Hits assay

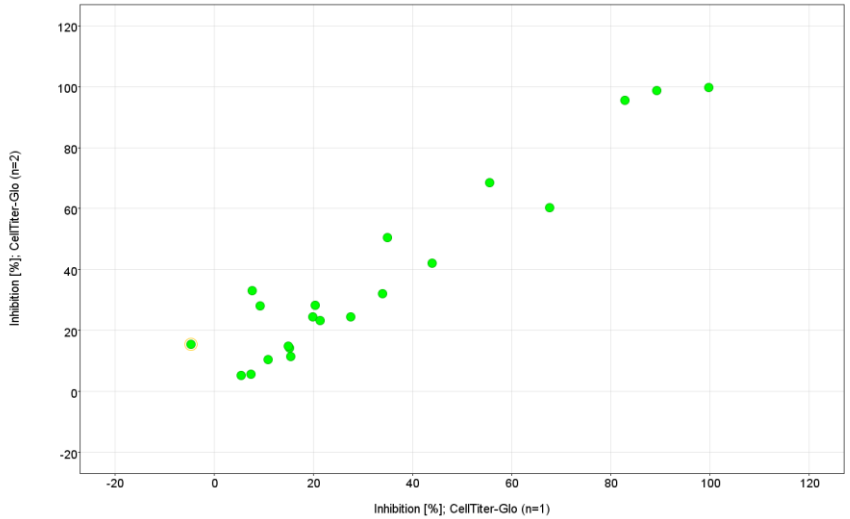


Assay performance:

