GCN5

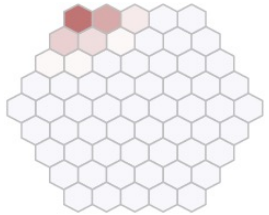
TCP 1

Wellcome Sanger Institute,
DDU, GSK

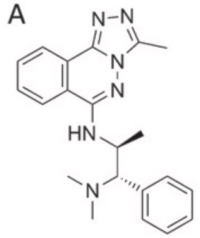
MalDA leads: Marcus Lee, Javier Gamo, Beatriz Baragana

Phase: Assay Development/
HitGen Del Screen

Metaprint:



Tool compound(s):



***L*-45** (*L*-Moses)

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	0.6 μM (1)
Liver (Pbluc)	1.2 μM (1)
Tox (HepG2)	>25 μM
Gametocyte (stage 2-3)	>50 μM (1)

Gene/ protein information	• **PF3D7_0823300** • **bromodomain and histone acetyltransferase**
Resistance	• Low resistance propensity – no mutations observed in GCN5 when selecting with L45 (1) • Minimum Inoculum for Resistance: 10^9 (ramping selection using DNApol line) using L45 (1) • Low diversity in patient isolates – only 1 SNV in bromodomain across 3400 patient isolates (2) • Resistance observed in vivo? ND. • Any comments on fitness and transmissibility of mutants? NA
Genetic validation	• PlasmoGEM phenotype: essential in *P. berghei* (3); piggyBac insertion mutagenesis screen phenotype: essential in *P. falciparum* (4) • Conditional knock out: DiCre-loxP excision of bromodomain results in death. Can only complemented with extra copy with a functional bromodomain. (1)
Chemical validation	• In vitro enzymatic assay (does SAR correlate with whole-cell data?): L-45 (5) • In vivo efficacy: ND • PRR: ND
Activity across species	• Close homology to other organisms or pathogens? Yes, conserved across eukaryotes. • Bromodomain is highly conserved across *Plasmodium* species. Approx 91% identity in bromodomain between *P. falciparum* and *P. vivax* GCN5 (1)
Druggability	• Confidence that drug-like compounds can be identified: L-45 (L-Moses binds with nanomolar affinity)
Toxicity/Selectivity potential	• Approx. 64% identity with human BRD. • Human orthologues exists and assays available
Assays	• Recombinant expression of *Pf*GCN5 successful (5) • Apo or co-crystal structure solved? PfGCN5 co-crystallized with L-45 (5) • Biochemical HTRF assay and BLI binding assay developed • Cell-based (e.g. NanoBRET) assays developed for related BRD-Histone interactions.
Publications	• 1: Lee lab, unpublished data • 2: www.malariagen.net • 3: PMID: 28708996 • 4: PMID: 29724925 • 5: PMID: 27966810

F/GGPPS

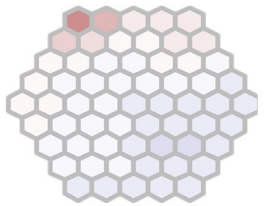
TCP 1

Stanford University (Ellen Yeh)

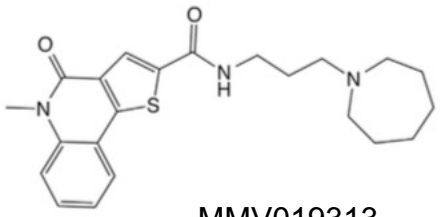
MalDA leads:
Sabine Ottilie, Nimisha Mittal (UCSD); Javier Gamo (GSK)

Phase: TCOLF HTS Screening/Hit ID DEL Screen

Metaprint:



Tool compound:



MMV019313

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	0.268 μM
Liver (Pbluc)	17.8 μM
Tox (HepG2)	> 25 μM
Gametocyte	n.d.

Gene/ protein information	• **Pf3D7_1128400** • **bifunctional farnesyl/geranylgeranyl diphosphate synthase**
Resistance	• Resistance mutations: S228T • EC_{50} shift of resistant mutants: 10-fold • SNPs existing in coding regions (Pf3k database) • MIR: 10^8 parasites, in the presence of EMS (alkylating agent) • *In vivo* resistance: undetermined • Mutants have a lower fitness score (PlasmoDB)
Genetic validation	• PlasmoGem phenotype: essential (PMID: 28708996) ; piggyBac insertion mutagenesis screen phenotype: non-mutable in CDS (PMID: 29724925) • Conditional knock down shows essentiality *in vitro* and GGPPS as drug target • S288T allelic replacement parasite lines recapitulate resistance phenotype of drug selected lines • Available genetic tools: cKD parasites; S228Tallelic replacement cell lines
Chemical validation	• *In vitro* enzymatic assay shows inhibition by MMV019313; mutant protein not inhibited • *(In vivo* studies show Risedronate, inhibitor of protein prenylation, leads to an 88.9% inhibition of *P. berghe*i in mice)
Activity across species	• No data is available about selective modulators having activity on other Plasmodium species • Orthologs in other plasmodium spp.; Bisphosphonate based compounds that are known inhibitors of FPPS, show activity against *T. brucei, T. cruzi, L. donovani, T. gondii* and *P. falciparum*
Druggability	• Confidence that drug-like compounds can be identified
Toxicity/Selectivity potential	• Human orthologues exists and assays are available • MMV019313 Inhibits the enzymatic activity of PfFPPS/ GGPPS but not human FPPS or GGPPS
Assays	• Recombinant expression of wt *P. falciparum* enzyme in *E. coli* successful • Recombinant *P. fal* S288T mutant enzyme 10-fold less susceptible to MMV019313 inhibition • Crystal structure available for Pv F/GGPPS • Homology model available for Pfal F/GGPPS
Publications	• PMID: 29276048; PMID: 29345110; PMID: 27564465 ; PMID: 26688062; PMID: 23734739

APP

TCP 1

Monash University (Sheena McGowan; Peter Scammels)

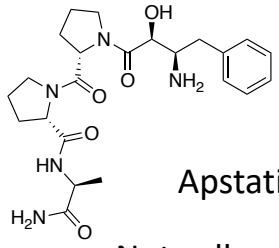
MalDA leads:
Sabine Ottilie, Miles Siegel

Phase: Screening (DEL-HitGen)/ Hit ID-Resynthesis

Metaprint:

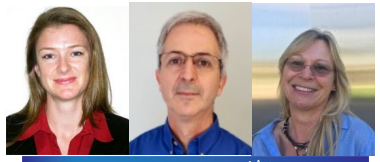
Not determined

Tool compound(s):



Apstatin

Not cell permeable



Lifecycle Dose-response Data	
ABS (3D7/Dd2)	NA
Liver (Pbluc)	NA
Tox (HepG2)	NA
Gametocyte (stage X)	NA

