Multiple Receptor Tyrosine Kinases Regulate Dengue Infection of Hepatocytes

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ABSTRACT

Multiple Receptor Tyrosine Kinases Mediate Dengue Infection of Hepatocytes

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Currently, there is no way to constrain dengue virus (DENV) infections in the clinic. My dissertation work explored the hypothesis that host kinase regulators of DENV infection have the potential to be therapeutically disrupted. I performed a thorough review of literature on host factors regulating dengue and summarized the growing body of evidence supporting kinase-targeted interventions for DENV infection and disease. I also conducted experimental work to elucidate kinases regulating DENV infection that can be targeted by existing drugs. Specifically, I performed kinase regression (KiR), an innovative tool that predicts kinase regulators of infection using existing drug-target information and a small drug screen. From this, thirty-six kinases were predicted to have a functional role in DENV infection. I investigated the role of the predicted receptor tyrosine kinases (RTKs) – EPH receptor A4 (EPHA4), EPH receptor B3 (EPHB3), EPH receptor B4 (EPHB4), erb-b2 receptor tyrosine kinase 2 (ERBB2), fibroblast growth factor receptor 2 (FGFR2), Insulin like growth factor 1 receptor (IGF1R), and ret proto-

oncogene (RET) – because there is already an existing repertoire of drugs against RTKs in the clinic. I found that predicted RTKs are expressed at higher levels in DENV infected cells, and that the activity of ERBB2 and IGF1R is induced following infection. I also demonstrated that knockdown of ERBB2, FGFR2 and IGF1R reduces DENV infection in some cases. I discuss therapeutic strategies that my work suggests could block dengue infection in the clinic and highlight further research that should be done to bolster these strategies. In addition to my primary dissertation research, I collaborated with colleagues to publish a research article uncovering how DENV interacts with mammalian target of rapamycin (MTOR) to support infection, as well as a review article highlighting how identification of host regulators across viral infections, malaria, and cancer have informed therapeutic strategies.

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Chapter 1

EVIDENCE FOR TARGETING KINASES TO CONTROL DENGUE

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Preface

This chapter is a literature review prepared for publication. Here, I discuss how identifying host factors with promise as interventional targets is crucial to combating the global health burden of dengue. This work briefly summarizes current knowledge on the global health burden and pathogenesis of dengue then thoroughly reviews opportunities for targeting kinases as a strategy for controlling dengue. I discuss work identifying the roles of kinases in dengue infection, tools for further identification of kinase targets, and kinase-targeting drugs which have the potential to be repurposed for dengue.

DENGUE IS A GLOBAL HEALTH CATASTROPHE

Dengue is a flavivirus caused by dengue virus (DENV). DENV is transmitted to humans from *Aedes* mosquitos in tropical and sub-tropical regions around the globe [1]. Over 4.2 million dengue cases were reported to the World Health Organization in 2019, a stark increase from the 500,000 cases reported in the year 2000 [2]. It is estimated that hundreds of millions more cases are evading surveillance each year [3]. Rise in incidence has unfortunately concurred with rise in both the number of nations affected as well as reports of death [2; 4]. Global warming and urbanization are increasing suitable habitats for dengue mosquito vector populations, lending to the prediction of further elevated transmission and incidence in the coming years [5; 6]. Dengue is a clear burden and threat to global health.

PATHOGENESIS AND CLINICAL MANIFESTATIONS

DENV first encounters cells as an enveloped capsid containing a single-stranded, positive-sense RNA genome encoding three structural proteins (envelope [Env], pre-membrane [PrM], and capsid [C]) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), all of which have been characterized for roles throughout each step of the viral life cycle (reviewed in [7]). First, Env attaches to the cell and fuses to the endosome, mediating entry and release of the nucleocapsid. DENV RNA is translated by host machinery as a polyprotein which is cleaved by host furin. Resulting NS4A and NS4B form the replication complex where NS5, DENV's RNA-dependent RNA polymerase, reproduces DENV RNA, which is translated into a polyprotein that is cleaved by NS2B/NS3 protease complex. PrM, C, NS1, and NS2A

regulate assembly, maturation, and exocytosis of the progeny virions. Following the bite of a DENV-infected *Aedes* mosquito, this infection cycle begins in skin cells and skin-resident antigen-presenting cells (APCs; namely Langerhans cells, dermal macrophages, blood-derived monocytes, and dermal dendritic cells); infected APCs migrate to the draining lymph node where viremia subsequently disseminates to many tissues and cell types (reviewed in [8]).

The innate immune response to DENV is predominantly characterized by robust interferon (IFN) signaling [9]. The adaptive response to DENV invokes both humoral and cell-mediated activity, chiefly involving the production of antibodies and deployment of Cytotoxic T Lymphocytes. In many dengue cases, natural immune control of infection and disease is successful, resulting in absent or mild symptoms, primarily involving rash, fever and gastrointestinal (GI) distress [10]. In other instances, DENV evades and manipulates the immune response, resulting in severe dengue which typically involves hemorrhagic fever, debilitating GI distress, and liver dysfunction; in the most severe cases, additional concurrent involvement of the brain, heart, lungs, and kidney occurs [10].

Previous research has investigated immune evasion and manipulation strategies employed by DENV (reviewed in [11]). For instance, NS1 has been shown to interfere with upstream kinase regulators of IFN signaling and vascular permeability as an innate immune evasion strategy [11], and severe dengue cases are linked with the absence of the IFN response typically observed in mild dengue cases [12]. Additionally, pro-inflammatory and -permeability cytokine levels are consistently elevated in severe dengue [13; 14; 15; 16; 17].

The adaptive immune response can also be manipulated by DENV. Human DENV comprises four serotypes (DENV1-4) which are antigenically distinct, meaning antibodies produced against one serotype are highly protective against subsequent infection by the same serotype, but they are typically sub-neutralizing in the face of other serotypes [18]. Thus, differing DENV serotypes can evade the adaptive response. Further, these sub-neutralizing heterotypic antibodies can mediate antibody-dependent enhancement (ADE) of DENV, in which sub-neutralizing antibodies facilitate viral entry through Fcγ receptors in addition to classical receptor-mediated endocytosis, which is thought to be responsible for amplified viral loads and increased risk of severe disease in secondary heterotypic infections [19].

Importantly, some severe dengue symptoms are associated with greater immune activation rather than higher viremia [20; 21], and findings have emerged that demonstrate a link between the early innate immune response and the outcome of disease, reviewed in [22]. This highlights the opportunity for interventions that tune the immune response to DENV as a strategy to control dengue.

CHALLENGES OF PREVENTION MEASURES

Intervention strategies that have been implemented for dengue thus far have included vector control and vaccination. Vector control, such as insecticide-treated curtains has unfortunately not resulted in adequate reduction of dengue burden [23; 24], and these efforts are predicted to be further challenged by the increasing impact of global warming and urbanization [25]. Vaccination has also met major challenges: there is major risk in a dengue vaccine which does not achieve broadly neutralizing protection

across all DENV serotypes, due to the potential of causing ADE in seronegative individuals who undergo subsequent DENV exposure [26].

This risk became a reality when the first vaccine for dengue, Sanofi Pasteur's Dengvaxia®, was licensed [27]. The vaccine was approved despite concerning safety signals for seronegative individuals in clinical trials. In the years following implementation of Dengvaxia®, an alarming number of deaths among vaccinated individuals was reported in parallel with a Sanofi study which indicated that seronegative children receiving the vaccine had higher likelihood of subsequent hospitalization [28]. In response, multiple officials involved faced criminal indictment, and the guidelines for the vaccine were modified. Currently, Dengvaxia® is only recommended for individuals over nine years of age with previous DENV exposure, leaving a highly vulnerable population – children – with no protection [29]. Additionally, only a limited amount of transmission and disease prevention efficacy has been observed [30; 31].

Clearly, a vaccine that induces highly neutralizing cross-serotype protection across all age groups will be essential to overcome this challenge. There are currently three vaccines that aim to do this in phase III clinical trials: TAK-003, TV003, and Butantan-DV (analogous to TV003); each has exhibited efficacy, albeit limited [32]. However, new dengue vaccines may face resistance in uptake due to vaccine hesitancy stimulated by the debacle of Dengvaxia's initial distribution [33]. With these major hurdles to overcome, identifying *therapeutic* interventions that curtail infection and disrupt disease will be critical to alleviating the burden of dengue.

SHORTCOMINGS OF ANTIVIRALS

Efforts towards identifying compounds that directly target DENV proteins are ongoing, reviewed in [34; 35]. To summarize, many inhibitors targeting DENV C, Env, NS5, and NS3/NS2B, as well as a smaller collection of compounds against NS4B, have interfered with viral infection *in vitro*. Approximately 15% of these went on to exhibit efficacy *in vivo*, and only one – balapiravir, an NS5 (polymerase) inhibitor – has proceeded to clinical trials. Unfortunately, no efficacy against viral load or inflammation in humans was observed [36]. Many efforts to generate dengue antivirals continue, with recent work investigating a variety of approaches, including novel production or repurposing of antiviral drugs [37; 38; 39; 40; 41; 42; 43; 44; 45; 46], bioactive extracts [45; 47; 48; 49; 50; 51; 52; 53; 54], and host-targeted drugs [55; 56; 57; 58; 59; 60; 61]. Furthest in the development pipeline is JNJ-1802, an NS3-NS4B inhibitor, which demonstrates safety in recently released phase I clinical trial reports, suggesting that efficacy in humans will soon be tested [38; 62].

POTENTIAL FOR HOST-DIRECTED THERAPIES

One potential explanation for the failure of a direct-acting antiviral as it transitions from animal models to human clinical trials is the lack of concurrent host immune modulation. This is particularly relevant because dengue animal models do not perfectly recapitulate the immune response in human DENV infection and disease, thus drugs may be efficacious in animal models where immune modulation is not critical then fail in humans where it is. Improved success in targeting both viral production and the immune response has been demonstrated in the clinic with human papilloma virus, hepatitis C

virus, hepatitis B virus, and human immunodeficiency virus, for which improved antiviral efficacy is achieved in the presence of a host-directed therapy (HDT) [63]. This strategy was originally pursued because these diseases do not yet have a vaccine and direct acting antivirals have stimulated drug resistance. Knowledge of host factors regulating HIV and HCV paved the path for implementing the addition of HDTs in treatment regimens. This landscape is very similar to what we currently face with dengue. Thus, sole use of antivirals is likely to continue to fall short for dengue intervention. Additionally, in the face of increasing antimicrobial resistance globally, constraining resistance to dengue antivirals must also be prioritized [64]. Because host cells do not replicate, and thus evolve, as rapidly as viruses, targeting the host decreases the likelihood of promoting drug resistance. Prioritizing the inclusion of host-directed interventions may be the key to bringing dengue interventions to success.

As we previously reviewed in [65], there are multiple ways in which HDTs can control viruses. HDTs could inhibit cellular processes on which DENV relies to stunt infection. Excitingly, HDTs can also modulate the immune response, providing the opportunity to protect individuals from disease independently from an effect on infection. For instance, HDTs could block regulators of severe disease manifestations such as hyper-inflammation and -permeability. HDTs could also interfere with differential host responses stimulated by ADE which are associated with progression to severe disease.

Extensive research demonstrates that DENV relies on host factors for infection and pathogenesis, reviewed in [66]. For instance, DENV has been shown to rely on host attachment receptors and regulators of endocytosis for entry [67; 68; 69; 70; 71; 72], regulators and structural components of host translation and secretory machinery for

viral genome replication [73; 74; 75; 76; 77; 78; 79], and factors controlling immune dysfunction in pathogenesis [80; 81; 82; 83]. These findings highlight exciting cellular pathways that could be targeted against dengue. However, many of these cellular factors do not yet have drugs targeting them in the clinic, and in fact may prove challenging to target due to concomitant requirements in host cells. Fortunately, targeting kinases that regulate these events could disrupt infection without causing intractable cell toxicity, as evidenced by the approval of dozens of kinase-targeted drugs for clinical use [84].

PRIORITIZING KINASES AS TARGETS AGAINST DENGUE

Kinase regulators are particularly valuable candidates to modulate signaling cascades that control these factors because kinases are global regulators of nearly all cellular processes, including those that viruses manipulate, such as endocytosis, replication and translation machinery, cell death, and the release of inflammatory cytokines. We illustrate interactions between host factors regulating dengue and kinases in Figure 1.1. To build this interaction network, we first performed a literature search using the terms “dengue” and “host”, “host response”, or “host factor”. Abstracts from results were read and a list of 218 host proteins that were concluded to be involved in dengue infection was compiled (Supplementary Table 1.1). Kinase phosphorylation events known to occur on each of these proteins were obtained using PhosphoSitePlus and these interactions were visualized using Cytoscape. The resulting interaction network comprises 68 non-kinases interacting with 147 kinases. As demonstrated,

Supplementary Table 1.1 Host Factors Regulating Dengue	
Factor(s)	**Ref**
5'-AMP-activated protein kinase subunit gamma-1 (AMPK); Serine, threonine-protein kinase mTOR	[85]
Acetyl-CoA Carboxylase	[55]
ALIX	[86]
AMPK	[87]
AP2-associated protein kinase 1	[88]
Apolipoprotein A-I (ApoA-I), scavenger receptor class B type I (SR-BI)	[89]
Apoptosis regulator Bcl-2/PI3K/AKT	[90]
ATP-dependent RNA helicase DDX25 (DDX25)	[91]
ATP-dependent RNA helicase DDX3X (DDX3X)	[92]
Aurora Kinase B	[93]
Beta-adrenergic receptor kinase 1 (GRK2)	[94]
BMP-2-inducible protein kinase (BIKE)	[95]
Caspase-1 (CASP1), Interleukin-1 beta (IL-1b)	[96]
Caveolin (Cav-1)	[97]
C-C motif chemokine 19 (CCL19)/Macrophage Inflammatory Protein (MIP)-3β	[98]
C-C chemokine receptor type 5 (CCR5)	[99]
CCR4-NOT transcription complex subunit 2 (CNOT2)	[100]
Clathrin heavy chain 1 (CLTC), AP-2 complex subunit beta (AP2B1), Dynamin-2 (DNM2), Beta-arrestin-1 (ARRB1), (V-type proton ATPase 116 kDa subunit a 1) ATP6V0A1, Actin-related protein 2/3 complex subunit 1B (ARPC1B), Ras-related protein Rab-5A (Rab5), Early endosome antigen 1 (EEA1), Actin cytoplasmic 1 (actin), MTs	[101]
Clathrin interactor 1 (CLINT1), BIKE	[102]
CMRF35-like molecule 8 (CD300a)	[103]
COX-2, eIF-2-alpha kinase GCN2 (GCN2)	[104]
Creatine kinase	[105]
C-type lectin domain family 5 member A (CLEC5A), NACHT, LRR and PYD domains-containing protein 3 (NLRP3), Interleukin-8 (IL8)	[106]
Cyclic GMP-AMP synthase (cGAS)	[107; 108]
Cyclin G Associated Kinase (GAK)	[109; 110]
Cyclin-dependent kinase 11B (CD11b)	[111]
Cyclin-Dependent Kinases 8 and 19	[112]
Cytochrome c oxidase subunit 2 (COX-2)	[113]
DDX3X, interferon b (IFNb), Interferon regulatory factor 3 (IRF3), Nuclear factor NF-kappa-B p105 subunit (NFKB)	[114]
DNA-dependent protein kinase catalytic subunit (PRKDC)	[115]
Dolichol-phosphate mannosyltransferase subunit 1 (DPM1), -3	[116]

Dopamine Receptor D4	[117]
ELKS/RAB6-Interacting/CAST Family Member 1 (ERC1)	[118]
Endoplasmin (grp94)	[119]
Endoribonuclease ZC3H12A (MCPIP1)	[120]
ER membrane protein complex subunit 4 (EMC4)	[121]
ERK1/2, NFKB	[122]
Eukaryotic initiation factor 4A-I (eIF4A), Eukaryotic translation initiation factor 4E-binding protein 2 (eIF4E), Eukaryotic translation initiation factor 4 gamma 1 (eIF4G1), Eukaryotic translation initiation factor 4 gamma 3 (eIF4G3), Eukaryotic translation initiation factor 4B (eIF4B), and Eukaryotic translation initiation factor 4E-binding protein 3 (eIF4E-BP3)	[123]
Eukaryotic initiation factor 4A-I (eIF4AI), Interferon-induced, double-stranded RNA-activated protein kinase (PKR)	[124]
Eukaryotic translation initiation factor 2A (eIF2α)	[125]
Exportin-1 (CRM1)	[126]
fatty acid synthase (FASN)	[127]
Fibroblast growth factor receptor 4 (FGFR4)	[128]
G3BP1, Ras GTPase-activating protein-binding protein 2 (G3BP2) and CAPRIN1	[129]
GBF1-ADP-ribosylation factor 1 (Arf1)	[79]
GBF1-Arf1/ADP-ribosylation factor 4 (Arf4)-Coatomer subunit beta (COPI)	[78]
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	[130]
Glycogen synthase kinase 3b (GSK-3b)	[131; 132; 133]
Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (GBF1)	[77; 78; 79]
GrpE protein homolog 1, mitochondrial (GrpEL1)	[134]
Heat Shock Factor Protein 1 (HSF1), Ubiquitin-like modifier-activating enzyme ATG7	[135]
Helicase With Zinc Finger 2, aryl hydrocarbon receptor (AHR)	[136]
heme oxygenase-1 (HMOX1)	[137]
Heparin sulfate, CD209 antigen (DC-SIGN), Low-density lipoprotein receptor (LDLR)	[138]
Hepatitis A virus cellular receptor 1/TIM-1	[139]
Heterogeneous nuclear ribonucleoprotein D0 (AUF1)	[140]
Heterogeneous nuclear ribonucleoprotein H (hn RNP-H), Protein disulfide-isomerase A3 (PDIA3), TNFa	[141]
Heterogeneous nuclear ribonucleoprotein K (hnRNP K)	[142]
HMG-CoA reductase, AMPK	[143]
Hsp70	[144]
IL-12, IFNg, TNFa, IL6, IL10, Interferon regulatory factor 1 (IRF1), Signal transducer and activator of transcription 1-alpha/beta (STAT1)	[145]
IL-6, IL-8, FGF-2, GM-CSF, G-CSF, TGF-α, GRO, RANTES, MCP-1 and MCP-3	[146]
iNOS	[147]
Integrin-linked kinase (ILK)	[148]

Interferon-inducible double-stranded RNA-dependent protein kinase activator A (PKR)	[149]
Interleukin enhancer-binding factor 3 (NF90)	[150]
IRF1	[151]
JNK1/2	[152]
Let-7c, BACH1, HO-1	[153]
Lymphocyte antigen 6 locus E (LY6E), Microtubule-associated protein RP/EB family member 3 (EB3)	[154]
Macrophage migration inhibitory factor (MIF)	[155]
miR-146a, TNF receptor-associated factor 6 (TRAF6)	[156]
miR-148a, Ubiquitin carboxyl-terminal hydrolase 33 (USP33), Cyclic AMP-dependent transcription factor ATF-3	[157]
miR-21	[158]
miR-223, Stathmin (STMN1), CCAAT/enhancer-binding protein alpha (C/EBPα) and transcription factor E2F1	[159]
miR-30e*	[160]
miR-34, GSK3β, Serine/threonine-protein kinase TBK1, interferon regulatory factor 3 (IRF3)	[161]
miR-383-5p, Cytosolic phospholipase A2 (PA24A, PLA2G4A)	[162]
miR-484 and miR-744	[163]
miRNA-133a	[164]
miRNA-155	[165]
Mitochondrial antiviral-signaling protein (MAVS), Inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKKe)	[166]
Mitogen-activated protein kinase 3 (ERK1)/Mitogen-activated protein kinase 1 (ERK2)	[167]
Mitogen-activated protein kinase 8 (JNK1)/ Mitogen-activated protein kinase 9 (JNK2)	[168]
mTOR	[169; 170; 171]
NADPH oxidase 1 (NOX), Nrf2, IRF-3, STAT1, Antiviral innate immune response receptor RIG-I (RIG-I), Interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), IkBa	[172]
NF-IL-6, RANTES	[173]
NF-kappa-B inhibitor epsilon (IKBe)	[174]
Non-receptor tyrosine-protein kinase TYK2, STAT1, STAT3	[175]
NRF2, CLEC5A, TNFa	[176]
Nuclear factor erythroid 2-related factor 2 (Nrf2)	[76]
Nuclear Paraspeckle Assembly Transcript 1 (NEAT1)	[177]
Nucleotide-binding oligomerization domain-containing protein 1 (NOD1), Receptor-interacting serine/threonine-protein kinase 2 (RIPK2), MHC class I polypeptide-related sequence B (MICB), 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase epsilon-1 (PLCE1), TNF, and Inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKBKE)	[178]
oligosaccharyltransferase complex (OSTC)	[179]
p38, MAPKAPK2, HSP27, and ATF2	[180]

p38MAPK and CD137	[181]
p38α (MK14) and p38β (MK11), MAPKAPK-2, HSP27	[182]
PAI1, MEK/ERK	[183]
Pex19	[184]
Phosphodiesterase 12 (PDE12)	[56]
PI3K	[185]
PI3K/AKT and GRP78	[186]
PI3K/Akt/MTOR	[187]
PKC	[188]
poly(A)-binding protein (PABP)	[189]
Probable ATP-dependent RNA helicase DDX6 (DDX6)	[190]
Prostaglandin G/H synthase 2 (COX-2)	[191]
Proto-oncogene tyrosine-protein kinase Src (c-Src)	[192]
Protein disulfide isomerase (PDI)	[193; 194]
Protein kinase-like ER resident kinase (PERK)/Eukaryotic translation initiation factor 2-alpha kinase 3, ATF6, inositol-requiring enzyme 1 (IRE1)/ Serine/threonine-protein kinase/endoribonuclease IRE1 (ERN1), Interferon-inducible double-stranded RNA-dependent protein kinase activator A (PKR), IRF3, NF-κB, IL-1β	[195]
P-TEFb (CDK9, Cyclin T1)	[196]
Pyruvate Kinase M2	[197]
Rab18	[198]
RAC-alpha serine/threonine-protein kinase (Akt)	[199]
RAC-alpha serine/threonine-protein kinase (Akt), Repressor of RNA polymerase III transcription MAF1 homolog (MAF1), PTEN, FOXO1	[200]
RACK1, SERBP1, vigilin	[73]
Ras GTPase-activating protein-binding protein 1 (G3BP1)	[70]
RBM10, SAT1	[83]
Reticulophagy regulator 1 (FAM134B)	[201]
Rho-associated protein kinase 1 (ROCK1), Rac1, Rac2, Cdc42, actin	[202]
Ribonuclease 3 (Drosha), Microprocessor complex subunit DGCR8 (DGCR8), and Endoribonuclease Dicer (Dicer)	[203]
RIG-I	[204; 205]
RIG-I, MDA5 and ISG15	[206]
RIG-I, MDA5 and TLR3	[207]
RIG-I/IRF3, phosphatidylinositol-3 kinase PI3K/NFKb	[208]
RNA Helicase A	[209]
RNASEK	[210]
ROCK	[211]
S1PR2	[212]
Small ribosomal subunit protein RACK1	[213]
SPCS1	[214]