average High	817820
average Low	2332
SD High	36289
SD Low	874
Z'	0.86

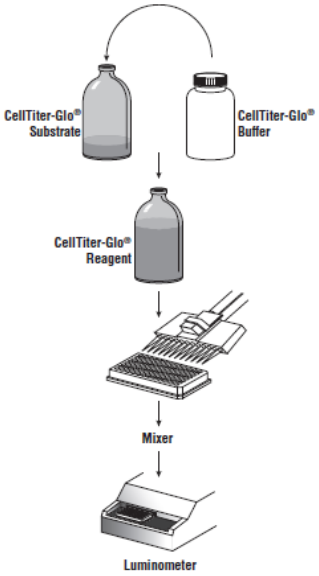


CellTiter-Glo assay

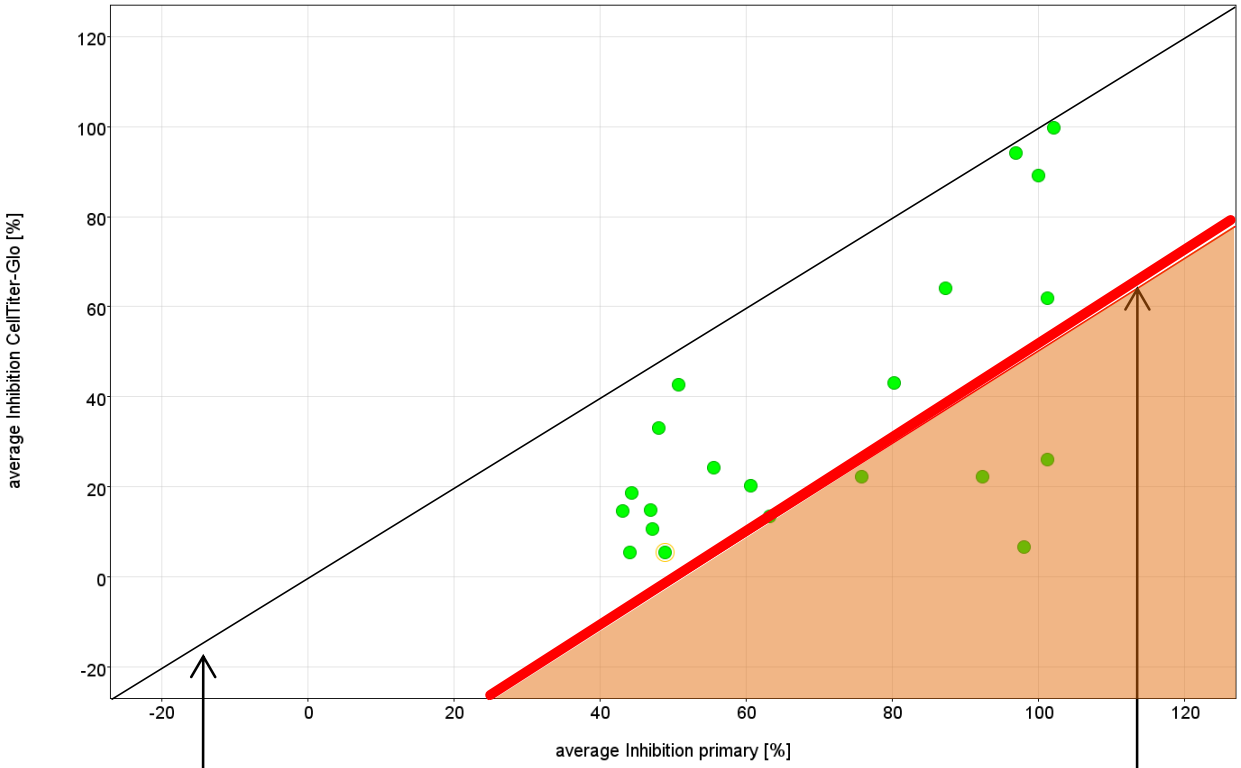


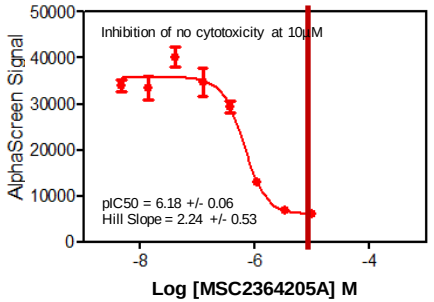
Assay performance:

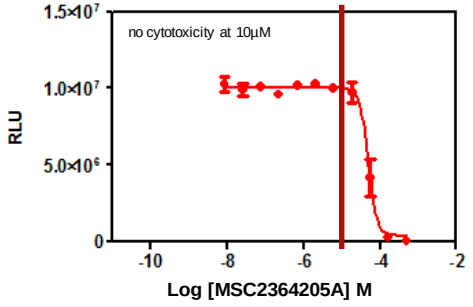
average High	10089125
average Low	23561
SD High	285866
SD Low	13881
Z'	0.91



PoC screen output (final analysis)







