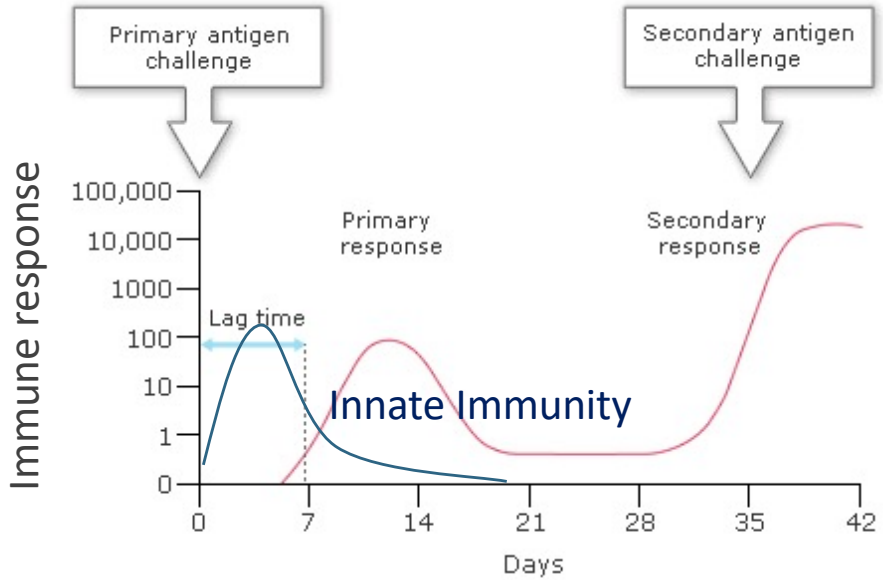
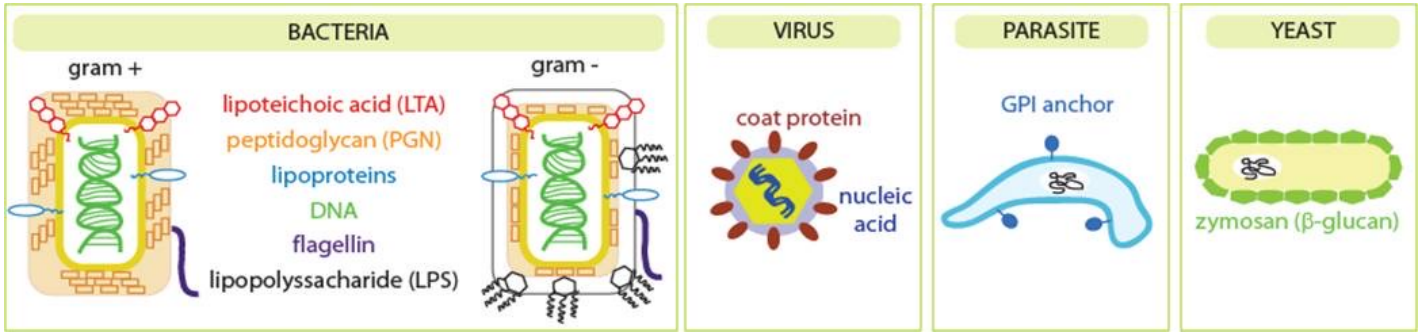
Immune Reporter Cell Lines –

Developing bioassays for
reliable compounds

Pathogens Associated Molecular Patterns (PAMPs)



Innate immunity: Quick, general, non-specific



British Society for Immunology

Innate immunity: Recognize “non-self” molecules known as PAMPs

PAMPs: structures found on pathogens

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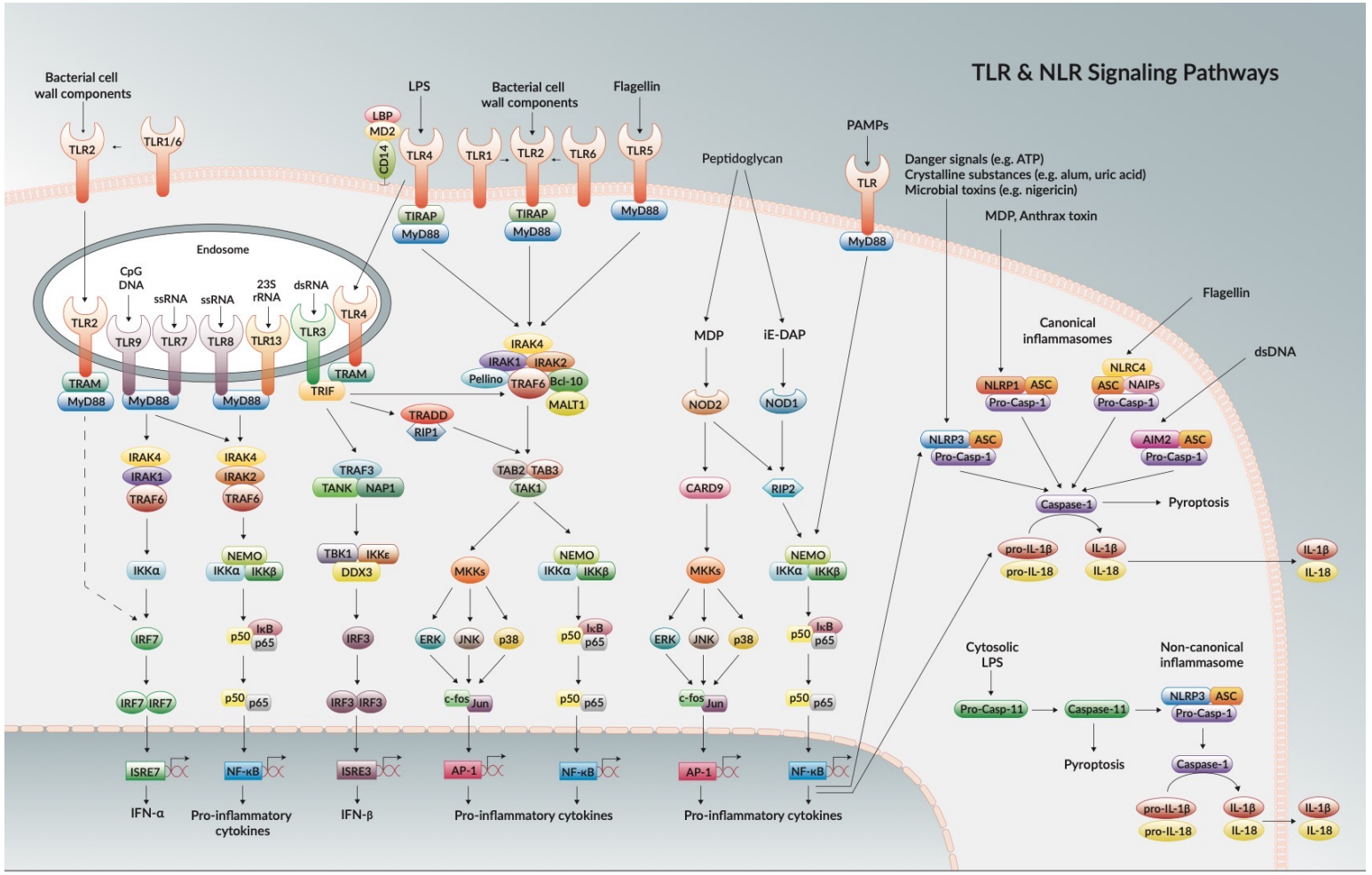
Pattern Recognition Receptors (PRRs)



PRRs: Receptors predominantly expressed in immune cells for PAMPs recognition

Can be on the cell surface, in the cytosol or in compartments like endosomes

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Immunomodulators in Clinical Trial

PRR ligands as new therapeutic approach:

Agonist – adjuvants
Antagonist – treatment for autoimmune diseases

Target	Compound	Application	Status
TLR7	Imiquimod	HBV vaccine	II, III
TLR9	CpG1018	HIV-1 vaccine	I
Inflammasome	Inzomelid	Cryopyrin-Associated Periodic Syndrome	I
TLR3	PolyICLC	Glioblastoma	II
STING	ADU-S100	Solid tumors	I
TLR7/8	R848	Brain tumors	II

Immunity. 2020 Jul 14;53(1):78-97. doi: 10.1016/j.immuni.2020.04.004.

Nucleic Acid Sensors as Therapeutic Targets

CellPress

Immunity

Review

Nucleic Acid Sensors as Therapeutic Targets for Human Disease

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https://doi.org/10.1016/j.immuni.2020.04.004

Table 1. Ongoing Clinical Trials Targeting Nucleic Sensor Agonists for Infectious Disease

Target	Drug Name	Developer	Disease	Phase	Combination Agent	Trial ID
TLR7	imiquimod (Aldara)	3M Pharmaceuticals	HBV	II/III	HBV vaccine	NCT04083157
			influenza	III	influenza vaccine	NCT04143451
TLR7	RO7020531 (RG7854)	Hoffmann-La Roche	HBV	I	none	NCT02956850
TLR7	TQ-A3334 (AL-034)	Janssen	HBV	II	RT inhibitor	NCT04180150
TLR7	GS-9620 (vesatolimod)	Gilead	HIV	I	ART	NCT03060447
TLR7/8	3M-052-AF	3M, IDRI	HIV	I	HIV-1 vaccine	NCT04177355
TLR8	GS-9688 (selgantolimod)	Gilead	HBV	II	oral antiviral agents	NCT03491553
			HBV	II	RT inhibitor	NCT03615066
TLR9	MGN1703 (lefitolimod)	Mologen AG	HIV	II	bNab	NCT03837756
TLR9	CpG 1018	Dynavax Technologies	HIV	I	HIV-1 vaccine	NCT04177355
RIG-I	SB-9200 (inarigivir soproxil)	Spring Bank Pharmaceuticals	HBV	II	RT inhibitor	NCT04023721
			HBV	II	none	NCT03932513
			HBV	II	RT inhibitor	NCT04059198
			HBV	II	RT inhibitor	NCT03434353

Source: ClinicalTrials.gov.
ART, antiretroviral therapy; bNab, broadly neutralizing antibody; HBV, hepatitis B virus; HIV, human immunodeficiency virus; RT, reverse transcriptase.

Table 2. Ongoing Clinical Trials Targeting Nucleic Sensor Agonists for Cancer

Target	Drug Name	Developer	Disease	Phase	ROA	Combination Agent	Trial ID
TLR3 Mda5	poly-ICLC (Hiltonol)	Oncovir	B cell lymphoma	I/II	IT	RT, Flt3 ligand	NCT01976585
			B cell lymphoma	I/II	IT	RT, Flt3 ligand and pembrolizumab	NCT03789097
			solid tumors	II	IT/i.m.	anti-PD1/L1	NCT02423863
			prostate cancer	I	IT/i.m.	n/a	NCT03262103
			prostate cancer	I	i.m.	SBRT, Flt3 ligand, anti-PD1	NCT03835533
			colorectal cancer	I/II	i.m.	anti-PD1	NCT02834052
			solid tumors	I/II	i.m.	anti-PD1/L1	NCT03721679
			solid tumors	I/II	IT/i.m.	anti-PD1 and anti-CTLA4	NCT02643303
			ovarian cancer	I/II	s.c.	CDX-1401[a], IDO1 inhibitor	NCT02166905
			MDS or AML	I	s.c.	CDX-1401[a], nivolumab, decitabine	NCT03358719
			melanoma	II	s.c.	CDX-1401[a], Flt3 ligand	NCT02129075
			lung cancer	I/II	unk	MUC1 peptide vaccine	NCT01720836
			solid tumors	I/II	s.c.	personal peptide vaccine, anti-PD1	NCT03633110
			breast cancer	I	unk	peptide vaccine, anti-PD1	NCT03362060
			multiple myeloma	I	i.m.	peptide vaccine, citarinostat, lenalidomide	NCT02886065
			lymphoma	I	unk	personal peptide vaccine, anti-PD1, rituxan	NCT03121677
			pediatric glioma	n/a	unk	glioma antigen peptide vaccine	NCT01130077
			glioma	I/II	unk	peptide vaccine, anti-PD1	NCT02960230
			glioblastoma	I/II	s.c.	peptide vaccine, anti-PD1	NCT03665545
			glioblastoma	I	s.c.	peptide vaccine, anti-CD27	NCT02924038
			glioblastoma	II	i.m.	glioma antigen peptide vaccine	NCT02358187
			glioblastoma	I	s.c.	glioma lysate vaccine	NCT02549833
			glioblastoma	I	unk	personal peptide vaccine	NCT03223103
			glioblastoma	I	i.m.	DC tumor cell lysate vaccine, anti-PD1	NCT04201873
			brain tumors	II	i.m.	DC vaccine	NCT01204684
			TNBC	I	s.c.	personal peptide vaccine	NCT02427581
			melanoma	I/II	s.c.	peptide vaccine, anti-CD27	NCT03617328
			kidney cancer	I	unk	personal peptide vaccine, anti-CTLA4	NCT02950766
			urothelial cancer	I	unk	personal peptide vaccine, anti-PDL1	NCT03359239
			lung cancer	I	s.c.	personal peptide vaccine, anti-PD1, chemotherapy	NCT03380871
			melanoma	I	s.c.	personal peptide vaccine, CPI, anti-CD40	NCT03597282
			pancreatic cancer	I	s.c.	personal peptide vaccine	NCT03956056
TLR3	Ampligen (rintatolimod)	Hemispherex Biopharma	ovarian cancer	I/II	i.p.	anti-PD1, cisplatin	NCT03734692
			prostate cancer	II	i.v.	IFN alpha 2B, aspirin	NCT03899987
			breast cancer	I	i.v.	IFNalpha-2B, celecoxib, chemotherapy	NCT04081389
			ovarian cancer	I/II	i.p.	DC vaccine, IFN alpha-2B, celecoxib	NCT02432378
			breast cancer	I	i.v.	anti-PD1, IFN alpha-2B, celecoxib	NCT03599453
			ovarian cancer	I	i.v.	tumor lysate vaccine	NCT01312389
			colorectal cancer	II	i.v.	anti-PD1, IFN alpha-2B, celecoxib	NCT03403634