Sphingosine Kinase 1 (SPHK1)	[215]
Sphingosine Kinase 2 (SPHK2)	[216; 217]
SphK1, eEF1A	[218]
Src, Fyn, Tyrosine-protein kinase Yes, Tyrosine-protein kinase CSK	[219]
Src/Fyn kinases	[220]
STAT2	[221; 222]
STAT3	[223]
Stress-70 protein, mitochondrial (GRP75)	[224]
TAP1, TBK1, IRF3	[225]
TBK-1	[226]
TBK1/IRF3	[227]
Thrombomodulin and ICAM-1, MIF, JNK MAPK, PI3K	[228]
TLR4/NFkB/MiR-155p/SOCS1	[229]
TNFR1- and TLR3-stimulated NF-κB activation by targeting Receptor-interacting serine/threonine-protein kinase 1 (RIPK1)	[230]
TRIM22, ERK/AMPK/MTOR	[231]
TRIM25	[232]
TRIM29, STC2, and IGFBP5	[233]
TRIM56	[234]
TRIM69	[235; 236]
TSG101	[237]
Tyrosine protein kinase Syk	[238]
Tyrosine-protein kinase ABL1	[239]
Tyrosine-protein kinase Fyn (Fyn)	[240]
Tyrosine-protein kinase JAK1 (JAK1)	[40; 130]
Tyrosine-protein kinase JAK2, Tyrosine-protein kinase JAK3, Signal transducer and activator of transcription 3 (STAT3)	[241]
UBXN1	[242]
UMP-CMP kinase 2, mitochondrial (CMPK2)	[243]
USP18	[244]
VE-Cadherin, RhoA	[245]
VEGF, VEGFR	[246]
VEGFR2/AMPK	[247]
Vimentin	[248]
α2-macroglobulin (α2M)	[249]

kinases are both regulators of non-kinase host factors mediating dengue in addition to being direct regulators of infection. Notably, due to limitations of existing knowledge on phospho-interactions, there may be many more interactions than what is shown here. Together with dozens of drugs targeting kinases already used in the clinic [250], kinases clearly have therapeutic potential against dengue. Furthermore, in addition to small

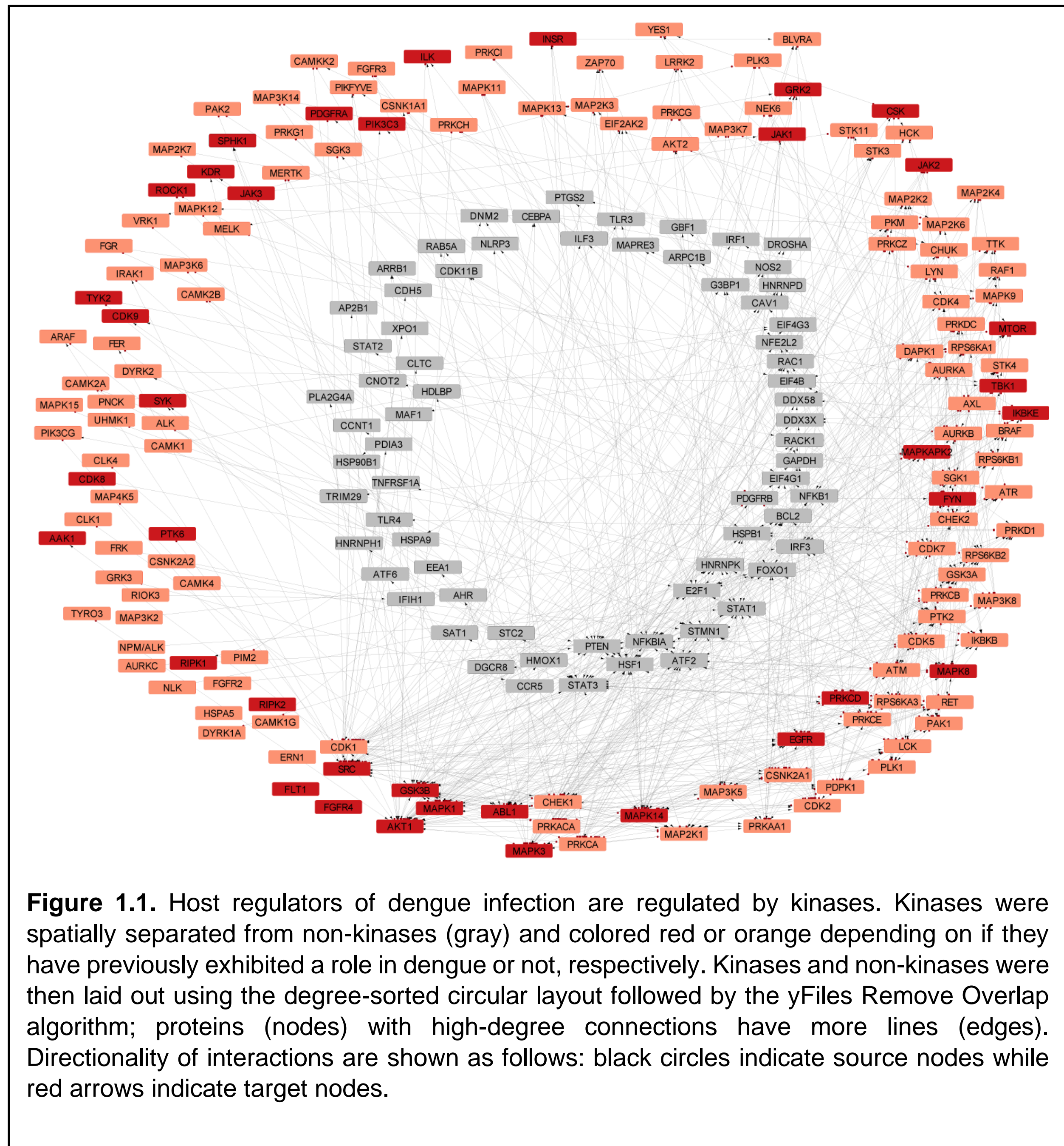

Figure 1.1. Host regulators of dengue infection are regulated by kinases. Kinases were spatially separated from non-kinases (gray) and colored red or orange depending on if they have previously exhibited a role in dengue or not, respectively. Kinases and non-kinases were then laid out using the degree-sorted circular layout followed by the yFiles Remove Overlap algorithm; proteins (nodes) with high-degree connections have more lines (edges). Directionality of interactions are shown as follows: black circles indicate source nodes while red arrows indicate target nodes.

molecule inhibitors against a range of kinases, monoclonal antibodies targeting receptor tyrosine kinases (RTKs) are also clinically approved [251]. Because RTKs are found at the surface of the cell, they may have a role in DENV entry, as has been seen with SARS-CoV-2 [252]. The potential to target DENV entry receptors – in addition to kinase activity mediating infection and pathogenesis – provides further support for kinases as promising dengue therapeutic targets.

As Figure 1.1 exemplifies, many kinases have already been investigated in the context of dengue. It is important to understand overlapping and differential roles of kinases to design an optimal treatment strategy, since kinase inhibitors exhibit target promiscuity through polypharmacology. To this end, we detail experimental evidence for kinase roles in dengue in Table 1.1. These kinases were studied either indirectly, as targets of a kinase inhibitor tested against DENV, or directly with mechanistic investigation into their involvement in DENV infection or pathogenesis. To build these tables, we performed a literature search using the terms “dengue” or “flavivirus” and “kinase”, scanned abstracts for inclusion of dengue and kinase(s). Papers selected from abstracts as well as their relevant references were reviewed. We summarize results pertaining to kinases for each study. This table provides comprehensive insight into what is known about kinases regulating dengue, despite the possibility that some studies were unaccounted in our literature search.

Table 1.1 Kinase Inhibitors and Regulators of Dengue

Kinase(s)	**System(s)**	**Results**	**Ref**
AAK1	Huh7, primary	Affinity-optimized AAK1 inhibitors reduce DENV infection	[88]

	human MDDCs		
AAK1, GAK	Murine model (IFNα/β and IFN-γ receptor–deficient)	Combined treatment with inhibitors *Sunitinib* and *Erlotinib* reduced viral load and mortality via AAK1 and GAK	[253]
ABL1	Huh7	GNF2 treatment disrupts ABL1 and Env-mediated infection	[239]
AKT	A549	DENV Capsid disrupted Akt-dependent apoptosis	[199]
AKT	Huh7	PTEN down-regulation of Akt blocks autophagy and promotes infection	[200]
AKT	Vero, BHK-21	DENV 3' UTR-derived subgenomic RNA induces PI3K/Akt-mediated apoptosis and toxicity	[90]
AKT	MEG-01	Infection reduced PI3K, Akt, and MTOR activity; AKT inhibitor treatment on infection reduces megakaryopoesis	[187]
AKT, PDK1	Huh7 and murine model	PDK1 inhibitor *AR-12* reduced PI3K/AKT, infection, and mortality	[186]
AMPK	Vero	AMPK activator PF-06409577 reduced DENV infection	[87]
AMPK, mTORC1, S6K	HepG2	AMPKα1 siRNA knockdown decreased infection and infection-induced autophagosome production and lipid droplet depletion; AMPK inhibitor (compound C) treatment decreased infection and infection-induced autophagosome production and lipid droplet depletion; enzymatically inactive (D156A) AMPK1 siRNA treatment decreases infection while enzymatically active AMPK complementation restores infection; constitutive mTORC1 expression via TSC1-siRNA treatment decreased infection; infection increased p-AMPK while decreasing mTORC1 target p-S6K	[85]
BIKE, AAK1	MDDCs[1], Huh7[2]	5Z-7-oxozeaenol reduced DENV2 infection partially via BIKE inhibition[1], BIKE SiRNA reduced DENV2 infection[2]	[95]
BIKE	Huh7	BIKE-phosphorylated CLINT1 mediated DENV infection	[102]
CaMKII	Human neuronal BE(2)C	CaMKII inhibitor *BSA 9* decreases infection[1] and mortality[2]	[254]

	cells[1], murine model (STAT-/-)[2]		
CDK8, CDK19	Huh7	CDK8, CDK19 shRNA knockdown or treatment with Senexin A decreased infection	[112]
CDK9	Huh7	DRB (53-85-0) inhibitor against CDK9 treatment reduced DENV-induced IL8 expression; CDK9 colocalizes with DENV in the nucleus; CDK9 partner Cyclin T1 silencing decreases DENV infection	[196]
CMPK2	BMDC[1], A549[2], THP-1[3], BMDM[4], DC[5]	Infection increases CMPK2 mRNA and protein expression[1,2,3]; CMPK knockdown increased infection[1,4,5]; CMPK overexpression decreased infection[2]; CMPK KO decreased DENV-induced IFN-α, IFN-λ1, TLR9, cytosolic mtDNA, 8-OHdG, mtROS, IL-1β, and CCR7-mediated cell migration[3]	[243]
CSK, EPHB2, INSR, PTK6, PTK9L, DDR1, DDR2, SRC, FYN, YES, PKA	Huh7, iMEF[2]	CSK, EPHB2, INSR, PTK6, and PTK9L siRNA knockdown reduced infection; DDR1, DDR2, and ERBB3 siRNA knockdown increased infection; CSK inhibitor ASN-2324598 decreased infection; CSK localizes with DENV dsRNA; triple depletion of Src/Fyn/YES (which CSK negatively regulates) decreases DENV infection[2]; CSK phosphorylation is increased 24 hours-post infection; PKA inhibitor H-89 treatment decreased CSK phosphorylation and infection; CSK overexpression decreases infection, only when kinase domain and SH3 domain are intact	[219]
DNA-PK	Huh7	Infection increased nuclear localization and phosphorylation of DNA-PK; DNA-PK RNAi decreased DENV-induced IFN	[115]
EGFR	Human monocytes (CD14+ primary)	EGFR inhibitor *Gefitinib* decreased infection and infection-induced IFNα/β, TNFα, IL-12, and IL-18	[255]
EGFR, ERBB2/4	HepG2	EGFR/ErbB-2/ErbB-4 inhibitor II decreased infection	[256]
ERK, JNK, P13K	HUVEC[1], THP-1[2]	DENV infection increased thrombomodulin and ICAM-1 via MIF[1,2]; Erk1/2 inhibitor (U0126), JNK inhibitor (SP600125) or PI3K inhibitor (LY294002) decreased MIF-induced thrombomodulin and ICAM-1[2]	[228]

ERK, JNK, p38	MDM	DENV infection increased p-JNK, p-p38, and p-ERK; inhibitors for JNK (SP60025) or p38 (SB203580) decreased DENV infection	[168]
ERK1/ERK2	Murine model	ERK1/2 and apoptosis are increased after DENV infection; ERK1/2 inhibitor FR180204 decreases caspase-3, hepatocyte apoptosis, and liver injury while improving other clinical parameters	[167]
ERK1/ERK2	A549	ERK1/2 phosphorylation is decreased after infection	[122]
FGFR4, PI3K	Huh7	FGFR4 siRNA decreased DENV infection; FGFR4 phosphorylation is decreased after DENV infection; FGFR4 inhibitor PD173074 decreased DENV replication but increased production of infectious virus; FGFR4 activation by ligand FGF19 increased DENV replication but decreased production of infectious virus; treatment with FGFR inhibitor (PD173074) or PI3K inhibitor (LY294002) increase DENV maturation	[128]
Fyn	Huh7	Inhibitors AZD0530 and dasatinib decrease DENV infection via Fyn (not Src/Abl); mutation in DENV NS4B overcame decrease	[240]
GAK	Huh7	Treatment with isothiazolo[4,3-b]pyridines decrease DENV infection via GAK	[109]
GCN2, IκB	MEF, HepG2[2]	GCN2-/- cells have decreased infection and COX-2 expression1; GCN2 stimulation with halofuginone decrease IKK-α/β-mediated NFκB activation[2]; GCN2-/- cells have increased Ikβ phosphorylation and NFκB	[104]
GRK2	Huh[7], iMEF[2]	GRK2 siRNA knockdown decreases infection[1]; GRK2-/- cells have decreased infection[2]	[94]
GSK3β	HMEC-1[1], murine model[2]	GSK-3β inhibitor SB415286 decreased anti-DENV NS1 antibody-induced NFκB activation and iNOS expression[1]. GSK-3β inhibitor LiCl decreased anti-DENV NS1 Ab-induced apoptosis in the liver[2]	[131]
GSK3β	Huh7[1], Vero[2]	GSK3β phosphorylation is increased shortly after DENV infection[1]; GSK3β inhibitor Kin-001-157 decreased DENV infection[1,2]. GSK3β siRNA or shRNA knockdown did not decrease DENV infection[1,2]	[132]
GSK3β	HBMEC[1], HUVEC[2], murine model[3]	GSK-3β Inhibitor GPI decreased DENV NS1-induced hyperpermeability[1,2] and vascular leak[3]	[133]

ILK, AKT, ERK	A549, murine model[2]	ILK shRNA knockdown or ILK inhibitor OSU-T315 decreased DENV infection; NFκB inhibitor Bay 11–7082, ERK inhibitor U0126, or Akt1/2 kinase inhibitor decreased DENV infection; OSU-T315 decreased DENV infection and mortality[2]	[148]
JAK1	Serum from DF or DHF patients in Brazil	JAK1 polymorphisms (rs11208534, rs2780831, rs310196, rs310222, and rs310216) were associated with DHF	[130]
JAK2, JAK3	HepG2	JAK2 inhibitor (AG490) or JAK3 inhibitor (WHI-P131) decrease DENV-induced IL8 and RANTES expression; only JAK2 inhibitor decreased DENV infection; JAK2 or JAK3 inhibitor-treated infection supernatant stimulated decreased U937 chemotaxis	[241]
JNK	Murine model	JNK inhibitor (SP600125) did not decrease DENV infection but did decrease DENV-induced ALT, AST, TNFα, TRAIL, and p-p53 while increasing bcl2 in the liver	[152]
MAPKAPK2	HUVEC	DENV NS1 treatment increased p-p38, p-MAPKAPK-2, and p-HSP27; p38 inhibitor (SB203580) decreased DENV NS1-induced vascular permeability	[182]
MAPKAPK2	Murine model	p38 inhibitor (SB203580) did not decrease DENV infection but did decrease infection-induced leukopenia, thrombocytopenia, liver pathology, AST, ALT, GGT, caspase-3/8/9-mediated apoptosis, TNF-α, IL-6, IL-10, RANTES, IP-10, p-MAPKAPK2, p-HSP27, and p-ATF2	[180]
MEK	Vero	MEK inhibitors Selumetinib and trametinib decreased DENV2 and DENV3 infection	[257]
MEK, ERK	Huh7	DENV EIII increased PAI-1, a factor associated with adverse outcomes in DSS; DENV EIII increased p-ERK; MEK (activator of ERK) inhibitor (U0126) or overexpression of Ras or Raf (activators of MEK) abrogated DENV EIII-induced PAI-1	[183]
MEK, ERK	BHK-21[1], murine model (AG129)[2]	MEK/ERK inhibitors U0126 and AZD6244 decreased DENV2/3 infection[1]; AZD6244 decreased viral load, infection-induced mortality and DHF-like disease[2]	[258]
MTOR	HUVEC	Infection decreased p-mTOR, p-Atg13, and p-ULK1 and increased autophagy marker LC3-II	[171]

MTOR, AKT	293FT[1], HepG2[2]	DENV NS5 coprecipitated with mTOR, raptor, and rictor[1]; immunopurified mTOR from infected cells probed positively for DENV NS52[2]; Rictor and raptor but not mTOR shRNA knockdown decreased infection[2]; infection increased mTORC2 target p-AKT (ser473)[2]; rictor shRNA knockdown decreased infection-induced p-AKT and increased caspase-3 and cell death[2]	[169]
MTOR, AKT	HepG2	*Eupatorium Perfoliatum* extract, quercetin, caffeic acid and eupafolin decreased infection; *EP* decreased infection-induced p-AKT, p-mTOR and autophagosomes; *EP* has high *in silico* affinity for TIM-1	[170]
NTRK1, MAPKAPK5	Huh7	NTRK1 and MAPKAPK5 inhibitor *SFV785* decreased infection; NTRK1 siRNA knockdown decreased infection; *SFV785* disrupted colocalization of DENV Env with NS3 and dsRNA	[259]
p38α	HepG2	p38α siRNA knockdown decreased infection- or DENV capsid-induced apoptosis; CD137 siRNA knockdown decreased infection-induced p-p38, apoptosis, and TNFα; p38 inhibitor (SB203580) decreased infection-induced TNFα and apoptosis while addition of recombinant TNFα partially restored apoptosis;	[181]
PERK, PKR	THP-1	GRP78, master regulator of unfolded protein response, inhibitor (VER-155008) decreased DENV infection while increasing PERK and PKR	[195]
PI3K	A549	Infection increased IFNβ and IRF3 via RIG-I and NFκB	[208]
PKC	BHK-21[1], HepG2[2]	PKC siRNA knockdown or inhibitor (BisI) decreased DENV p-NS5[1]; PKC siRNA knockdown or inhibitor increased infection while PKC activator (PMA) decreased infection and cell viability[2]	[188]
PKM2	U937	Infection increases p-PKM2; PKM2 inhibitor decreased infection while PKM2 activator had no effect	[197]
PKR	A549	PKR siRNA knockdown decreased infection-induced IFN-β and increased infection-induced JNK, p38, and IRF3 via RIG-I and IPS-1; PKR siRNA knockdown did not change infection but RIG-I siRNA knockdown decreased infection	[149]

PKR	A549	EIF4AI knockdown increased infection and decreased infection-induced p-PKR and p-eIF2α	[124]
RIPK1	Huh7[1], MDDC[2], A549[3], HepG2[4], Vero[5], U937[6], HEK293T[7], U937 DC-SIGN[8]	Infection decreased RIPK1[1-6]; DENV NS2B3 or NS3 overexpression plasmid decreased RIPK1[7] ; catalytically inactive NS2B3-S135A did not decrease RIPK1 but NS3 did[7]; RIPK1 co-precipitated with DENV NS3, NS2B3, and catalytically inactive NS2B3 plasmids[7]; NS3 coprecipitated with RIPK1 and vice versa from infected cells[7]; DENV infection or NS3 plasmid expression decreased TNFR1- and TLR3-mediated NFκB activation via RIPK1[7]; infection abrogated increase in RIPK1 downstream necroptosis mediator p-MLKL following treatment with necroptosis inducers (TNFα, Smac mimetic and ZVAD)	[230]
RIPK2	HepG2	RIPK2 inhibition by SB203580 decreased infection- or DENV capsid-induced apoptosis; RIPK2 siRNA decreased infection-induced apoptosis	[260]
ROCK1	Huh7	Infection increased PI3K/AKT activation and actin reorganization; inhibitors for PI3K/Akt/Rho GTPases, actin microfilaments or downstream effectors ROCK and Rac1 decreased infection;	[202]
RTKs	Murine ADE model (AG129 + 2H2 anti-DENV mAb)	Receptor Tyrosine Kinase inhibitor *Sunitinib* decreased DENV-induced serum TNF, hematocrit, WBC count, and mortality but did not change viral load; combined *Sunitinib* and anti-TNF ab decreased mortality to a greater extent and decreased vascular leakage	[261]
SPHK1	HEK293	Infection decreased SPHK1 activity	[262]
SPHK1	HEK293[1], BHK-21[2], C6/36[3], K562[4], MDM[5]	SPHK1 overexpression or SphK1 inhibitor (SKi) did not change infection[1]; DENV-induced decreased cell viability was further decreased by Ski[1]; Infection decreased SPHK1 activity at 30hpi[1-5]; NS3 plasmid expression did not change SPHK1 activity[1]; DENV 3' UTR plasmid expression decreased SPHK1 activity[1]; DENV RNA colocalized and coprecipitated with eEF1A at the time of SPHK1 activity reduction[1]	[218]