Gene/ protein information	• **PF3D7_1454400** • **PfAPP (Aminopeptidase P)**
Resistance	• Tool compound is not active in whole-cell assays; resistance alleles and potential unknown
Genetic validation	• Scored as essential in Piggybac screen (PMID: 29724925) • Conditional knock down shows essentiality • Available genetic tools to help screening or MoA work: cKD parasites, episomal cytosolic overexpression transgenic parasites
Chemical validation	• In vitro enzymatic assay amenable to HTS • cKD line available
Activity across species	• Evidence of activity on Pv, Po, Pm and Pk: unknown • ~32% sequence homology between human, *E.coli*, and *P. falciparum* enzymes, but highly conserved 3-dimensional structure
Druggability	• Currently do not have whole-cell active inhibitor; peptidases/proteases have been successfully drugged for other diseases (DPP4, type 2 diabetes; HIV-1 and HIV-2, etc.)
Toxicity/Selectivity potential	• Structural studies comparing human, *E. coli*, and *P. falciparum* enzymes indicate opportunity for selective inhibitors • Mammalian orthologues exist and assays available for counter-screening
Assays	• Pf and human protein and biochemical assay available • Pf and human apo and apstatin-bound structure solved
Publications	• PMID:27462122

ACβ

TCP1

TropIQ

Wellcome Sanger Institute

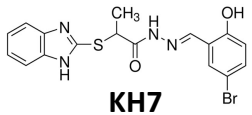
MalDA leads: Koen Dechering, Youri van Nuland, Sabine Ottilie,

Phase: Assay Development
HTS Screening

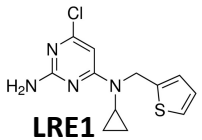
Metaprint:

N/A

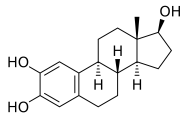
Tool compound(s):



KH7

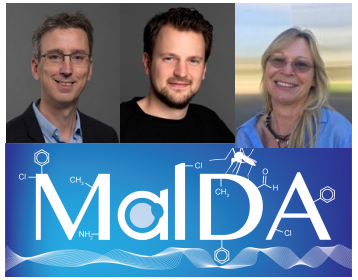


LRE1



2-hydroxy estradiol

Lifecycle Dose-response Data (KH7)	
ABS (3D7/Dd2)	6 μM
Liver (Pbluc)	? μM
Tox (HepG2)	? μM
Gametocyte (stage III/IV)	18 μM



Gene/ protein information	• **PF3D7_0802600,** • **adenylate cyclase β**
Resistance	• *In vitro* resistance: TBD
Genetic validation	• Confirmed with conditional knockdown • piggyBac and RMgm indicate target is essential (PMID: 29724925) • Conditional knockdown screen reveals essentiality
Chemical validation	• Robust correlation between antiparasitic activity of reference compounds and decrease in cAMP levels • In vivo efficacy: ND • PRR: ND
Activity across species	• Conserved across *Plasmodium* sp. • Close homology to *Toxoplasma*
Druggability	• Confidence that drug-like compounds can be identified: high
Toxicity/Selectivity potential	• Low homology to human orthologue • Assay available for human orthologue (Levin/Buck lab @ Cornell)
Assays	• AlphaScreen cAMP homogeneous assay developed (lysates) • Homology model available?
Publications	• PMID: 22761895

cGMP-dependent protein kinase (PKG)

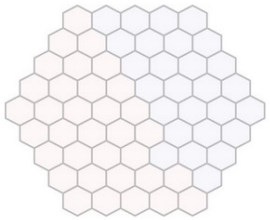
TCP 1,4

H3D, University of Cape Town

MalDA leads: David Fidock, Kelly Chibale, Lauren Arendse

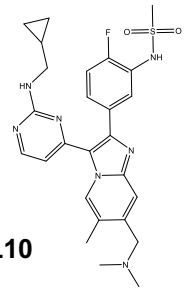
Phase: Formal hit assessment

Metaprint:

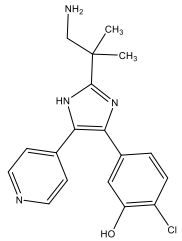


Determined for MMV030084

Tool compound:



ML10



MMV030084

Lifecycle Dose-response Data (IC50 µM) (MMV030084)

	ML10	084
ABS (3D7)	0.002	0.109
Liver (Pbluc)	n.d.	0.199
Gametocyte (early & late)	n.d.	> 40
Male sexual gamete stage	n.d.	0.141
Transmission blocking (SMFA)	0.041	n.d.

Gene/ protein information	• **PF3D7_1436600** • ***Pf*PKG (serine/threonine protein kinase)**
Resistance (MMV030084)	• Low resistance propensity – no resistant mutation identified in PKG • Resistance mutation: TKL3 (PF3D7_1349300) T1268R • EC_{50} shift of resistant mutant: 2.9 -fold (validated by CRISPR/Cas9 editing) • Continuous exposure of 10^9 Dd2-B2 TKL3KO parasites did not result in parasite recrudescence. • Pulsing procedure on Dd2-B2 TKL3KO line resulted in 1.5 to 2.2 fold EC_{50} shift with mutations in PP1 (PF3D7_1414400) and URP(PF3D7_0808300). • cKD experiments suggest that TKL3, PP1 and URP are not direct targets • Attempts to raise resistant mutants against ML10 were reported as unsuccessful
Genetic validation	• piggyBac insertion mutagenesis screen phenotype: essential (PMID: 29724925) • Conditional knock down shows essentiality *in vitro* • PKG T618Q gatekeeper mutant parasite lines (ABS & Gametocyte stage V rounding-up) recapitulate resistance phenotype observed for *in vitro* enzymatic assays (ML10)
Chemical validation	• Subnanomolar *in vitro* inhibition of recombinant *Pf*PKG (ML10 & ‘084) • *In vitro* liver stage, ABS and transmission blocking activity demonstrated for PKG inhibitors • *In vitro* parasite reduction rate (PRR) assay showed slow rate of killing for ML10 (24h. lag) • Twice daily oral administration of ML10 at 50 or 100 mg/kg for 4 days resulted in a dramatic reduction in parasitemia in humanized SCID mouse model of *Pf* infection with 50 mg/kg dose resulting in complete parasite clearance from the peripheral blood.
Activity across species	• *In vitro* inhibition of recombinant *P. falciparum* and *P. vivax* PKG (ML10)
Druggability	• Confidence that drug-like compounds can be identified
Toxicity/Selectivity potential	• ~30% similarity to human PRKG1 & PRKG2 • Both human PKGs and most serine/threonine kinases have a large gatekeeper residue • ML10 showed > 600-fold selectivity for *Pf*PKG when tested against 80 human protein kinases (including 14 small gatekeeper kinases) at 100 nM concentration, highest inhibition was observed for human MLK3 (40% at 100 nM) • ML10 EC_{50} > 10 µM against human cell lines A549, HT-29 & MCF7 • 084 may also inhibit *Pf*CDPK1 (Kinobead studies) but cKD studies show that CDPK1 is not the primary target
Assays	• Recombinant expression of full-length *Pf*PKG in *E. coli* or baculovirus-insect cells • Biochemical assay established (ADP-Glo Kinase assay) • *Pf*PKG and *Pv*PKG crystal structures available • Target has been characterised in detail
Publications	• PMID: 28874661; PMID: 32359426