Nucleic Acid Sensors as Therapeutic Targets

Table 2. *Continued*

Target	Drug Name	Developer	Disease	Phase	ROA	Combination Agent	Trial ID
TLR3/ MDA5	BO-112	Bioncotech Therapeutics	solid tumors	I	IT	anti-PD1	NCT02828098
TLR7	imiquimod (Aldara)	3M Pharmaceuticals	lentigo maligna	III	topical	n/a	NCT02394132
			squamous cell carcinoma	III	topical	5-fluorouracil	NCT03370406
			VIN (pre-cancer)	III	topical	n/a	NCT01861535
			anal cancer	III	topical	5-fluorouracil	NCT02135419
			anal cancer	III	topical	n/a	NCT02059499
			cervical cancer	II	topical	n/a	NCT03233412
			melanoma	I	topical	pembrolizumab	NCT03276832
			CIN (pre-cancer)	I	topical	fluorouracil	NCT03196180
			CIN (pre-cancer)	I	topical	DNA vaccine	NCT00788164
			vulvar cancer	II	topical	DNA vaccine	NCT03180684
			basal cell carcinoma	II	topical	sonidegib	NCT03534947
			ependymoma	n/a	unk	synthetic tumor antigen	NCT01795313
			CIN (pre-cancer)	II	topical	HPV vaccine	NCT02864147
			melanoma	I	topical	anti-PD1	NCT03276832
			solid tumors	I	unk	anti-PD1, FUSA treatment	NCT04116320
			CTCL	I	topical	doxycycline	NCT03116659
			leukemia (ALL)	I/II	topical	GM-CSF, personal peptide vaccine	NCT03559413
			leukemia (CLL)	II	topical	personal peptide vaccine	NCT02802943
			solid tumors		topical	tumor-targeting antibody	NCT03872947
TLR7	resiquimod	Galderma Pharma	melanoma	II	topical	peptide vaccine	NCT00960752
			melanoma	I/II	topical	peptide vaccine	NCT02126579
			brain tumors	II	i.m.	DC vaccine	NCT01204684
TLR 7/8	NKTR-262	Nektar	solid tumors	I/II	IT	PEGylated IL-2, anti-PD1	NCT03435640
TLR 7/8	BDC-1001	Bolt Biotherapeutics	HER2-positive tumors	I	i.v.	anti-HER2 antibody[b], anti-PD1	NCT04278144
TLR7	LHC165	Novartis	solid tumors	I	IT	anti-PD1	NCT03301896
TLR8	VTX-2337	Celgene	HNSCC	I	IT or s.c.	anti-PD1	NCT03906526
			ovarian cancer	I/II	s.c.	durvalumab, Doxil	NCT02431559
TLR7/ 8, RIG-I	CV8102	CureVac	solid tumors	I	IT	anti-PD1	NCT03291002
TLR9	SD-101	Dynavax Technologies	melanoma, HNSCC	I/II	IT	anti-PD1	NCT02521870
			lymphoma	I/II	IT	BTK inhibitor and RT	NCT02927964
			prostate	II	IT	anti-PD1, SBRT	NCT03007732
			solid tumors	I/II	IT	IDO inhibitor and RT	NCT03322384
			lymphoma	I	IT	anti-OX40 and RT	NCT03410901
			solid tumors	I	IT	anti-OX40	NCT03831295
			pancreatic cancer	I	IT	anti-PD1 and RT	NCT04050085
TLR9	IMO-2125 (tilsotolimod)	Idera Pharmaceuticals	melanoma	II	IT	anti-CTLA4, anti-PD1	NCT02644967
			melanoma	III	IT	anti-CTLA4	NCT03445533
			solid tumors	II	IT	anti-CTLA4, anti-PD1	NCT03865082
			melanoma	II	i.d.	none	NCT04126876
			HNSCC	I	IT	anti-OX40, anti-PD1, chemotherapy	NCT04196283

Table 2. *Continued*

Target	Drug Name	Developer	Disease	Phase	ROA	Combination Agent	Trial ID
TLR9	MGN1703	Mologen AG	solid tumors	I	IT, s.c.	anti-CTLA4	NCT02668770
			colorectal cancer	III	s.c.	none	NCT02077868
TLR9	CMP-001	Checkmate Pharmaceuticals	melanoma	I	IT	anti-PD1	NCT02680184
			melanoma	II	IT	anti-PD1	NCT03618641
			melanoma	I	s.c., IT	anti-PD1	NCT03084640
			lung cancer	I	s.c., IT	anti-PDL1, RT	NCT03438318
			colorectal cancer	I	s.c., IT	anti-PD1, anti-CTLA4, RT	NCT03507699
			HNSCC	II	IT	anti-PDL1, anti-41BB, anti-OX40	NCT02554812
RIG-I	MK-4621	Merck	solid tumors	I	IT	anti-PD1	NCT03739138
STING	ADU-S100 (MIW815)	Aduro Biotech, Novartis	solid tumors	I	IT	none	NCT02675439
			solid tumors	I	IT	anti-PD1	NCT03172936
			HNSCC	II	IT	anti-PD1	NCT03937141
STING	MK-1454	Merck	solid tumors	I	IT	anti-PD1	NCT03010176
			HNSCC	II	IT	anti-PD1	NCT04220866
STING	MK-2118	Merck	solid tumors	I	IT or s.c.	anti-PD1	NCT03249792
STING	GSK3745417	GlaxoSmithKline	solid tumors	I	i.v.	anti-PD1	NCT03843359
STING	BMS-986301	Bristol-Myers Squibb	solid tumors	I	IT or i.m.	anti-PD1 and anti-CTLA4	NCT03956680
STING	IMSA101	ImmuneSensor Therapeutics	solid tumors	I	IT	CPI	NCT04020185
STING	SB 11285	Spring Bank Pharmaceuticals	solid tumors	I	i.v.	anti-PD1	NCT04096638
STING	SYNB1891	Synlogic	solid tumors	I	IT	anti-PDL1	NCT04167137

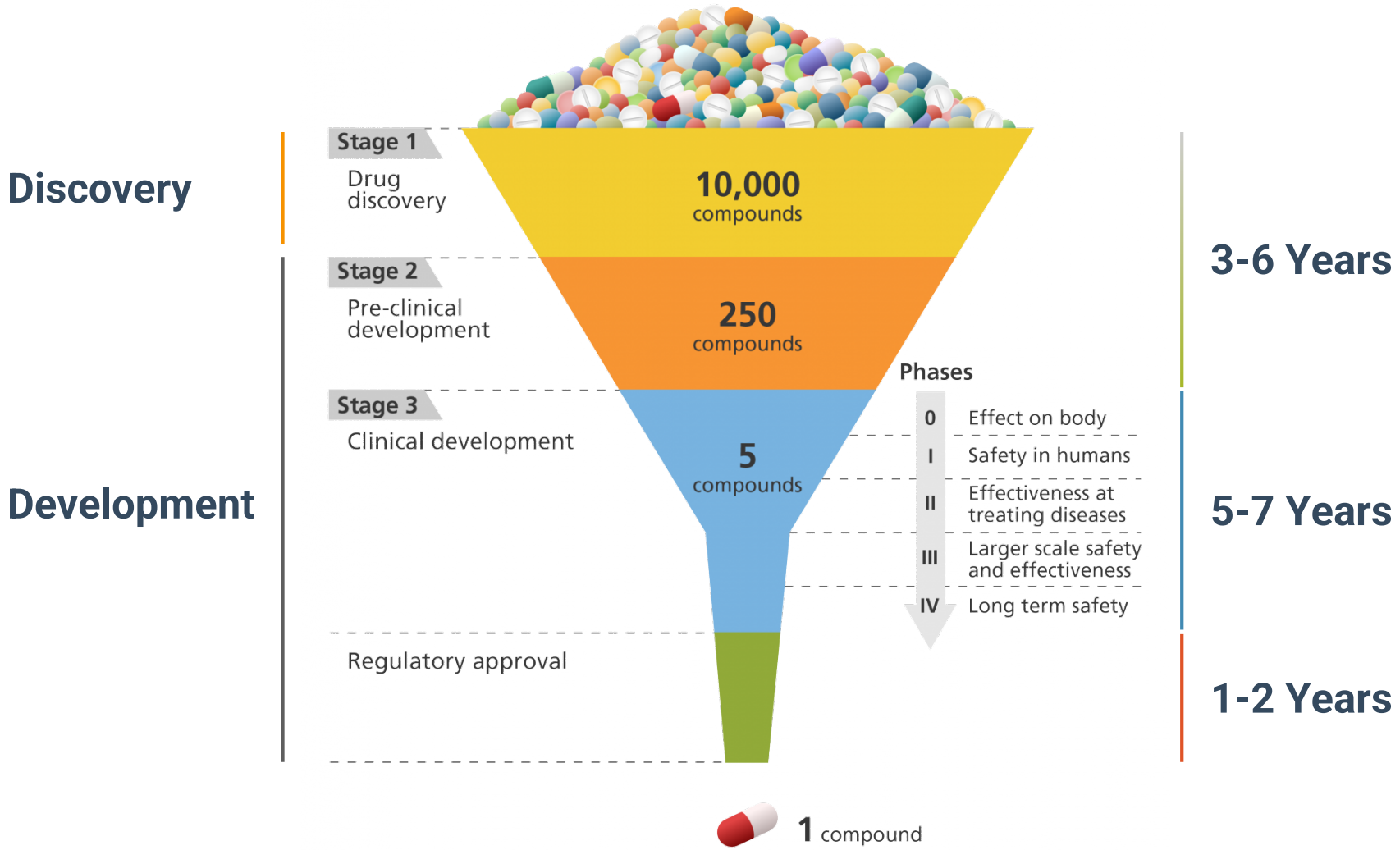
Source: ClinicalTrials.gov.

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CIN, cervical intraepithelial neoplasia; CLL, chronic lymphatic leukemia; CTCL, cutaneous T cell lymphoma; FUSA, focused ultrasound ablation; HNSCC, head and neck squamous cell carcinoma; i.d., intradermal; i.m., intramuscular; i.p., intraperitoneal; IT, intratumoral; i.v., intravenous; MDS, myelodysplastic syndrome; n/a, not applicable; ROA, route of administration; RT, radiation therapy; SBRT, stereotactic body radiation therapy; s.c., subcutaneous; sonidegib, Hedgehog signaling pathway inhibitor; TNBC, triple-negative breast cancer; unk, unknown; VIN, vulvar intraepithelial neoplasia.

[a]DEC-205/NY-ESO-1 fusion protein.

[b]anti-HER2 monoclonal antibody conjugated to TLR 7/8 dual agonist.

To Identify Novel Immunomodulator Drugs

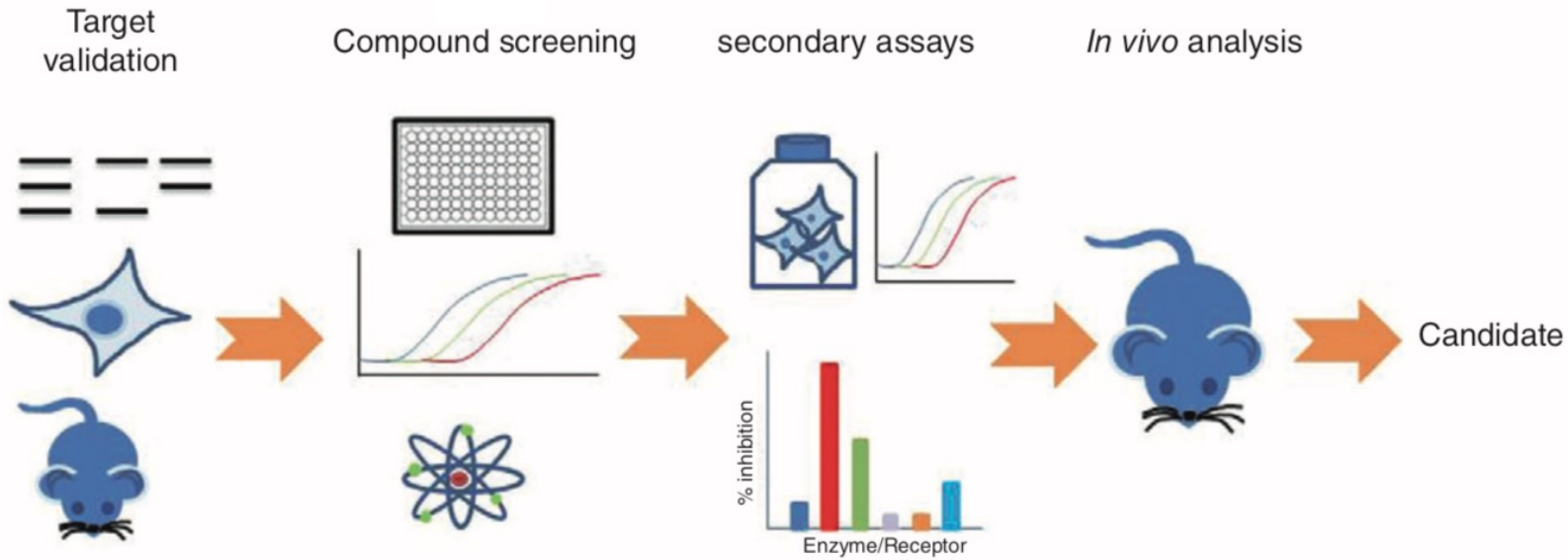


Tools to save time and resources in stage 1, so you can focus on later stages

InvivoGen

Compound Discovery

Cell based assay



Target validation	Compound screening	secondary assays	*In vivo* analysis	
•Genetic, cellular and *in vivo* experimental models to identify and validate target	•HTS & selective library screens; structure based design •Reiterative directed compound synthesis to improve compound properties	•*in vitro* & *ex vivo* secondary assays (mechanistic) •Selectivity & liability assays	•Compound pharmacology •Disease efficacy models •Early safety & toxicity studies	•Preclinical safety & toxicity package

Br J Pharmacol. 2011 Mar;162(6):1239-49.doi: 10.1111/j.1476-5381.2010.01127.x.

InvivoGen

Compound Discovery

Activators	Inhibitors	Target Identity	QC platform
Identify immunostimulatory compounds	Identify immune inhibitors	Identify target for novel compounds	Assess microbial contaminations in biologics

Allows for identification of better molecules

InvivoGen

Case I: Screening for Immunoactive Compounds

Activators

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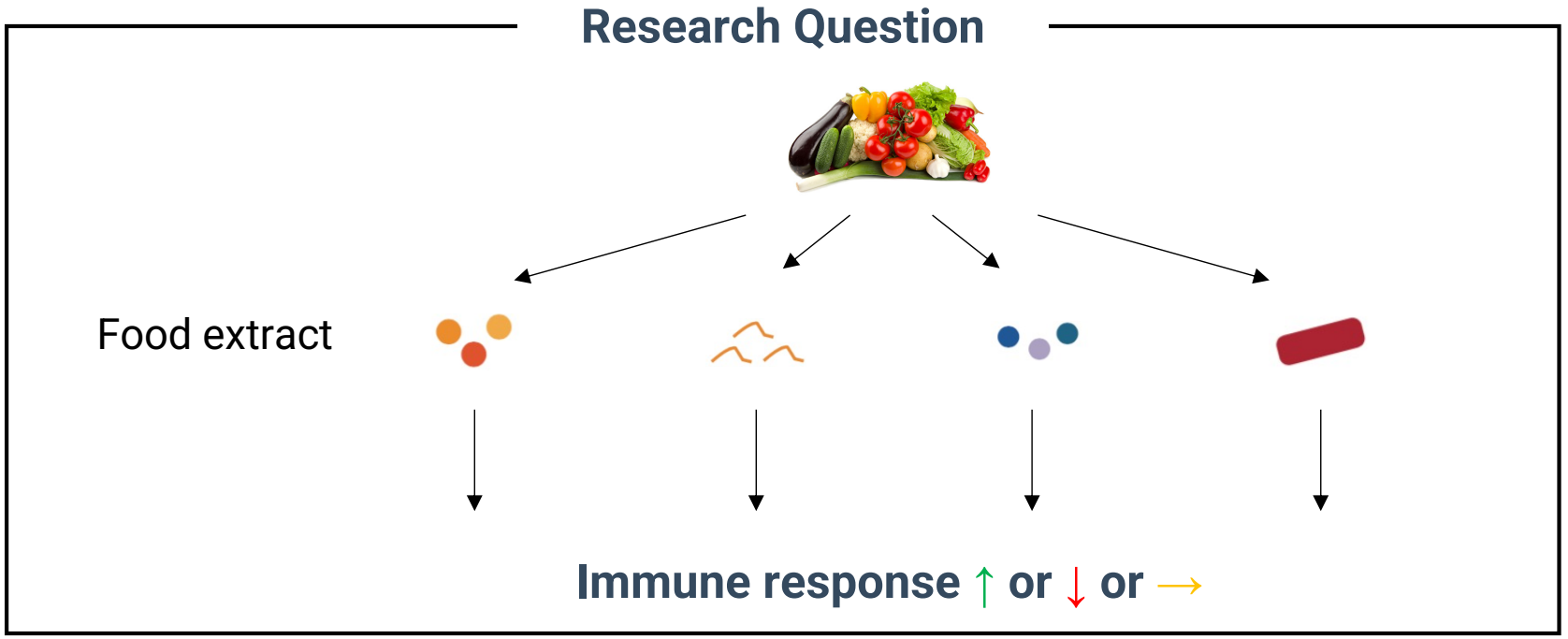
J Biomed Transl Res 2018;19(4):092-102
https://doi.org/10.12729/jbtr.2018.19.4.092
Received 23 Sep. 2018, Revised 19 Dec. 2018, Accepted 19 Dec. 2018