SPHK1	HEK293[1], iMEF[2], pMEF[3]	SPHK1 overexpression did not change infection[1]; infection did not change SPHK1 activity within 24hpi; SPHK1/2 inhibitor (Ski) decreased infection[1]; SPHK1-/- decreased infection[2]; DENV infection increased IFNβ, viperin, IFIT1, IRF7 and CXCL10 expression[1,2], which was abrogated by SPHK1-/-[2] or SK1 inhibitor (SK1-I)[1]; basal levels of CXCL10, OAS1 and IRF7 are increased in SPHK1-/- compared to WT[2]; [pMEF] SPHK1-/- increased infection and did not increase ISGs [unlike in iMEFs][3]	[215]
SPHK1	pMEF	Infection decreased IFNAR1 surface protein expression and increased IFNAR2 mRNA and p-STAT; SPHK1-/- increased infection; infection increased total and p-STAT1, SPHK1-/- had less infection-induced p-STAT1, IRF1, and IRF7	[263]
SPHK2	HEK293 c18[1], HepG2[2], iMEF[3], murine model[4],	SPHK2 inhibitors (ABC294640[1] or K145[1-2]) did not change infection; SPHK2-/- reduced infection[3]; infection-induced IFNβ, viperin, IFIT1, and CXCL10 was decreased in SPHK2-/-[3]; SPHK2-/- did not change infection or survival but infection-induced body weight reduction was increased[4]; infection increased CD8 T cells in the brain but this was not changed by SPHK2-/-[4]	[216]
SPHK2	Huh7[1], HepG2[2], A549[3]	Infection increased caspase-3 and cell death [apoptosis][1-3]; SPHK2 siRNA decreased DENV1-4 infection-induced apoptotic cell death and caspase-3[1-2], -9[1]; SPHK2 inhibitor (ABC294640) decreased infection-induced apoptotis[1]; SPHK2 siRNA did not change infection[1];	[217]
SRC	Vero[1], Huh7[2], C63/36[3]	Imatinib, GNF2, AZD0530[1-3], dasatinib[1-3], SU11652, Lavendustin A, SU5271, Kenpaullone, Lavendustin C, MC7, Tyrphostin46, as well as inhibtors for CDKs, c-Raf, JAK1/2/3, Kdr, CKII, Src, and Abl decreased infection by at least 50%[1]; AZD0530 and dasatinib decreased DENV1, 3, and 4 serotypes[1]; SRC siRNA knockdown decreased infection[2]; dasatinib decreased DENV in ER lumen and secretory vesicles[1]	[192]

SYK	CD14+ monocytes with anti-DENV prM mAb 5G22	DENV ADE increases Syk and ERK1/2 activity; Syk inhibitor (BAY 61-3606) decreased DENV ADE-induced p-ERK1/2, IL1β, TNF, and IL6 but had no effect on infection; ERK1/2 inhibitor (PD98059), p38 inhibitor SB (203580) and JNK inhibitor (SP600125) also decreased DENV-induced IL1β	[238]
TBK1	HepG2	PIKA adjuvant decreased infection via IFNβ increase; TBK1 inhibitor abrogated PIKA-mediated infection decrease	[226]
TBK1	A549[1], iMEF[2]	RIG-I activator (5′pppRNA) decreased infection whereas IFNα/β, IL-6, and IL-1α were increased; RIG-I or MAVS siRNA knockdown[1] or TBK1-/-[2] abrogated anti-DENV activity	[172]
TBK1, IKKε	HEK293T	DENV1, 2, and 4 NS2A or NS4B plasmid expression decreased IFNβ and ISRE promoter activity via RIG-I/MDA5/MAVS/TBK1/IKKε and decreased p-TBK1 and p-IRF3; NS4B N terminus but not C terminus decrease TBK1-mediated IFNβ; DENV1 NS4A decreased RIG-I- and TBK1-mediated p-IRF3 and IFNβ	[227]
TYK2	MDDC	Recombinant IFNα or IFNγ pre-inoculation treatment decreased infection but only IFNγ post-inoculation treatment decreased infection; DENV decreases IFNα- but not IFNγ-induced p-STATs; infection decreased IFNα-mediated p-TYK2	[175]
VEGFR1	Serum from DF and DHF patients 21 or older in Singapore	sVEGFR1 was increased in DHF compared to DF	[246]
VEGFR2, AMPK	HUVEC[1], MDA-MB-231[2], BHK-21[3], primary mouse peritoneal macrophages cells[4], murine model (IFNAR-/-)[5]	DENV1-4 increased VEGFR2; VEGFR2 overexpression increased infection[1]; VEGFR2 agonist VEGF-A increased infection while VEGFR2 blocking antibody decreased infection[1]; VEGFR2 shRNA knockdown decreased infection[1-2]; VEGFR2 inhibitor (brivanib) decreased infection-induced cytopathic effects and DENV1-4 [and ZIKV] infection[3]; Brivanib increased infection-suppressed p-AMPK; AMPK inhibitor (Compound C) abrogated brivanib's reduction of infection; VGFR2 shRNA knockdown	[247]

		decreased infection and did not increase brivanib's reduction of infection or p-AMPK[1]; brivanib decreased infection-induced p-IRF3, IFNβ secretion, p-STAT1/2 and ISGs transcription which AMPK inhibitor (Compound C) abrogated[4]; brivanib decreased infection, mortality, transcriptional levels of, IL-6, IFN-α, IL-1β and CXCL10, and abrogated p-AMPK suppression while AMPK inhibitor abrogated each of these phenotypes[5]	

Abbreviations: AAK1: AP2 Associated Kinase 1, GAK: cyclin-G-associated kinase, ABL1: ABL proto-oncogene 1, AKT: RAC-alpha serine threonine-protein kinase, PDK1: 3-phosphoinositide-dependent kinase 1, AMPK: 5'-AMP-activated protein kinase subunit gamma-1, mTORC1: mammalian target of rapamycin complex 1, S6K: p70 S6 kinase, BIKE: BMP-2-inducible protein kinase, CAMKII: calmodulin-dependent protein kinase II, CDK8: Cyclin-Dependent Kinases 8, CDK19: Cyclin-Dependent Kinases 19, CDK9: Cyclin-Dependent Kinases 9, CMPK2: cytidine/uridine monophosphate kinase 2, CSK: c-terminal Src kinase, EPHB2: ephrin type B receptor-2, INSR: insulin receptor, PTK6: protein tyrosine kinase 6, PTK9L: protein tyrosine kinase 9-like, DDR1: discoidin domain receptor tyrosine kinase 1, DDR2: discoidin domain receptor tyrosine kinase 2, ERBB3: receptor tyrosine-protein kinase erbB-3, SRC: Proto-oncogene tyrosine-protein kinase Src, FYN: Proto-oncogene tyrosine-protein kinase Fyn, YES: Proto-oncogene tyrosine-protein kinase Yes, PKA: Protein Kinase A, DNAPK: DNA-dependent protein kinase catalytic subunit, EGFR: epidermal Growth Factor Receptor, ERBB2: receptor tyrosine-protein kinase erbB-2, ERBB4: receptor tyrosine-protein kinase erbB-4, ERK1/2: mitogen-activated protein kinase 1/3, JNK: mitogen-activated protein kinase 8, PI3K: phosphatidylinositol-3 kinase, p38: mitogen-activated protein kinase 14, FGFR4: Fibroblast growth factor receptor 4, GCN2: eIF-2-alpha kinase, IκB: Iκβ kinase, GSK3β: Glycogen synthase kinase 3b, ILK: integrin-linked kinase, JAK1/2/3: Janus kinase 1/2/3, MAPKAPK2: MAPK activated protein kinase 2, MEK: mitogen-activated protein kinase kinase, MAPAPK5: MAPK activated protein kinase 5, PERK: Protein kinase-like ER resident kinase, PKR: interferon-inducible double-stranded RNA-dependent protein kinase activator A, PKC: protein kinase C, PKM2: pyruvate kinase M2, RIPK1/2: receptor-interacting serine/threonine-protein kinase 1/2, ROCK1: rho-associated protein kinase 1, RTKs: receptor tyrosine kinases, SPHK1/2: sphingosine Kinase 1/2, SYK: spleen tyrosine kinase, TBK1: TANK Binding Kinase 1, IKKε: IκB kinase epsilon, TYK2: tyrosine kinase 2, VEGFR1/2: vascular endothelial growth factor receptor 1/2; MDDC: monocyte-derived dendritic cell, BMDC: bone marrow-derived dendritic cell, BMDM: bone marrow-derived macrophage, DC: dendritic cell, iMEF: immortalized murine embryonic fibroblast, pMEF: primary murine embryonic fibroblast, MDM: monocyte-derived macrophage.

As Table 1.1 demonstrates, there are diverse roles of kinases in DENV infection and pathogenesis, with some kinases acting as restriction factors, some as dependency

factors, and some regulating the immune response to DENV. While some kinases have already demonstrated efficacy as targets of inhibitors against DENV, many others play a mechanistic role in dengue but have not yet been tested as therapeutic targets. Taken together, we demonstrate that kinases are promising candidate targets for DENV HDTs, and kinase combinations which can be targeted to block infection and disease should be carefully evaluated.

TOOLS FOR IDENTIFYING KINASE CANDIDATES FOR DENGUE

While these works highlight exciting elucidations towards kinase-directed therapies against dengue, there are still many kinases that have not been investigated in the context of dengue. We summarize tools that could be useful in further identification of kinases regulating dengue infection. Firstly, it is important to note that whole-genome depletion screens often fail to suggest kinase regulators of infection [116; 128; 214; 264; 265; 266; 267; 268]. This is likely due to rewiring capabilities of kinase networks which make a single gene depletion insufficient to disrupt the activity necessary for infection. Certain interrogation approaches, such as kinase regression and synthetic lethality provide the opportunity to specifically screen kinases.

Kinase regression (KiR) is a screening method that uses a small panel of kinase inhibitors (20-40 inhibitors) as chemical probes to screen kinase activity regulating any quantifiable phenotype [269; 270]. The enzymatic inhibition activity of this inhibitor panel against a total of 300 kinases spanning the human kinome has been collected. KiR uses elastic net regularization to deconvolve which shared targets of the inhibitor panel are

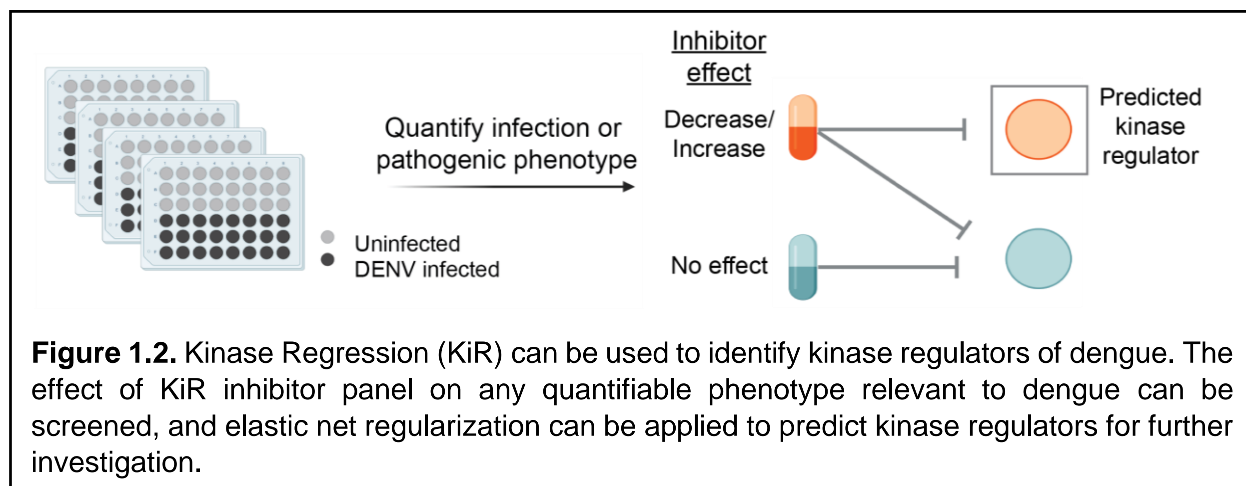

Figure 1.2. Kinase Regression (KiR) can be used to identify kinase regulators of dengue. The effect of KiR inhibitor panel on any quantifiable phenotype relevant to dengue can be screened, and elastic net regularization can be applied to predict kinase regulators for further investigation.

involved in an impact on a measured phenotype. In other words, the likelihood of a kinase being a regulator of the phenotype measured is evaluated based on the activity across multiple inhibitors and the known targets of those inhibitors. Figure 1.2 illustrates the functionality of KiR with a simplified example. KiR was originally established to investigate kinases that regulate cell migration during cancer [270], but it could be used to evaluate infection, inflammatory cytokines, ADE, or many other phenotypes important to dengue. Notably, this approach has been successfully utilized to identify key host factors regulating malaria liver infection and disruption of the blood-brain barrier [271; 272]. More recently, this approach has been further developed as Temporally REsolved KInase Network Generation (TREKING), which resolves kinase regulation at differing time points [273] and could be utilized to better inform multi-kinase drug strategies.

Another novel approach to identify druggable kinase targets for DENV is by investigating synthetic lethality. Synthetic Lethality is a phenomenon in that a cell will die when two proteins – synthetic lethal partners – are simultaneously disrupted, but no cell death occurs when only one of the proteins is disrupted [274]. To this end, another potential strategy to control dengue infection is by inhibiting kinases which are synthetic lethal partners with host factors disrupted during infection. Targeting synthetic lethal partners has exhibited efficacy against SARS-CoV-2- and poliovirus-infected cells [79] and tumor cells [275], and drugs developed based on this concept are currently in the clinic for cancer [276]. Using combined kinase inhibitor treatment and gene depletion, screening the survival of infected versus uninfected cells could reveal synthetic lethal interactions that selectively kills DENV-infected cells.

CONCLUSIONS AND FUTURE DIRECTIONS

These existing literature findings, along with the work presented in the chapters that follow, provide clear evidence that targeting kinases is a promising therapeutic strategy for controlling dengue. We highlight kinases that have already shown efficacy as drug targets against infection *in vitro* and *in vivo*, those which can prevent disease, and those that regulate infection and disease but have not yet been therapeutically investigated. We also suggest tools for further expanding our knowledge on kinase regulation of dengue. Ultimately, identifying kinase mediators of dengue that can be drugged may be key to combating the burden of dengue through therapeutic intervention.

Chapter 2

MULTIPLE RECEPTOR TYROSINE KINASES MEDIATE DENGUE INFECTION OF HEPATOCYTES

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Preface

This chapter includes my primary dissertation research that is in peer review with Frontiers and is available on bioRxiv: https://www.biorxiv.org/content/10.1101/2023.07.30.549949v1?rss=1 (differences from what is on bioRxiv are due to changes made in response to suggestions by my graduate committee or co-authors after submission). In this work, I aimed to identify druggable kinase activity regulating dengue. I did this by investigating kinases important for DENV infection in hepatocytes through a series of computational and protein perturbation approaches. Ultimately, a role in infection for multiple kinases was elucidated, granting progress towards the field's need for host-directed interventions to dengue.

ABSTRACT

Dengue is an arboviral disease causing severe illness in over 500,000 people each year. Currently, there is no way to constrain dengue in the clinic. Host kinase regulators of dengue virus (DENV) infection have the potential to be disrupted by existing therapeutics to prevent infection and/or disease progression. To evaluate kinase regulation of DENV infection, we performed kinase regression (KiR), a machine learning approach that predicts kinase regulators of infection using existing drug-target information and a small drug screen. We infected hepatocytes with DENV *in vitro* in the presence of a panel of 38 kinase inhibitors then quantified the effect of each inhibitor on infection rate. We employed elastic net regularization on these data to obtain predictions of which of 300 kinases are regulating DENV infection. Thirty-six kinases were predicted to have a functional role. Intriguingly, seven of the predicted kinases – EPH receptor A4 (EPHA4), EPH receptor B3 (EPHB3), EPH receptor B4 (EPHB4), erb-b2 receptor tyrosine kinase 2 (ERBB2), fibroblast growth factor receptor 2 (FGFR2), Insulin like growth factor 1 receptor (IGF1R), and ret proto-oncogene (RET) – belong to the receptor tyrosine kinase (RTK) family, which are already therapeutic targets in the clinic. We demonstrate that predicted RTKs are expressed at higher levels in DENV infected cells. Knockdown of ERBB2, FGFR2 and IGF1R reduces DENV infection in hepatocytes. Finally, we observe differential temporal induction of ERBB2 and IGF1R following DENV infection, highlighting their unique roles in regulating DENV. Collectively, our findings underscore the significance of multiple RTKs in DENV infection and advocate further exploration of RTK-oriented interventions against dengue.

INTRODUCTION

Dengue is a neglected tropical disease caused by dengue virus (DENV), a mosquito-borne flavivirus [277]. Dengue incidence has increased at an alarming rate, with over 4.2 million dengue cases reported to the World Health Organization in 2019 compared to the 500,000 cases reported in the year 2000 [2]. Strikingly, it is estimated that hundreds of millions more dengue cases are evading surveillance each year [3]. Global warming and urbanization are expanding suitable habitats for mosquito vector populations, lending to the prediction of further escalated dengue incidence in the coming years [5]. In the absence of an effective vaccine or specific therapeutics for DENV, instances of severe disease have also been rising [2]. Identifying effective interventions against infection is imperative to combat the growing global health burden of dengue.

Efforts towards identifying compounds that directly target DENV proteins are ongoing. DENV comprises an enveloped positive-sense single-stranded RNA genome which encodes three structural proteins – envelope (Env), pre-membrane (PrM), and capsid (C) – and seven non-structural proteins – NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (reviewed in [7]). Many compounds targeting these proteins exhibit efficacy *in vitro* and *in vivo* (reviewed in [278]). However, none of these have demonstrated efficacy against viral load or disease in clinical trials [36; 279; 280; 281].

On the other hand, host-targeting compounds have provided some protection against dengue disease in recent clinical trials [35]. Importantly, targeting the host has proven to be a successful therapeutic strategy for other infectious diseases, including human papilloma virus, hepatitis C virus, hepatitis B virus, and human

immunodeficiency virus [63; 282]. In addition to curbing disease in clinical trials, this strategy has also demonstrated promise for blocking DENV infection, with synergistic effects demonstrated against viral load *in vitro* and *in vivo* when combining a host-targeting α-glucosidase inhibitor and the broad antiviral ribavirin, a guanosine analog [283]. However, a comprehensive understanding of druggable host molecules that are critical for successful DENV infection is unavailable, limiting the pool of candidates for host targeted intervention against dengue.

Ample evidence demonstrates that DENV relies on host factors for infection and pathogenesis, as extensively reviewed in [66]. For instance, DENV requires host attachment receptors and regulators of endocytosis for entry [67; 68; 69; 70; 71; 72], exploits regulators and structural components of host translation machinery for viral genome replication [73; 74; 75; 76], and manipulates factors involved in the immune response [80; 81; 82; 83]. Notably, protein kinases serve as upstream regulators of each of these events. Taken together with the existence of hundreds of kinase-targeting drugs already existing in the clinic [250], kinases are promising candidate targets for DENV therapeutics.

Evidence for a role of kinase activity in DENV infection and pathogenesis continues to accumulate [112; 128; 169; 230; 284], and multiple kinase inhibitors have been shown to restrict dengue infection *in vitro* and *in vivo* [152; 220; 239; 285; 286]. Despite numerous high-throughput screens aiming to identify host factors regulating DENV [116; 128; 214; 264; 265; 266; 267], these attempts have failed to identify kinase regulators of infection, perhaps due to the extensive compensatory roles of other kinases or insufficient degree of depletion. Here, we overcome this shortcoming of prior

screening methods by employing Kinase Regression (KiR) on DENV infection. KiR is an innovative computational tool that uses a panel of 38 kinase inhibitors – with known enzymatic inhibition activity against a total of 300 kinases spanning the human kinome – as chemical probes to screen kinase activity regulating infection [269; 270]. KiR uses elastic net regularization to decipher which shared targets of the inhibitor panel influence infection (Figure 2.1A). Notably, this approach has been successfully utilized to identify key host factors regulating malaria liver infection and disruption of the blood-brain barrier [271; 272].

We performed KiR on DENV infection in HepG2 cells and predicted 36 kinase regulators. We further investigated a subset of these predictions, namely the receptor tyrosine kinases (RTKs), since many predicted kinases in this family are targeted by drugs that are already in clinical use. To validate and characterize their involvement during DENV, we determined the impact of DENV infection on their expression and activity, and we tested the impact of their knockdown on multiple DENV factors. We show that levels of KiR-predicted kinases are significantly higher in infected cells both in total and at the surface. Knockdown of a subset of these kinases significantly disrupts DENV infection, and their phosphorylation is induced at different times throughout infection. Our findings provide support for further investigation into targeting RTKs as a as a promising avenue for combating dengue infection and pathogenesis.

MATERIALS AND METHODS

Cell Culture and Maintenance

All cells used in this study were propagated from commercial passage 0 stock and stored at low passage in 10-90% FBS/10% DMSO in LN_2. Sterility of cell cultures was maintained through the use of a biosafety cabinet. Cell cultures were validated for absence of mycoplasma contamination before, during, and after experimental work using the MycoStrip™ - Mycoplasma Detection Kit (Invivogen #rep-mys). All experimental cell lines were grown under specific conditions and handled following precise procedures to ensure optimal growth and reproducibility. Typing and authentication for each cell line was provided by the American Type Culture Collection (ATCC). A maximum of 25 passages was maintained for each cell line.

HepG2 cells were received from ATCC (#HB-8065) and grown in primocin (100 µg/mL)-supplemented Complete Hepatocyte Media (CHM): DMEM Glutamax (Gibco™ #10566016) with 10% heat-inactivated FBS (SeraPrime #F31016HI), and 4 mM L-glutamine filtered through a 0.22 µM polyethersulfone (PES) membrane. Cells were maintained in tissue culture-treated flasks at 37 °C, 5% CO_2. Upon reaching 90% confluency, cells were washed once with 1X PBS then detached with 0.25% Trypsin-EDTA (Gibco™ #25200072) for 5 min at 37 °C, 5% CO_2. Detached cells were centrifuged at 158 rcf for 3 min to remove trypsin, resuspended in primocin-supplemented CHM, then filtered through a 40 µM nylon mesh cell strainer to reduce cell clumps for improved growth and counting consistency.

Vero cells were received from ATCC (#CCL-81) and grown in primocin (100 µg/mL)-supplemented Complete Vero Media (CVM): DMEM Glutamax with 10% FBS,

1X MEM Non-Essential Amino Acid solution (NEAA, Gibco™ #11140050) and filtered through a 0.22 µM PES membrane. Cells were grown in tissue culture-treated flasks at 37 °C, 5% CO_2. Upon reaching 100% confluency, cells were washed once with 1X PBS then detached with 0.25% Trypsin-EDTA for 5 min at 37 °C, 5% CO_2. Detached cells were centrifuged at 158 rcm for 3 min to remove trypsin then resuspended in primocin-supplemented CVM.