MRS

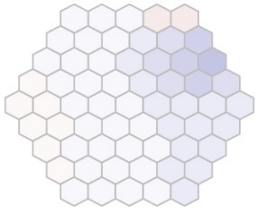
TCP1,4

UCSD, MIT, Lgenia, GSK

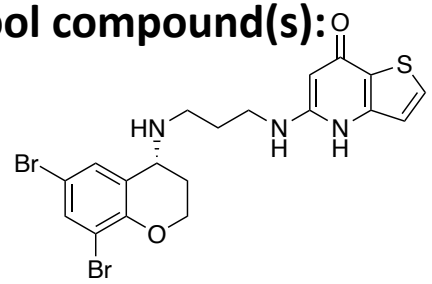
MalDA leads: Sabine Ottilie, Charisse Flerida Pasaje, Jacquin Niles, Miles Siegel, Javier Gamo

Phase: Target Validation

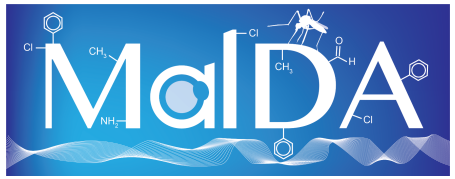
Metaprint:



Tool compound(s):



MMV1578884
REP3123 (Replidyne)
CRS3123 (Crestone)



Lifecycle Dose-response Data	
ABS (3D7/Dd2)	1.95 – 1.28 μM
Liver (Pbluc)	1 – 2 μM
Tox (HepG2)	> 5 μM
Gametocyte (stage X)	

Gene/ protein information	• **PF3D7_1034900** • **Methionyl tRNA synthetase, cytosolic**
Resistance	• Resistance mutations: n/a • MIR: n/a • Resistance observed in vivo (Clinical resistance, resistance in model organism): n/a • Any comments on fitness and transmissibility of mutants: n/a
Genetic validation	• Plasmogem data, knock out (piggybac) to suggest: essential (PMID: 28708996); (PMID: 29724925) • Conditional knock down: essential • Knock in, allelic replacements: n/a • Available genetic tools to help screening or MoA work: cKD line
Chemical validation	• In vitro enzymatic assay: cell lysate • In vivo efficacy: n/a • PRR: n/a
Activity across species	• Evidence that selective modulators have activity on Pv, Po, Pm and Pk: n/a • Close homology to other organisms or pathogens: *Clostridium difficile*
Druggability	• Confidence that drug-like compounds can be identified: yes
Toxicity/Selectivity potential	• Intrinsic issues with the target e.g. close homology to human targets, nature of toxicity if non-selective : n/a • Mammalian orthologues exist and assays available: yes
Assays	• conditional knockdown approach to identify inhibitors • Pf and other species protein and biochemical or cellular target assay available: in vitro lysate • Apo or co-crystal structure solved?: no • Homology model available?: *Clostridium difficile*
Publications	• PMID: 33431834; PMID: 2558372; PMID: 19258353

cyto *Pf*SRS

TCP 1

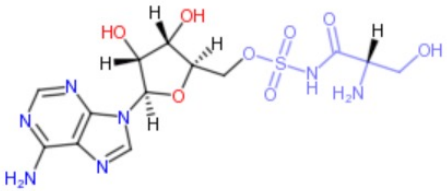
SDDC/DDU

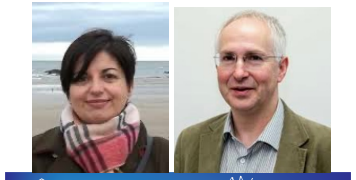
MalDA leads: Beatriz Baragana, Ian Gilbert

Phase:
DEL Screening-Hit Validation/
TCOLF Screen

Metaprint: N/A

Tool compound(s):





MalDA

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	6.4 μM
Liver (Pbluc)	TBD
Tox (HepG2)	5.8 μM
Gametocyte (stage X)	TBD

Gene/ protein information	• **PF3D7_0717700.1** • **Cytosolic Seryl-tRNA synthetase**
Resistance	• TBD
Genetic validation	• PiggyBac insertion mutagenesis: essential (PMID: 29724925) • Knock down shows strong effect in parasite growth (J. Niles' lab)
Chemical validation	• Seryl sulfamoyl adenylate use as tool compound • Validation of new drug like inhibitors underway • PRR: TBD
Activity across species	• Not tested; high homology
Druggability	• AARS are druggable targets; however hit rates are usually low • Fragments identified by NMR and Xchem screens • Validation of DEL hits underway • HTS screens planned
Toxicity/Selectivity potential	• Some active site residues are different between Pf and Hs SerRS; overall identity 30% • Biochemical assay developed for Human cytosolic SerRS • Recombinant mitochondrial SerRS available
Assays	• Biochemical assay for PfSerRS • Crystal structure of chimera *T.brucei* like PfSerRS – all active site residues are *Pf*SerRS like. This crystallography platform allows for soaking • Homology model available? Yes
Publications	N/A

Acetate CoA Ligase (PfAcAS)

TCP 1,4

Harvard University
University of Dundee

MalDA leads: Amanda Lukens, Beatriz Baragaña, Sabine Ottilie

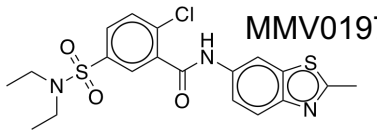
Phase: HTS Screening/Hit ID

Metaprint:

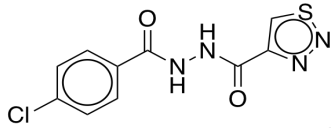
MMV019721

Tool compounds:

MMV019721



MMV084978



Fast killing

Lifecycle Dose-response Data (MMV019721)	
ABS (Dd2)	0.40 µM
Liver (Pbluc)	2.1 µM
Gametocycte (Stage V)	> 25 µM
Cytotox (HepG2)	27 µM