Original Article

Screening of immunoactive ingredients in frequently consumed food in Korea

Na-Young Gil[1], Sang-Myeong Lee[2], Ji-Young Mun[1], Soo-Hwan Yeo[1], So-Young Kim[1*]

[1]*Fermented and Processed Food Science Division, National Institute of Agricultural Sciences, RDA, Wanju 55365, Korea,*
[2]*Division of Biotechnology, College of Environmental and Bioresource Sciences, Chonbuk National University, Iksan 54596, Korea.*



InvivoGen

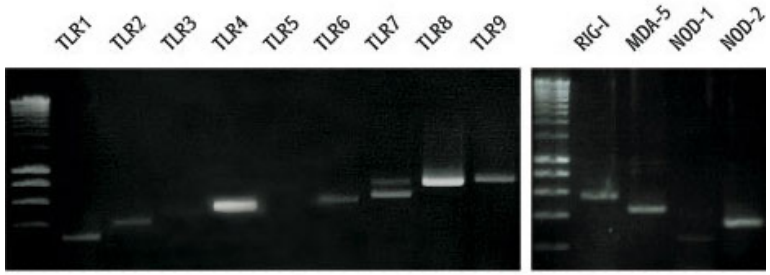
Case I: Screening for Immunoactive Compounds

Activators

RAW-Blue™ Cells

RAW-Blue™ cells	Unit size	Cat. code
NF-κB-SEAP Reporter Cell Line	3-7 x 10e6 cells	raw-sp

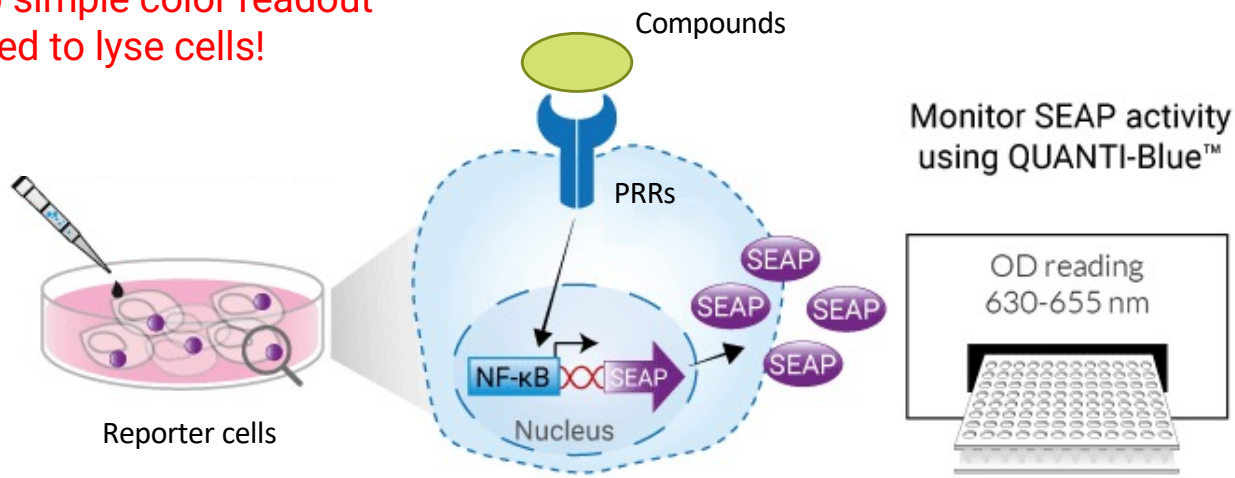
NFκB reporter cells derived from RAW264.7



RAW cells endogenous expression of PRRs

RAW-Blue™ Screening Setup

- 1-step simple color readout
- No need to lyse cells!



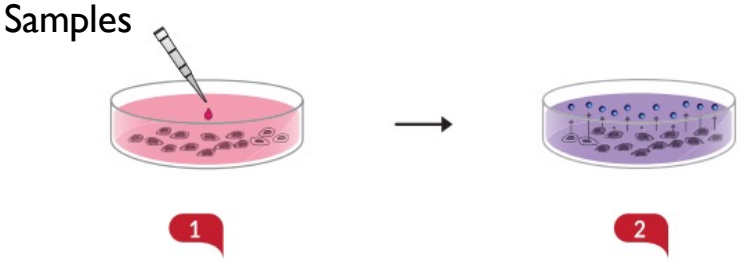
InvivoGen

Case I: Screening for Immunoactive Compounds

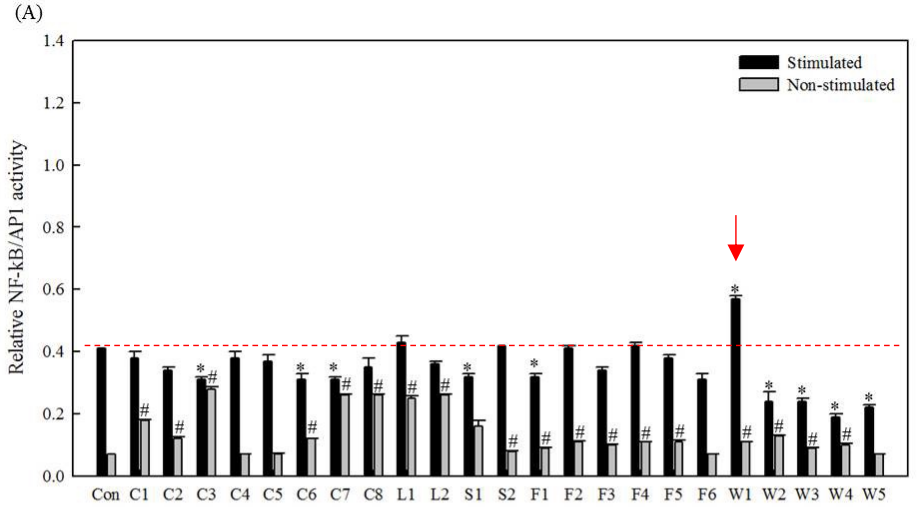
Activators

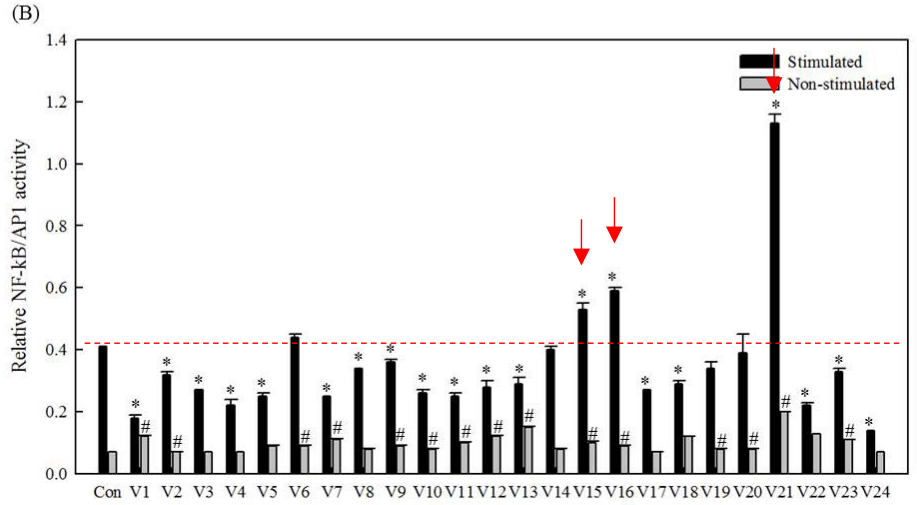
Group			Food and Description	Origin
Fruits	F1	밀감	Mandarin orange	Jeju
	F2	바나나	Banana	Philippines
	F3	배	Pear	Jochiwon
	F4	사과	Apple	Cheongsong
	F5	수박	Water melon	Korean
	F6	포도	Grape	Sangju
Wild plant	W1	가죽나무	*Ailantias altissima*	Korean
	W2	꿀풀	*Prunella vulgaris* L.	Korean
	W3	다래	*Actinidia arguta*	Korean
	W4	머위	*Petasites japonicus*	Korean
	W5	참취	*Aster scaber*	Korean
	V1	고사리	Bracken	Yeoju
	V2	깻잎	Perilla leaves	Daegu
	V3	파리고추	Green young hot pepper	Jinju
	V4	당근	Carrot	Jeju
	V5	대파	Welsh onion	Yeoju
	V6	마늘	Garlic	Seosan
	V7	무	Korean radish	Jeju
	V8	미나리	Water dropwort	Gijang

Examples of food samples for screening



Quantify NFκB activity by OD630





Lead compounds identified (e.g. W1, V15/16/21)

InvivoGen

Case II: Screening Inhibitory Compounds

Inhibitors

jove Journal of Visualized Experiments www.jove.com

Video Article

Screening Bioactive Nanoparticles in Phagocytic Immune Cells for Inhibitors of Toll-like Receptor Signaling

Hong Yang[1], Shan Yu Fung[2], Aihua Bao[1], Qiang Li[1], Stuart E. Turvey[2]

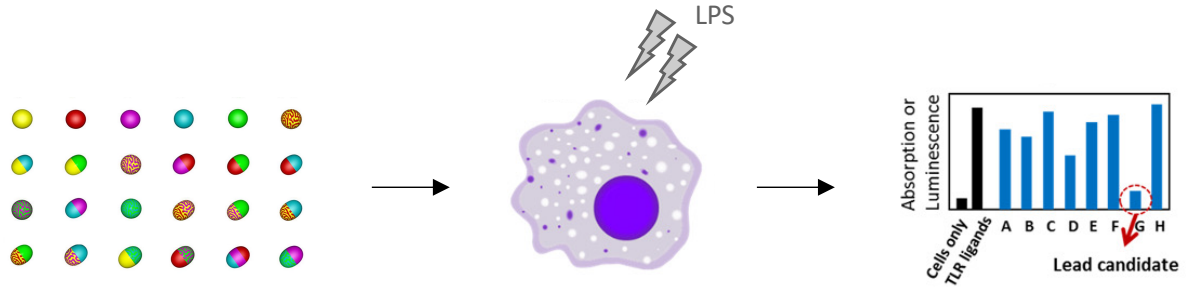
[1]Department of Respiratory Medicine, Shanghai First People's Hospital, Shanghai Jiaotong University School of Medicine

[2]Department of Pediatrics, BC Children's Hospital and University of British Columbia

Research Question

No clinically approved TLR inhibitors

(except for hydroxychloroquine)



Nanoparticles library

Reporter cell screening platform

Identify novel compounds

InvivoGen

Case II: Screening Inhibitory Compounds

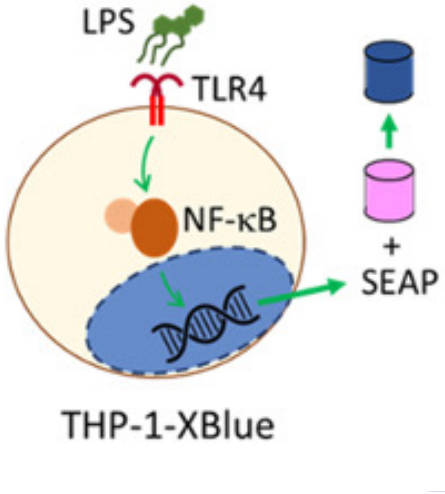
Inhibitors

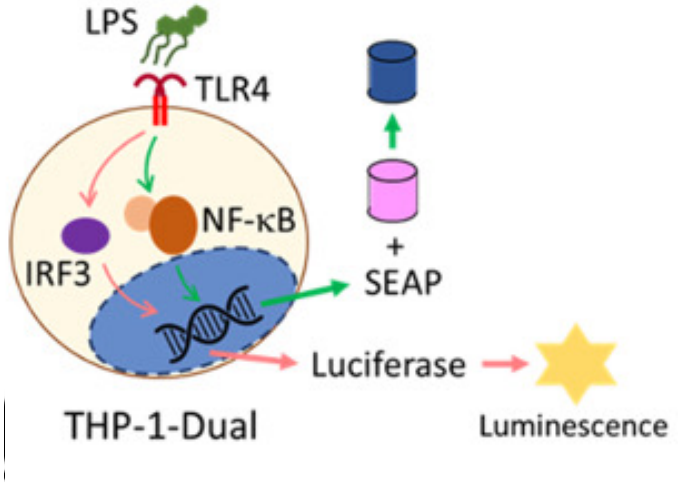
THP1-Blue™ NF-κB Cells

THP1-Blue™ NF-κB Cells	Unit size	Cat. code
NF-κB SEAP Reporter Monocytes	3-7 x 10e6 cells	thp-nfkb

THP1-Dual™ Cells

THP1-Dual™ Cells	Unit size	Cat. code
NF-κB-SEAP IRF-Luc Reporter Monocytes	3-7 x 10e6 cells	thpd-nfis