C6/36 cells were received from ATCC (#CRL-1660) and grown in Complete C6/36 Media (CCM): MEM with 10% FBS, 1X MEM NEAA, and 100 µg/mL primocin filtered through a 0.22 µM PES membrane. Cells were grown in tissue culture-treated flasks at 28 °C, 5% CO_2. Upon reaching 100% confluency, cells were washed once with 1X PBS then detached with 0.25% Trypsin-EDTA for 5 min at 28 °C, 5% CO_2. Detached cells were centrifuged at 158 rcf for 3 min to remove trypsin then resuspended in CCM

HEK293FT cells were a gift from Alan Aderem (Seattle Children's Research Institute) and grown in primocin (100 µg/mL)-supplemented Complete HEK Media (CHKM): DMEM with 10% FBS, 25 mM HEPES, 1X MEM NEAA and filtered through a 0.22 µM PES membrane. Cells were grown in tissue culture-treated flasks at 37 °C, 5% CO_2. Upon reaching 100% confluency, cells were gently washed once with 1X PBS then detached with 0.025% Trypsin-EDTA (diluted in 1X PBS) for 5 min at room temperature. Detached cells were centrifuged at 158 rcf for 3 min to remove trypsin then resuspended in primocin-supplemented CHKM.

shRNA-mediated Gene Knockdown

Non-replicating shRNA lentivirus were generated in HEK293FT cells transfected with MISSION plasmids procured from Sigma-Aldrich (details in Supplementary Table 2.3). Transfection involved the combination of MISSION plasmid (6 µg), pCMV-VSV-G (3 mg/ml), psPax2 (6 mg/ml), and Polyethylenimine Hydrochloride (PEI Max, 1 mg/mL) in 500 µl serum free-DMEM. The resulting solution was vortexed and incubated at room temperature for 10 min before being added dropwise to HEK293FT cells at 70% confluency in 10 cm^2 TC-treated dishes. Following overnight incubation at 37 °C, 5% CO_2, media was replaced, and cells were incubated overnight. Lentivirus-containing supernatant was harvested and filtered through a 0.45 µm PVFD membrane over the subsequent two days.

Supplementary Table 2.3 shRNA Constructs					
Clone ID	**Oligo Seq**	**RefSeq ID**	**Gene ID**	**Taxon ID**	**Gene**
TRCN0000010165	CCGGTCAGTCCGTGTGTTCTATAAACTCGAGTTTATAGAACACACGGACTGATTTTT	NM_004438.3	2043	9606	EPHA4
TRCN0000006427	CCGGCCCAAACCTCTTCATATTGAACTCGAGTTCAATATGAAGAGGTTTGGGTTTTT	NM_004443.3	2049	9606	EPHB3
TRCN0000006428	CCGGGCAGTACATTGCTCCTGGAATCTCGAGATTCCAGGAGCAATGTACTGCTTTTT	NM_004443.3	2049	9606	EPHB3
TRCN0000001773	CCGGCAATGGGAGAGAAGCAGAATACTCGAGTATTCTGCTTCTCTCCCATTGTTTTT	NM_004444.4	2050	9606	EPHB4
TRCN0000001774	CCGGTGATCTGAAGTGGGTGACATTCTCGAGAATGTCACCCACTTCAGATCATTTTT	NM_004444.4	2050	9606	EPHB4
TRCN0000039878	CCGGTGTCAGTATCCAGGCTTTGTACTCGAGTACAAAGCCTGGATACTGACATTTTTG	NM_001005862.1,NM_004448.2	2064	9606	ERBB2
TRCN0000039881	CCGGCAGTGCCAATATCCAGGAGTTCTCGAGAACTCCTGGATATTGGCACTGTTTTTG	NM_001005862.1,NM_004448.2	2064	9606	ERBB2

TRCN0000000366	CCGGGCACACACTTACAGAGCACAACTCGAGTTGTGCTCTGTAAGTGTGTGCTTTTT	NM_000141.4, NM_001144914.1,NM_001144915.1,NM_001144916.1,NM_001144917.1,NM_001144918.1,NM_022970.3	2263	9606	FGFR2
TRCN0000000367	CCGGGCCACCAACCAAATACCAAATCTCGAGATTTGGTATTTGGTTGGTGGCTTTTT	NM_000141.4, NM_001144913.1,NM_001144914.1,NM_001144917.1,NM_022970.3	2263	9606	FGFR2
TRCN0000000368	CCGGCCGAATGAAGAACACGACCAACTCGAGTTGGTCGTGTTCTTCATTCGGTTTTT	NM_000141.4, NM_001144913.1,NM_001144914.1,NM_001144915.1,NM_001144916.1,NM_001144918.1,NM_001144919.1, NM_022970.3	2263	9606	FGFR2
TRCN0000000424	CCGGGCTGATGTGTACGTTCCTGATCTCGAGATCAGGAACGTACACATCAGCTTTTT	NM_000875.3	3480	9606	IGF1R
TRCN0000000425	CCGGCCTTGGACGTTCTTTCAGCATCTCGAGATGCTGAAAGAACGTCCAAGGTTTTT	NM_000875.3	3480	9606	IGF1R
TRCN0000000404	CCGGCCGCTGGTGGACTGTAATAATCTCGAGATTATTACAGTCCACCAGCGGTTTTT	NM_020630.4, NM_020975.4	5979	9606	RET
TRCN0000000405	CCGGGCTGCATGAGAACAACTGGATCTCGAGATCCAGTTGTTCTCATGCAGCTTTTT	NM_020630.4, NM_020975.4	5979	9606	RET

For the knock-down of host kinases of interest, HepG2 cells were reverse-transduced with the generated shRNA lentivirus. Detached HepG2 cells were mixed

with lentivirus (1 ml lentivirus-containing supernatant per 4×10^6 cells) in antibiotic-free CHM supplemented with 1 µg/ml polybrene (EMD Millipore TR-1003-G) then plated in 10 cm^2 TC-treated dishes. Following overnight incubation at 37 °C, 5% CO2, cells were replenished with CHM then incubated overnight. For the next seven days, transduced cells were selected in CHM supplemented with 1 µg/ml puromycin (replenished daily). Non-transduced cells were always included in parallel as a positive control for puromycin killing. After puromycin selection, knockdown was verified at the protein level and cells were utilized for experimentation.

Viral Production, Propagation, and Storage

DENV2 MON601, a molecular clone of DENV-2 New Guinea strain C, was generated by transfecting *in vitro*-transcribed RNA into Vero cells [77]. Virus was propagated by interchangeably infecting 80% confluent C6/36 or Vero cell monolayers with low-passage stock virus at an MOI of 0.01 in Dengue Stock Media (DSM), comprising DMEM Glutamax supplemented with 2% FBS, 1X MEM NEAA, 25 mM HEPES, and 4 mM L-glutamine. Supernatants, collected twice weekly between 5-14 days post-infection, were cleared of cellular debris by centrifugation at 632 rcf followed by filtration through a 0.2 µM CA filter and stored at −80 °C. The viral titer of stocks was enumerated as described below.

Viral Stock Titer Quantification

Virus stocks were titrated using a flow cytometry approach as previously described [287]. Briefly, Vero cells were infected with serially diluted virus stocks and analyzed at 24 hours post-infection by flow cytometry. Fixed cells were stained with antibody specific to flavivirus envelope protein (4G2) conjugated to AlexaFluor488 and the percentage of cells that stained positive for 4G2-AlexaFluor488 was quantified. The titer, expressed as fluorescence forming units (FFU) per m of virus stock, was derived from the percentage of infected cells relative to the virus volume and cell count. The titer was used to calculate the volume of virus stock necessary to achieve desired MOI.

Viral Infection

For experimental infections, plated cells were washed once with 1X PBS then titered virus stock diluted in Opti-MEM to an MOI of 2 was added to cells at the minimum volume per well. Cells were incubated for 90 min at 37 °C, 5% CO_2 then washed once with 1X PBS. Cells were then replenished with DSM and incubated at 37 °C, 5% CO_2 for the indicated time (initial addition of virus is the start time).

DENV Detection

Pan-flavivirus envelope (Env) antibodies were prepared from hybridoma 290 supernatants and purified by protein A/G chromatography [169]. Anti-Env was conjugated to Alexa Fluor 488 (Env-488) and titrated to determine the optimal concentration between 1:500 and 1:5000. Antibodies against DENV non-structural

protein 3 (NS3) were obtained from GeneTex (GTX124252), conjugated to Alexa Fluor 647 (NS3-647), and titrated as with 4G2-488. Fluorophores were conjugated to these primary antibodies using Thermo Scientific™ Antibody Labeling Kits according to the manufacturers protocol.

RTK Detection

Primary antibodies against EphA4 (Thermo Scientific™ #PA5-14578), EphB3 (Santa Cruz Biotechnology, Inc. #sc-100299), EphB4 (GeneTex #GTX108595), ErbB2 (Cell Signaling Technology, Inc. (CST) # 2165), FGFR2 (CST #11835), IGF-1R (R&D Systems #MAB391) and RET (CST #3220) were used at a concentration of 1:100 then stained with anti-rabbit-PE or anti-mouse Pacific Blue secondary antibody (BioLegend® #406421, Life Technologies #P10993) for flow cytometry.

For western blots, primary RTK antibodies were used at a 1:1000 concentration followed by anti-rabbit or -mouse-HRP (R&D Systems #HAF008, #HAF007). Primary antibodies p-ErbB2 (MilliporeSigma #04-293) and p-IGF-1R (MilliporeSigma #ABE332) were used at a 1:1000 concentration , p-FGFR2 (CST #3471) was used at a 1:500 concentration. Anti-mouse or anti-rabbit GAPDH were used for loading control staining.

Flow Cytometry

To harvest cells for flow cytometry, HepG2s were washed once with 1X PBS then treated with TrypLE (Gibco™ #12604021) and incubated for 5 min at 37 °C, 5% CO_2. Detached cells were diluted in CHM, transferred to a 96-well U-bottom plate, then

centrifuged at 158 rcf for 5 min to pellet. The supernatant was discarded and the pellet was resuspended in 3.7% paraformaldehyde (PFA, VWR #100503-917) then incubated at room temperature on a shaker for 15 min for chemical fixation. Fixed cells were further prepared and assayed for flow cytometry as described below or stored at 4 °C for no more than one week.

For analysis of total protein levels in cells, fixed cells were washed twice with 1X PBS then incubated in 0.1% Triton-X-100/1X PBS at room temperature on a shaker for 10 min. Permeabilized cells were washed twice with 1X PBS then resuspended in 0.01% Triton-X-100/2% BSA/1X PBS (GoldBio #A-420) at room temperature on a shaker for 1 hour or overnight at 4 °C to block non-specific protein binding. Blocked cells were pelleted at 632 rcf for 5 min and then resuspended in indicated detection antibody. Cells were stained for at least 2 h at room temperature or overnight at 4 °C. For cells stained with unconjugated primary, cells were washed twice with 1X PBS after primary antibody incubation and then resuspended in secondary antibody solution for 1-2 h at room temperature. For analysis of surface proteins levels in cells, fixed cells were washed twice with 1X PBS then resuspended in 2% BSA/1X PBS to block non-specific binding. After staining for kinase detection as described above, cells were permeabilized, blocked, and stained for DENV. Stained cells were washed twice with 1X PBS then assayed on an 18-color FACS analyzer harboring 405, 488, 532 and 640 nm lasers.

The collected events were analyzed using FlowJo. Events were gated for cells by size on FSC-A x SSC-A (Supplementary Figure 2.1A). Cells were gated for infection using uninfected sample stained with Env-488 or NS3-647 (Supplementary Figure 2.1B-

C). The percentage of cells stained positive for Env-488 or NS3-647 is denoted as % infection. Kinase protein levels were obtained by adding the Geometric Mean statistic to the relevant fluorescence channel and denoted as Mean Fluorescence Intensity (MFI).

Western Blot

To collect cells for analysis by western blot, HepG2s were washed once with 1X PBS then lysed in SDS lysis buffer (50 mM Tris HCl/2% SDS/5% glycerol/5 mM EDTA/1mM NaF/dH_2O) supplemented with cOmplete Protease Inhibitor Cocktail Tablets (Roche #11836170001), Phosphatase Inhibitor Cocktail 2 (Sigma-Aldrich® #P5726), B-GP (Sigma-Aldrich® #G9422), PMSF (Sigma-Aldrich® #10837091001), Na_3VO_4 (Sigma-Aldrich® #567540-5GM) and DTT (Sigma-Aldrich® # D9779). Lysates were transferred to QIAshredder tubes (QIAGEN #79656) then centrifuged at 1935 rcf for 5 min to separate proteins from genetic material. Bolt Sample Reducing Agent (Invitrogen™ B0009) was added to the eluate and incubated at 70 °C for 10-20 min to denature proteins.

Reduced lysates or pre-stained protein ladder (Thermo Scientific™ #26619) were loaded into the specified lanes of a Bolt 4-12% Bis-Tris Plus gel (Invitrogen™ #NW04125BOX) in Bolt™ MES SDS Running Buffer (Invitrogen™ #B0002). A 180 V current was applied for 60 min to separate proteins by electrophoretic mobility (i.e. size). Separated proteins were transferred onto an iBlot™ 2 Transfer Stacks PVFD membrane (Invitrogen™ IB24002) using dry transfer (as described previously [288]) in an iBlot™ 2 system.

Following transfer, the PVFD membranes were incubated at room temperature on a shaker for 1 h or overnight at 4 °C in 5% BSA-supplemented TBS/0.1% Tween-20 (TBST) to block non-specific protein binding during subsequent antibody staining. Blocked membranes were then incubated at 4 °C overnight in primary antibody solution. Blots were then washed 3 x 5 min each in TBST and then incubated at room temperature for 1 hour in secondary antibody solution.

Labeled blots were washed 4 x 5 min each then incubated in SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Scientific™ #34577) at room temperature for 3 min. Developed blots were scanned for chemiluminescence on a BioRad ChemiDoc Imaging System. ImageJ was used to enhance raw images for display and to quantify band intensity. Band intensity is reported as chemiluminescence of the protein of interest, normalized to the GAPDH loading control.

For phosphorylation time course (Figure 2.5), 0.5 – 1.5 hpi were compared to uninfected 0.5 hour sample, 6 – 8 hpi to uninfected 6 hr sample, and 16-24 hpi to uninfected 16 hr sample.

Prediction of Kinases

HepG2 cells were pre-treated with the KiR inhibitor panel in Opti-MEM (Supplementary Table 2.1) at 500 nM for 1 h, after which media was removed, and cells were replenished with either DSM or DENV2 MON601 at an MOI of 2 in the presence of the inhibitor panel. After 90 min, cells were washed once with 1X PBS then replenished with inhibitor-supplemented DSM. 24 h post-infection, cells were fixed and stained with

Env-488. The percentage of cells staining positive for Env was quantified by flow cytometry. Inhibitor-induced background fluorescence was measured on uninfected, inhibitor-treated samples and subtracted from infection values. Resulting values below zero were adjusted to zero. Grubb's Test was used to remove outlier data.

Supplementary Table 2.1 Kinase Regression Inhibitor Panel

Inhibitor ID	CAS #
Aminopurvanolol A	220792-57-4
AMPK Inhibitor; Compound C (Dorsomorphin)	866405-64-3
Bosutinib	380843-75-4
Casein kinase I inhibitor D4476	301836-43-1
Cdk1/2 Inhibitor III	443798-55-8
CDK2 inhibitor IV; NU6140	444723-13-1
CDK4 inhibitor	546102-60-7
Dasatinib	302962-49-8
Dovitinib	405169-16-6
EGFR/ErbB2/ErbB4 inhibitor	881001-19-0
Erlotinib	183321-74-6
Gefitinib	184475-35-2
Go 6976	136194-77-9
Go 6983	133053-19-7
GSK inhibitor IX (BIO)	667463-62-9
GSK-3 Inhibitor X	740841-15-0
GSK-3 Inhibitor XIII	404828-08-6
GSK-3b inhibitor I (TDZD-6)	327036-89-5
H89	130964-39-5
Imatinib	152459-95-5
JAK inhibitor I	457081-03-7
JNK inhibitor II (SP600125)	129-56-6
K252a	99533-80-9
Lapatinib	388082-78-8
Lck inhibitor	213743-31-8
Masitinib	790299-79-5
Nilotinib	641571-10-0
PKR inhibitor	608512-97-6
ROCK inhibitor (Y-27632)	129830-38-2
SB218078	135897-06-2
Sorafenib	284461-73-0
Staurosporine	62996-74-1
Staurosporine n benzoyl	120685-11-2
SU11274	658084-23-2
SU6656	330161-87-0
Tofacitinib	477600-75-2
TWS119	601514-19-6

Vandetanib	443913-73-3

The elastic net regularization algorithm used for this study was published previously [272; 273]. Briefly, normalized percent infection data from five independent kinase inhibitor screens and existing biochemical data of the kinase inhibitors against 300 recombinant protein kinases [269], were input into the elastic net regularization algorithm using a condition-specific cross-validation strategy. The glmnet package (version 2.2.1, https://github.com/bbalasub1/glmnet_python) in Python (version 3.7.6, https://www.python.org) was used for performing elastic net regression, with the elastic net mixing parameter α, which confers the stringency of model selection, scanned from 0.1 to 1.0 in steps of 0.1. Predictions using an α of 0.8 are reported in this manuscript. The regularization path was computed for the elastic net penalty at a grid of values for the regularization parameter λ of 10^3. The Python code used for this analysis is provided in Supplementary Files (Supplementary File 1).

Building Kinase Interaction Networks

Phosphosignaling networks were built to identify upstream/downstream kinases of the KiR predicted kinases and infer the connections between them. Searches for two layers upstream/downstream of the KiR predicted kinases were done using the kinase-substrate phosphorylation database PhosphoSitePlus® [289]. These data were visualized using Cytoscape 3.9.1 [290]. L0, L1, and L2 nodes were manually spatially organized, then the degree-sorted circular layout algorithm was applied to spatially organize predicted RTKs and their interactions based on degree of connection.

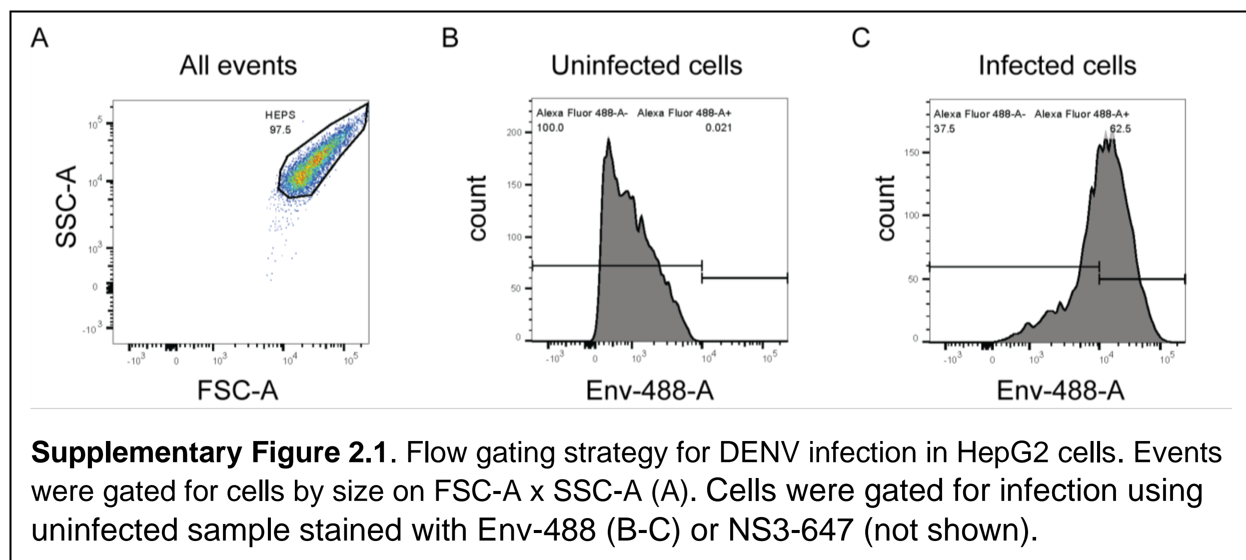
Statistical Analyses

Sample set details and method of statistical analysis are reported for each experiment in the corresponding figure legend. For multiple comparison analyses for data with normal distribution, One-Way ANOVA was used, while data with skewed distribution were analyzed by Brown-Forsythe.

RESULTS

Kinase Regression predicts multiple receptor tyrosine kinases that regulate DENV infection of hepatocytes

The liver is directly infected by DENV and can be critically damaged in severe dengue cases (reviewed in [291]). Additionally, kinase regulation of dengue-induced liver injury has been reported [152; 292]. Considering this, we chose to apply kinase regression (KiR) to DENV infection of HepG2 hepatoma cells, a widely used model for studying DENV infection of the liver. We pre-treated HepG2 cells with a panel of 38



Supplementary Figure 2.1. Flow gating strategy for DENV infection in HepG2 cells. Events were gated for cells by size on FSC-A x SSC-A (A). Cells were gated for infection using uninfected sample stained with Env-488 (B-C) or NS3-647 (not shown).

inhibitors collectively targeting 300 kinases (Supplementary Table 2.1, see methods) with known target overlap at 500 nM. An hour after treatment, we infected the cells with DENV2 MON601 at an MOI of 2 and continued inhibitor treatment. Twenty-four hours post-infection, we fixed the cells and stained them with a 488-conjugated Env (Env-488) antibody solution. The percentage of cells staining positive for Env was quantified by flow cytometry (Supplementary Figure 2.1). Several kinase inhibitors dramatically decreased DENV infection while other inhibitors had little effect; in contrast, no inhibitors dramatically elevated levels of infection (Figure 2.1B). To predict kinases important for DENV infection, we input these data, along with existing information on kinase-substrate inhibition, into an elastic net regularization algorithm (see Methods) [269; 270]. This analysis led us to identify 36 kinases as potentially crucial in regulating dengue infection (Figure 2.1C, Supplementary Table 2.2).

Supplementary Table 2.2 KiR-predicted Kinases

Predicted Kinase	**Coefficient of Correlation**
ACK1	-0.04048
CHK1	-0.02247
CK1g3	-0.05048
CTK_MATK	-0.00949
DYRK4	0.294582
EPHA4	0.020529
EPHB3	0.020855
EPHB4	0.009052
ERBB2/HER2	0.076245
ERK1	-0.129
FGFR2	-0.0591
HIPK1	-0.06499
IGF1R	0.294032
IKKa/CHUK	0.251537
JAK3	0.07556
KHS_MAP4K5	0.048963
LKB1	-0.01933
MAPKAPK5/PRAK	0.171559
MARK1	0.00079
MARK4	0.022757
NEK11	0.036702

NEK3	-0.03688
NIK/MAP3K14	0.013453
P38b/MAPK11	0.014872
P38d/MAPK13	-0.02163
PAK1	0.178078
PAK4	-0.0126
PAK5	-0.00049
PIM3	0.066172
PKCepsilon	-0.01607
PKG1a	0.039417
RET	-0.03284
ROCK1	-0.00073
SIK2	0.10975
SRPK1	0.016612
TTK	-0.01278

To prioritize predictions for subsequent investigation, we explored which of the predicted kinases are targets of FDA approved drugs. Strikingly, the majority of KiR-predicted kinases with available drugs were receptor tyrosine kinases (RTKs), despite the fact that the RTK family represents only about 10% of all human kinases (Figure 2.1C) [293]. The predicted RTKs included Ephrin type-A receptor 4 (EPHA4), Ephrin type-B receptor 3 (EPHB3), Ephrin type-B receptor 4 (EPHB4), Receptor tyrosine-protein kinase erbB-2 (ERBB2), Fibroblast growth factor receptor 2 (FGFR2), Insulin-like growth factor 1 receptor (IGF1R), and Proto-oncogene tyrosine-protein kinase receptor Ret (RET).