Gene/ protein information	• **PF3D7_0627800** • **PfAcAS (Acetate CoA ligase/Acetyl CoA synthetase)**
Resistance	• Resistance mutations: AcAS: A597V, T648M, Y607C, A652S, A652T, and CNV; ACS11 mutations also seen (D269G, ΔL24-D30, T767I, S74L) • EC_{50} shift of resistant mutants: 3 to 25-fold • Resistance mutations not present in Pf3K database • MIR: 10^7 • *In vivo* resistance: undetermined • A597V mutant has slight fitness defect (relative to WT) in competitive growth assays; T648M mutant appears as fit as WT; affects on transmission yet to be evaluated
Genetic validation	• Plasmogem phenotype: essential (PMID: 28708996) ; piggyBac insertion mutagenesis screen phenotype: essential (PMID: 29724925) • Conditional knock down shows essentiality and AcCoAS as drug target • A597V and T648M allelic replacement parasite lines recapitulate resistance phenotype of drug selected lines • Available genetic tools: cKD parasites; A597V and T648M allelic replacement cell lines
Chemical validation	• *In vitro* enzymatic assay: SAR correlates with whole-cell data • *In vivo* efficacy: undetermined; literature reports predict *in vivo* efficacy • *In vitro* PRR: MMV084978, fast; MMV019721, undetermined
Activity across species	• *P. vivax* and *P. berghei* constructs designed
Druggability	• Confidence that drug-like compounds can be identified
Toxicity/Selectivity potential	• Mammalian orthologues exist and assays available • *In vitro* assays with tool compound MMV019721 show > 100-fold selectivity between *P. falciparum* and human enzyme
Assays	• Recombinant expression of WT *P. falciparum* enzyme and activity assay EnzChek (continuous kinetic assay) and RapidFire (Mass spec, HTS amenable) formats successful; recombinant *P. fal* A597V mutant enzyme less active than WT; T648M recombinant enzyme also generated • Human enzyme purified and assayed (with control inhibitors) • *Theileria parva* structure solved (*Tp*AcAS-AcCoA-AMP complex) • *P. falciparum* homology model available
Publications	• PMID: 34348113

DPCK

TCP 1

TropIQ Health Sciences
Sean Prigge, John Hopkins

MalDA leads: TropIQ Health Sciences (Koen Dechering), Marnix Vlot

Phase: Assay Development – on hold

Metaprint:

Tool compound(s):

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	NA
Liver (Pbluc)	NA
Tox (HepG2)	µM
Gametocyte (stage X)	µM

Gene/ protein information	• **PF3D7_1443700,** • **dephospho-CoA kinase, putative**
Resistance	• No mutations found in pantothenamide selections • No other resistance selections performed as no chemical matter is available
Genetic validation	• Piggyback screen suggests essentiality (PMID: 29724925) • Conditional knockdown screen reveals essentiality • Knockout attempts unsuccessful, suggesting essentiality in ASB
Chemical validation	• No chemical matter available • Downstream pathway validated through pantothenamides and AcCS inhibitors
Activity across species	• No information available
Druggability	• Druggable target class, non protein kinase, crystal structures from other species may provide guidance
Toxicity/Selectivity potential	• Human orthologue shows structural similarity but low AA identity • Recombinant protein for human enzyme is available
Assays	• Recombinant protein available • Rudimentary assay shows good prospects for homogeneous assay format • Homology model available • cKD line for inhibitor identification
Publications	

Hexose transporter (HT)

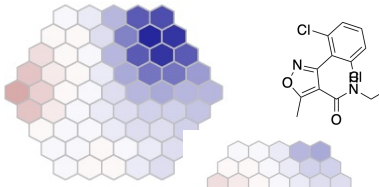
TCP 1

MIT (Jacquin Niles)

MalDA leads: Jacquin Niles, Charisse Flerida Pasaje, Sabine Ottilie, Miles Siegel

Phase: Assay Development
Screening/Hit ID
Hit validation

Metaprint:

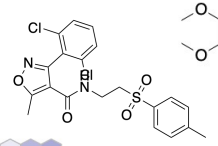


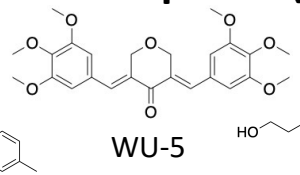
WU-5

MMV009085

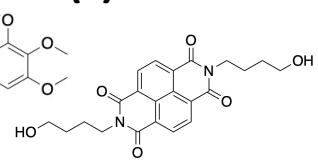
Tool compound(s):

WU-1





WU-5



MMV009085

Lifecycle Dose-response Data

	WU-1	WU-5	MMV009085
ABS (Dd2)	12.7, 6.64 µM	1.57, 1.39 µM	0.540, 0.771 µM
Liver (Pbluc)	ND	ND	>50 µM
Tox (HepG2)	ND	ND	>50 µM
Gametocyte (stage V)	ND	ND	81% inhibition at 12.5 µM

Gene/ protein information	• **PF3D7_0204700** • **PfHT (hexose transporter)**
Resistance	• *In vitro* resistance TBD.
Genetic validation	• Conditional knockdown system reveals essentiality and PfHT as a drug target. • Essential in RMgm and piggyBac screens. (PMID: 28708996); (PMID: 29724925) • Available genetic tools to help screening or MoA work: cKD parasite line hypersensitization to compounds.
Chemical validation	• *In vitro* enzymatic assay: biochemical activity assays exist for validation. • Inhibitor (PMID 31071153) exists to validate cKD phenotypic screen • *In vivo* efficacy: undetermined • PRR: undetermined
Activity across species	• PfHT is highly conserved inter-species.
Druggability	• Confidence that drug-like compounds can be identified: high
Toxicity/Selectivity potential	• PfHT is divergent from human GLUT transporters, potential for selective inhibition. PfHT is the sole glucose transporter bioinformatically identified in parasite. • Heterologous expression in yeast, Xenopus, Leishmania, mammalian cell lines (HEK296) exist to assay for specificity.
Assays	• Intracellular glucose FRET sensor and glucose uptake (accumulation, kinetics) to validate phenotypic effect. • Heterologous expression in yeast, Xenopus, Leishmania, mammalian cell lines. • Crystal structure recently solved (PMID 31996846), PDB ID 6RW3 • cKD line for inhibitor identification
Publications	• PMID: 31071153; PMID: 33402433; PMID: 32860739

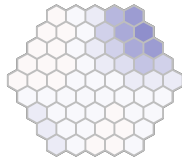
NEK3

TCP 1,4

Goldberg lab; UCT
Collaborators:
D. Chakrabarti
N. Gray
L. Birkholtz

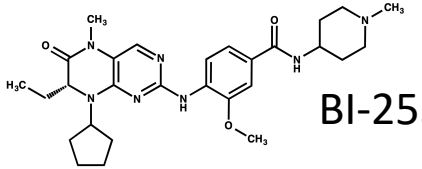
MalDA leads: Eva Istvan, Lauren Arendse, Sabine Ottilie
Phase: Target Validation

Metaprint:



hemoglobin catabolism disruption

Tool compound(s):



BI-2536

Human kinase targets: PLK1, PLK2, PLK3, RPS6KA4, CAMKK1, CAMKK2, MYLK (potency <100nM *in vitro*)

Lifecycle Dose-response Data µM	
ABS (3D7/Dd2)	0.06/0.15
Liver (Pbluc)	0.29/0.34
Tox (HepG2)	>50
Gametocyte V	0.064

Gene/ protein information	• **PF3D7_1201600** • **NIMA related kinase 3 (dual-specificity serine/threonine and tyrosine kinase)**
Resistance	• Resistance mutations: NEK1 (synonymous); 4x EC_{50} fold change *in vitro*

Genetic validation	• Conflicting results on essentiality in ABS:
	  • Non-essential in *Pf*3D7 based on homologous recombination, single crossover gene disruption strategy
	  • Essential in Pb based on homologous recombination, double crossover gene disruption strategy
	  • piggyBac insertion mutagenesis (Mutagenesis Index score 0.64) (PMID: 29724925)
	• Conditional knock down: in the works