NFκB reporter cells derived from THP-1

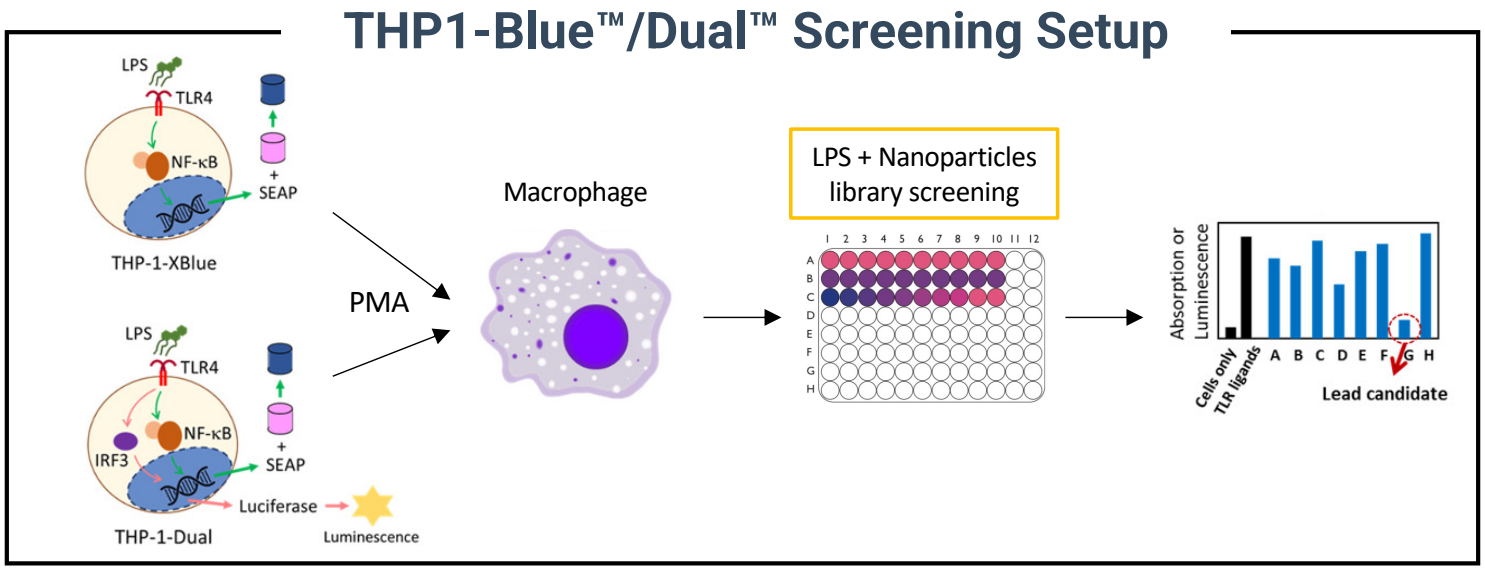
SEAP: NFκB reporter

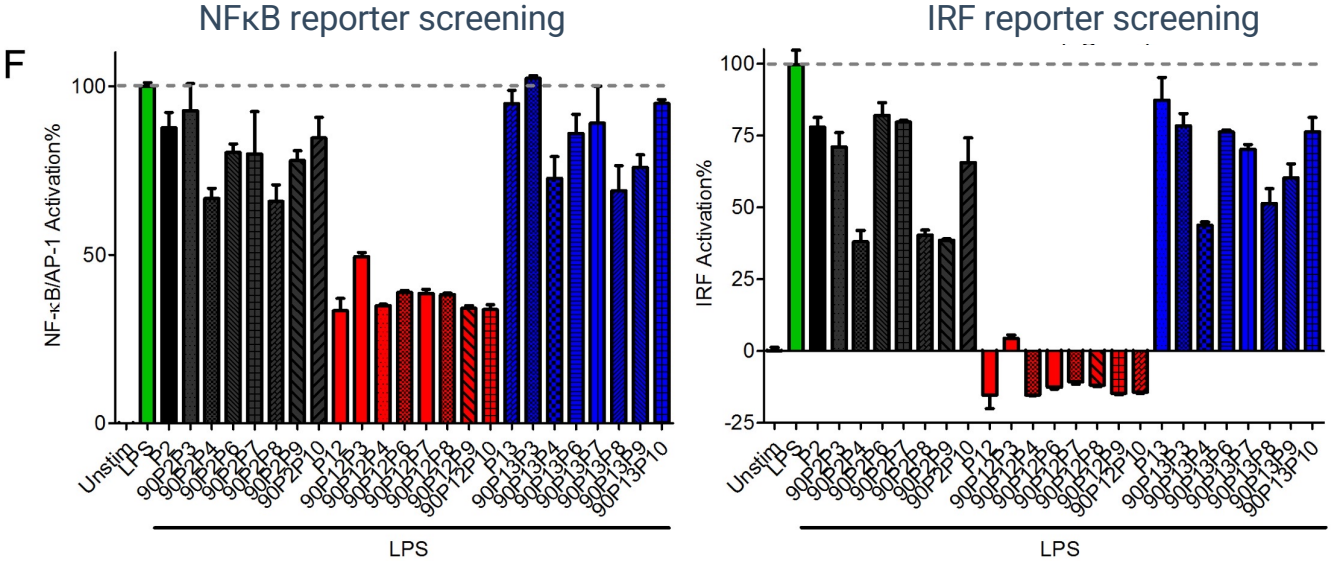
Lucia luciferase: IRF reporter

InvivoGen

Case II: Screening Inhibitory Compounds

Inhibitors



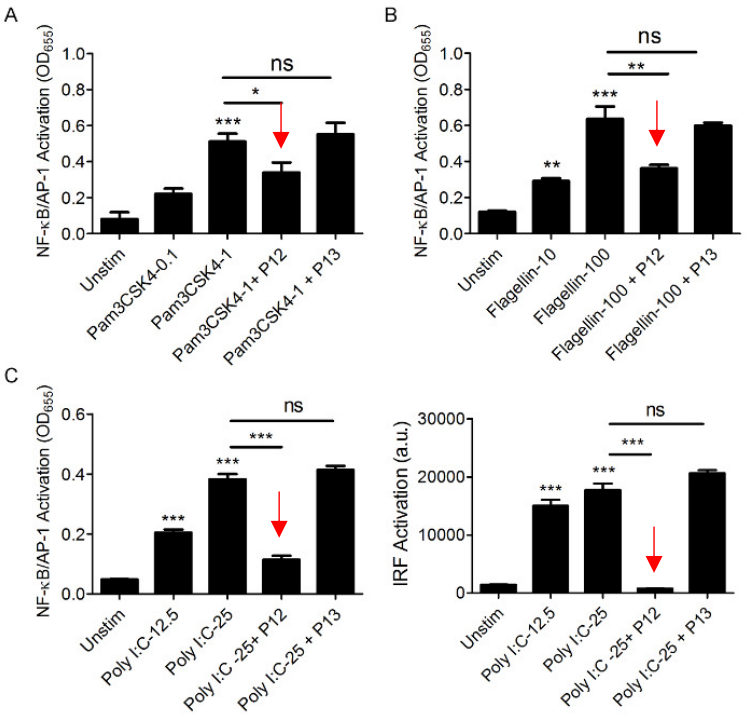


Lead compounds identified (e.g. 90P12P group)

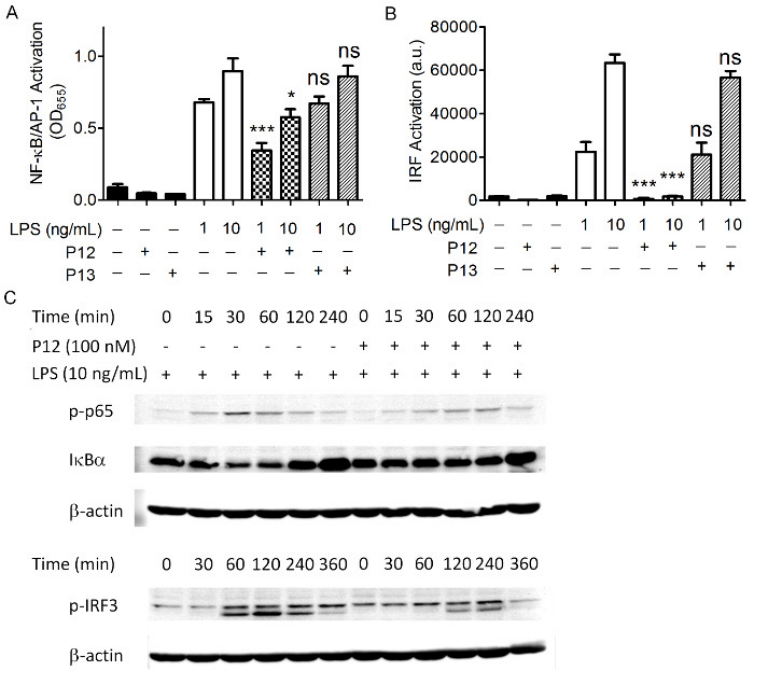
InvivoGen

Case II: Screening Inhibitory Compounds

Inhibitors



P12 inhibits TLR2, TLR3, TLR5 activity

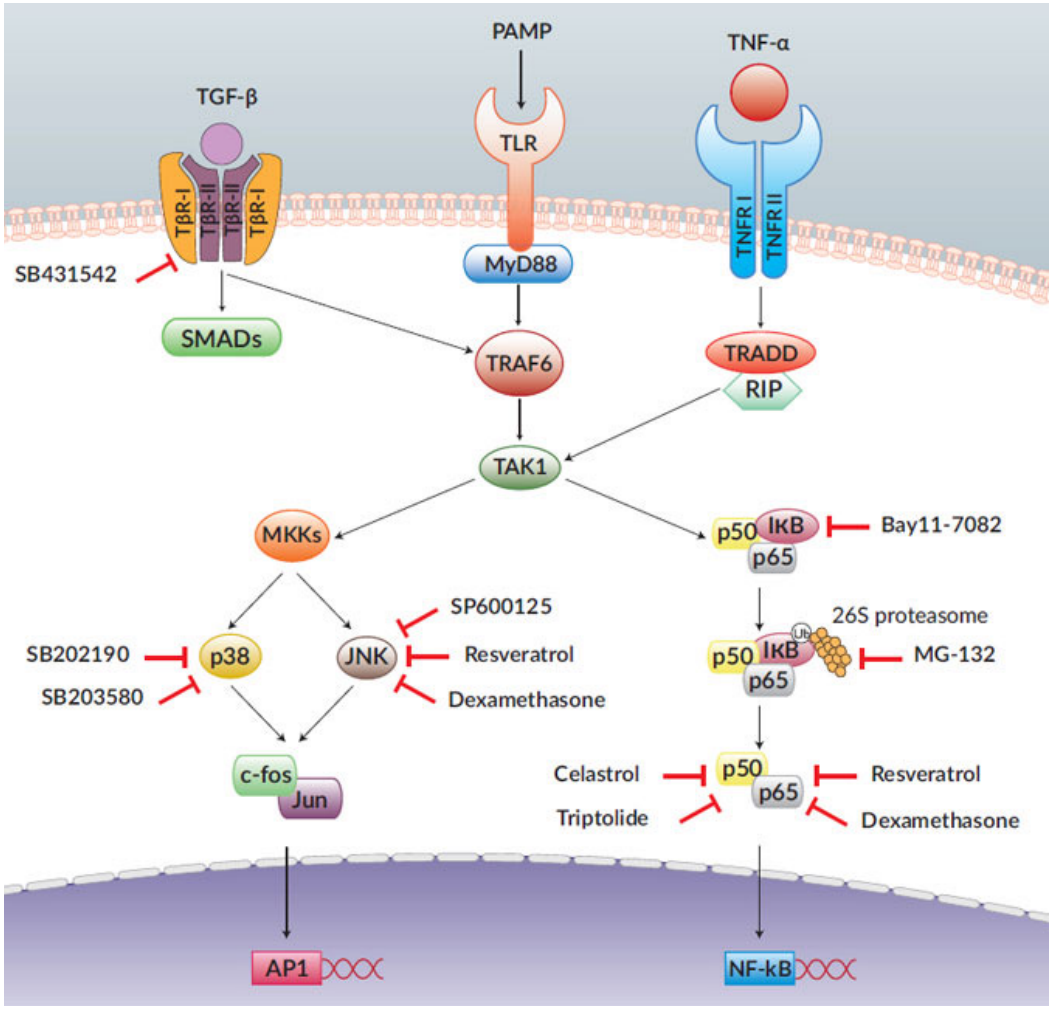


P12 delays p65 and IRF phosphorylation

Lead compounds function verified by further experiments

InvivoGen

Reporter Cell Lines



Multiple activators/inhibitors targeting the same pathway

Target Identification:

1. Pick a target of interest, then ask yes/no

2. Screening to identify specific target for novel drugs

InvivoGen

Case III: HTS for Specific TLR Inhibitors

Target Identity

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 20 NOVEMBER 2017 | DOI: 10.1038/NCHEMBIO.2518

Small-molecule inhibition of TLR8 through stabilization of its resting state

Shuting Zhang[1-3,7], Zhenyi Hu[2,7], Hiromi Tanji[4,7], Shuangshuang Jiang[1], Nabanita Das[2], Jing Li[5], Kentaro Sakaniwa[4], Jin Jin[6], Yanyan Bian[6], Umeharu Ohto[4], Toshiyuki Shimizu[4]* & Hang Yin[1,2]*

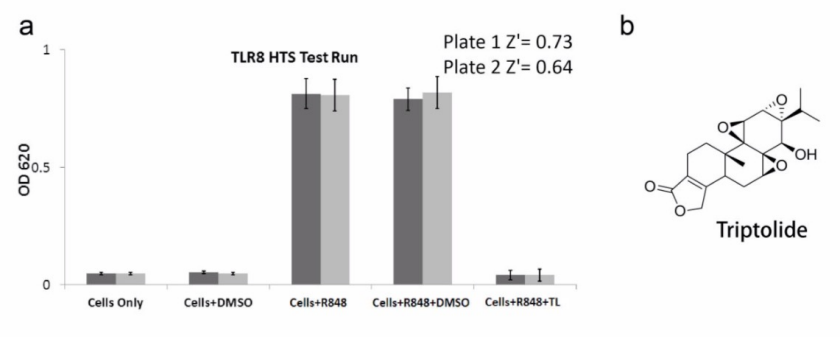
Verifying HEK-Blue™ assay is robust for HTS

384-well HTS

Supplementary Table 1: Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	Cell-based
	Target	Toll-like Receptor 8 signaling pathway
	Primary measurement	Detection of TLR8-inducted secreted embryonic alkaline phosphatase (SEAP) concentration in the culture media
	Key reagents	R848, QUANTI-Blue™, Triptolide (Invitrogen)
	Assay protocol	http://www.invivogen.com/quanti-blue
Library	Library size	14,400
	Library composition	Drug-like molecules
	Source	Maybridge
Screen	Format	384-well plate
	Concentration(s) tested	4 µM, 0.04% DMSO

HEK-Blue™ cells verification



Supplementary Figure 2: Z'-factor for HTS assay. (a) An average Z'-factor of 0.68 was determined with two independent experiment to demonstrate that the HTS assay is robust. A previously established NF-κB inhibitor, triptolide (TL), was employed as the positive control. Data are mean ± *SD*; n =60, technical replicates). **(b)** Chemical structure of triptolide.

InvivoGen

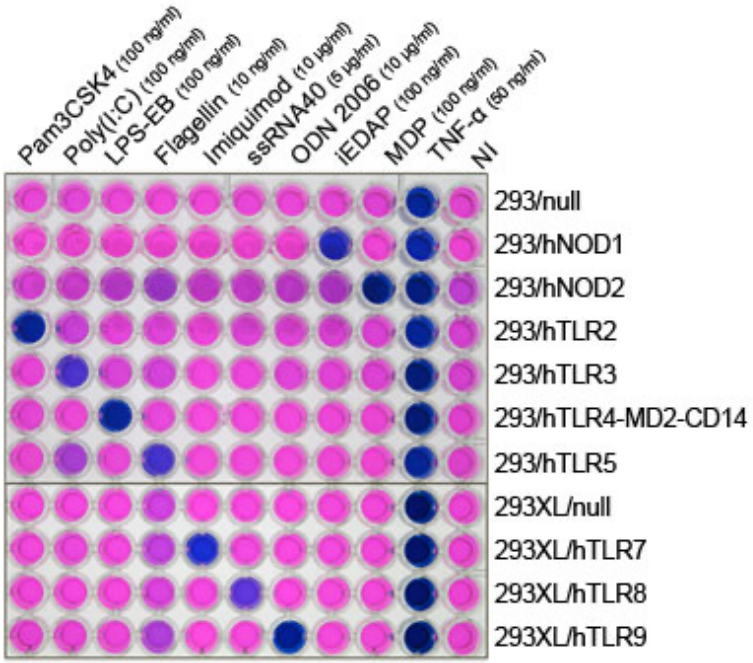
Case III: HTS for Specific TLR Inhibitors

Target Identity

HEK-Blue™ hTLR8

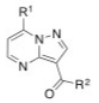
HEK-Blue™ hTLR8 cells	Unit size	Cat. code
Human TLR8-expressing HEK 293 cells	3-7 x 10e6 cells	hkb-htlr8

TLR8-NFκB reporter cells derived from HEK293



HEK293 expresses limited TLRs, so the response is highly specific to overexpressed receptors

Supplementary Table 2: Representative structure-activity relationship (SAR) results for inhibitory activities of pyrazolo[1,5-*a*]pyrimidine derivatives in HEK-Blue hTLR8 cells.