To further assess the potential of RTKs as regulators of DENV infection, we examined if the seven predicted RTKs interacted with other KiR predictions or with kinases previously shown to be important for DENV infection [85; 90; 94; 122; 131; 132; 133; 148; 149; 161; 167; 168; 169; 170; 171; 180; 181; 182; 183; 187; 188; 192; 199; 219; 228; 231; 238; 240; 255; 256; 258; 294; 295]. We utilized the kinase-substrate phosphorylation database PhosphoSitePlus® [289] to identify interacting kinases and

substrates of the predicted RTKs and then visualized these interactions using Cytoscape 3.9.1 (Fig 1D). Known interactions for EPHA4 and EPHB4 are entirely composed of autophosphorylation events within the PhosphoSitePlus® database, so they are not included in this network. Notably, the PhosphoSitePlus® database does not

A

KI #1 → KI #38

Uninfected
Infected

24h infection analyzed by flow cytometry

Decreases infection

No effect on infection

Kinase 1

Predicted kinase regulator

Kinase 2

Not predicted kinase regulator

B

% infection of DMSO control

0 50 100 150 200

Staurosporine, K252a, Cdk1/2 inhibitor III, CDK4 inhibitor, SB218078, Staurosporine n benzoyl, EGFR/ErbB2/ErbB4 inhibitor, Bosutinib, Dasatinib, AMPK inhibitor, CDK2 inhibitor IV, Casein Kinase I inhibitor, PKR inhibitor, Gefitinib, SU11274, JAK inhibitor I, Tofacitinib, Vandetanib, Dovitinib, Aminopurvanol A, Go 6976, ROCK inhibitor, GSK-3 Inhibitor X, Erlotinib, Lapatinib, Nilotinib, JNK inhibitor II, GSK-3 inhibitor XIII, Masitinib, GSK inhibitor, H89 inhibitor, Imatinib, SU6656, Sorafenib, GSK-3b inhibitor I, Go 6983, Lck inhibitor, TWS119

C

TK, TKL, STE, CK1, AGC, CAMK, CMGC

FGFR2, EphA4, EPHB4, RET, IGF1R, ErbB2, EphB3, JAK3, ACK, CTK, KH81, DYRK4, HIPK, NIK, PAK1, PAK4, PAK5, TTK, SRPK1, CK1g3, Erk1, P38d, p38b, IKKa, LKB, CHK1, NEK11, NEK3, PIM3, PKG1, PKCe, ROCK1, MAPKAPK5, SIK, MARK1, MARK4

KiR predicted

KiR predicted and drug target

D

TTK, AXL, CDK5, STK4, EPHA2, CSK, AKT2, MAPKAPK2, MAP2K2, WNK1, LIMK1, MAP3K8, DAPK1, MAPK7, RPS6KA5, MAP3K11, MYLK, BMX, RPS6KB1, SGK1, CDK2, PRKCD, PRKCE, RPS6KA1, RAF1, PRKAA1, MTOR, FGFR1, MAP2K4, MET, GSK3A, PAK1, CHEK1, MAP3K5, LCK, RPS6KA3, BRAF, EGFR, CSNK2A1, MAP2K1, GSK3B, NUAK1, TRIO, PKN2, SGK2, TP53RK, SRPK2, FGR, CLK2, AKT3, HSPB8, ROCK1, PAK6, IRAK1, PRKCZ, SGK3, DYRK1B, PDGFRA, STK3, MKNK1, PRKCG, PTK2B, BTK, CHUK, RPS6KA4, PDK1, PKN1, MST1R, PDGFRB, EIF2AK2, MAPKAPK5, CDK4, YES1, WEE1, ZAP70

PRKCA, PTK2, MAPK14, PRKACA, PDPK1, GRK2, IGF1R, FGFR2, ERBB2, MAPK3, MAPK8, EPHB3, RET, SRC, MAPK9, GRK6, MAPK1, AKT1

KiR predicted RTK

KiR predicted

Literature reported

L0

L1

L2

Figure 2.1. Kinase Regression (KiR) on dengue (DENV) infection predicts seven receptor tyrosine kinases (RTKs) as regulators of infection. (A) HepG2 cells were pre-treated with KiR kinase inhibitor (KI) panel (Supplementary Table 2.1) at a concentration of 500 nM for one hour, followed by infection with DENV2 MON601 at a MOI of 2 under continuous inhibitor exposure. 24 h post-infection, cells were fixed and stained with antibody against DENV envelope (Env) protein. Flow cytometry analysis subsequently determined the percentage of cells staining positively for Env. (B) Percent difference in infection rates in response to each inhibitor compared to the mock-treated control (DMSO). Data below the dashed horizontal line indicates lower infection rates than the mock control and vice versa. (C) KiR predicted 36 kinases as potential regulators of dengue infection in HepG2s. The phylogenetic kinase family tree depicts KiR-predicted kinases and the subset that are targets of existing therapeutics in light or dark orange, respectively. (D) Interaction network denotes predicted RTKs (in orange), their the direct (L1, gray) or indirect (L2, light gray) interactors, other predicted kinases (orange outline), and kinases implicated in existing DENV literature (blue outline). The directionality of interactions is shown, where black circles indicate source kinase and red arrows indicate target substrate.

exhaustively catalog all kinase-substrate interactions, so additional interactions beyond those illustrated in Figure 2.1D likely exist. Nevertheless, we observed that EPHB3, ERBB2, FGFR2, IGF1R, and RET are upstream regulators both of kinases shown to be important in previous dengue research and of kinases predicted by KiR. We therefore hypothesized that the KiR-predicted RTKs are key mediators of DENV infection.

Elevated levels of RTKs are observed in DENV-infected cells

RTKs are stationed on the plasma membrane of cells, where they mediate growth factor signaling and cell-cell communication (reviewed in [296]). After interacting with ligand, RTKs are often endocytosed and activate signaling cascades that orchestrate cell function. There is extensive evidence that viruses can alter canonical RTK expression to hijack endocytosis or manipulate replication and cell death machinery, as reviewed in [297]. We thus investigated whether the KiR predicted RTKs – EPHA4, EPHB3, EPHB4, ERBB2, FGFR2, IGF1R, and RET – were present at higher levels in infected cells.

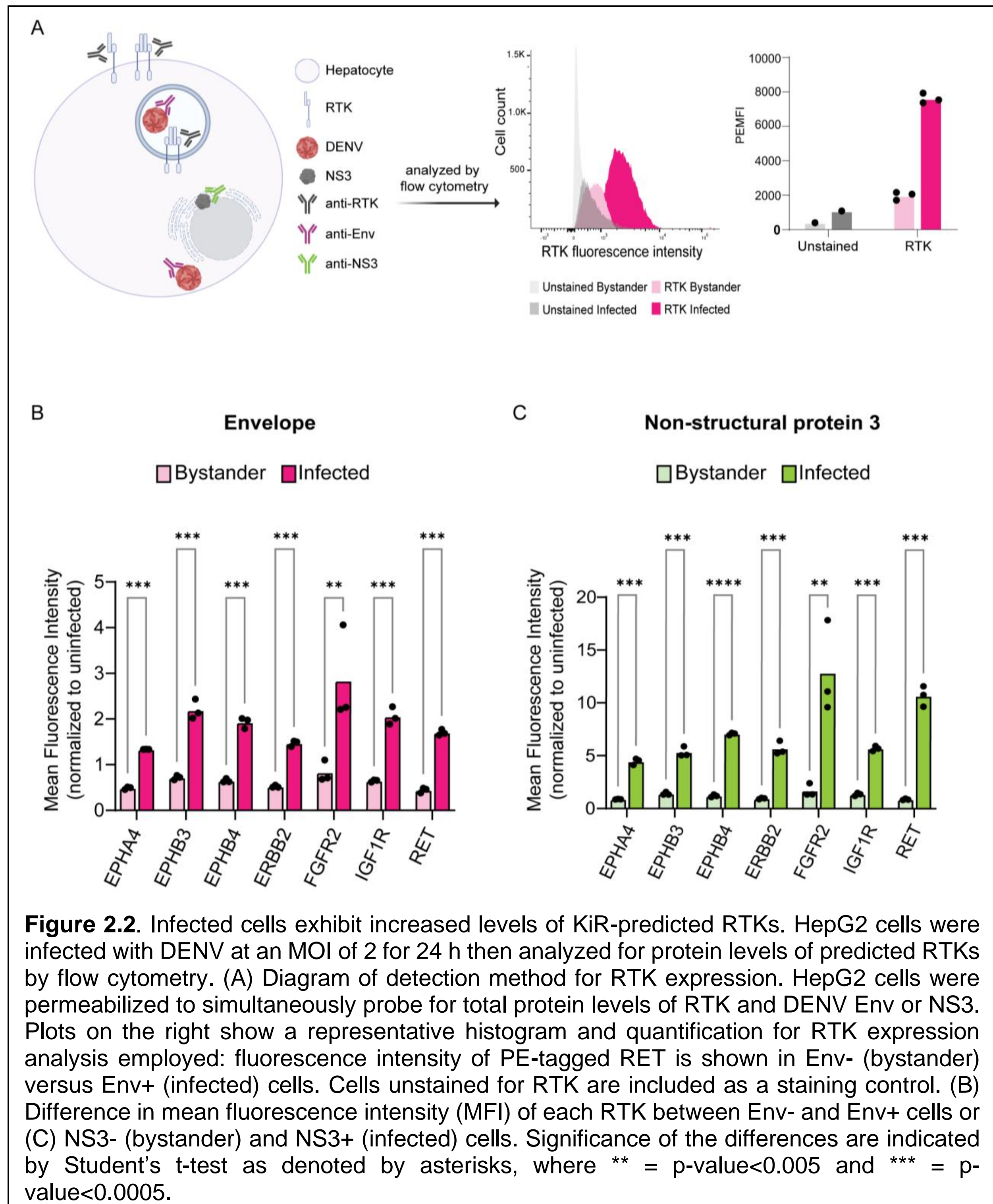
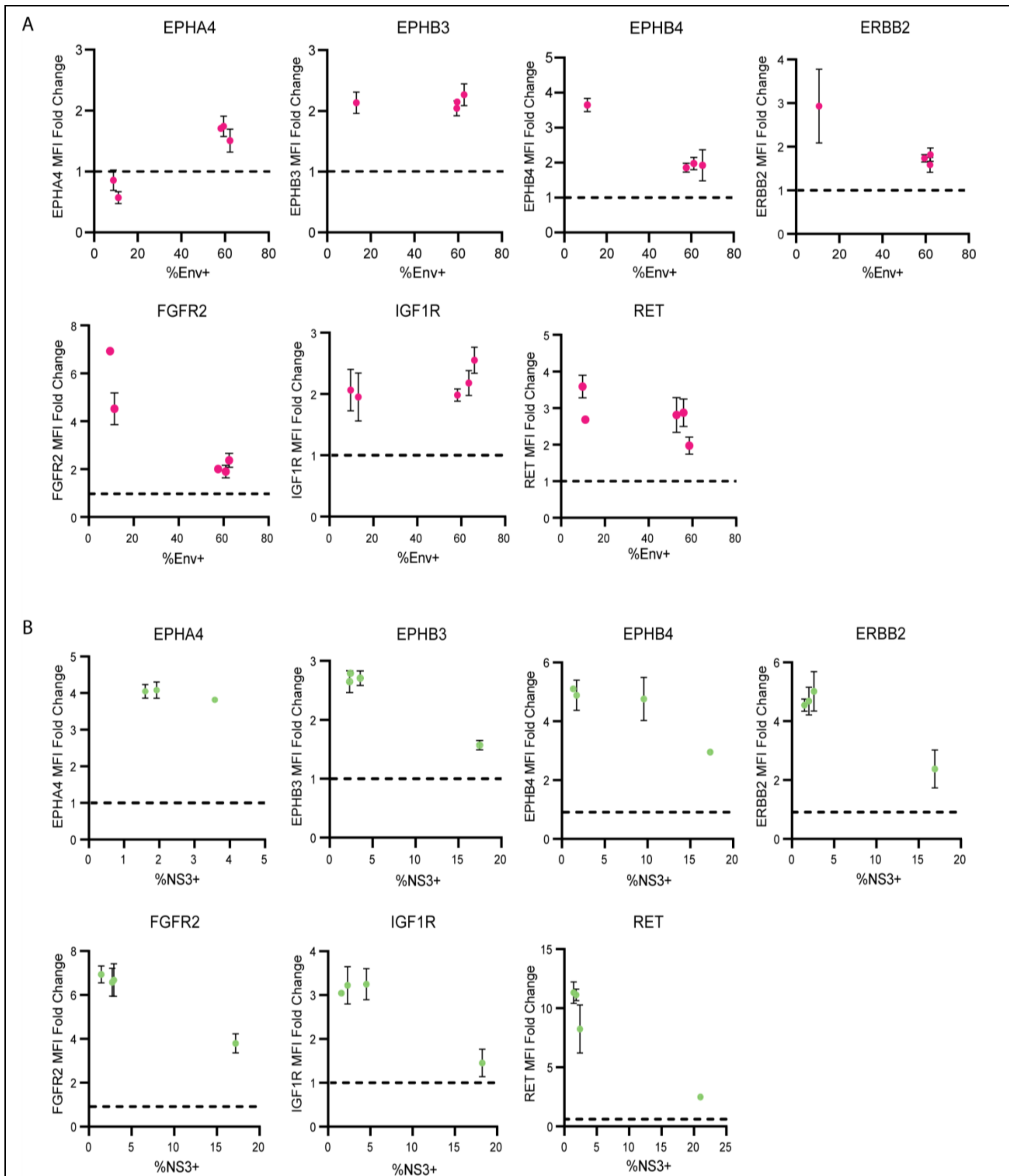


Figure 2.2. Infected cells exhibit increased levels of KiR-predicted RTKs. HepG2 cells were infected with DENV at an MOI of 2 for 24 h then analyzed for protein levels of predicted RTKs by flow cytometry. (A) Diagram of detection method for RTK expression. HepG2 cells were permeabilized to simultaneously probe for total protein levels of RTK and DENV Env or NS3. Plots on the right show a representative histogram and quantification for RTK expression analysis employed: fluorescence intensity of PE-tagged RET is shown in Env- (bystander) versus Env+ (infected) cells. Cells unstained for RTK are included as a staining control. (B) Difference in mean fluorescence intensity (MFI) of each RTK between Env- and Env+ cells or (C) NS3- (bystander) and NS3+ (infected) cells. Significance of the differences are indicated by Student's t-test as denoted by asterisks, where ** = p-value<0.005 and *** = p-value<0.0005.

We infected HepG2 cells with DENV2 MON601 for 24 h and then stained them with Phycoerythrin (PE)- or Pacific Blue-labeled RTK antibodies in parallel with Env-488



Supplementary Figure 2.2. Difference in total RTK MFI in DENV-infected cells across biological replicates with varying infection levels. The fold change in total RTK MFI between bystander and infected cells is shown against the average infection level for each experiment for Env (A) and NS3 (B). Dashed line denotes fold change = 1 to draw attention to the difference in scale for fold change across the RTKs.

and NS3-647; RTK-unstained samples were included as a control (Figure 2.2A). The

mean fluorescence intensity (MFI) of each labeled RTK was first analyzed in uninfected cells to establish the baseline expression of each RTK. We then quantified the MFI of the Env- (bystander) and the Env+ (infected) cell populations and normalized these data to the MFI of uninfected cells for each RTK (Figure 2.2B). The same analysis was

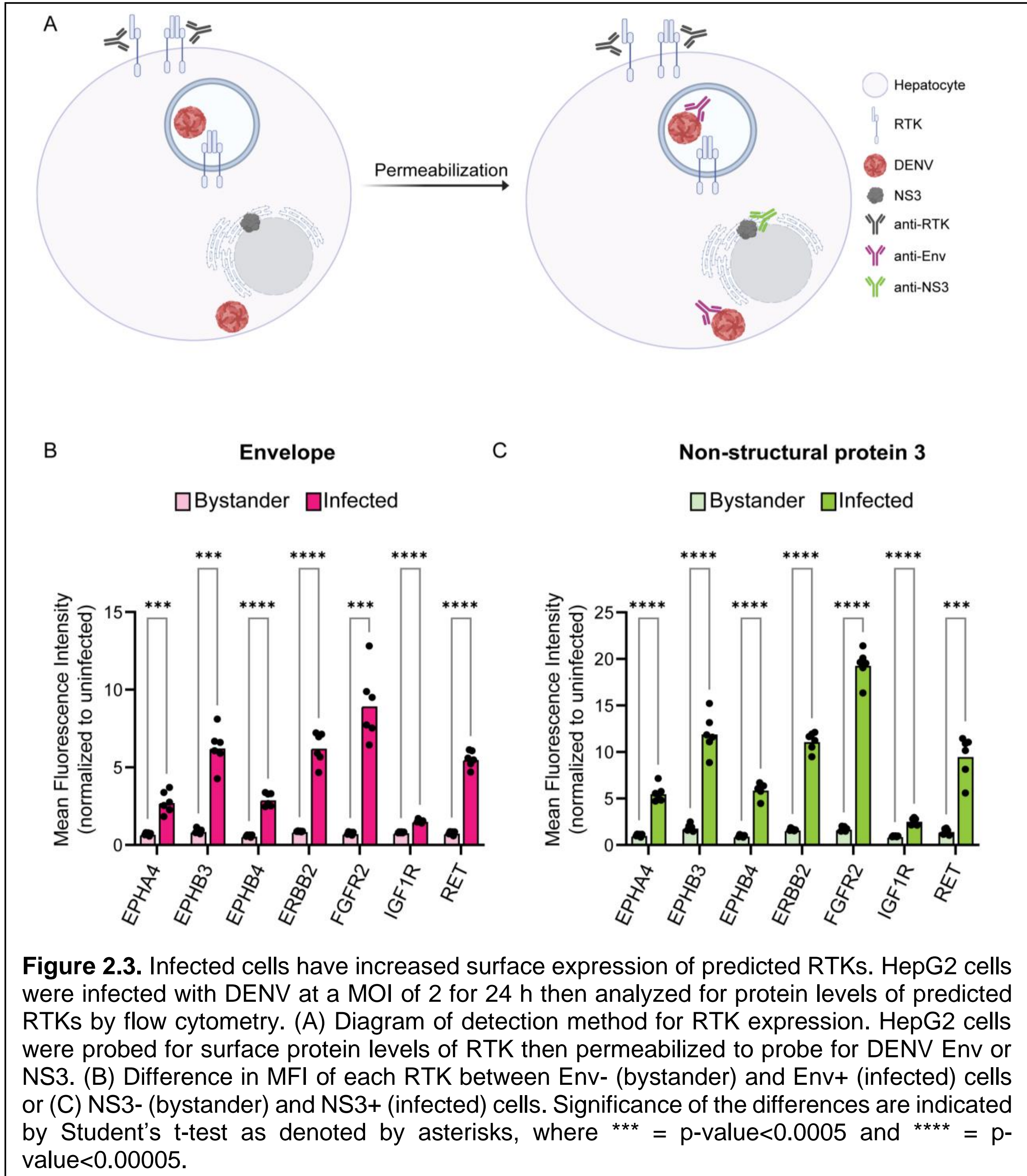
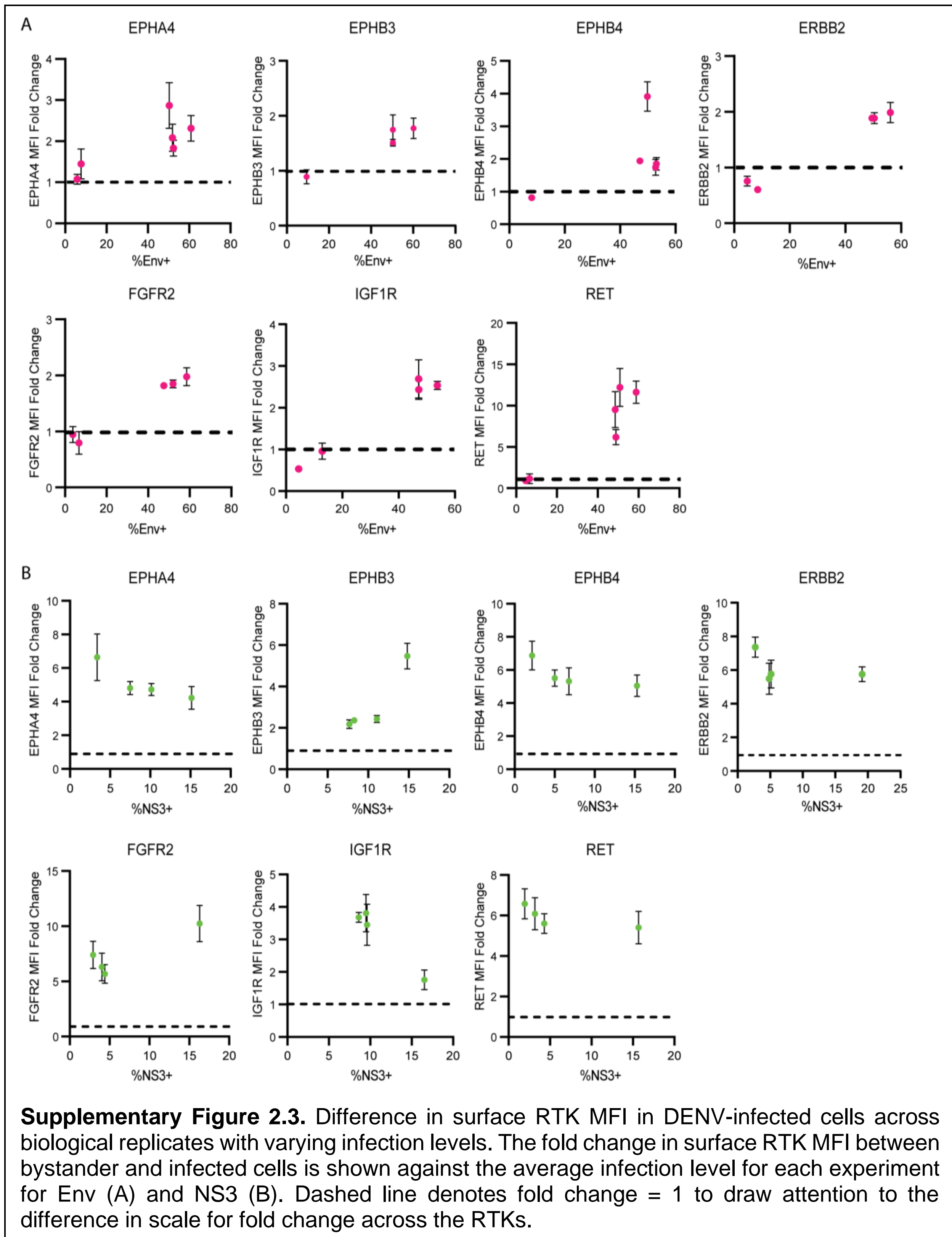


Figure 2.3. Infected cells have increased surface expression of predicted RTKs. HepG2 cells were infected with DENV at a MOI of 2 for 24 h then analyzed for protein levels of predicted RTKs by flow cytometry. (A) Diagram of detection method for RTK expression. HepG2 cells were probed for surface protein levels of RTK then permeabilized to probe for DENV Env or NS3. (B) Difference in MFI of each RTK between Env- (bystander) and Env+ (infected) cells or (C) NS3- (bystander) and NS3+ (infected) cells. Significance of the differences are indicated by Student's t-test as denoted by asterisks, where *** = p-value<0.0005 and **** = p-value<0.00005.