Expression (ABS)	• schizonts & gametocytes; highly expressed in sexual stages • NEK1 & NEK3 implicated in *Pf* atypical MAPK cascade and mitosis
Chemical validation	• **Not confirmed**, *in vitro* NEK3 binding assay: 95% inhibition @ 2.5 µM BI-2536 • Other/additional targets could be responsible for ABS activity
Activity across species	• Unknown activity on Pv, Po, Pm and Pk
Druggability	• Confidence that drug-like compounds can be identified
Toxicity/Selectivity potential	• NEK3 is an atypical NEK, divergent from other kinases in this family • Closest human homologue: 36% sequence conservation with NEK9 & unknown protein (AK128693.1); ~30% sequence conservation with other huNEKs • BI-2536 in a potent human polo-like kinase (PLK) inhibitor, *P. falciparum* lack PLKs
Assays	• *In vitro* NEK3 binding assay • *In vitro* catalytic assay reported: IC50 for BI-2536 to be determined • No closely related high-resolution structures (27% identity with huNEK2 structure; ~23% identity with huPLK1 structure)
Publications	PMID: 23462523; PMID: 17662247; PMID: 11322879; PMID: 22116321; PMID: 22127061

Cyclin Like Kinase 3 (CLK3)

TCP 1,4

University of Glasgow (Andrew Tobin)

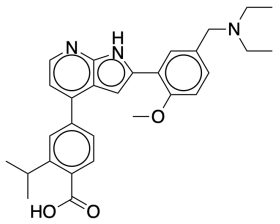
MalDA leads: Ian Gilbert, Beatriz Baragana, Avinash Punekar, Andrew Plater

Phase: HTS Screening/GHIT/HitGen

Metaprint:

undetermined

Tool compound:



Fast killing TCMDC-135051

Lifecycle Dose-response Data	
ABS (3D7)	0.11 µM
Liver (Pbluc)	0.40 µM
Gametocycte (Stage II)	0.80 µM
Cytotox (HepG2)	10 µM

Gene/ protein information	• **PF3D7_1114700** • **PfCLK3 (serine/threonine protein kinase)**
Resistance	• Resistance mutations: P196R, H259P, G449P • EC_{50} shift of resistant mutants: 5 to 13-fold • Resistance mutations not present in Pf3K database • MIR: 10^9 • *In vivo* resistance: undetermined • G449P mutant parasites have no apparent fitness defect compared to WT in asexual growth assays
Genetic validation	• Plasmogem phenotype: essential (PMID: 28708996) ; piggyBac insertion mutagenesis screen phenotype: intermediate (PMID: 29724925) • Conditional knock down shows essentiality *in vitro* • G449P allelic replacement mutant parasite lines recapitulate resistance phenotype observed in *in vitro* enzymatic assays
Chemical validation	• *In vitro* inhibition of recombinant *P. falciparum*, *P. berghei* and *P. vivax* CLK3 by TCMDC-135051 • Twice- daily intraperitoneal dosing of TCMDC-135051 into mice infected with *P. berghei* resulted in a dose-related reduction in parasitemia over a 5-day infection period, where the maximal dose (50 mg/kg) resulted in near-complete clearance of parasites from peripheral blood
Activity across species	• *In vitro* inhibition of recombinant *P. falciparum*, *P. berghei, P.knowlesi,* and *P. vivax* CLK3 by TCMDC-135051
Druggability	• Confidence that drug-like compounds can be identified
Toxicity/Selectivity potential	• ~50% similarity to human PRP4 kinase • no evidence of TCMDC-135051 interacting with the human ortholog of Pf CLK3, PRPF4B • Liver tox: ~ 10 uM
Assays	• Recombinant expression of WT *P. falciparum* enzyme in *E. coli* successful • Recombinant *P. fal* H259P mutant enzyme less active than WT (biochemical assay) • High selectivity for CLK3 over other kinases • *P. falciparum* structural homology model available
Publications	• PMID: 31467193

Phenylalanine tRNA Synthetase (FRS)

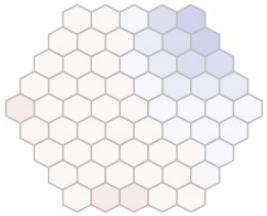
TCP 1,4

GHDDI; Lgenia
Harvard University/Broad Institute

MalDA leads:
Nobutaka Kato, Sabine Ottilie, Miles Siegel

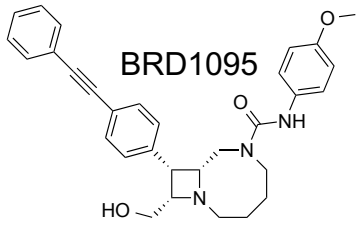
Phase: DEL Screening/Hit ID

Metaprint:



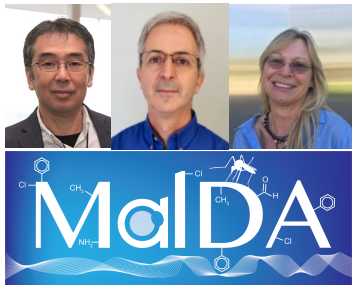
BRD3914

Tool compound:



Moderate killing

Lifecycle Dose-response Data	
ABS (Dd2)	0.01 µM
Liver (Pbluc)	0.140 µM
Gametocycte (Stage V)	0.660 µM
Cytotox (HepG2)	> 16 µM



Gene/ protein information	• **PF3D7_0109800 and PF3D7_1104000** • **PfcFRS (cytoplasmic phenylalanine tRNA ligase)**
Resistance	• Resistance mutations: M316I, G512E & V545I, L550V • EC_{50} shift of resistant mutants: 4 to 100-fold • Resistance mutations not present in Pf3K database • MIR: 10^9 • *In vivo* resistance: undetermined • Fitness and transmissibility of mutants unknown
Genetic validation	• Plasmogem phenotype: essential (PMID: 28708996); piggyBac insertion mutagenesis screen phenotype: essential (PMID: 29724925) • Conditional knock down shows essentiality and PfcFRS as drug target • Allelic replacements unsuccessful • Available genetic tools to help screening or MoA work: cKD parasites; drug-selected mutant parasites
Chemical validation	• *In vitro* enzymatic assay: SAR correlates with whole-cell data • *In vivo* efficacy: single oral 25 mg kg^{-1} dose cure in *P. berghei* model; single oral 12.5 mg kg^{-1} dose cure in *P.falciparum* SCID model; potent causal prophylaxis activity • PRR: Moderate (*in vitro*)
Activity across species	• *In vitro* potency against *P. falciparum, P. berghei*, and *P. cynomolgi* • *P. vivax* enzymatic activity demonstrated
Druggability	• Confidence that drug-like compounds can be identified
Toxicity/Selectivity potential	• *In vitro* assays with tool compound BRD7929 show > 100-fold selectivity between *P. falciparum* and human enzyme • Mammalian orthologues exist and assays available
Assays	• BRD1095 inhibited the aminoacylation activity of recombinant PfPheRS in a concentration-dependent manner (half-maximal inhibitory concentration (IC_{50}) = 46 nM) (TranScreen AMP^2 TR-TRET red assay, Bellbrook, amenable to HTS) • *P. falciparum* structural homology model available
Publications	• PMID: 27602946

cIRS

TCP 1

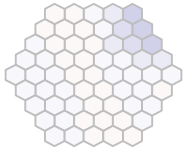
Washington University (Daniel Goldberg), UCSD, GHDDI