No.	R^1	R^2	IC_{50} [nM]
5 (CU-CPT8m)	3-Me-C_6H_4-	-NH_2	67 ± 10
6	3-CF_3-C_6H_4-	-OH	> 20,000
9	3-CF_3-C_6H_4-	-OEt	210 ± 43
10	3-Me-C_6H_4-	-OEt	92 ± 15
11	3-Me-C_6H_4-	-OH	> 20,000
12	3-CF_3-C_6H_4-	-NH_2	130 ± 30
13	2-CF_3-C_6H_4-	-NH_2	230 ± 140
14	4-CF_3-C_6H_4-	-NH_2	1,220 ± 200
15	Phenyl-	-NH_2	760 ± 230
16	3-NO_2-C_6H_4-	-NH_2	220 ± 70
17	3-F-C_6H_4-	-NH_2	450 ± 90
18	3-Cl-C_6H_4-	-NH_2	280 ± 100
19	3,5-diCF_3-C_6H_3-	-NH_2	250 ± 130
20	2-OMe-C_6H_4-	-NH_2	2,310 ± 220
21	3-OMe-C_6H_4-	-NH_2	1,200 ± 200
22	3-Pyridyl	-NH_2	>20,000
23	3-Et-C_6H_4-	-NH_2	120 ± 23
24	3-CF_3-C_6H_4-	-NHMe	1,700 ± 300
25	3-CF_3-C_6H_4-	-NEt_2	>20,000

*IC_{50} values and corresponding standard deviations were determined from at least three biological replicates.

HTS Screening results

InvivoGen

Case III: HTS for Specific TLR Inhibitors

Target Identity

nature chemical biology
ARTICLE
PUBLISHED ONLINE: 20 NOVEMBER 2017 | DOI: 10.1038/NCHEMBIO.2518

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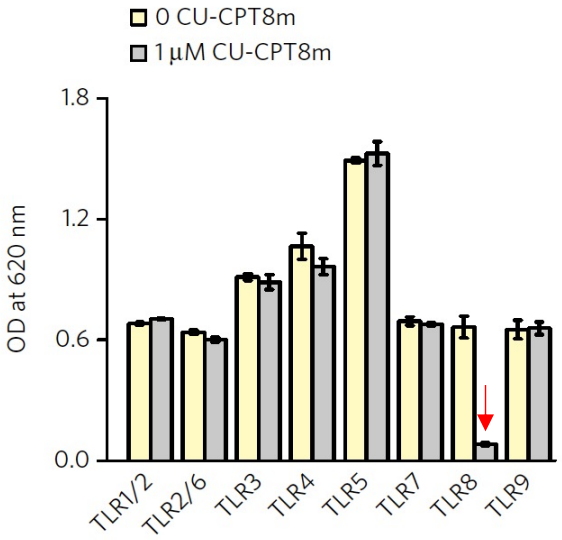
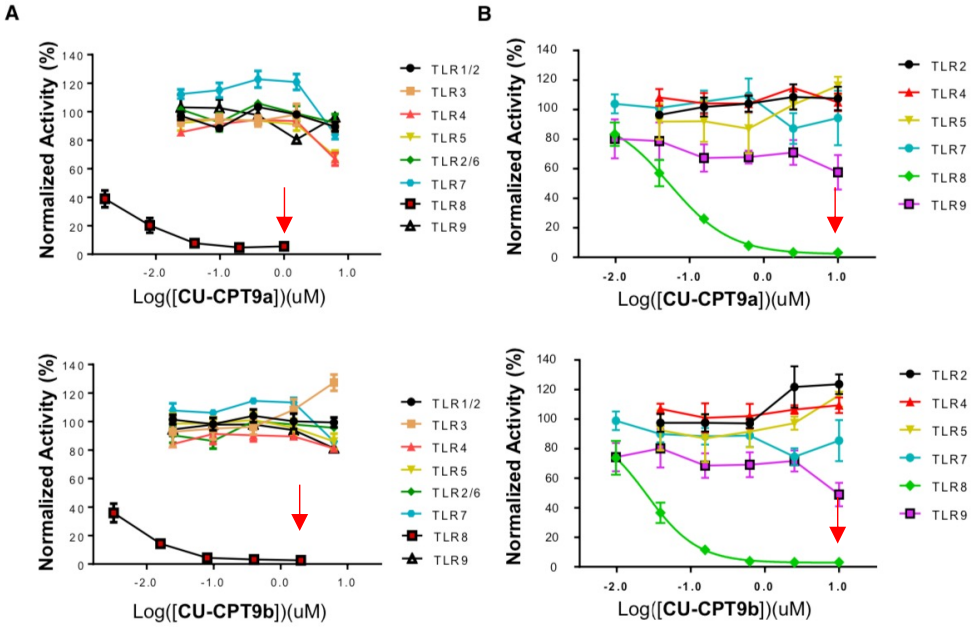


Verifying TLR8 specificity for lead compound

Cell Chemical Biology
Brief Communication
CellPress

Small-Molecule TLR8 Antagonists via Structure-Based Rational Design

Zhenyi Hu,[1,2] Hiromi Tanji,[3] Shuangshuang Jiang,[2] Shuting Zhang,[2] Kyoin Koo,[1] Jean Chan,[4] Kentaro Sakaniwa,[3] Umeharu Ohto,[3] Albert Candia,[4] Toshiyuki Shimizu,[3] and Hang Yin[1,2,5,*]
[1]Department of Chemistry and Biochemistry and BioFrontiers Institute, University of Colorado Boulder, Boulder, CO 80309, USA
[2]School of Pharmaceutical Sciences, Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Tsinghua University, Beijing 100082, China
[3]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan
[4]Dynavax Technologies Corporation, Berkeley, CA 94710, USA



Verifying TLR8 specificity for lead compound (serial dilution)

TLR reporter cells panel for specificity test

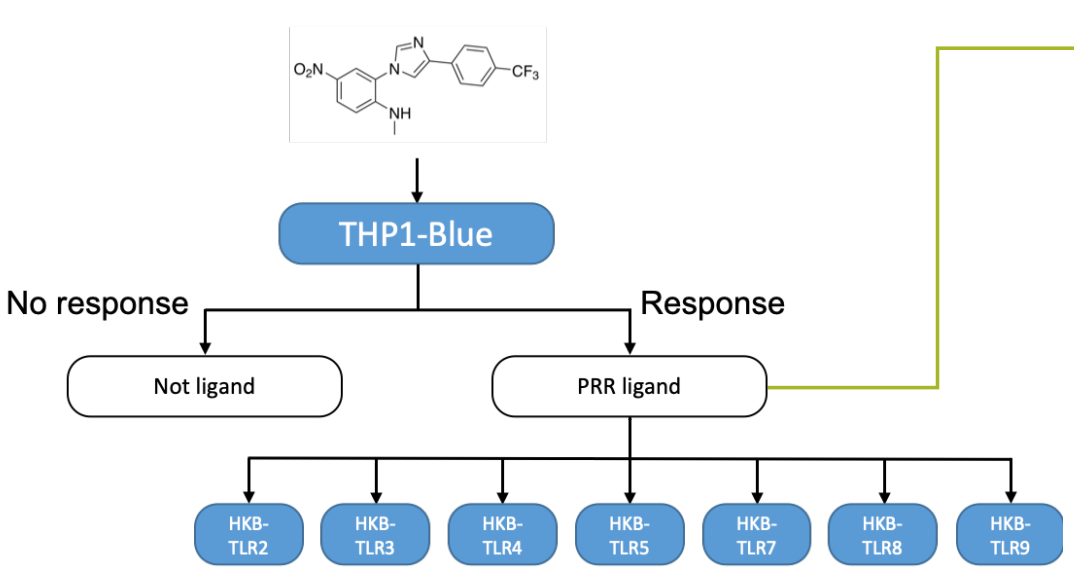
InvivoGen

Screening Specific PRR Ligands

Target Identity

PRR Ligands Screening Platform Scheme

**PRR target identification for novel compounds



Identify specific TLR target

MyD88 dependent	MyD88 independent
TLR2	TLR3
TLR4	NOD1
TLR5	NOD2
TLR7	Dectin-1
TLR8	Dectin-2
TLR9	Mincle
	STING
THP1-Blue ✓	THP1-Blue ✓
THP1-KO MyD88 ✗	THP1-KO MyD88 ✓

InvivoGen

Case IV: Reporter Cell Lines for QC

QC platform

PRODUCT	QUANTITY	CAT. CODE
HUMAN		
HEK-Blue™ hTLR2 Cells	3-7 x 10^6 cells	hkb-htlr2
HEK-Blue™ hTLR3 Cells	3-7 x 10^6 cells	hkb-htlr3
HEK-Blue™ hTLR4 Cells	3-7 x 10^6 cells	hkb-htlr4
HEK-Blue™ hTLR5 Cells	3-7 x 10^6 cells	hkb-htlr5
HEK-Blue™ hTLR7 Cells	3-7 x 10^6 cells	hkb-htlr7
HEK-Blue™ hTLR8 Cells	3-7 x 10^6 cells	hkb-htlr8
HEK-Blue™ hTLR9 Cells	3-7 x 10^6 cells	hkb-htlr9
MOUSE		
HEK-Blue™ mTLR2 Cells	3-7 x 10^6 cells	hkb-mtlr2
HEK-Blue™ mTLR3 Cells	3-7 x 10^6 cells	hkb-mtlr3
HEK-Blue™ mTLR4 Cells	3-7 x 10^6 cells	hkb-mtlr4
HEK-Blue™ mTLR5 Cells	3-7 x 10^6 cells	hkb-mtlr5
HEK-Blue™ mTLR7 Cells	3-7 x 10^6 cells	hkb-mtlr7
HEK-Blue™ mTLR8 Cells	3-7 x 10^6 cells	hkb-mtlr8
HEK-Blue™ mTLR9 Cells	3-7 x 10^6 cells	hkb-mtlr9
HEK-Blue™ mTLR13 Cells	3-7 x 10^6 cells	hkb-mtlr13
CONTROL		
HEK-Blue™ Null1 Cells	3-7 x 10^6 cells	hkb-null1
HEK-Blue™ Null1-k Cells	3-7 x 10^6 cells	hkb-null1k
HEK-Blue™ Null1-v Cells	3-7 x 10^6 cells	hkb-null1v
HEK-Blue™ Null2 Cells	3-7 x 10^6 cells	hkb-null2
HEK-Blue™ Null2-k Cells	3-7 x 10^6 cells	hkb-null2k

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2009, p. 3427–3434 Vol. 47, No. 11
0095-1137/09/$12.00 doi:10.1128/JCM.00373-09

Use of Toll-Like Receptor Assays To Detect and Identify Microbial Contaminants in Biological Products▽

Li-Yun Huang,[1] James L. DuMontelle,[2] Melissa Zolodz,[2] Aparna Deora,[2] Ned M. Mozier,[2] and Basil Golding[1]*

Division of Hematology, Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892,[1] and Research and Development, Pfizer Global Biologics, Chesterfield, Missouri 63017[2]

US FDA and Pfizer propose HEK-Blue™ assays to detect microbial contaminants in biological products

	0	TLR2	TLR3	TLR4	TLR5	TLR7	TLR8	TLR9
mycoplasma		+						
Gram +		+			+			+
Gram -		+		+	+			+
Fungi		+		+		+	+	+
Virus	+							+
Flagellin	+				+			
dsRNA	+		+					
ssRNA						+	+	
LPS				+				

Proposed Algorithm for microbial contaminant detection using InvivoGen cell lines

InvivoGen

Case IV: Reporter Cell Lines for QC

QC platform

SCIENTIFIC REPORTS

OPEN

Cell based assay identifies TLR2 and TLR4 stimulating impurities in Interferon beta

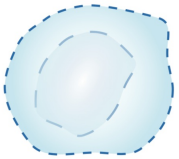
Received: 15 March 2017
Accepted: 1 August 2017
Published online: 05 September 2017

Lydia Asrat Haile, Swamy Kumar Polumuri, Roshni Rao, Logan Kelley-Baker, Dimitri Kryndushkin, Rajesh Rajaiah, Tomer Israely, V. Ashutosh Rao & Daniela Verthelyi

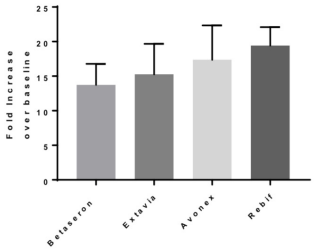
Using reporter cells for QC

Parameters	Rebif (IFNβ-1a)	Avoxnex (IFNβ-1a)	Avonex (IFNβ-1a)	Betaseron (IFNβ-1b)	Extavia (IFNβ-1b)
Produced in	CHO	CHO	CHO	E.Coli	E.Coli
Manufactured by	Serono Inc.	Biogen	Biogen	Bayer	Novartis