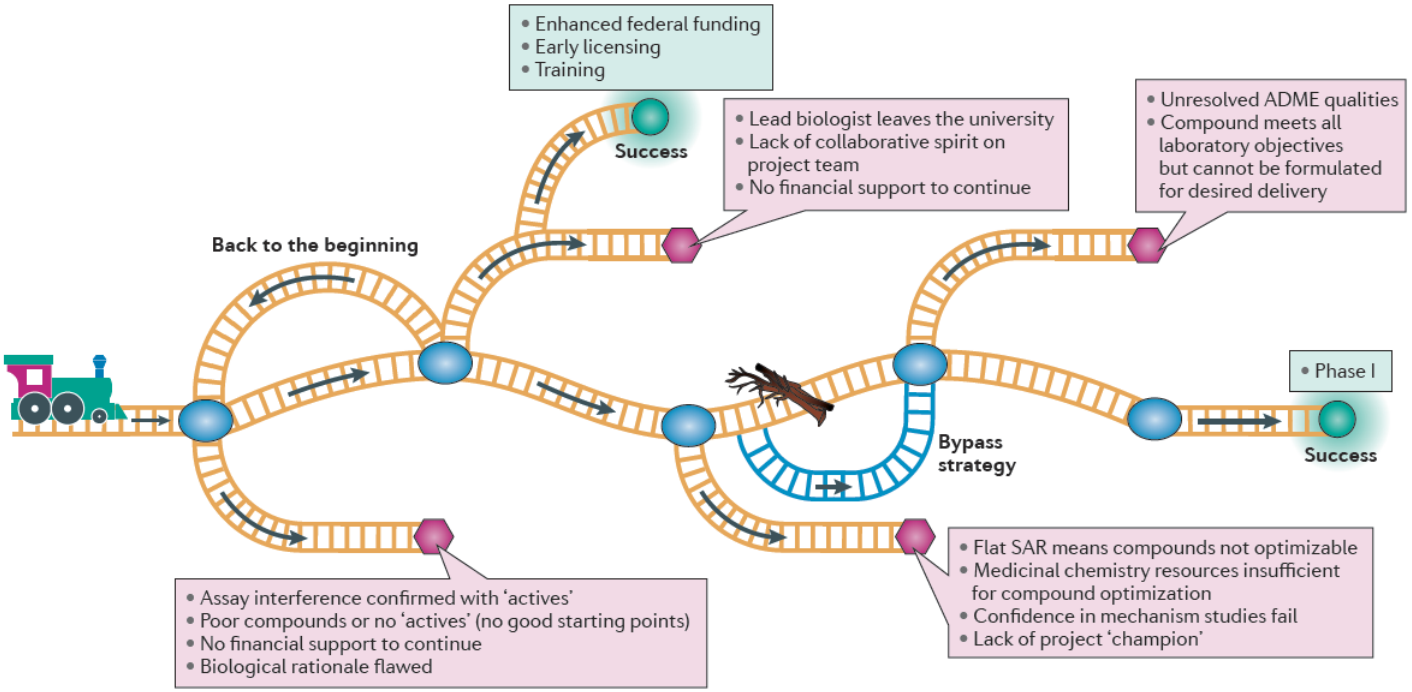
[compound] = 10µM

1:1 correlation NIK vs cytotoxicity

Compounds below this line give >50% inhibition in NIK assay over cytotoxicity

Workflows to identify problem compounds from small molecule screens

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

Use panels of assays (HDAC)

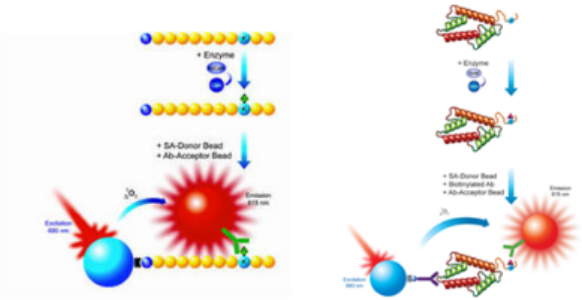
AlphaLISA Epigenetic Tool Box

- AlphaLISA® 5X Epigenetics Buffer 1
- AlphaLISA® Anti-acetyl-Histone H3 Lysine 27 (H3K27ac) Acceptor Beads
- AlphaLISA® Anti-acetyl-p53 Lysine 382 (p53K382ac) Acceptor Beads
- AlphaLISA® Anti-di/mono-methyl-Histone H3 Lysine 27 (H3K27me2-1) Acceptor Beads
- AlphaLISA® Anti-di-methyl-Histone H3 Lysine 36 (H3K36me2) Acceptor Beads
- AlphaLISA® Anti-methyl Histone H3 Arginine 2 (H3R2me) Acceptor Beads
- AlphaLISA® Anti-tri-methyl-Histone H3 Lysine 27 (H3K27me3) Acceptor Beads
- AlphaLISA® Anti-unmodified Histone H3 Lysine 4 (H3K4) Acceptor Beads

AlphaLISA Epigenetic Tool Box

Highly sensitive no wash detection for measuring peptide and protein modification by epigenetic enzymes.