Supplementary Figure 2.3. Difference in surface RTK MFI in DENV-infected cells across biological replicates with varying infection levels. The fold change in surface RTK MFI between bystander and infected cells is shown against the average infection level for each experiment for Env (A) and NS3 (B). Dashed line denotes fold change = 1 to draw attention to the difference in scale for fold change across the RTKs.

performed on NS3+ infected cells and NS3- bystander cells (Figure 2.2C). Interestingly,

we found that the level of each RTK was significantly higher in infected cells compared to bystander cells. These results were consistent across biological replicates with varied infection rates (Supplementary Figure 2.2).

Surface levels of RTKs are also elevated in DENV-infected cells

RTKs can regulate infection either on the surface as a mediator of viral endocytosis or within the cell as a signal transducer. This led us to investigated whether RTK levels specifically at the cell surface was altered in infected cells. We probed 24 hr DENV-infected HepG2 cells for RTK prior to permeabilization such that only surface exposed RTK would be measured (Figure 2.3A). We compared the RTK MFI of bystander and infected cells for DENV Env (Figure 2.3B) and NS3 (Figure 2.3C). We found that, similar to what was observed for total RTK levels, the amount of RTK specifically at the surface was significantly increased in infected cells compared to bystander cells.

Knockdown of ERBB2, FGFR2, or IGF1R impairs DENV infection

Higher levels of RTK does not necessarily translate to a functional role, so we next determined if genetically reducing RTK levels affected DENV infection. We generated individual lentivirus clones carrying a puromycin selection marker and shRNA against RTK or scrambled shRNA, as a control (Supplementary Table 2.3). In total, we generated a single scrambled control clone and at least one clone for each RTK. We transduced HepG2 cells with each lentivirus clone and selected cells with puromycin for

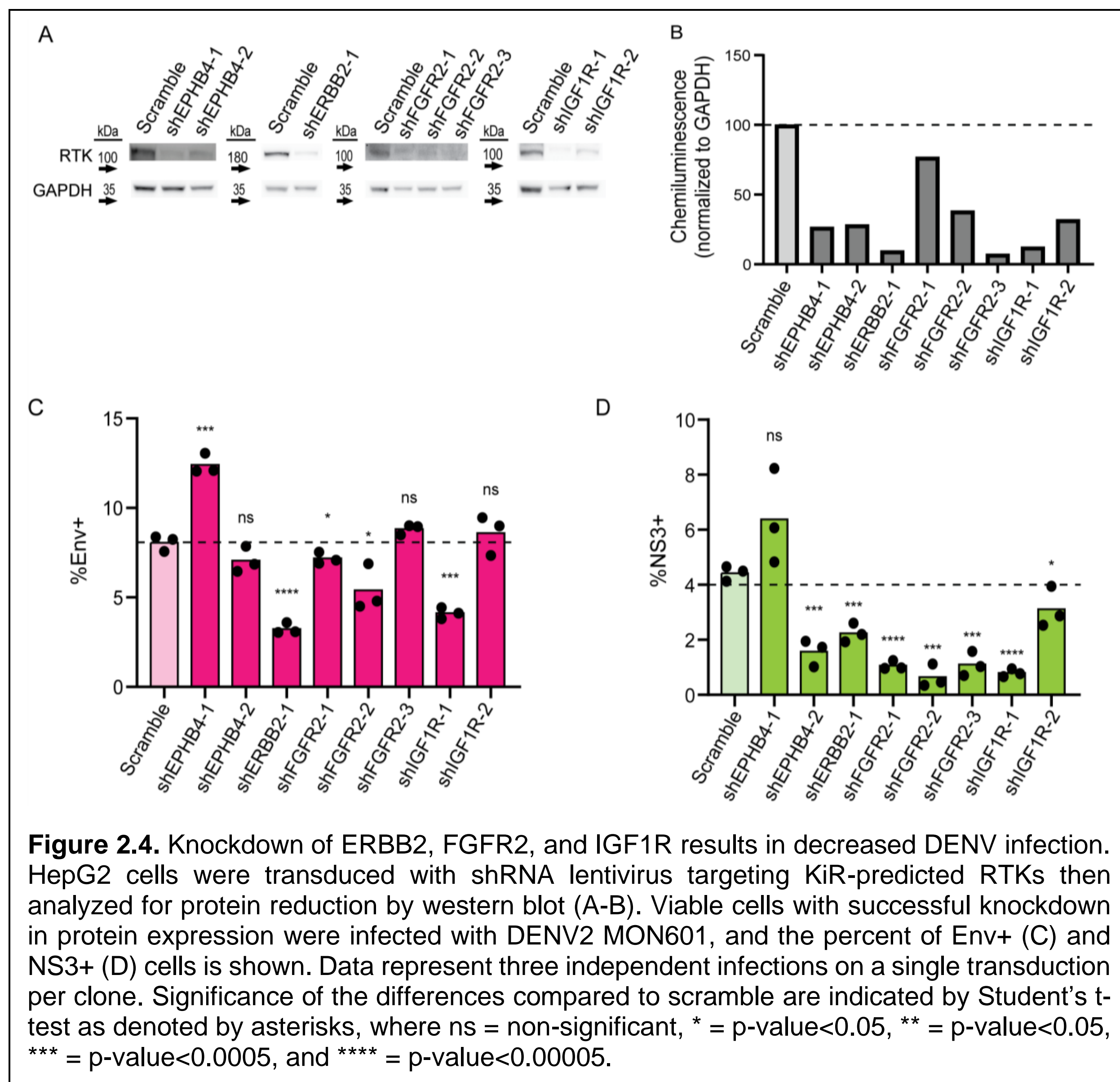


Figure 2.4. Knockdown of ERBB2, FGFR2, and IGF1R results in decreased DENV infection. HepG2 cells were transduced with shRNA lentivirus targeting KiR-predicted RTKs then analyzed for protein reduction by western blot (A-B). Viable cells with successful knockdown in protein expression were infected with DENV2 MON601, and the percent of Env+ (C) and NS3+ (D) cells is shown. Data represent three independent infections on a single transduction per clone. Significance of the differences compared to scramble are indicated by Student's t-test as denoted by asterisks, where ns = non-significant, * = p-value<0.05, ** = p-value<0.05, *** = p-value<0.0005, and **** = p-value<0.00005.

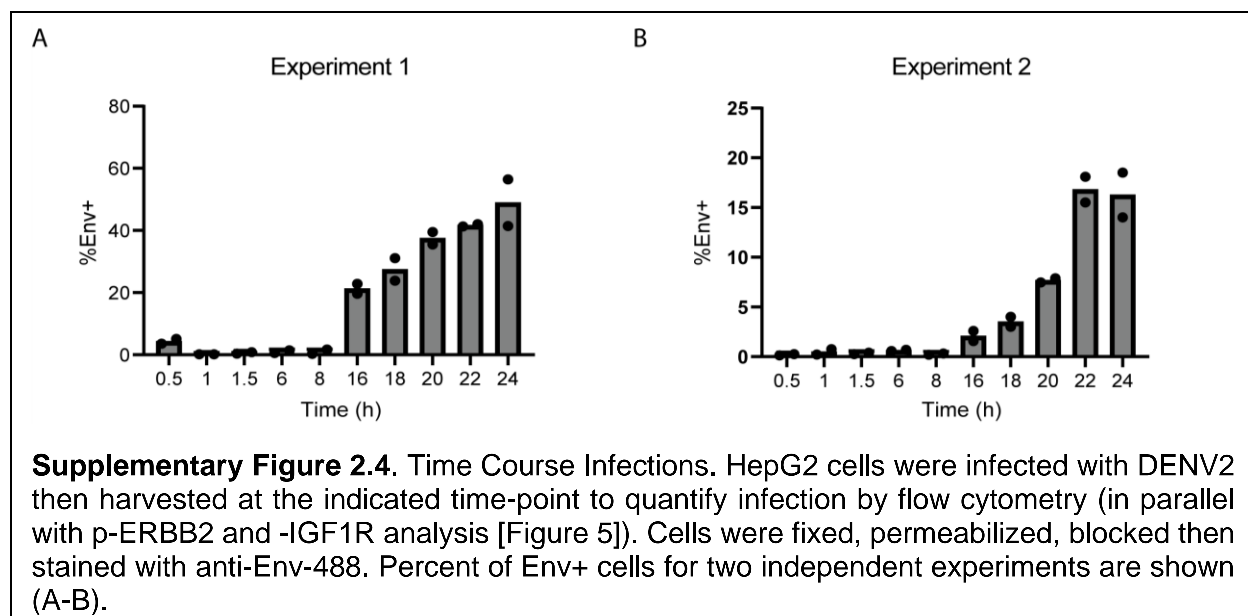
seven days. Cells with <10% loss in viability were analyzed by western blot to confirm protein knockdown. We successfully obtained one or more knockdown lines for EPHB4, ERBB2, FGFR2, and IGF1R (Figure 2.4A-B), but we were unable to procure knockdown lines for EPHA4, EPHB4, or RET, due to either loss in viability or observing no reduction in protein levels after transduction.

To test whether reduced protein levels of EPHB4, ERBB2, FGFR2, or IGF1R impacted infection, we infected control and knockdown cell lines with DENV2 MON601

for 24 h. We then quantified the percentage of Env+ or NS3+ cells by flow cytometry. We calculated infection in each knockdown line as a percentage of the scramble control (Figure 2.4C-D). Remarkably, both ERBB2 and FGFR2 knockdown led to significantly fewer Env- and NS3-positive cells in each cell line. One IGF1R knockdown line had significantly fewer Env- and NS3-positive cells while the other knockdown line only had significantly fewer NS3-positive cells. The effect of EPHB4 knockdown on infection varied, with one knockdown line having increased infection while the other knockdown line had decreased infection, making its role in infection unclear. Together, our data suggest that depletion of ERBB2, FGFR2 and IGF1R can interfere with DENV infection in hepatocytes.

DENV infection induces ERBB2 and IGF1R phosphorylation

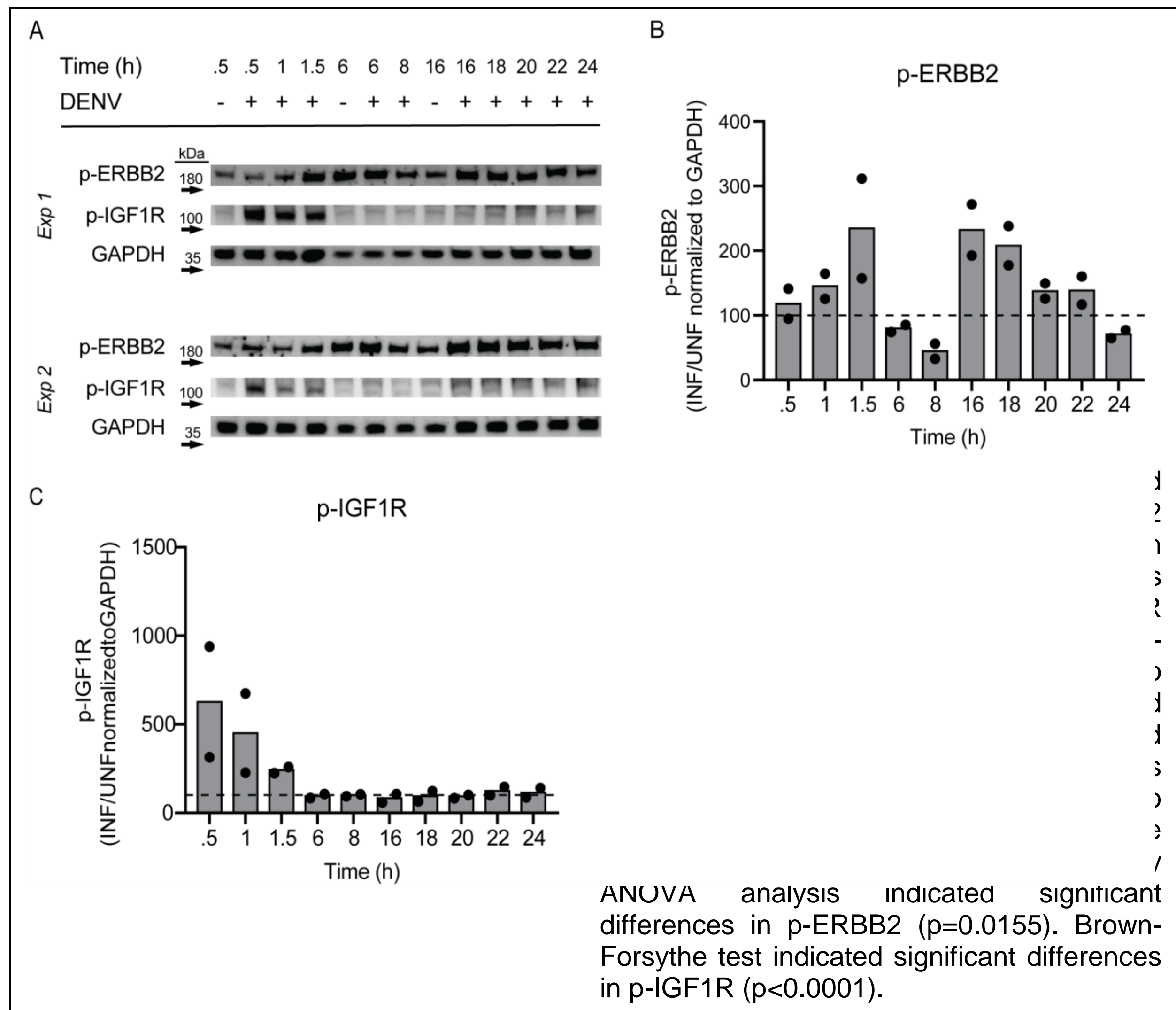
We next investigated if DENV infection impacts phosphorylation of the RTKs whose knockdown reduced infection. After infecting HepG2 cells with DENV2 MON601,



Supplementary Figure 2.4. Time Course Infections. HepG2 cells were infected with DENV2 then harvested at the indicated time-point to quantify infection by flow cytometry (in parallel with p-ERBB2 and -IGF1R analysis [Figure 5]). Cells were fixed, permeabilized, blocked then stained with anti-Env-488. Percent of Env+ cells for two independent experiments are shown (A-B).

we collected cell lysates at various time increments throughout infection. We probed cell lysates for p-ERBB2 (Thr686), p-IGF1R (Tyr1161/Tyr1165/Tyr1166), p-FGFR2 (Tyr653/654) and GAPDH; however, we did not detect a robust signal for phosphorylated FGFR2 in infected or uninfected cells (data not shown). Infection was also monitored at each time point to ensure robust infection (Supplementary Figure 2.4).

We observed elevated levels of phosphorylated ERBB2 and IGF1R with differing kinetic patterns during infection (Figure 2.5A-C). Interestingly, phosphorylated ERBB2 increased gradually from 0.5 to 1.5 hpi, having a greater than 2-fold increase at 1.5 hpi



ANOVA analysis indicated significant differences in p-ERBB2 (p=0.0155). Brown-Forsythe test indicated significant differences in p-IGF1R (p<0.0001).

on average, then declined from 6 to 8 hpi. ERBB2 phosphorylation was increased at 16 hpi but then gradually declined through 24 hpi. In contrast, phosphorylated IGF1R was induced from 0.5 to 1.5 hpi then returned to baseline for the remainder of infection. These kinetic differences in RTK activation suggest that different RTKs have roles at distinct stages of DENV infection in hepatocytes.

DISCUSSION

Dengue poses a significant global health threat. To mitigate this, we sought to identify host regulators of DENV infection to inform therapeutic interventions against DENV infection. We chose to focus on kinases in our study, as there is an extensive library of pharmacological tools to modulate their activity in the clinic and thus could provide useful therapeutic targets for dengue. Using kinase regression (KiR), an approach consisting of a small inhibitor screen and linear regression modeling, we predicted 36 kinases that could regulate DENV infection in HepG2 cells (Figure 2.1A-C). These 36 kinases, likely significant in various cell types due to the universality of kinase networks, represent potential targets for dengue interventions.

We narrowed our focus to a subset of the predicted kinases, the receptor tyrosine kinases (RTKs) – EPHA4, EPHB3, EPHB4, ERBB2, FGFR2, IGF1R, and RET – since many of these are targets of drugs already used in the clinic (Figure 2.1C). Interestingly, we discovered that DENV-infected HepG2 cells have elevated expression of both total and surface RTK (Figures 2.2-2.3). A limitation of this approach is that flow cytometric fluorescence measurements are not always linear and thus we cannot quantify the absolute magnitude of the change in levels of RTK between infected and uninfected

cells. Additionally, previous proteomics analysis of DENV-infected cells has demonstrated that many kinases are upregulated by infection, so being present at higher levels in infected cells may not be indicative of a role as a functional regulator of DENV [197; 298; 299; 300]. Given this, we investigated the impact of RTK knockdown on DENV infection. We observed that depletion of ERBB2 or FGFR2 led to reduced levels of both Env+ and NS3+ cells, suggesting that these RTKs can be targeted to interrupt DENV infection (Figure 2.4). The dependence of infection on IGF1R is unclear due to differences in the impact of one IGF1R knockdown line on Env+. Of note, the absolute value of Env+ and NS3+ cells was relatively low and thus an accurate biological interpretation of these data may be constrained. We are currently repeating this experiment with additional infection time-points and improved infection detection in an effort to strengthen our conclusions for manuscript resubmission.

Strikingly, we observe DENV infection leads to phosphorylation of ERBB2 and IGF1R at different times during infection (Figure 2.5). This is particularly intriguing given that our data indicated that no single kinase knockdown or kinase inhibitor can, on its own, completely abolish DENV infection of HepG2 cells (Figure 2.1, Figure 2.4), and previous studies have demonstrated that combination kinase inhibitor treatment leads to increased efficacy against DENV [253; 286]. Therefore, additional research is needed to examine the different functional kinetics of ERBB2 and IGF1R during DENV infection. One hypothesis is that IGF1R activity is solely important early during infection, while ERBB2 has roles throughout infection. In this case, combination treatment to block both IGF1R and ERBB2 could result in more robust inhibition than targeting either one individually.

Of note, IGF1R and ERBB2 drug combinations are already being investigated in the context of cancer [301; 302]. Additionally, both IGF1R and ERBB2 interact with other host factors known to play a role in dengue infection or pathogenesis, such as phosphoinositide 3-kinase (PI3K)/RAC-alpha serine threonine-protein kinase (AKT) [90; 169; 186; 187; 199; 200; 202; 303], proto-oncogene tyrosine-protein kinase Src [192; 219; 220; 304], and mitogen-activated protein kinases (ERK) [71; 183; 231; 258; 305; 306]. Thus, the activity of ERBB2 and IGF1R during DENV infection should be further investigated to understand their utility as dual targets of dengue therapeutics.

In addition to small molecule inhibitors, RTKs can also be targeted by monoclonal antibodies in the clinic [307; 308]. Elevated surface levels of RTKs in infected cells (Figure 2.3) suggests that they could have roles as receptors for viral entry, in which case blocking receptor interaction would inhibit DENV infection. If further investigation demonstrates their role as entry receptors, approved monoclonal antibodies such as Teprotumumab (IGF1R) and Trastuzumab (ERBB2) could prove useful for blocking DENV infection.

Importantly, since kinase inhibitors have the propensity to inhibit multiple kinases through polypharmacology, single kinase inhibitors that block multiple kinase regulators of dengue should also be explored. For instance, FGFR inhibitor Futibatinib has the potential to block both FGFR2 activity demonstrated in the present study (Figure 2.4) in addition to FGFR4 activity previously shown to regulate DENV infection [128]. Promiscuous inhibitors targeting a combination of kinases identified in this study and others could enhance anti-DENV activity without generating toxicity often observed in multi-drug regimens.

While we focused on only a subset of predicted kinases here, the remaining predictions are also promising as druggable regulators of DENV infection. A different down-selection strategy would be to investigate kinase interaction pathways that are statistically enriched for KiR-predicted or literature-reported kinases [309]. Additionally, how downstream targets of KiR predicted kinases (Figure 2.1D) may mediate detrimental DENV immune responses which could be therapeutically blocked with RTK-targeting drugs should be investigated. For instance, KiR predicted liver kinase B1 (LKB1) is part of the regulation pathway for alanine transaminase (ALT), a liver enzyme that is significantly elevated in severe dengue cases [13]. Inhibitor of nuclear factor kappa-B kinase subunit alpha (IKK-α) and NF-kappa-B-inducing kinase (NIK) are upstream regulators of TNFα which is implicated in DENV pathogenesis [310]. Additionally, rho associated coiled-coil containing protein kinase 1 (ROCK1), which is a target of Fasudil – used in the clinic for cerebral vasospasm – is known to regulate the migration and adhesion of inflammatory cells [245]. These examples highlight pathways that could be targeted to block infection while simultaneously preventing immune-mediated disease. The network analysis we utilize provides a framework for forming such hypotheses on the systematic mechanism of kinase involvement. Notably, it is possible for infection to rewire canonical kinase signaling pathways [311; 312; 313; 314], so taking an unbiased enrichment approach such as Kinase Enrichment Analysis 3 (KSEA3) may be useful in identifying novel pathways that result from infection [315; 316].

In conclusion, this study provides novel insight into kinase regulators of DENV infection and highlights the potential of receptor tyrosine kinases as therapeutic targets against dengue.

ACKNOWLEDGEMENTS

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Chapter 3

BRIDGING THE GAP TO THE CLINIC

Preface

In this chapter, I discuss how I would like the culmination of my dissertation work to impact the field. I suggest specific next steps which my work supports, and I describe my vision of how further work could ultimately put dengue drugs in the clinic.

As my previous chapters demonstrate, recent work and my own experimental research support the hypothesis that kinases are promising targets for dengue therapeutics. However, further efforts are needed to potentiate this line of work and ultimately decrease the burden of dengue.

Firstly, the limitations of my work should be considered when exploring immediate next steps. In addition to those discussed in Chapter 2, taking a broader perspective on the limitations of this research is important.