MalDA leads:
Eva Istvan, Lan Xu

Phase: Assay Development

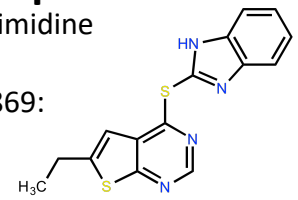
Metaprint:

MMV019869



Tool compounds:

Thienopyrimidine series MMV019869:



Fast killing

MalDA

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	0.3-0.6µM
Liver (Pbluc)	7µM
Tox (HepG2)	>50µM
Gametocyte (stage 5)	0.5µM

Gene/ protein information	• **PF3D7_1332900** • **Cytoplasmic Isoleucine-tRNA synthetase**
Resistance	• Resistance mutations:E180D/Q, S288I, S269K, W395L, V500A, C502Y, L810F • EC_{50} fold change *in vitro*: 3 to 50-fold • MIR: 10^8-10^9 • *In vivo* resistance: undetermined • Fitness defects compared to Wt in asexual growth assays not observed
Genetic validation	• piggyBac insertion mutagenesis screen: essential (PMID: 29724925) • Conditional knock down shows essentiality *in vitro* • Allelic replacement mutant parasite lines recapitulate resistance phenotype • ABS selected resistance mutations shift dose-response in gametocyte assay
Chemical validation	• Sensitivity to compounds is dependent on isoleucine concentration • Observation which needs to be addressed: Thienopyrimidine potency is attenuated by CoA
Activity across species	• Close homology to other organisms
Druggability	• Confidence that drug-like molecules can be identified
Toxicity/Selectivity potential	• PRR: Very fast (*in vitro)* • Not toxic to HepG2 cells • Unclear if selectivity can be achieved
Assays	• Biochemical assay for Pv cIRS available (GHDDI) • Homology model available
Publications	• PMID: 21205898

NCR1

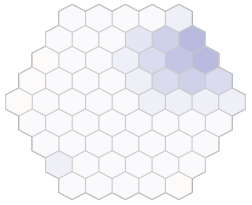
TCP 1

Washington University (Daniel Goldberg); UCSD; Calibr

MalDA leads:
Eva Istvan, Dan Goldberg, Case McNamara

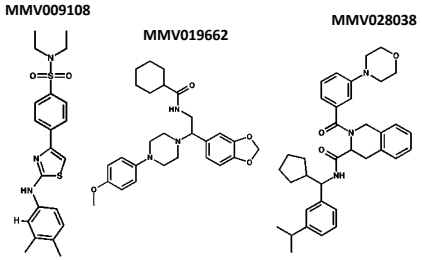
Phase: on hold – MIR concern

Metaprint:



MMV009108

Tool compounds:



Slow killing

Lifecycle Dose-response Data	
ABS (3D7)	0.3-0.6μM
Liver (Pbluc)	0.8 μM
Tox (HepG2)	>1 μM
Gametocyte (stage 5)	0.9 μM

Gene/ protein information	• **PF3D7_0107500** • **Niemann-Pick type C1-related protein**
Resistance	• Resistance mutations: M398I, S490L, A1108T; A1208E, F1436I; 3-10x EC_{50} fold change *in vitro* • 12 amplification events in selections with MMV019662 and MMV028038 • MIR: 10^8 • Selected resistant mutants do not have reduced fitness *in vitro*
Genetic validation	• plasmoGEM phenotype: essential (PMID: 28708996) ; piggyBac insertion mutagenesis screen: intermediate (PMID: 29724925) • Conditional knock down shows essentiality *in vitro* • Allelic replacement mutant parasite lines recapitulate resistance phenotype • Conditional knock down hypersensitizes to compounds
Chemical validation	• PRR: Slow (*in vitro*)
Activity across species	• Protein is highly conserved in *Plasmodium*
Druggability	• Confidence that drug-like compounds can be identified
Toxicity/Selectivity potential	• Compound that inhibits distant human orthologue is not toxic to parasites • Essentiality unique in Pfal versus human suggesting opportunities for specificity
Assays	• Whole cell assay with knockdown parasites has been validated
Publications	• PMID: 30888318

V-type ATPase

TCP 1,4

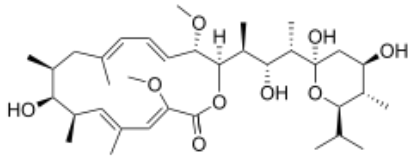
Calibr, UCSD, Harvard University
MIT, Columbia

MalDA leads:
Case McNamara, Charisse Flerida Pasaje, Amanda Lukens, Sabine Ottilie, Robert Summers, John Okombo, David Fidock

Phase: Screening/Hit ID

Metaprint:

Tool compound(s):



Bafilomycin A

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	10 nM
Liver (Pbluc)	1 nM
Tox (HepG2)	2 nM
Gametocyte (stage X)	

Gene/ protein information	• **PF3D7_1311900, PF3D7_0406100, PF3D7_1341900, PF3D7_0934500, PF3D7_0106100, PF3D7_1140100, PF3D7_1306600, PF3D7_1323200, PF3D7_0806800, PF3D7_0519200, PF3D7_1354400** • **V-type proton ATPase catalytic subunit A, B, D, E, C, F, H, G, a, 16 kDa proteolipid subunit, 21 kDa proteolipid subunit**
Resistance	• Have not performed selections with tool compound • Subunits demonstrate fairly high population level conservation • V-type ATPase G29V mutant selected with AZ412 compound was less robust under standard in vitro growth condition relative to WT suggesting mutants might not be very fit at a population level
Genetic validation	• V1 subunits A, B, F, & 16 kDa proteolipid subunit scored as dispensable in PiggyBac screen (PMID: 29724925) ; v0 subunits d, c, e, h, a, & 21 kDa proteolipid subunit were scored essential in PiggyBac screen (PMID: 29724925) ; subunit g showed an intermediate phenotype (PMID: 29724925) • Conditional knock downs of subunits A, B, D, and E demonstrate essentiality • A, B, D, and E subunit cKD parasite lines, D subunit G29V allelic replacement line
Chemical validation	• Cell-based functional assay (vacuolar pH readout) to validate on-target effects.
Activity across species	• Unknown activity on Pv, Po, Pm and Pk
Druggability	• Confidence that drug-like compounds can be identified unknown
Toxicity/Selectivity potential	• Close homology to human ortholog, selectivity may be a challenge • Mammalian orthologues exist and assays available
Assays	• Cell-based functional assay in Pfal and yeast • Yeast structure of complex published
Publications	• PMID: 7935619; PMID: 30910982; PMID: 30952699; PMID: 29526695; PMID: 33741963

NMT

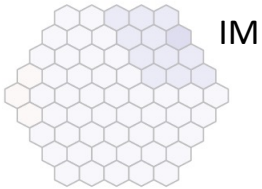
TCP 1,4

Columbia University Med Ctr
Imperial College, Ed Tate

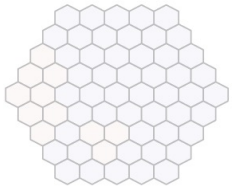
MalDA leads:
David Fidock

Phase: Screening/Hit ID
Hit to Lead/TCOLF?