Use convenient, versatile AlphaLISA technology to detect specific methylation and acetylation states for epigenetics research. In addition to providing high-quality data and robust performance, AlphaLISA assays are simple and quick to optimize. Easy to automate and miniaturize, AlphaLISA are fully validated for mark specificity and cross reactivity.



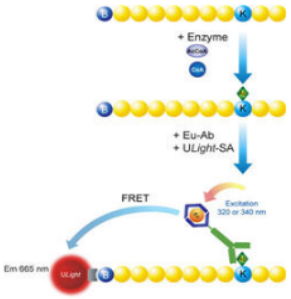
LANCE® TR-FRET Epigenetics Tool Box

- LANCE® *Ultra* Europium-anti-acetyl-Histone H3 Lysine 9 (H3K9ac)
- LANCE® *Ultra* Europium-anti-methyl-Histone H3 Lysine 4 (H3K4me1-2)
- LANCE® *Ultra* Europium-anti-methyl-Histone H3 Lysine 9 (H3K9me2)
- LANCE® *Ultra* Europium-anti-unmodified-Histone H3 Lysine 9/Lysine 27 (H3K9/K27) Antibody
- LANCE® Ultra Europium-anti-acetyl-Histone H3 Lysine 27 (H3K27ac) Antibody
- LANCE® Ultra Europium-anti-acetyl-p53 Lysine 382 (p53K382ac) Antibody
- LANCE® Ultra Europium-anti-di/mono-methyl-Histone H3 Lysine 27 (H3K27me2-1) Antibody
- LANCE® Ultra Europium-anti-di-methyl-Histone H3 Lysine 36 (H3K36me2) Antibody
- LANCE® Ultra Europium-anti-tri-methyl-Histone H3 Lysine 27 (H3K27me3) Antibody

LANCE® TR-FRET Epigenetics Tool Box

Consistent, validated solutions for your most demanding epigenetics applications.

Proven, cost-efficient LANCE *Ultra* reagents can be used to quantitate peptide ifications, detecting specific meythylation and acetylation states. No wash, homogenous LANCE *Ultra* reagents are HTS friendly - design your own epigenetics screening strategy for greatest efficiency.

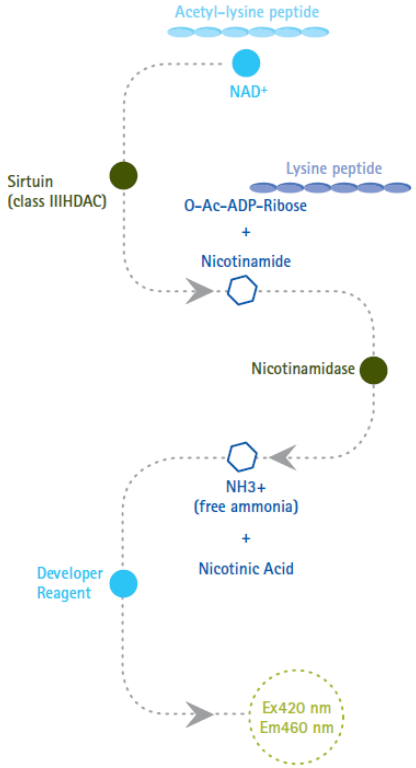


Use panels of assays (HDAC)

Data Sheet

SIRTainty™ Class III HDAC Assay

The most flexible and reliable assay for measuring sirtuin activity



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Product Information

Ammonia Assay Kit

Catalog Number **AA0100**
Storage Temperature 2–8 °C
DO NOT FREEZE

Product Description

This kit is for the quantitative, enzymatic determination of ammonia in food and biological samples. Ammonia reacts with α-ketoglutaric acid (KGA) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of L-glutamate dehydrogenase (GDH) to form L-glutamate and oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) as follows:

$$KGA + NH_4^+ + NADPH \xrightarrow{GDH} L\text{-}Glutamate + NADP^+ + H_2O$$

Use panels of assays (HDAC)