For instance, KiR is an excellent tool for specifically screening kinase activity, but it is only powered to investigate 300 kinases, which excludes over 200 kinases from assessment. These unassessed kinases could also be regulators of dengue. This highlights an important limitation of studying kinases in general: a portion of human kinases have not been well studied or do not have sufficient tools to explore [317]. When considering systematically investigating kinase cascades, it is vital to keep in mind that there may be important kinase interactions that evade detection by traditional tools.

It is also important to expand on the limitations of our investigation into the role of kinases in DENV infection by shRNA knockdown. We were unable to obtain knockdown lines of KiR-predicted RTKs EPHA4, EPHB3, EPHB4, or RET with the tools we employed. However, these kinases are still valuable predictions, since their expression levels are elevated in DENV-infected cells, and they are targets of drugs either already in the clinic or in the development pipeline. Therefore, refined attempts to investigate the effect of their knockdown on DENV infection should be pursued. On the other hand, while we showed that ERBB2 or FGFR2 knockdown decreases infection, we did not test

the effect of complementation of each gene in restoring infection. Therefore, it is possible that the reduction in infection observed was caused by an off-target effect of the shRNA rather than depletion of its intended RTK target. Determining if restoration of kinase levels by complementation returns infection levels to that of the scrambled control would provide confirmation that the effect on infection was directly due to respective RTK knockdown. An additional approach that would provide insight into the necessity of these RTKs on optimal infection would be to investigate infection in cells with catalytically inactive kinase knocked in. Not only would this enable further determination of the importance of RTKs, but it would also elucidate the importance of the kinase's phosphorylation activity versus the presence of its protein.

While these studies are critical to identify kinases required for optimal DENV infection, and thus illuminate candidates of kinase-targeted drugs against dengue, these mechanistic approaches do not recapitulate therapeutic strategies in the clinic; drugs in the clinic act on the functional activity of kinases through direct interaction at the protein level, rather than genetically depleting or substituting transcripts. Given this, there are specific *in vitro* experiments that I think should also be pursued to deepen our understanding of the potential efficacy of drugs targeting these RTKs.

When considering follow-up experiments, it is important to keep in mind that, as mentioned in Chapter 2, there are two types of RTK-targeting drugs: small molecule inhibitors and monoclonal antibodies. Inhibitors block the phosphorylation activity of RTKs while monoclonal antibodies bind RTKs at the cell surface. This is important because RTKs could be functioning at the cell surface as DENV entry receptors or as signal transducers of critical downstream cellular functions that support replication,

maturation, and egress. In fact, of the studies involving host factors mediating dengue highlighted in Chapter 1, (Supplementary Table 1.1, Table 1.1) some identified specific roles only during certain stages of infection [68; 69; 71; 89; 94; 95; 102; 103; 115; 121; 193; 219; 252; 253; 318]. Thus, insight would be gained on the optimal therapeutic strategy for each RTK by investigating the stage of DENV infection for which the RTKs are critical. We have demonstrated that phosphorylation of ERBB2 and IGF1R is induced at different times throughout infection (Figure 2.5), suggesting that IGF1R may only be important during the first 90 minutes of infection while ERBB2 may have roles throughout infection. This beckons the hypothesis that IGF1R's function in DENV infection is as a cell surface receptor whereas ERBB2 regulates infection through the maintained activation of signaling cascades.

To more precisely examine a differential role of RTKs during viral entry, a direct interaction of each RTK with DENV Env in the first 30 minutes of infection should be investigated, since it is known that Env mediates DENV entry during this time *in vitro* [69; 319]. For this, an Enzyme-Linked Immunosorbent Assay in which a plate coated with recombinant RTK is incubated with supernatant containing DENV could be utilized. Detecting DENV after washes that eliminate any non-specific interaction would indicate that the virus was able to bind to the RTK. One potential limitation of this approach is that weak interactions can be missed through the many wash steps of this methodology. Determining if RTKs are binding partners of DENV Env following co-immunoprecipitation from *in vitro* infection lysate would assess a more biologically intact system. However, this methodology does not enable distinguishing between direct or

indirect interaction. If RTKs are indirectly binding DENV Env, success of monoclonal antibody treatment to block interaction may not be as fruitful.

Alternatively, investigating the effect of blocking downstream substrates of RTKs on dengue infection would reveal if their primary involvement is through signaling cascades. For instance, targets of RTKs, such as those illustrated in Figure 2.1D, would likely be activated in infected cells. Assaying the phosphorylation status of RTK substrates and downstream effectors by western blot would enable this investigation. If results suggest that specific cascades are activated, interrogating infection in cells bearing knockdown of the key signal transducers would extend our understanding of their role for signaling during infection. Further delineation of what aspect of DENV reproduction the kinase cascade promotes would also provide valuable insight into therapeutic design. For instance, investigating differences in lipid sequestration, ER-Golgi transport, and production of infectious progeny could explain if replication, maturation, or egress is affected. These studies could aid in understanding which RTKs can be targeted to block specific aspects of infection, in addition to understanding how RTKs may be regulating the critical host factors identified in previous dengue studies (Supplementary Table 1.1, Figure 1.1).

Importantly, these experiments have the capacity to investigate how to block infection, but a successful dengue therapeutic must also prevent disease. To bolster the efficacy of kinase-targeting therapeutics in this way, it would be beneficial to perform KiR on disease phenotypes, thereby identifying kinases specifically mediating events that occur during severe dengue. KiR could be employed on the release of pro-inflammatory cytokines in DENV-infected monocytic cells or of aspartate transaminase

(AST), alanine transaminase (ALT), and adipokines in primary hepatocytes, all of which are elevated in severe dengue cases and can be measured *in vitro* [320; 321]. This could also assist in understanding differential or adjacent roles of kinases regulating viral load versus disease phenotypes which is important to strategize the best therapeutic regimen. Because of the suspected role of ADE in causing severe disease, KiR on infection in the presence of sub-neutralizing antibody is also of interest. Elucidation of kinases that are important in both DENV infection and disease would supply the field with a comprehensive list of candidate kinases for dengue therapeutics.

Ideally, clinically available promiscuous inhibitors that best collectively target these candidate kinases – and therefore are likely to interrupt multiple aspects of DENV infection – could be tested on dengue infection and disease. Additionally, barring drug toxicity, combinations of kinase inhibitors could also be investigated to achieve optimal coverage of multiple kinases regulating DENV infection and disease. A checkerboard synergy assay, which is useful in determining the impact of drug combinations on potency, could help identify kinase inhibitors that have a synergistic effect on hindering dengue [322]. Cepharanthine, a drug approved for a diversity of ailments (leukopenia, snake bites, xerostomia, alopecia), has exhibited broad antiviral activity through AMPK-modulated inflammatory responses – including reducing dengue infection and inflammation – and therefore represents a particularly exciting candidate for investigating combinatorial approaches [59; 294; 323; 324; 325].

Of course, another promising avenue for drug repurposing against dengue would be to test existing KiR-predicted RTK therapeutics.

Table 3.1 Drugs Targeting KiR-predicted RTKs			
Drug	**RTK**	**Pediatric Indication**	**Toxicity**
Abiraterone	EPHA4 [326]	NI	Cardiovascular [327]
Cyproheptadine		I	OD [328]
Ergoloid		U	-
Nilotinib		I	Cardiovascular, hepatic [329; 330]
Retapamulin		I	-
Afatinib	ERBB2	I	Cutaneous, hepatic, pulmonary [330; 331; 332]
Dacomitinib		U	Cutaneous, hepatic [330; 333]
Fam-trastuzumab deruxtecan		U	Cardiovascular, embryonic, GI, hematological, pulmonary [332; 334]
Hyaluronidase/trastuzumab		U	None [335]
Lapatinib		U	Cutaneous, GI, hepatic [330; 332; 336]
Margetuximab		U	Cardiovascular [332]
Neratinib		U	Embryonic, hepatic [330; 332]
Pertuzumab		U	Cardiovascular, embryonic [332]
Pyrotinib		U	Hepatic [337]
Trastuzumab		U	Cardiovascular, pulmonary [332]
Tucatinib		U	Embryonic, GI, hepatic [330; 338]
Erdafitinib	FGFR2	U	Hepatic, ocular [330; 339]
Futibatinib		U	Ocular [340]
Infigratinib		U	Embryonic, hepatic, ocular [330; 341]
Pemigatinib		U	Cutaneous, GI, hepatic, ocular [330; 342]
Regorafenib	FGFR2, RET	U	Cutaneous, GI, hepatic [330; 343]
Ceritinib	IGF1R	U	Cardiovascular, GI hepatic [330; 344]
Teprotumumab-trbw		U	GI [345]
Alectinib	RET	U	Cardiovascular [346]
Cabozantinib		I	GI, hepatic [330; 347]
Lenvatinib		U	GI, hepatic [330; 348]

Ponatinib		U	Cardiovascular, hepatic [329; 330]
Pralsetinib		>11 yo	Hematological, hepatic [330; 349]
Selpercatinib		>11 yo	GI, hepatic [330; 350]
Sunitinib		U	GI, hepatic [330; 351]
Vandetanib		I	Cutaneous, GI, hepatic [330; 352]

Abbreviations: NI: not indicated; I: indicated; U: unknown if safe and effective; yo: years old; OD: toxicity only in the case of overdose; -: no toxicity information available; GI: gastrointestinal.

Table 3.1 summarizes these along with their utility in children; because dengue infection results in more severe disease in children in several studies [353; 354; 355; 356; 357; 358], selecting drugs for testing with minimal toxicity is vital. In this table, drugs listed for EPHA4 have exhibited inhibition separately from their FDA-approved use. As shown, toxicity is possible for many of these KiR-predicted RTK therapeutics, although most are mild or rare. Further delineation of which drugs have the least toxicity, particularly in children, should be done when considering building a panel to test against dengue.

Incorporating kinase-targeted therapeutics with direct antivirals is likely to boost the efficacy of kinase-directed interventions. Identifying synergism of kinase inhibitors or monoclonal antibodies with available broad-spectrum antivirals such as the viral polymerase inhibitor Favipiravir and its derivatives, which have demonstrated anti-dengue activity, is another critical avenue to explore [359; 360]. As potent treatment regimens are revealed, serial viral passage in the presence of treatment regimens should be undertaken to test for the capacity of driving drug resistance, and drugs which promote resistance should be eliminated.

Testing promising inhibitor combinations in clinically relevant models will be the best way to understand the utility of kinase-directed drugs against dengue. There are

several animal models that have historically been used to evaluate different aspects of dengue infection, including mice, non-human primates, the Yucatan miniature pig, and the tree shrew (reviewed in [361]). There are both immunocompetent and immunodeficient murine models for dengue, where mice with relatively intact immune responses (C57BL/6, BALB/c) would prove useful in the study of how treatment modulates dengue-induced immune activity, while mice that do not mount a robust immune response (AG129, IFNAR-/-) can be used to test an effect on lethal dengue disease and ADE. Importantly, while immunodeficient mice enable testing for efficacy against lethal disease, they harbor the major limitation of not having the immune response on which HDTs are likely to function, thus the efficacy of kinase-targeted interventions could be overlooked in this model. Humanized mice, on the other hand, more closely recapitulate the immune response and clinical manifestations seen in humans and thus may be more useful for drug testing of dengue. Non-human primates, although more costly, classically represent a more physiologically similar system to humans for testing infection and disease. Rhesus macaques, bonnet macaques, marmosets, and chimpanzees have more similar infection course, viremia, and immune response to humans than mice. However, these monkeys do not undergo clinical dengue disease and therefore could not be used to specifically test the efficacy of kinase-targeted drug interventions on preventing severe dengue. Excitingly, preliminary studies in the Yucatan miniature pig and the tree shrew also demonstrate greater physiological similarity to humans than rodents *and* exhibit some disease manifestations observed in humans. However, further work is needed to validate these models for drug testing for dengue.

Despite this lack of an ideal animal model for comprehensively studying infection and disease, these models can still be used to provide valuable insight into whether specific kinase-directed drugs control infection and beneficially modulate pathogenesis. As part of these studies, it will be critical to investigate the effects of kinase-targeted drugs on each DENV serotype and on ADE infection. Similarly, because children are more at risk of severe disease manifestations, testing for sustained efficacy in a pediatric dengue model, such as C57BL/6 suckling mice, is critical [362].

Finally, when considering an even broader impact of my work and the next steps, it is particularly interesting that kinase regulators of infection have the potential to be shared across many diseases (appendix, [65]). Extending this work to other pathogens, particularly the other flaviviruses, could elucidate central kinase pathways that universally get utilized or rewired during infection and disease.

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Diagrams used in this work were adapted from BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

REFERENCES

[1] L. Cattarino, I. Rodriguez-Barraquer, N. Imai, D.A.T. Cummings, and N.M. Ferguson, Mapping global variation in dengue transmission intensity. Science Translational Medicine 12 (2020) eaax4144.
[2] Z. Zeng, J. Zhan, L. Chen, H. Chen, and S. Cheng, Global, regional, and national dengue burden from 1990 to 2017: A systematic analysis based on the global burden of disease study 2017. EClinicalMedicine 32 (2021) 100712.
[3] S. Bhatt, P.W. Gething, O.J. Brady, J.P. Messina, A.W. Farlow, C.L. Moyes, J.M. Drake, J.S. Brownstein, A.G. Hoen, O. Sankoh, M.F. Myers, D.B. George, T. Jaenisch, G.R.W. Wint, C.P. Simmons, T.W. Scott, J.J. Farrar, and S.I. Hay, The global distribution and burden of dengue. Nature 496 (2013) 504-507.
[4] J.P. Messina, O.J. Brady, N. Golding, M.U.G. Kraemer, G.R.W. Wint, S.E. Ray, D.M. Pigott, F.M. Shearer, K. Johnson, L. Earl, L.B. Marczak, S. Shirude, N. Davis Weaver, M. Gilbert, R. Velayudhan, P. Jones, T. Jaenisch, T.W. Scott, R.C. Reiner, and S.I. Hay, The current and future global distribution and population at risk of dengue. Nature Microbiology 4 (2019) 1508-1515.
[5] A. Anwar, N. Khan, M. Ayub, F. Nawaz, A. Shah, and A. Flahault, Modeling and Predicting Dengue Incidence in Highly Vulnerable Countries using Panel Data Approach. Int J Environ Res Public Health 16 (2019).
[6] J.P. Messina, O.J. Brady, D.M. Pigott, N. Golding, M.U.G. Kraemer, T.W. Scott, G.R.W. Wint, D.L. Smith, and S.I. Hay, The many projected futures of dengue. Nature Reviews Microbiology 13 (2015) 230-239.
[7] S. Nasar, N. Rashid, and S. Iftikhar, Dengue proteins with their role in pathogenesis, and strategies for developing an effective anti-dengue treatment: A review. J Med Virol 92 (2020) 941-955.
[8] F. Begum, S. Das, D. Mukherjee, S. Mal, and U. Ray, Insight into the Tropism of Dengue Virus in Humans. Viruses 11 (2019).
[9] B.H. Kok, H.T. Lim, C.P. Lim, N.S. Lai, C.Y. Leow, and C.H. Leow, Dengue virus infection - a review of pathogenesis, vaccines, diagnosis and therapy. Virus Res 324 (2023) 199018.
[10] A. Tayal, S.K. Kabra, and R. Lodha, Management of Dengue: An Updated Review. Indian Journal of Pediatrics 90 (2023) 168-177.
[11] M.F. Lee, G.Z. Voon, H.X. Lim, M.L. Chua, and C.L. Poh, Innate and adaptive immune evasion by dengue virus. Front Cell Infect Microbiol 12 (2022) 1004608.
[12] C. Fernandes-Santos, and E.L. Azeredo, Innate Immune Response to Dengue Virus: Toll-like Receptors and Antiviral Response. Viruses 14 (2022).
[13] K. Yuan, Y. Chen, M. Zhong, Y. Lin, and L. Liu, Risk and predictive factors for severe dengue infection: A systematic review and meta-analysis. PLOS ONE 17 (2022) e0267186.
[14] S. Sivasubramanian, S. Mohandas, V. Gopalan, K. Govindan, P. Varadarajan, K. Kaveri, and K.M. Ramkumar, Serum levels of matrix metalloproteinases as prognostic markers for severe dengue with plasma leakage. Exp Mol Pathol 128 (2022) 104821.

[15] S. Mukherjee, B. Saha, and A. Tripathi, Clinical significance of differential serum-signatures for early prediction of severe dengue among Eastern Indian patients. Clin Exp Immunol 208 (2022) 72-82.
[16] J.D. Nanda, C.J. Jung, R.D. Satria, M.K. Jhan, T.J. Shen, P.C. Tseng, Y.T. Wang, T.S. Ho, and C.F. Lin, Serum IL-18 Is a Potential Biomarker for Predicting Severe Dengue Disease Progression. J Immunol Res 2021 (2021) 7652569.
[17] P.C. Sigera, R. Amarasekara, C. Rodrigo, S. Rajapakse, P. Weeratunga, N.L. De Silva, C.H. Huang, M.K. Sahoo, B.A. Pinsky, D.R. Pillai, H.A. Tissera, S. Jayasinghe, S. Handunnetti, and S.D. Fernando, Risk prediction for severe disease and better diagnostic accuracy in early dengue infection; the Colombo dengue study. BMC Infect Dis 19 (2019) 680.
[18] R. Narayan, and S. Tripathi, Intrinsic ADE: The Dark Side of Antibody Dependent Enhancement During Dengue Infection. Front Cell Infect Microbiol 10 (2020) 580096.
[19] L.C. Katzelnick, L. Gresh, M.E. Halloran, J.C. Mercado, G. Kuan, A. Gordon, A. Balmaseda, and E. Harris, Antibody-dependent enhancement of severe dengue disease in humans. Science 358 (2017) 929-932.
[20] D.H. Libraty, T.P. Endy, H.-S.H. Houng, S. Green, S. Kalayanarooj, S. Suntayakorn, W. Chansiriwongs, D.W. Vaughn, A. Nisalak, F.A. Ennis, and A.L. Rothman, Differing Influences of Virus Burden and Immune Activation on Disease Severity in Secondary Dengue-3 Virus Infections. The Journal of Infectious Diseases 185 (2002) 1213-1221.
[21] J.G. Low, A. Ong, L.K. Tan, S. Chaterji, A. Chow, W.Y. Lim, K.W. Lee, R. Chua, C.R. Chua, S.W. Tan, Y.B. Cheung, M.L. Hibberd, S.G. Vasudevan, L.C. Ng, Y.S. Leo, and E.E. Ooi, The early clinical features of dengue in adults: challenges for early clinical diagnosis. PLoS Negl Trop Dis 5 (2011) e1191.
[22] G.N. Malavige, C. Jeewandara, and G.S. Ogg, Dysfunctional Innate Immune Responses and Severe Dengue. Front Cell Infect Microbiol 10 (2020) 590004.
[23] A. Lenhart, A.C. Morrison, V.A. Paz-Soldan, B.M. Forshey, J.J. Cordova-Lopez, H. Astete, J.P. Elder, M. Sihuincha, E.E. Gotlieb, E.S. Halsey, T.J. Kochel, T.W. Scott, N. Alexander, and P.J. McCall, The impact of insecticide treated curtains on dengue virus transmission: A cluster randomized trial in Iquitos, Peru. PLOS Neglected Tropical Diseases 14 (2020) e0008097.
[24] L.R. Bowman, S. Donegan, and P.J. McCall, Is Dengue Vector Control Deficient in Effectiveness or Evidence?: Systematic Review and Meta-analysis. PLoS Negl Trop Dis 10 (2016) e0004551.
[25] A.B.B. Wilke, J.C. Beier, and G. Benelli, Complexity of the relationship between global warming and urbanization – an obscure future for predicting increases in vector-borne infectious diseases. Current Opinion in Insect Science 35 (2019) 1-9.
[26] J. Bigay, R. Le Grand, F. Martinon, and P. Maisonnasse, Vaccine-associated enhanced disease in humans and animal models: Lessons and challenges for vaccine development. Front Microbiol 13 (2022) 932408.
[27] S.J. Thomas, and I.-K. Yoon, A review of Dengvaxia®: development to deployment. Human Vaccines & Immunotherapeutics 15 (2019) 2295-2314.

[28] D. The Lancet Infectious, Infectious disease crisis in the Philippines. Lancet Infect Dis 19 (2019) 1265.
[29] J. Park, J. Kim, and Y.-S. Jang, Current status and perspectives on vaccine development against dengue virus infection. Journal of Microbiology 60 (2022) 247-254.
[30] D. Tully, and C.L. Griffiths, Dengvaxia: the world's first vaccine for prevention of secondary dengue. Ther Adv Vaccines Immunother 9 (2021) 25151355211015839.
[31] H. Salje, M.T. Alera, M.N. Chua, T. Hunsawong, D. Ellison, A. Srikiatkhachorn, R.G. Jarman, G.D. Gromowski, I. Rodriguez-Barraquer, S. Cauchemez, D.A.T. Cummings, L. Macareo, I.-K. Yoon, S. Fernandez, and A.L. Rothman, Evaluation of the extended efficacy of the Dengvaxia vaccine against symptomatic and subclinical dengue infection. Nature Medicine 27 (2021) 1395-1400.
[32] J.M. Torres-Flores, A. Reyes-Sandoval, and M.I. Salazar, Dengue Vaccines: An Update. BioDrugs 36 (2022) 325-336.
[33] K. Fatima, and N.I. Syed, Dengvaxia controversy: impact on vaccine hesitancy. J Glob Health 8 (2018) 010312.
[34] A.M. Bifani, K.W.K. Chan, D. Borrenberghs, M.J.A. Tan, W.W. Phoo, S. Watanabe, O. Goethals, S.G. Vasudevan, and M.M. Choy, Therapeutics for flaviviral infections. Antiviral Research 210 (2023) 105517.
[35] M. Palanichamy Kala, A.L. St John, and A.P.S. Rathore, Dengue: Update on Clinically Relevant Therapeutic Strategies and Vaccines. Curr Treat Options Infect Dis 15 (2023) 27-52.
[36] N.M. Nguyen, C.N. Tran, L.K. Phung, K.T. Duong, A. Huynh Hle, J. Farrar, Q.T. Nguyen, H.T. Tran, C.V. Nguyen, L. Merson, L.T. Hoang, M.L. Hibberd, P.P. Aw, A. Wilm, N. Nagarajan, D.T. Nguyen, M.P. Pham, T.T. Nguyen, H. Javanbakht, K. Klumpp, J. Hammond, R. Petric, M. Wolbers, C.T. Nguyen, and C.P. Simmons, A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. J Infect Dis 207 (2013) 1442-50.
[37] O.V. Maltsev, K.V. Kasyanenko, K.V. Zhdanov, N.A. Malyshev, E.V. Kolomoets, and V.K. Konomou, [The experience in treatment of dengue fever using antiviral drug riamilovir in the Republic of Guinea (case report)]. Ter Arkh 95 (2023) 85-89.
[38] O. Goethals, S.J.F. Kaptein, B. Kesteleyn, J.F. Bonfanti, L. Van Wesenbeeck, D. Bardiot, E.J. Verschoor, B.E. Verstrepen, Z. Fagrouch, J.R. Putnak, D. Kiemel, O. Ackaert, R. Straetemans, S. Lachau-Durand, P. Geluykens, M. Crabbe, K. Thys, B. Stoops, O. Lenz, L. Tambuyzer, S. De Meyer, K. Dallmeier, M.K. McCracken, G.D. Gromowski, W. Rutvisuttinunt, R.G. Jarman, N. Karasavvas, F. Touret, G. Querat, X. de Lamballerie, L. Chatel-Chaix, G.N. Milligan, D.W.C. Beasley, N. Bourne, A.D.T. Barrett, A. Marchand, T.H.M. Jonckers, P. Raboisson, K. Simmen, P. Chaltin, R. Bartenschlager, W.M. Bogers, J. Neyts, and M. Van Loock, Blocking NS3-NS4B interaction inhibits dengue virus in non-human primates. Nature 615 (2023) 678-686.
[39] K.K. Roy, D. Jyothi, U. Paul, and S. Sukla, Identification and validation of novel non-nucleoside class of molecules inhibiting the dengue virus replication. J Biomol Struct Dyn (2023) 1-10.