Commercial biological products



Reporter Cells

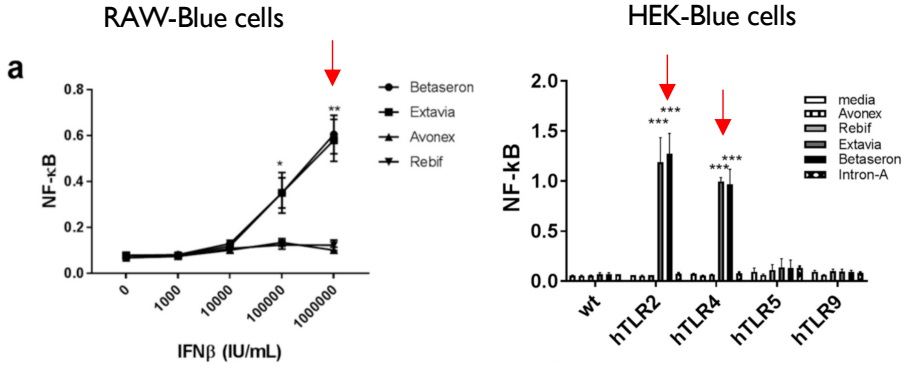


Measurement of immunoactive impurities

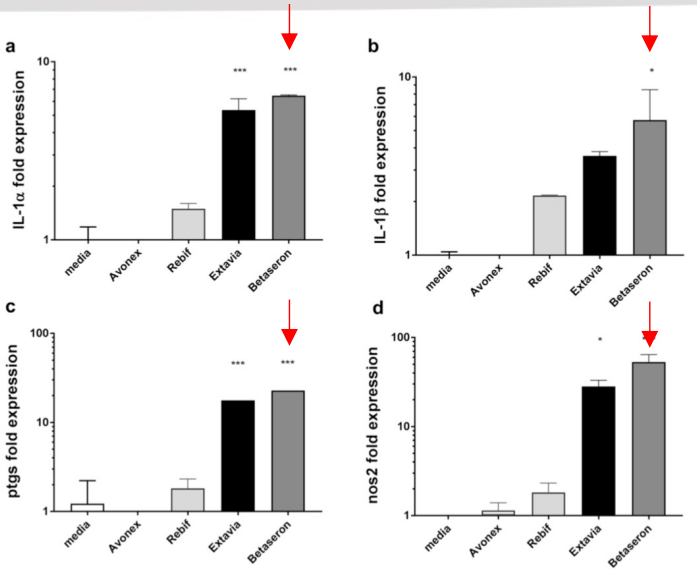
InvivoGen

Case IV: Reporter Cell Lines for QC

QC platform

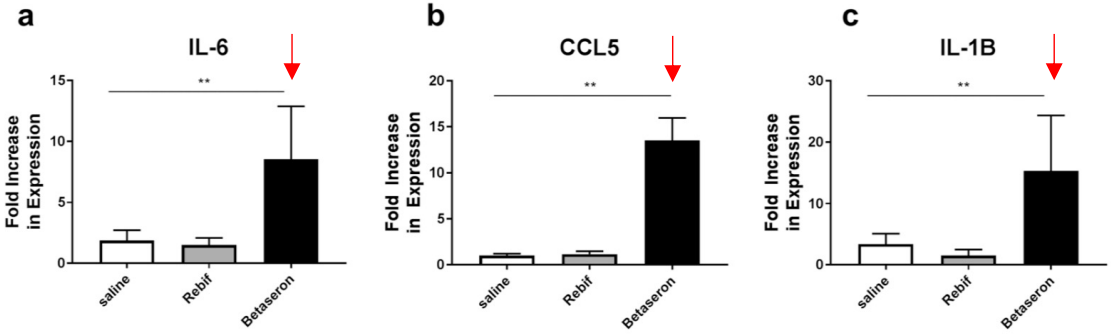


RAW-Blue and HEK-Blue cells suggest Betaseron contains TLR2/4 impurities



Test in mouse primary splenocytes suggest presence of immunoactive impurities

Commercial LAL-based tests showed no endotoxin (TLR4) contamination



In vivo experiments showed presence of immunoactive impurities

FDA comments: … the method exclusively detected low levels of IIRMIs
… that were not evident using the LAL test

InvivoGen

Other Research Hot Topics

Case V: STING Ligands Screening

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

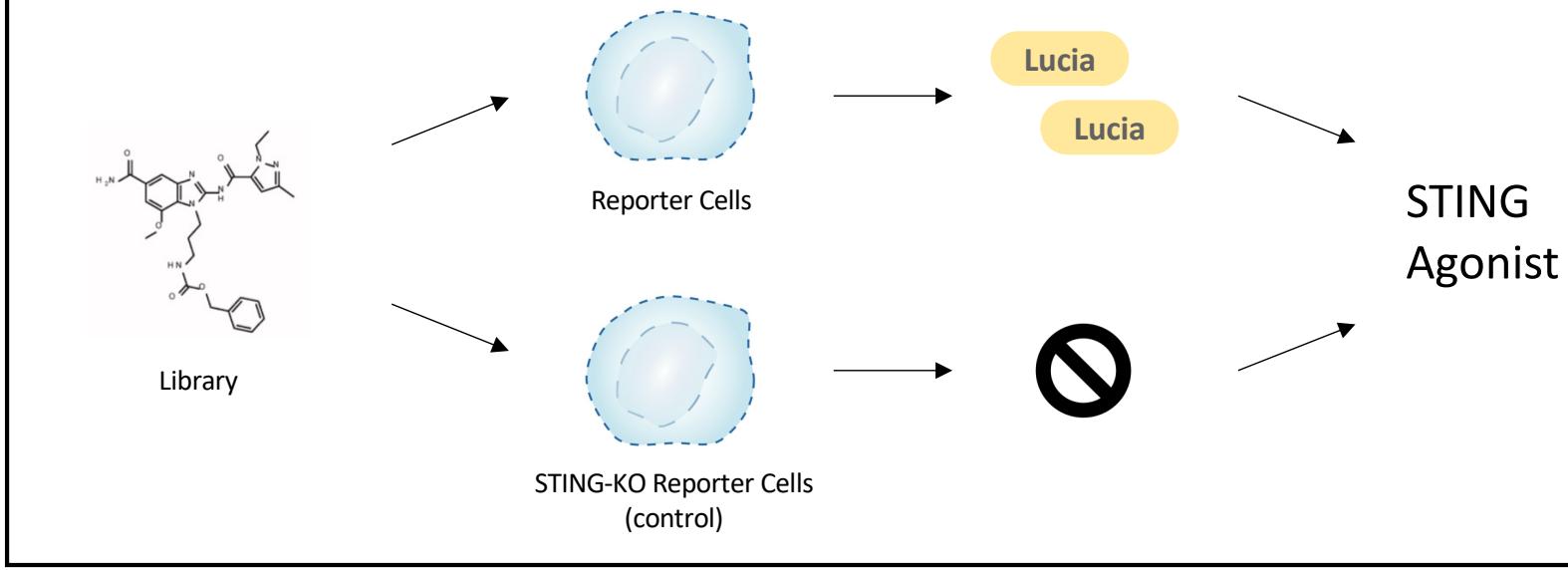
Research paper

A synthetic STING agonist inhibits the replication of human parainfluenza virus 3 and rhinovirus 16 through distinct mechanisms

Qingyuan Zhu *, Hui Hu, Haixia Liu, Hong Shen, Zhipeng Yan **, Lu Gao ***

Roche Innovation Center Shanghai, Shanghai, 201203, China

Screening for STING Ligands



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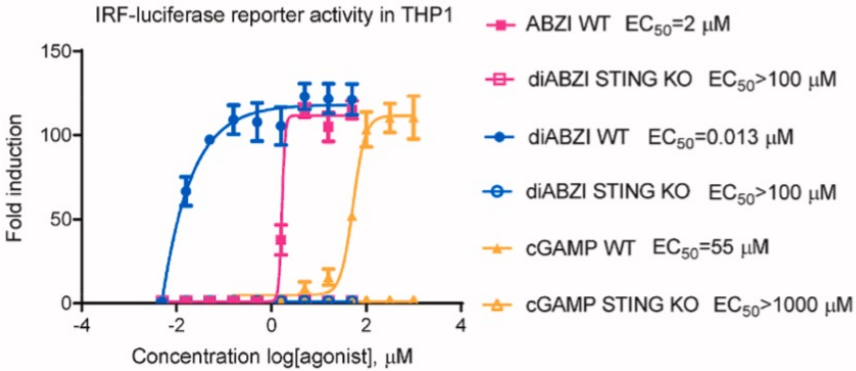
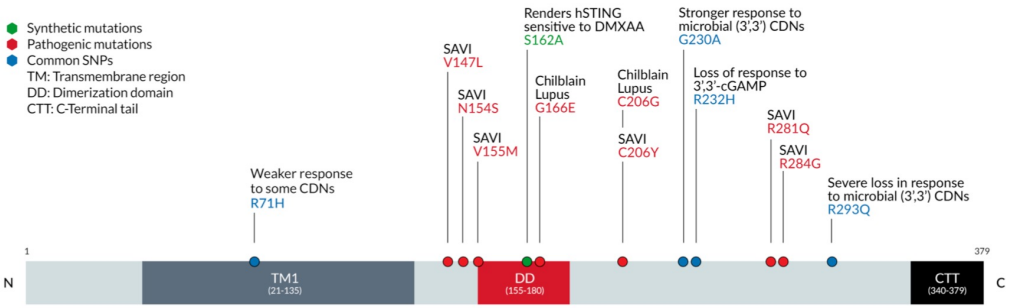
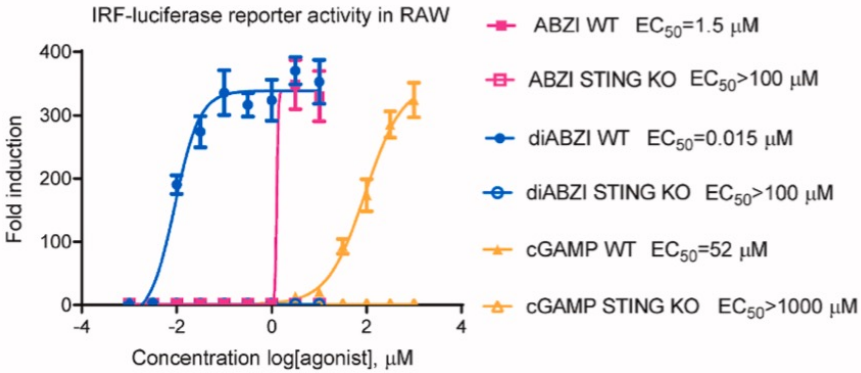
Case V: STING Ligands Screening





STING exists as multiple variants



Reporter cells identify ABZI and diABZI as potent and specific STING agonists

CELL LINE	PRODUCTS
B16	B16-Blue™ ISG Cells
	B16-Blue™ ISG-KO-STING Cells
HEK293	HEK-Blue™ ISG Cells
	HEK-Blue™ ISG-KO-STING Cells
HEK293T	293T-Dual™ hSTING-A162 Cells
	293T-Dual™ hSTING-H232 Cells
	293T-Dual™ hSTING-R232 Cells
	293T-Dual™ mSTING Cells

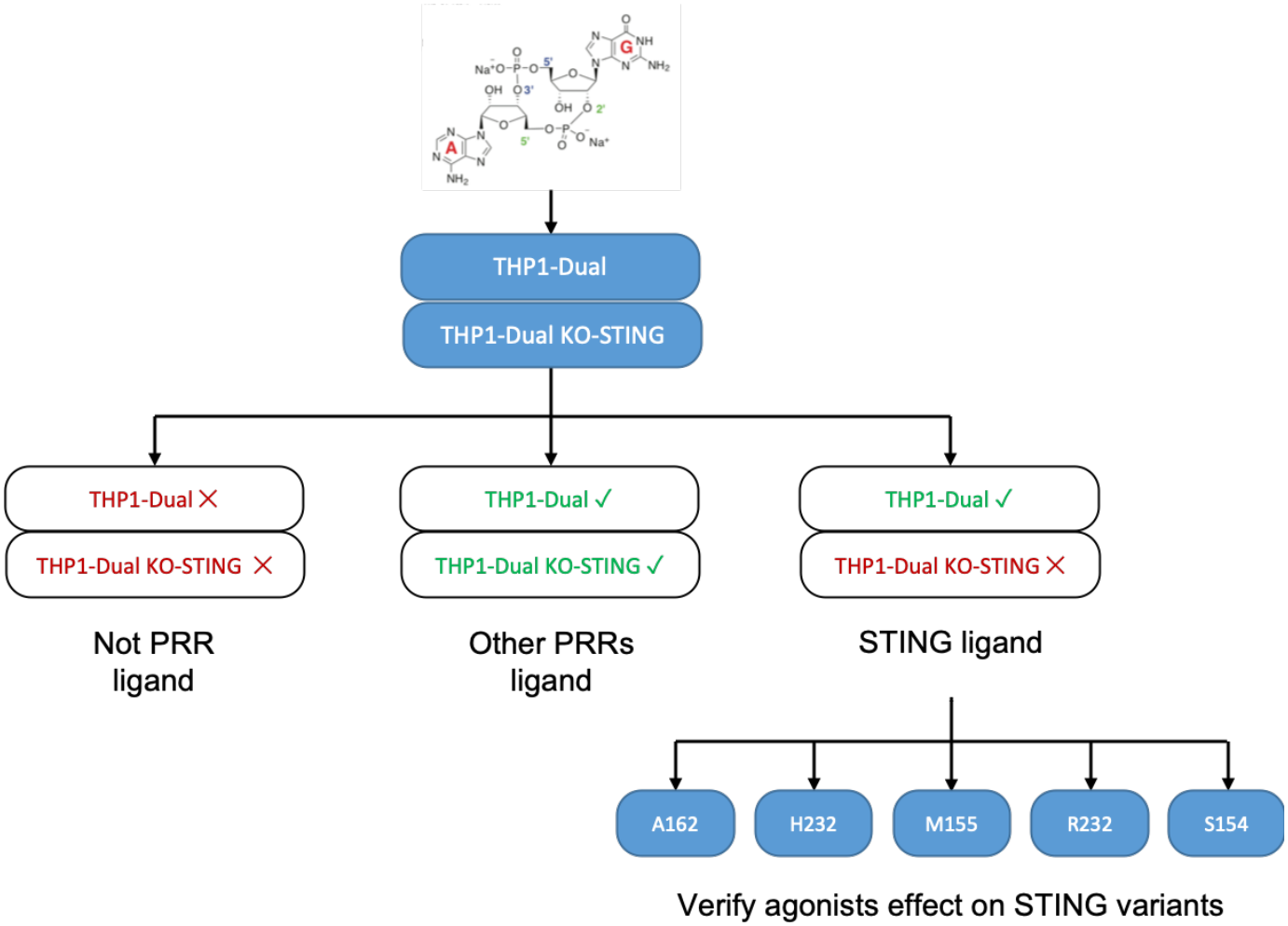
CELL LINE	PRODUCTS
RAW 264.7	RAW-Lucia™ ISG Cells
	RAW-Lucia™ ISG-KO-cGAS Cells
	RAW-Lucia™ ISG-KO-IRF3 Cells
	RAW-Lucia™ ISG-KO-STING Cells
THP-1	THP1-Dual™ Cells
	THP1-Dual™ KO-cGAS Cells
	THP1-Dual™ KO-STING Cells
	THP1-Dual™ KI-hSTING-A162 Cells
	THP1-Dual™ KI-hSTING-H232 Cells
	THP1-Dual™ KI-hSTING-M155 Cells
	THP1-Dual™ KI-hSTING-R232 Cells
	THP1-Dual™ KI-hSTING-S154 Cells

Panels of reporter cells with different STING variants

InvivoGen

Case V: STING Ligands Screening

STING Ligands Screening Platform Scheme



Case VI: Inflammasome Ligands Screening

Cell

CellPress

Article

A Translocation Pathway for Vesicle-Mediated Unconventional Protein Secretion

Min Zhang,[1,3] Lei Liu,[1,3] Xubo Lin,[2] Yang Wang,[1] Ying Li,[1] Qing Guo,[1] Shulin Li,[1] Yuxin Sun,[1] Xuan Tao,[1] Di Zhang,[1] Xiachen Lv,[1] Li Zheng,[1] and Liang Ge[1,4,*]

[1]State Key Laboratory of Membrane Biology, Tsinghua University-Peking University Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China

[2]Beijing Advanced Innovation Center for Biomedical Engineering, Beihang University, Beijing 100191, China