Journal of Biomolecular Screening
http://jbx.sagepub.com/

A Substrate-Independent TR-FRET Histone Deacetylase Inhibitor Assay

Bryan D. Marks, Stephen A. Fakhoury, William J. Frazee, Hildegard C. Eliason and Steven M. Riddle

J Biomol Screen published online 21 September 2011
DOI: 10.1177/1087057111422102

TR-FRET Histone Deacetylase Binding Assay

Table 1. Values (nM) for 18 Known HDAC Inhibitors Were Determined Using the Panel of HDAC Binding Assays

Compound	*Class*	*HDAC1*	*HDAC2*	*HDAC3*	*HDAC6*	*HDAC8*	*HDAC10*
LBH589 (Panobinostat)	Hydroxamate	0.9 ± 0.3	5.3 ± 1.1	1.2 ± 0.3	2.4 ± 0.5	250 ± 40	2.3 ± 0.7
LAQ824 (Dacinostat)	Hydroxamate	1.2 ± 0.6	7 ± 3	1.2 ± 0.4	2.3 ± 0.7	230 ± 30	2.0 ± 0.6
Trichostatin A	Hydroxamate	1.4 ± 0.6	7 ± 2	2.4 ± 0.8	2.0 ± 0.3	700 ± 200	54 ± 19
CUDC-101	Hydroxamate	2.8 ± 1.3	10 ± 4	2.9 ± 0.5	10 ± 4	370 ± 70	60 ± 20
M344	Hydroxamate	9.6 ± 0.2	80 ± 8	4.1 ± 0.3	0.53 ± 0.08	1380 ± 60	18 ± 3
HDAC-42, (*S*)-	Hydroxamate	13 ± 6	90 ± 20	4.7 ± 1.4	2.8 ± 1.0	190 ± 20	1800 ± 400
PXD101 (Belinostat)	Hydroxamate	17 ± 6	120 ± 30	7 ± 2	4 ± 2	220 ± 40	51 ± 20
BML-281	Hydroxamate	17 ± 6	71 ± 20	12 ± 5	2.9 ± 1.0	1300 ± 400	52 ± 18
SAHA (Vorinostat)	Hydroxamate	20.7 ± 1.0	215 ± 3	7.0 ± 0.9	1.8 ± 0.3	2690 ± 130	39 ± 6
Scriptaid	Hydroxamate	24 ± 3	148 ± 13	4.6 ± 0.8	0.66 ± 0.07	1100 ± 200	310 ± 70
Oxamflatin	Hydroxamate	30 ± 11	151 ± 50	9 ± 5	19 ± 13	1800 ± 600	310 ± 140
Suberoyl *bis*-hydroxamic acid	Hydroxamate	500 ± 30	5100 ± 200	100 ± 40	20 ± 6	7400 ± 700	15 ± 4
MC 1293	Hydroxamate	5300 ± 600	>10000	2600 ± 800	55 ± 12	2100 ± 300	410 ± 90
HC Toxin	Cyclic peptide	0.35 ± 0.05	2.6 ± 0.3	0.52 ± 0.04	500 ± 80	30 ± 3	>10 000
Apicidin	Cyclic peptide	0.51 ± 0.06	4.7 ± 0.6	1.27 ± 0.07	320 ± 20	380 ± 90	>10 000
MS-275 (Entinostat)	Benzamide	180 ± 50	1900 ± 500	420 ± 60	>10 000	>10 000	>10 000
CI-994	Benzamide	520 ± 130	4800 ± 700	590 ± 90	>10 000	>10 000	>10 000
BML-210	Benzamide	1630 ± 70	7500 ± 500	200 ± 40	>10 000	>10 000	>10 000

CONCLUSIONS

- 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