Metaprint:

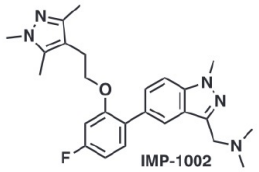


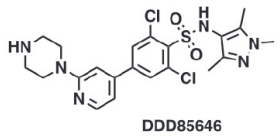
IMP-1002



MMV1545650

Tool compound(s):





Slow killing

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	0.034µM
Liver (Pbluc)	µM
Tox (HepG2)	µM
Gametocyte (stage X)	µM

Gene/ protein information	• **PF3D7_1412800** • **N-myristoyltransferase**
Resistance	• PfNMT[G386E] parasites obtained from *in vitro* selections with IMP-1002. Eight-fold IC_{50} shift • MIR: $3x10^6$ parasites. • Parasite treatment at rings with 4× IC_{50} concentration of both IMP-1002 and DDD85646 caused parasites to stall at a morphologically distinct “pseudoschizont” stage with 4–6 nuclei.
Genetic validation	• Essentiality determined in *P. falciparum* piggyBac screen. (PMID: 29724925)
Chemical validation	• *In vitro* inhibition of recombinant PvNMT[WT] and PvNMT[G386E] protein correlated SAR with whole-cell data (Anja et al Cell Chem Biol. 2019. PMID: 31080074)
Activity across species	• Pf and Pv active. • PvNMT possesses 80% sequence identity and 93% similarity to PfNMT.
Druggability	• Inhibitor series that can overcome parasite resistance to NMT inhibition available (Anja et al Cell Chem Biol. 2019. PMID: 31080074).
Toxicity/Selectivity potential	• Mammalian homologue exists but selective toxicity against parasite protein still achievable.
Assays	• Thiol-selective fluorescent dye (CPM)-based enzymatic assay, surface plasmon resonance and direct binding thermal shift assays available. • PvNMT[WT] and PvNMT[G386E] crystal structures available.
Publications	• PMID: 31080074; PMID: 29760414

cPRS

TCP 1,4

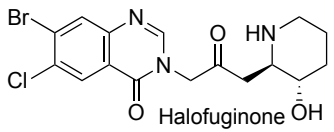
Harvard University
Mass General Hospital (Ralph Mazitschek)

MalDA leads: Dyann Wirth, Amanda Lukens, Sabine Ottilie

Phase: TCOLF HTS Screening/Hit ID; Hit to Lead

Metaprint:

Tool compound(s):



Slow killing

HFG Lifecycle Dose-response Data	
ABS (3D7/Dd2)	0.0007μM
Liver (Pbluc)	0.008μM
Tox (HepG2)	>1μM
Gametocyte	ND

Gene/ protein information	• **PF3D7_0925300** • **Cytoplasmic Prolyl tRNA Synthetase (cPRS)**
Resistance	• Resistance mutations: PfcPRS:L482H, L482F; MFR4 LoF; elevated intracellular proline; 20-30 x EC_{50} fold change *in vitro* • No pre-existing resistant mutants • MIR: ND • Resistance *in vivo* unknown
Genetic validation	• Essential in Plasmogem and piggybac screens (PMID: 28708996); (PMID: 29724925) • Conditional knock down also demonstrates essentiality • PRS allelic replacements unsuccessful (mutant thought to have a fitness defect); MFR4 KO parasites generated • cKD and MFR4 KO parasite lines; transgenic yeast system with WT and mutant PfcPRS knocked-in
Chemical validation	• *In vitro* enzymatic assay; SAR correlates with whole-cell data • *In vivo* efficacy of HFG demonstated against liver and blood stages
Activity across species	• Close homology to other organisms and pathogens; Halofuginone used in veterinary applications to treat coccidiosis and cryptosporidiosis
Druggability	• Confidence that drug-like compounds can be identified (Takeda working on a series)
Toxicity/Selectivity potential	• Close homology to human target • Protein from mammalian orthologues exist and assays are available
Assays	• Pf and other species protein and biochemical or cellular target assay available • Crystal structures for *Pfal* and human enzymes solved
Publications	• PMID: 25995223; PMID: 22327401; PMID: 22438279

Plasmepsin X

TCP 1,4

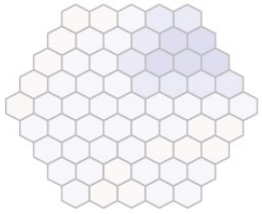
Washington University
(Dan Goldberg)

MalDA leads: Dan Goldberg

Phase: Screening/Hit ID

Status: on hold

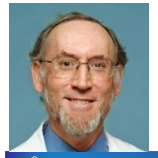
Metaprint:



CWHM-117

Tool compound(s):
CWHM-117-49c

Lifecycle Dose-response Data	
ABS (3D7)	200/0.6 nM
Liver (Pbluc)	6 nM
Tox (HepG2)	µM
Gametocyte (stage X)	Gametocyte egress in vivo 100mg/kg



Gene/ protein information	• **PF3D7_0808200** • **plasmepsin X**
Resistance	• Resistance mutations: F305Y confers 15x resistance in isolated enzyme and confers resistance in cultured Toxoplasma. Resistance in P. falciparum up to 3X with amplification of PM IX/X • Degree of pre-existing resistant mutants: none • *In vivo* resistance: undetermined
Genetic validation	• Scored as dispensible in Adams screen (PMID: 29724925) ; • Conditional knock down: Lethal • Available genetic tools to help screening or MoA work: Regulated KD line
Chemical validation	• In vitro enzymatic assay (does SAR correlate with whole-cell data?) YES • In vivo efficacy: YES in P. berghei
Activity across species	• Evidence that selective modulators have activity on Pv, Po, Pm and Pk • Close homology to other organisms or pathogens? TOXO
Druggability	• Confidence that drug-like compounds can be identified: HIGH – efforts at Merck underway
Toxicity/Selectivity potential	• Intrinsic issues with the target, e.g. close homology to human targets, nature of toxicity if non-selective: Cathepsin D • Mammalian orthologues exist and assays available: Yes
Assays	• Pf and other species protein and biochemical or cellular target assay available: yes • Apo or co-crystal structure solved? yes
Publications	• PMID: 29074774; PMID: 29074775