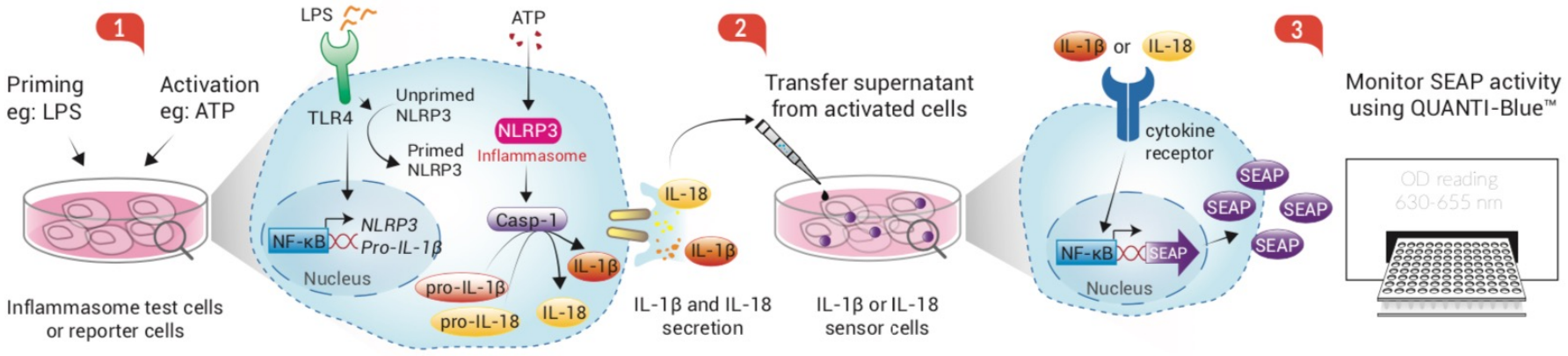
[3]These authors contributed equally

[4]Lead Contact

Testing the effect of protein candidate on IL-1β secretion

1. Treatment on test cells

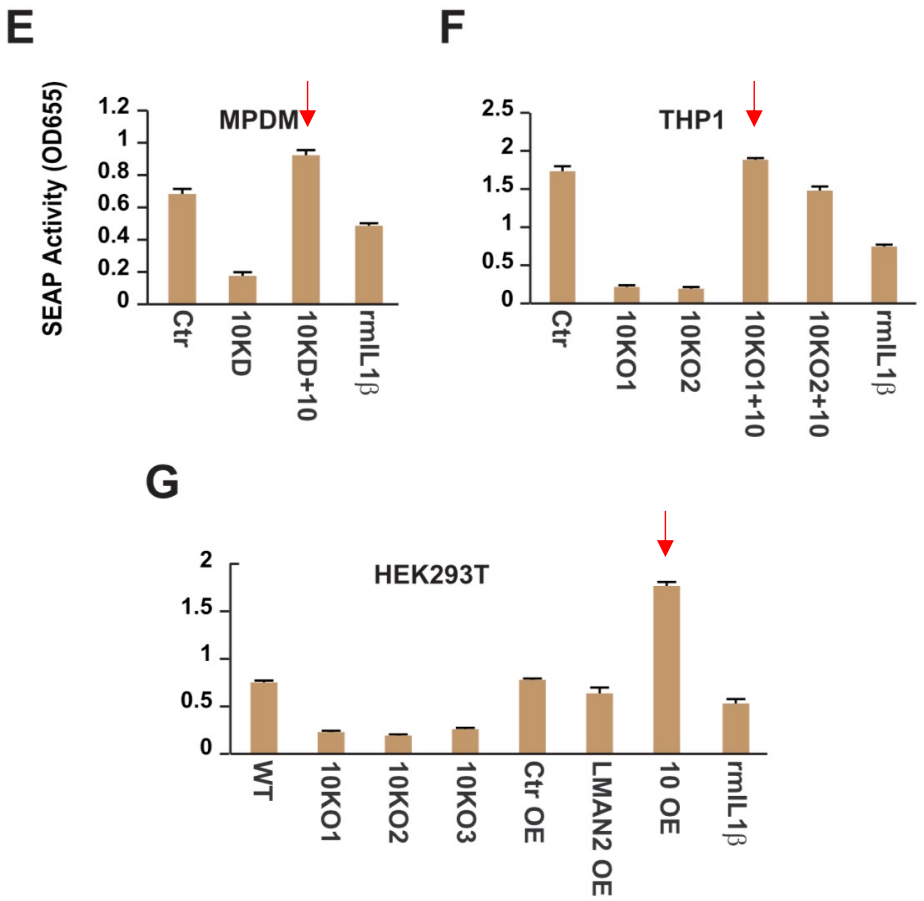
2. Quantify IL-1β using reporter cells



Principle of IL-1β or IL-18 detection and quantification in the supernatant of inflammasome-activated cells using the HEK-Blue™ IL-1β, HEK-Blue™ IL-1R, and HEK-Blue™ IL-18 cells.

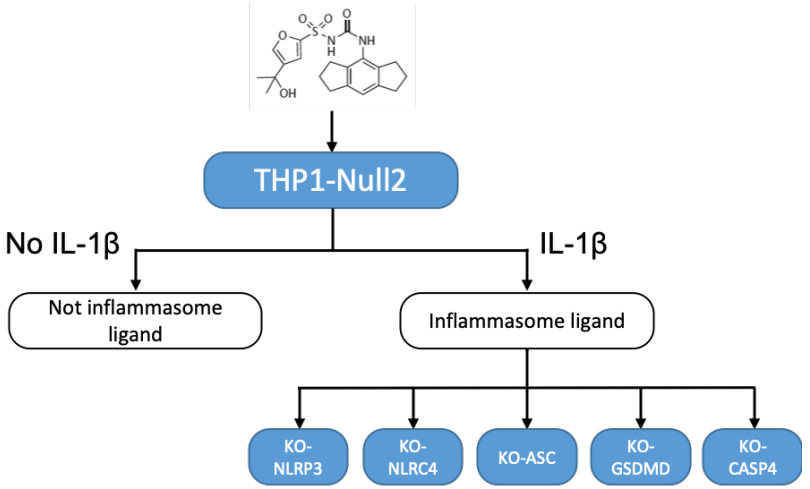
InvivoGen

Case VI: Inflammasome Ligands Screening



Demonstrating candidate protein TMED10's effect to activate inflammasome

Proposed Inflammasome Screening Platform



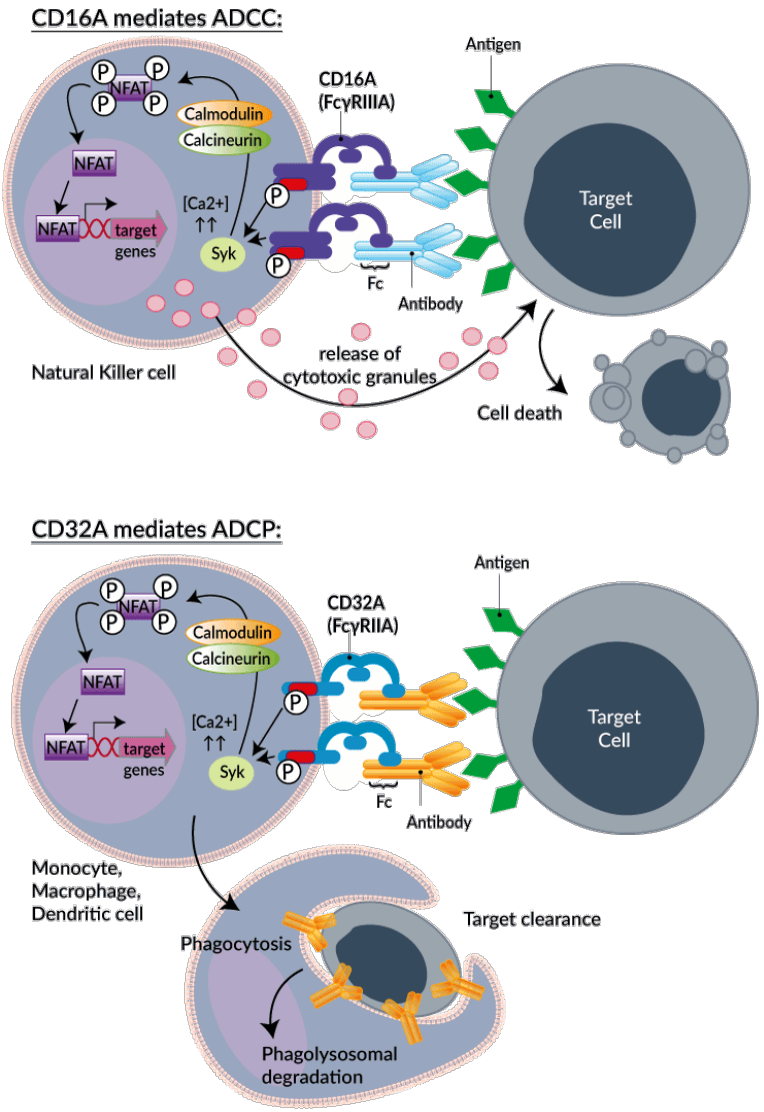
Identify inflammasome target

Proposed Algorithm for inflammasome compound screening using InvivoGen cell lines

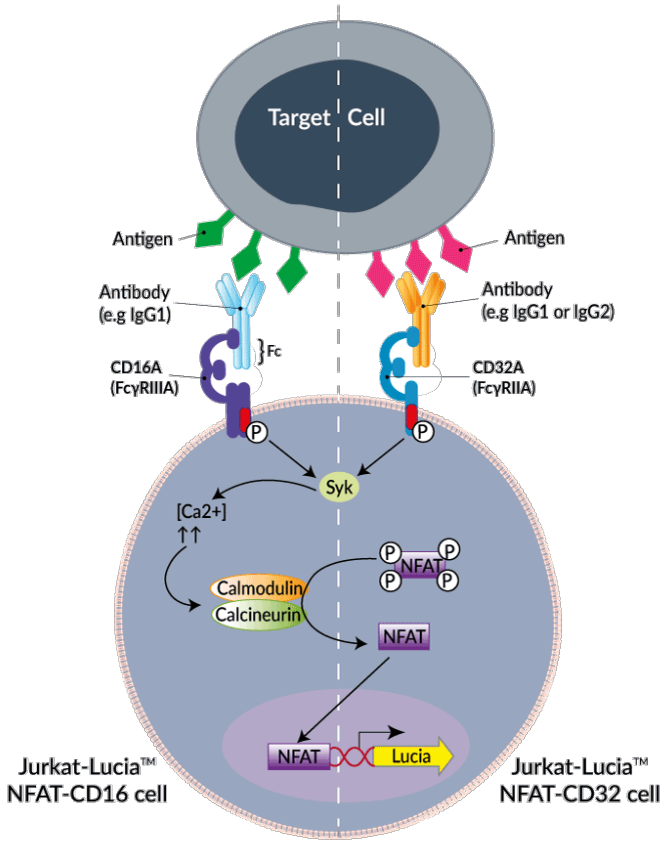
InvivoGen

Case VII: ADCC, ADCP

CD16A and CD32A mediated ADCC and ADCP



ADCC and ADCP cell reporter assays

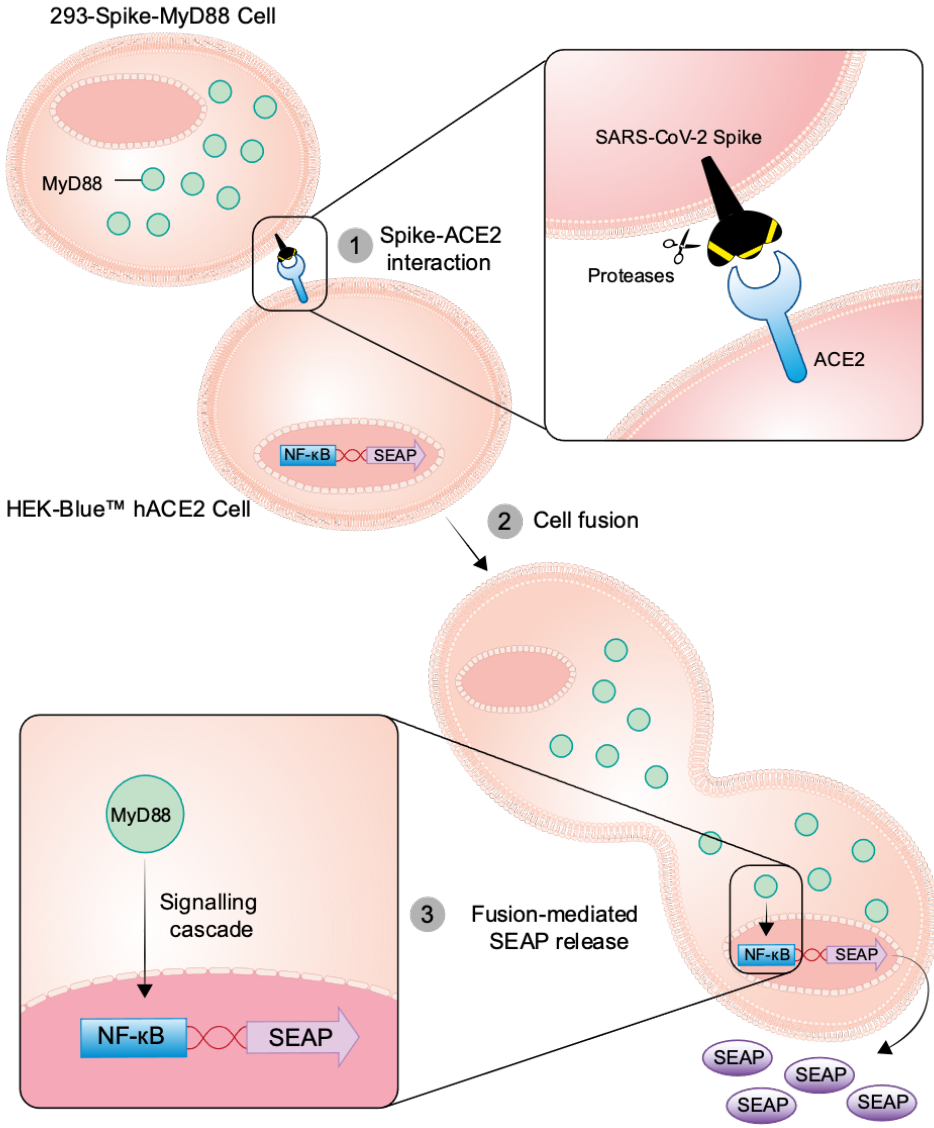


	Traditional	InvivoGen
Cell Line	NK cells / Macrophages	Jurkat cell line
Readout	Target cell death / Phagocytosis	Luciferase reporter

InvivoGen

Case VIII: COVID-19

(Or without Spike for you to express Spike variants)