P. falciparum monoacylglycerol lipase (MAL)

TCP 1

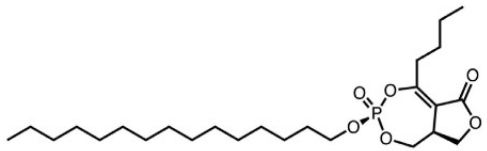
Columbia University
Stanford University
MalDA leads: David Fidock

Phase: Target Validation – Assay Develoment

Metaprint:

Not Available

Tool compound(s):



Salinipostin A

Lifecycle Dose-response Data	
ABS (W2)	0.05 μM
Liver (Pbluc)	–
Tox (HEK-293T)	>50 μM
Gametocyte (stage X)	–

Gene/ protein information	• **PF3D7_1038900** • **monoacylglycerol lipase (putative esterase)**
Resistance	• Target has no known isoforms within same species and in vitro resistance difficult
Genetic validation	• Essentiality has been shown in *P. falciparum* piggyBac screen (PMID: 29724925)
Chemical validation	• In vitro inhibition of recombinant protein correlated SAR with whole-cell data (Yoo et al Cell Chem Biol. 2020. pii: S2451-9456(20)30001-5)
Activity across species	• No data on cross-species activity
Druggability	• A synthetic set of potent small molecule inhibitors has been designed that show whole-cell and anti-enzymatic activity (PMID: 31978322)
Toxicity/Selectivity potential	• Mammalian homolog exists but selective toxicity against parasite protein still achievable
Assays	• *P. falciparum* activity-based protein profiling assay available • Homology model available
Publications	• PMID: 25584395; PMID: 31978322

HSP90

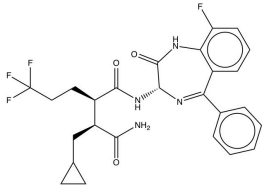
TCP 1,4

UCSD, Harvard Chan School
Duke University (Emily Derbyshire)

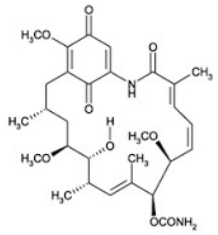
MalDA leads: Sabine Ottilie, Amanda Lukens
Phase: Assay Optimization, POC Screen

Metaprint:

Tool compound(s):



BMS-983970



Geldanamycin

MalDA

Lifecycle Dose-response Data (BMS)	
ABS (Dd2)	0.07 µM
Liver (Pbluc)	0.03µM
Tox (HepG2)	> 50µM
Gametocyte (stage X)	ND

Gene/ protein information	• **PF3D7_0708400** • **Heat Shock Protein 90 (HSP90)**
Resistance	• Resistance mutations: N9K, A41S, D88Y, G112W; 4-10 x EC_{50} fold change *in vitro* • No pre-existing resistant mutants • MIR: unknown • Resistance observed in vivo: not determined • Some mutants demonstrate hypersensitivity to radicicol
Genetic validation	• Essential in Plasmogem and piggybac screens (PMID: 28708996); (PMID: 29724925) • Impaired growth in genome-wide studies • Conditional knock down ongoing • A41S allelic replacement validates resistance phenotype, D88Y allelic replacement in progress • Allelic replacement clones; cKD clones in preparation
Chemical validation	• In vitro enzymatic assays developed for Hs, Sc, Pf • In vivo efficacy demonstrated in humans for cancer therapy (Geldanamycin) • PRR: fast killing
Activity across species	• Pb activity demonstrated • Close homology to orthologs including human protein
Druggability	• Confidence that drug-like compounds can be identified
Toxicity/Selectivity potential	• Close homology to human target • Mammalian orthologues exist and assays are available
Assays	• Pf and other species protein and biochemical and cellular target assays available • Co-crystal structure solved for *Pfal* and human proteins
Publications	• PMID: 31978322; PMID: 29339390

Ftase

TCP1,4

TropIQ
GSK

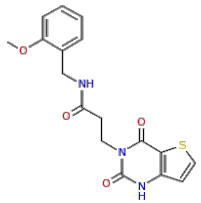
MalDA leads: Koen Dechering, Youri van Nuland, Javier Gamo

Phase:
On hold

Metaprint:

N/A

Tool compound(s):



MMV019066

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	0.07 μM
Liver (Pbluc)	0.318 μM
Tox (HepG2)	> 5μM
Gametocyte (stage X)	μM

Gene/ protein information	• **PF3D7_1242600 (alpha subunit); PF3D7_1147500 (beta subunit)** • **Farnesyltransferase**
Resistance	• Resistance mutations A515V; >10 x EC_{50} fold change *in vitro*
Genetic validation	• piggyBac and RMgm indicate that both subunits are essential (PMID: 28708996); (PMID: 29724925) • cKD shows no growth inhibition
Chemical validation	• Enzymatic assay with peptide confirms activity • Tetrahydroquinoline (THQ) series potently inhibits FTase, dramatic reduction in parasitemia *P. berghei* model (PMID: 15916422) • THQ compound inhibit Pf Ftase at sub-nanomolar levels
Activity across species	• Conserved across *Plasmodium* sp.
Druggability	• Many drug-like compounds developed, sub-nanomolar IC50
Toxicity/Selectivity potential	• GGTase could potentially compensate prenyltransferase activity • Mammalian orthologues exist, assays available • THQ compounds are selective
Assays	• Bead-based fluorescence assay developed, assays available for PPi formation • Homology model available
Publications	• PMID: 15916422

PDEβ

TCP 1

TropIQ

MalDA leads: Koen Dechering, Youri van Nuland, Sabine Ottilie

Phase:

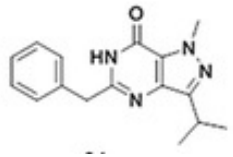
Assay Development

HTS Screening

Metaprint:

N/A

Tool compound(s):



BIPPO

Lifecycle Dose-response Data	
ABS (3D7/Dd2)	2 μM
Liver (Pbluc)	? μM
Tox (HepG2)	? μM
Gametocyte (stage III/IV)	0.3 μM

Gene/ protein information	• **PF3D7_1321500** • **phosphodiesterase beta**
Resistance	• TBD
Genetic validation	• Conditional knock down confirms essentiality (PMID: 30794532) • piggyBac and RMgm indicate that both subunits are essential (PMID: 28708996); (PMID: 29724925)
Chemical validation	• In vitro assay confirms activity on cAMP, preference for cAMP over cGMP • Robust correlation between antiparasitic activity of BIPPO analogues and increase in cAMP levels • In vivo efficacy: ND • PRR: ND
Activity across species	• Conserved across species • BIPPO active against *T. gondii*
Druggability	• Confidence that drug-like compounds can be identified: High
Toxicity/Selectivity potential	• Low homology to human orthologue (~35%) • Selectivity might be an issue due to many human orthologues
Assays	• AlphaScreen homogeneous cAMP assay available • No crystal structure available
Publications	• PMID: 30794532