SARS-CoV2 binding analysis

1. Binding : SEAP
2. No binding: no SEAP

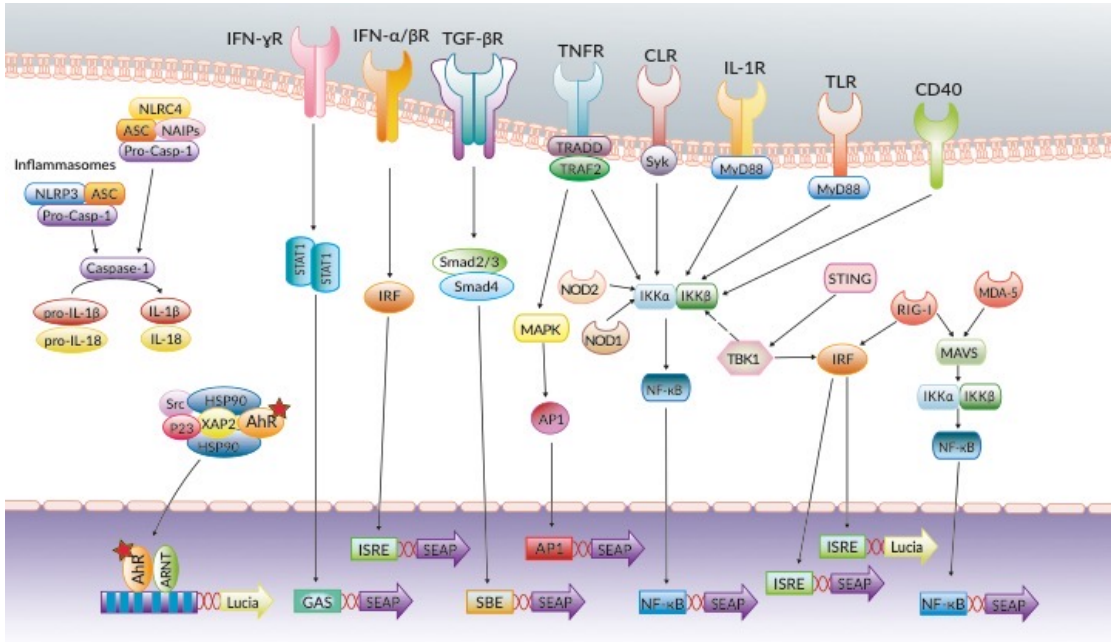
<u>Screen for inhibitors and neutralizing antibodies</u>

<u>Test the effect of different Spike variants</u>

Products	
Wuhan-Hu-1 Spike NEW	Wuhan-Hu-1 Spike Expression vectors
Wuhan-Hu-1 (D614G) NEW	Wuhan-Hu-1 Spike (D614G) Expression vectors
United Kingdom Variant (B.1.1.7) NEW	United Kingdom (B.1.1.7) Spike Expression vectors
South African Variant (B.1.351) NEW	South African (B.1.351) Spike Expression vectors
Californian (US) Variant (B.1.429) NEW	Californian (B.1.429) Spike Expression vectors

InvivoGen

InvivoGen Reporter Cells Collections



Cell Line Family	Examples
PRR reporter cells	TLR reporter STING-KO
Inflammasome test cells	IL-1b reporter NLPR3-KO
Transcription reporter cells	TBK1-KO IRF3-KO
Cytokine reporter cells	IFNα/β IL-6 IL-17 TNFα
ADCC / ADCP	NFAT-CD16 / CD32
COVID-19	ACE2

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InvivoGen Reporter Cells and Screening Service

“Do it yourself” vs “Let InvivoGen help you”

CELL CULTURE

REPORTER CELL LINES

See-through innate immunity signaling pathways

- PRR, transcription factor, inflammasome, cytokine, & autophagy reporter cells
- Thoroughly tested for viability and biological activity
- Compatible with high-throughput screening

InvivoGen offers a large panel of human and mouse cell lines that are stably transfected with innate immunity-specific pathway reporter constructs. Our cells express one or two reporter proteins, the secreted embryonic alkaline phosphatase (SEAP) and/or the Lucia luciferase. Both proteins offer the great advantage of being secreted, allowing for multiple and non-destructive readings over time. InvivoGen has developed easy-to-use detection reagents: QUANTI-Blue™ for SEAP monitoring using a spectrophotometer, and QUANTI-Luc™ for Lucia luciferase monitoring with a luminometer.

A product range for flexible assays

- SEAP, Lucia or Dual™ reporter cells
- PRRs, Inflammasomes, Transcription factors, Cytokines and Autophagy reporter cells
- PRR and IFN signaling knockout reporter cells
- Optimized detection reagents
- Selective antibiotics
- Anti-contamination reagents

WWW.INVIVOGEN.COM/REPORTER-CELLS

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www.invivogen.com +33 (5)62 71 69 39 +33 (5)62 71 69 30 info.eu@invivogen.com

SCREENING

PRR LIGAND SCREENING SERVICE

Finding the needle just got easier

- Our expertise will bring you the data you need
- Cost effective
- Screening flexibility, Reliable & Recognized

There is a growing interest in the targeting of Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) for drug discovery research. As a recognized industry leader in innate immunity, InvivoGen provides a high quality immunomodulatory compound screening service to assist our clients' drug discovery and development needs.

Screening Service for Ligands of PRRs:

TLRs (2,3,4,5,7,9),
NOD1, NOD2,
RIG-I, MDA-5,
STING
Dectin-1 & Mincle

www.invivogen.com/custom-tlr-screening

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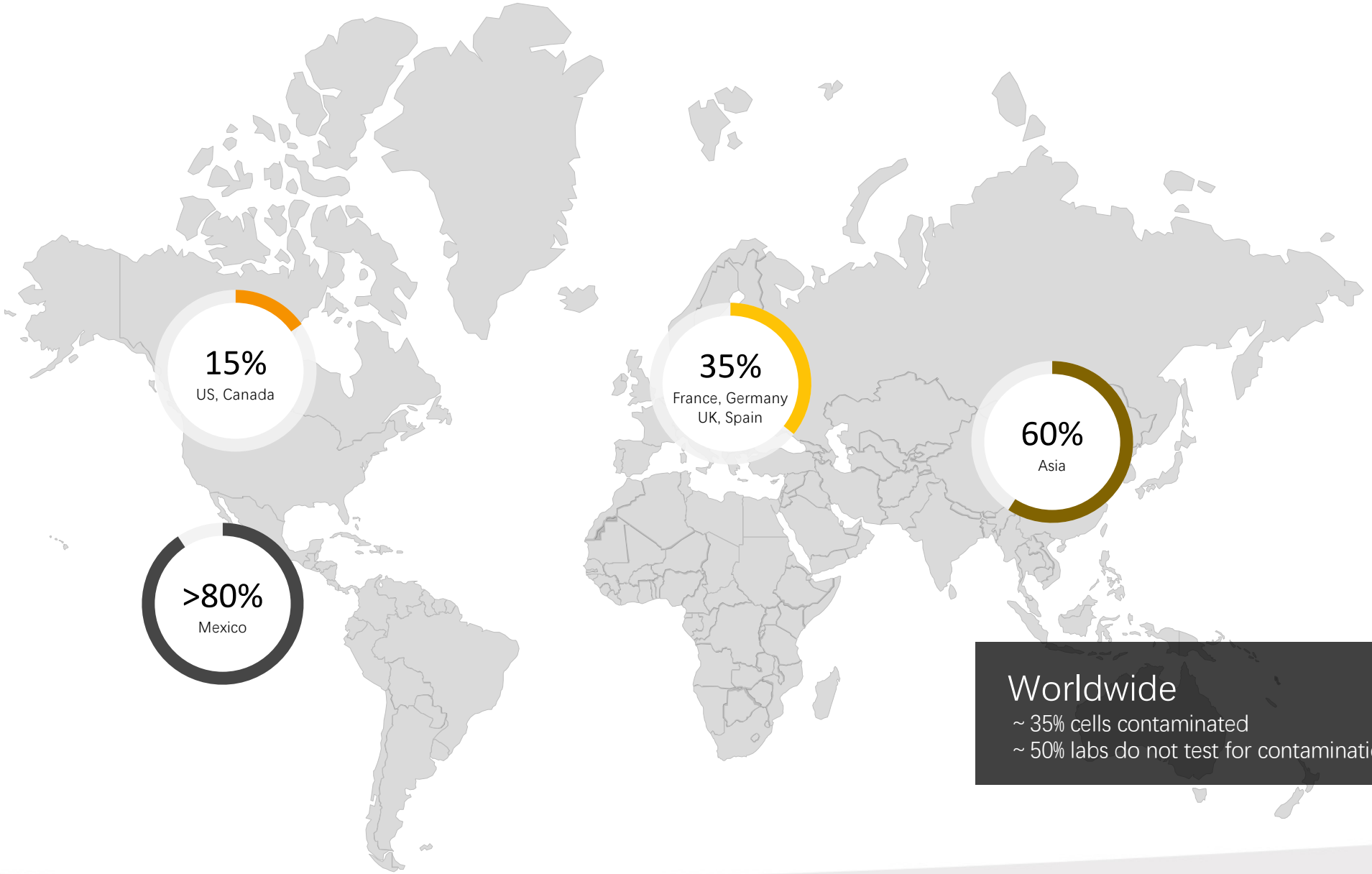
Mycoplasma Contamination



Worldwide

~ 35% cells contaminated

~ 50% labs do not test for contamination

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Mycoplasma Detection

Required by journals for publication

Publication

nature microbiology

ARTICLES

https://doi.org/10.1038/s41564-020-00802-x

Check for updates

Metallodrug ranitidine bismuth citrate suppresses SARS-CoV-2 replication and relieves virus-associated pneumonia in Syrian hamsters

Shuofeng Yuan[1,6], Runming Wang[2,6], Jasper Fuk-Woo Chan[1,3,6], Anna Jinxia Zhang[1], Tianfan Cheng[4], Kenn Ka-Heng Chik[1], Zi-Wei Ye[1], Suyu Wang[2], Andrew Chak-Yiu Lee[1], Lijian Jin[4], Hongyan Li[2], Dong-Yan Jin[5], Kwok-Yung Yuen[1,3]✉ and Hongzhe Sun[2]✉

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Human colon epithelial (Caco2) cells, African green monkey kidney (VeroE6) cells and human lung epithelial cell (Calu-3) were purchased from American Type Culture Collection (ATCC).HEK293-hACE2 stable cells were kindly provided by Dr. Li Liu (AIDS Institute, The University of Hong Kong).
Authentication	The cell lines used in this study were not authenticated since they were purchased from commercial source and were extensively validated before shipping.
Mycoplasma contamination	The cell lines used in this study were comfirmed to be free of mycoplasma contamination by PlasmoTest (InvivoGen).
Commonly misidentified lines (See ICLAC register)	No cell lines used in this study were is listed in ICLAC.

Declaration form to be submitted for publication

Mycoplasma changes cell properties

Protect data

Oncogene (2008) 27, 4521–4531

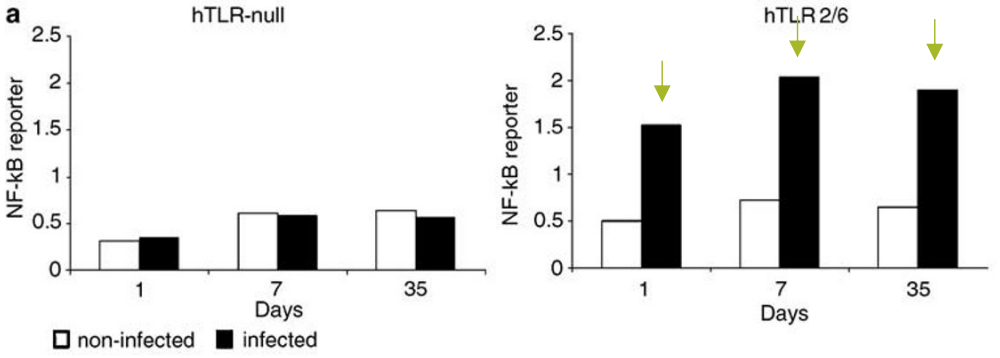
www.nature.com/onc

ORIGINAL ARTICLE

Mycoplasma infection suppresses p53, activates NF-κB and cooperates with oncogenic Ras in rodent fibroblast transformation

DY Logunov[1], DV Scheblyakov[1], OV Zubkova[1], MM Shmarov[1], IV Rakovskaya[1], KV Gurova[2], ND Tararova[2], LG Burdelya[2,3], BS Naroditsky[1], AL Ginzburg[1] and AV Gudkov[2,3]

[1]Gamaleya Research Institute for Epidemiology and Microbiology, Moscow, Russia; [2]Cleveland BioLabs Inc., Buffalo, NY, USA and [3]Department of Cell Stress Biology, Roswell Park Cancer Institute, Buffalo, NY, USA



Mycoplasma activates NFkB (Luciferase experiment)

Mycoplasma detection is needed / advantage for publication

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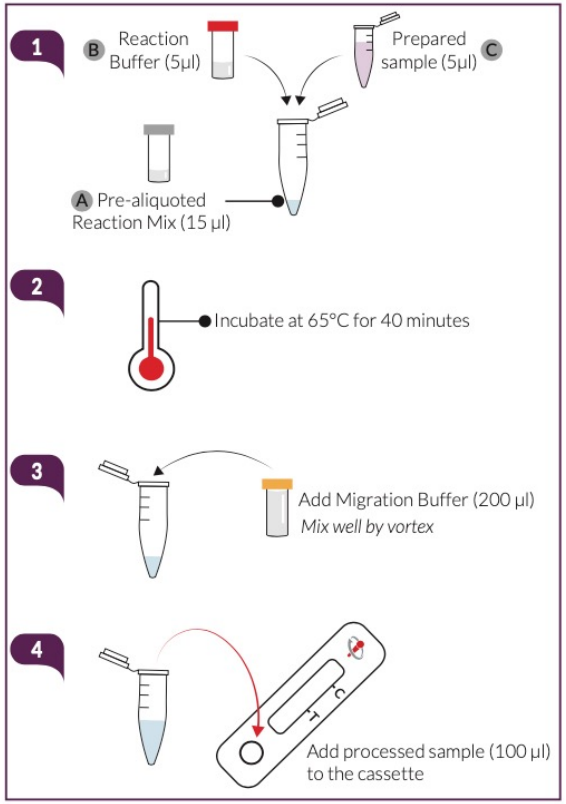
MycoStrip™

Protocol overview



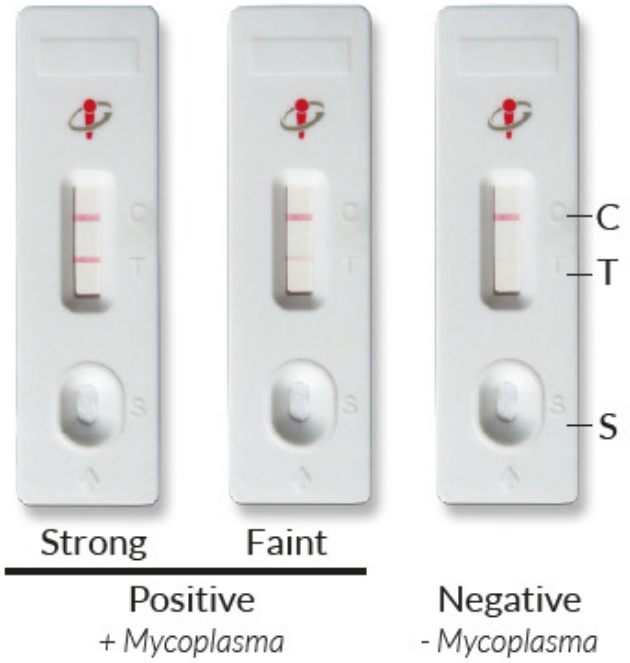
MycoStrip™ Advantages :

- Simple (no special equipment needed)
- Rapid (1hr, 15min hands-on time)
- Sensitive (10-100 CFU/ml)

Detection mechanism

Isothermal PCR of the 16S rRNA gene

Read out



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Thank you!

tech.hk@invivogen.com

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