[40] T. Delgado-Maldonado, A. Moreno-Herrera, G. Pujadas, L.K. Vázquez-Jiménez, A. González-González, and G. Rivera, Recent advances in the development of methyltransferase (MTase) inhibitors against (re)emerging arboviruses diseases dengue and Zika. Eur J Med Chem 252 (2023) 115290.
[41] M. Feracci, C. Eydoux, V. Fattorini, L. Lo Bello, P. Gauffre, B. Selisko, P. Sutto-Ortiz, A. Shannon, H. Xia, P.Y. Shi, M. Noel, F. Debart, J.J. Vasseur, S. Good, K. Lin, A. Moussa, J.P. Sommadossi, A. Chazot, K. Alvarez, J.C. Guillemot, E. Decroly, F. Ferron, and B. Canard, AT-752 targets multiple sites and activities on the Dengue virus replication enzyme NS5. Antiviral Res 212 (2023) 105574.
[42] S. Behrouz, N. Kühl, and C.D. Klein, N-sulfonyl peptide-hybrids as a new class of dengue virus protease inhibitors. Eur J Med Chem 251 (2023) 115227.
[43] M. Del Rosario García-Lozano, F. Dragoni, P. Gallego, S. Mazzotta, A. López-Gómez, A. Boccuto, C. Martínez-Cortés, A. Rodríguez-Martínez, H. Pérez-Sánchez, J. Manuel Vega-Pérez, J. Antonio Del Campo, I. Vicenti, M. Vega-Holm, and F. Iglesias-Guerra, Piperazine-derived small molecules as potential Flaviviridae NS3 protease inhibitors. In vitro antiviral activity evaluation against Zika and Dengue viruses. Bioorg Chem 133 (2023) 106408.
[44] Y.J. An, S.M. Choi, E.R. Choi, Y.E. Nam, E.W. Seo, S.B. Ahn, Y. Jang, M. Kim, and J.H. Cho, Synthesis and biological evaluation of new β-D-N(4)-hydroxycytidine analogs against SARS-CoV-2, influenza viruses and DENV-2. Bioorg Med Chem Lett 83 (2023) 129174.
[45] S. Boonyasuppayakorn, T. Saelee, T.N.T. Huynh, R. Hairani, K. Hengphasatporn, N. Loeanurit, V. Cao, V. Vibulakhaophan, P. Siripitakpong, P. Kaur, J.J.H. Chu, C. Tunghirun, O. Choksupmanee, S. Chimnaronk, Y. Shigeta, T. Rungrotmongkol, and W. Chavasiri, The 8-bromobaicalein inhibited the replication of dengue, and Zika viruses and targeted the dengue polymerase. Sci Rep 13 (2023) 4891.
[46] V. Jarerattanachat, C. Boonarkart, S. Hannongbua, P. Auewarakul, and R. Ardkhean, In silico and in vitro studies of potential inhibitors against Dengue viral protein NS5 Methyl Transferase from Ginseng and Notoginseng. J Tradit Complement Med 13 (2023) 1-10.
[47] Y. Feng, Y. Yang, S. Zou, S. Qiu, H. Yang, Y. Hu, G. Lin, X. Yao, S. Liu, and M. Zou, Identification of alpha-linolenic acid as a broad-spectrum antiviral against zika, dengue, herpes simplex, influenza virus and SARS-CoV-2 infection. Antiviral Res 216 (2023) 105666.
[48] B. Kesteleyn, D. Bardiot, J.F. Bonfanti, B. De Boeck, O. Goethals, S.J.F. Kaptein, B. Stoops, E. Coesemans, J. Fortin, P. Muller, F. Doublet, G. Carlens, M. Koukni, W. Smets, P. Raboisson, P. Chaltin, K. Simmen, M. Van Loock, J. Neyts, A. Marchand, and T.H.M. Jonckers, Discovery of Acyl-Indole Derivatives as Pan-Serotype Dengue Virus NS4B Inhibitors. J Med Chem 66 (2023) 8808-8821.
[49] B. Yi, B.X.Z. Chew, H. Chen, R.C.H. Lee, Y.D. Fong, W.X. Chin, C.K. Mok, and J.J.H. Chu, Antiviral Activity of Catechin against Dengue Virus Infection. Viruses 15 (2023).
[50] M. Mukhtar, H.A. Khan, and N. Zaidi, Exploring the inhibitory potential of Nigella sativa against dengue virus NS2B/NS3 protease and NS5 polymerase using computational approaches. RSC Adv 13 (2023) 18306-18322.

[51] L.Z. Shi, X. Chen, H.H. Cao, C.Y. Tian, L.F. Zou, J.H. Yu, Z.B. Lu, W. Zhao, J.S. Liu, and L.Z. Yu, N-Butanol Extract of Glycyrrhizae Radix et Rhizoma Inhibits Dengue Virus through Targeting Envelope Protein. Pharmaceuticals (Basel) 16 (2023).
[52] C.R.A. Samy, K. Karunanithi, J. Sheshadhri, M. Rengarajan, P. Srinivasan, and P. Cherian, (R)-(+)-Rosmarinic Acid as an Inhibitor of Herpes and Dengue Virus Replication: an In Silico Assessment. Rev Bras Farmacogn 33 (2023) 543-550.
[53] K.A. Yamamoto, K. Blackburn, M.B. Goshe, D.T. Brown, E. Migoswski, I.B. Campanhon, M.F. Moreira, D.F. Ferreira, and M.R. Soares, Tizoxanide Antiviral Activity on Dengue Virus Replication. Viruses 15 (2023).
[54] R.K. Joshi, S. Agarwal, P. Patil, K. Alagarasu, K. Panda, S. Cherian, D. Parashar, and S. Roy, Anti-Dengue Activity of Lipophilic Fraction of Ocimum basilicum L. Stem. Molecules 28 (2023).
[55] W. Wu, R. Chen, Y. Wan, L. Li, J. Han, Q. Lei, Z. Chen, S. Liu, and X. Yao, Acetyl-CoA Carboxylase (ACC) Inhibitor, CP640186, Effectively Inhibited Dengue Virus (DENV) Infection via Regulating ACC Phosphorylation. Molecules 27 (2022).
[56] M. Thursz, F. Sadiq, J.A. Tree, P. Karayiannis, D.W.C. Beasley, W. Dejnirattisai, J. Mongkolsapaya, G. Screaton, M. Wand, M.J. Elmore, M.W. Carroll, I. Matthews, and H. Thomas, Inhibition of phosphodiesterase 12 results in antiviral activity against several RNA viruses including SARS-CoV-2. J Gen Virol 104 (2023).
[57] V.C. Roa-Linares, M. Escudero-Flórez, M. Vicente-Manzanares, and J.C. Gallego-Gómez, Host Cell Targets for Unconventional Antivirals against RNA Viruses. Viruses 15 (2023).
[58] T. Felicetti, C.P. Gwee, M.S. Burali, K.W.K. Chan, S. Alonso, M.C. Pismataro, S. Sabatini, M.L. Barreca, V. Cecchetti, S.G. Vasudevan, and G. Manfroni, Functionalized sulfonyl anthranilic acid derivatives inhibit replication of all the four dengue serotypes. Eur J Med Chem 252 (2023) 115283.
[59] C. Bailly, Cepharanthine: An update of its mode of action, pharmacological properties and medical applications. Phytomedicine 62 (2019) 152956.
[60] M.O. Pohl, L. Martin-Sancho, R. Ratnayake, K.M. White, L. Riva, Q.Y. Chen, G. Lieber, I. Busnadiego, X. Yin, S. Lin, Y. Pu, L. Pache, R. Rosales, M. Déjosez, Y. Qin, P.D. De Jesus, A. Beall, S. Yoh, B.G. Hale, T.P. Zwaka, N. Matsunaga, A. García-Sastre, S. Stertz, S.K. Chanda, and H. Luesch, Sec61 Inhibitor Apratoxin S4 Potently Inhibits SARS-CoV-2 and Exhibits Broad-Spectrum Antiviral Activity. ACS Infect Dis 8 (2022) 1265-1279.
[61] V.V. Costa, M.A. Sugimoto, J. Hubner, C.S. Bonilha, C.M. Queiroz-Junior, M.H. Gonçalves-Pereira, J. Chen, T. Gobbetti, G.O. Libanio Rodrigues, J.L. Bambirra, I.B. Passos, C.E. Machado Lopes, T.P. Moreira, K. Bonjour, R.C.N. Melo, M.A.P. Oliveira, M.V.M. Andrade, L.P. Sousa, D.G. Souza, H.D.C. Santiago, M. Perretti, and M.M. Teixeira, Targeting the Annexin A1-FPR2/ALX pathway for host-directed therapy in dengue disease. Elife 11 (2022).
[62] O. Ackaert, F. Vanhoutte, N. Verpoorten, A. Buelens, S. Lachau-Durand, L. Lammens, R. Hoetelmans, M. Van Loock, and G. Herrera-Taracena, Safety, tolerability and pharmacokinetics of JNJ-1802, a pan-serotype dengue direct antiviral small molecule, in a Phase 1, double-blind, randomized, dose-escalation study in healthy volunteers. Clin Infect Dis (2023).

[63] S. Chaudhuri, J.A. Symons, and J. Deval, Innovation and trends in the development and approval of antiviral medicines: 1987-2017 and beyond. Antiviral Res 155 (2018) 76-88.
[64] D.C. Jones, E.L. LaMartina, J.R. Lewis, A.J. Dahl, N. Nadig, A. Szabo, R.J. Newton, and T.A. Skwor, One Health and Global Health View of Antimicrobial Susceptibility through the "Eye" of Aeromonas: Systematic Review and Meta-Analysis. Int J Antimicrob Agents (2023) 106848.
[65] K. Vijayan, L. Wei, E.K.K. Glennon, C. Mattocks, N. Bourgeois, B. Staker, and A. Kaushansky, Host-targeted Interventions as an Exciting Opportunity to Combat Malaria. Chem Rev 121 (2021) 10452-10468.
[66] S.K. Roy, and S. Bhattacharjee, Dengue virus: epidemiology, biology, and disease aetiology. Can J Microbiol 67 (2021) 687-702.
[67] Y. Chen, T. Maguire, R.E. Hileman, J.R. Fromm, J.D. Esko, R.J. Linhardt, and R.M. Marks, Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. Nat Med 3 (1997) 866-71.
[68] P. Che, H. Tang, and Q. Li, The interaction between claudin-1 and dengue viral prM/M protein for its entry. Virology 446 (2013) 303-13.
[69] O. Dejarnac, M.L. Hafirassou, M. Chazal, M. Versapuech, J. Gaillard, M. Perera-Lecoin, C. Umana-Diaz, L. Bonnet-Madin, X. Carnec, J.Y. Tinevez, C. Delaugerre, O. Schwartz, P. Roingeard, N. Jouvenet, C. Berlioz-Torrent, L. Meertens, and A. Amara, TIM-1 Ubiquitination Mediates Dengue Virus Entry. Cell Rep 23 (2018) 1779-1793.
[70] Z. Xia, Y. Ren, S. Li, J. Xu, Y. Wu, and Z. Cao, ML-SA1 and SN-2 inhibit endocytosed viruses through regulating TRPML channel expression and activity. Antiviral Res 195 (2021) 105193.
[71] M. Liang, Y. Li, K. Zhang, Y. Zhu, J. Liang, M. Liu, S. Zhang, D. Chen, H. Liang, L. Liang, S. An, X. Zhu, and Z. He, Host factor DUSP5 potently inhibits dengue virus infection by modulating cytoskeleton rearrangement. Antiviral Res 215 (2023) 105622.
[72] M. Laureti, D. Narayanan, J. Rodriguez-Andres, J.K. Fazakerley, and L. Kedzierski, Flavivirus Receptors: Diversity, Identity, and Cell Entry. Front Immunol 9 (2018) 2180.
[73] A. Brugier, M.L. Hafirrassou, M. Pourcelot, M. Baldaccini, V. Kril, L. Couture, B.M. Kümmerer, S. Gallois-Montbrun, L. Bonnet-Madin, P.O. Vidalain, C. Delaugerre, S. Pfeffer, L. Meertens, and A. Amara, RACK1 Associates with RNA-Binding Proteins Vigilin and SERBP1 to Facilitate Dengue Virus Replication. J Virol 96 (2022) e0196221.
[74] K. Wang, J. Wang, T. Sun, G. Bian, W. Pan, T. Feng, P. Wang, Y. Li, and J. Dai, Glycosphingolipid GM3 is Indispensable for Dengue Virus Genome Replication. Int J Biol Sci 12 (2016) 872-83.
[75] P. Krishnamoorthy, A.S. Raj, P. Kumar, N. Das, and H. Kumar, Host and viral non-coding RNAs in dengue pathogenesis. Rev Med Virol 32 (2022) e2360.
[76] M. Ferrari, A. Zevini, E. Palermo, M. Muscolini, M. Alexandridi, M.P. Etna, E.M. Coccia, A. Fernandez-Sesma, C. Coyne, D.D. Zhang, E.T.A. Marques, D. Olagnier, and J. Hiscott, Dengue Virus Targets Nrf2 for NS2B3-Mediated

Degradation Leading to Enhanced Oxidative Stress and Viral Replication. J Virol 94 (2020).
[77] L.N. Carpp, R.S. Rogers, R.L. Moritz, and J.D. Aitchison, Quantitative proteomic analysis of host-virus interactions reveals a role for Golgi brefeldin A resistance factor 1 (GBF1) in dengue infection. Mol Cell Proteomics 13 (2014) 2836-54.
[78] N.G. Iglesias, J.A. Mondotte, L.A. Byk, F.A. De Maio, M.M. Samsa, C. Alvarez, and A.V. Gamarnik, Dengue Virus Uses a Non-Canonical Function of the Host GBF1-Arf-COPI System for Capsid Protein Accumulation on Lipid Droplets. Traffic 16 (2015) 962-77.
[79] A.T. Navare, F.D. Mast, J.P. Olivier, T. Bertomeu, M.L. Neal, L.N. Carpp, A. Kaushansky, J. Coulombe-Huntington, M. Tyers, and J.D. Aitchison, Viral protein engagement of GBF1 induces host cell vulnerability through synthetic lethality. J Cell Biol 221 (2022).
[80] S.T. Chen, Y.L. Lin, M.T. Huang, M.F. Wu, S.C. Cheng, H.Y. Lei, C.K. Lee, T.W. Chiou, C.H. Wong, and S.L. Hsieh, CLEC5A is critical for dengue-virus-induced lethal disease. Nature 453 (2008) 672-6.
[81] H. Ye, X. Duan, M. Yao, L. Kang, Y. Li, S. Li, B. Li, and L. Chen, USP18 Mediates Interferon Resistance of Dengue Virus Infection. Frontiers in Microbiology 12 (2021).
[82] J.F. Osuna-Ramos, J.M. Reyes-Ruiz, and R.M. del Ángel, The Role of Host Cholesterol During Flavivirus Infection. Frontiers in Cellular and Infection Microbiology 8 (2018).
[83] B. Pozzi, L. Bragado, P. Mammi, M.F. Torti, N. Gaioli, L.G. Gebhard, M.E. García Solá, R. Vaz-Drago, N.G. Iglesias, C.C. García, A.V. Gamarnik, and A. Srebrow, Dengue virus targets RBM10 deregulating host cell splicing and innate immune response. Nucleic Acids Res 48 (2020) 6824-6838.
[84] P. Cohen, D. Cross, and P.A. Jänne, Kinase drug discovery 20 years after imatinib: progress and future directions. Nature Reviews Drug Discovery 20 (2021) 551-569.
[85] T.X. Jordan, and G. Randall, Dengue Virus Activates the AMP Kinase-mTOR Axis To Stimulate a Proviral Lipophagy. J Virol 91 (2017).
[86] C. Thepparit, S. Khongwichit, K. Ketsuwan, S. Libsittikul, P. Auewarakul, and D.R. Smith, Dengue virus requires apoptosis linked gene-2-interacting protein X (ALIX) for viral propagation. Virus Res 261 (2019) 65-71.
[87] N. Jiménez de Oya, A.B. Blázquez, J. Casas, J.C. Saiz, and M.A. Martín-Acebes, Direct Activation of Adenosine Monophosphate-Activated Protein Kinase (AMPK) by PF-06409577 Inhibits Flavivirus Infection through Modification of Host Cell Lipid Metabolism. Antimicrob Agents Chemother 62 (2018).
[88] S. Verdonck, S.Y. Pu, F.J. Sorrell, J.M. Elkins, M. Froeyen, L.J. Gao, L.I. Prugar, D.E. Dorosky, J.M. Brannan, R. Barouch-Bentov, S. Knapp, J.M. Dye, P. Herdewijn, S. Einav, and S. De Jonghe, Synthesis and Structure-Activity Relationships of 3,5-Disubstituted-pyrrolo[2,3- b]pyridines as Inhibitors of Adaptor-Associated Kinase 1 with Antiviral Activity. J Med Chem 62 (2019) 5810-5831.

[89] Y. Li, C. Kakinami, Q. Li, B. Yang, and H. Li, Human Apolipoprotein A-I Is Associated with Dengue Virus and Enhances Virus Infection through SR-BI. PLOS ONE 8 (2013) e70390.
[90] Y. Liu, H. Liu, J. Zou, B. Zhang, and Z. Yuan, Dengue virus subgenomic RNA induces apoptosis through the Bcl-2-mediated PI3k/Akt signaling pathway. Virology 448 (2014) 15-25.
[91] T. Feng, T. Sun, G. Li, W. Pan, K. Wang, and J. Dai, DEAD-Box Helicase DDX25 Is a Negative Regulator of Type I Interferon Pathway and Facilitates RNA Virus Infection. Front Cell Infect Microbiol 7 (2017) 356.
[92] S.N.Y. Yang, S.C. Atkinson, M.D. Audsley, S.M. Heaton, D.A. Jans, and N.A. Borg, RK-33 Is a Broad-Spectrum Antiviral Agent That Targets DEAD-Box RNA Helicase DDX3X. Cells 9 (2020).
[93] J.H. Pérez-Olais, F. Ruiz-Jiménez, E.J. Calderón-Garcia, L.A. De Jesús-González, R. Hernández-Rivas, and R.M. del Angel, The activity of Aurora kinase B is required for dengue virus release. Virus Research 274 (2019) 197777.
[94] C. Le Sommer, N.J. Barrows, S.S. Bradrick, J.L. Pearson, and M.A. Garcia-Blanco, G protein-coupled receptor kinase 2 promotes flaviviridae entry and replication. PLoS Negl Trop Dis 6 (2012) e1820.
[95] S. Pu, S. Schor, M. Karim, S. Saul, M. Robinson, S. Kumar, L.I. Prugar, D.E. Dorosky, J. Brannan, J.M. Dye, and S. Einav, BIKE regulates dengue virus infection and is a cellular target for broad-spectrum antivirals. Antiviral Res 184 (2020) 104966.
[96] T.Y. Tan, and J.J.H. Chu, Dengue virus-infected human monocytes trigger late activation of caspase-1, which mediates pro-inflammatory IL-1β secretion and pyroptosis. J Gen Virol 94 (2013) 2215-2220.
[97] J. García Cordero, M. León Juárez, J.A. González-Y-Merchand, L. Cedillo Barrón, and B. Gutiérrez Castañeda, Caveolin-1 in Lipid Rafts Interacts with Dengue Virus NS3 during Polyprotein Processing and Replication in HMEC-1 Cells. PLOS ONE 9 (2014) e90704.
[98] M.K. Jhan, T.J. Shen, P.C. Tseng, Y.T. Wang, and C.F. Lin, Signaling of Macrophage Inflammatory Protein (MIP)-3β Facilitates Dengue Virus-Induced Microglial Cell Migration. Viruses 10 (2018).
[99] R.E. Marques, R. Guabiraba, J.L. Del Sarto, R.F. Rocha, A.L. Queiroz, D. Cisalpino, P.E. Marques, C.C. Pacca, C.T. Fagundes, G.B. Menezes, M.L. Nogueira, D.G. Souza, and M.M. Teixeira, Dengue virus requires the CC-chemokine receptor CCR5 for replication and infection development. Immunology 145 (2015) 583-96.
[100] J. Liu, L. Yang, F. Liu, H. Li, W. Tang, X. Tong, and J. Zuo, CNOT2 facilitates dengue virus infection via negatively modulating IFN-Independent Non-Canonical JAK/STAT pathway. Biochem Biophys Res Commun 515 (2019) 403-409.
[101] F. Ang, A.P. Wong, M.M. Ng, and J.J. Chu, Small interference RNA profiling reveals the essential role of human membrane trafficking genes in mediating the infectious entry of dengue virus. Virol J 7 (2010) 24.
[102] S. Schor, S. Pu, V. Nicolaescu, S. Azari, M. Kõivomägi, M. Karim, P. Cassonnet, S. Saul, G. Neveu, A. Yueh, C. Demeret, J.M. Skotheim, Y. Jacob, G. Randall, and S. Einav, The cargo adapter protein CLINT1 is phosphorylated by the Numb-

associated kinase BIKE and mediates dengue virus infection. J Biol Chem 298 (2022) 101956.
[103] X. Carnec, L. Meertens, O. Dejarnac, M. Perera-Lecoin, M.L. Hafirassou, J. Kitaura, R. Ramdasi, O. Schwartz, and A. Amara, The Phosphatidylserine and Phosphatidylethanolamine Receptor CD300a Binds Dengue Virus and Enhances Infection. J Virol 90 (2016) 92-102.
[104] S. Afroz, S. Battu, J. Giddaluru, and N. Khan, Dengue Virus Induced COX-2 Signaling Is Regulated Through Nutrient Sensor GCN2. Front Immunol 11 (2020) 1831.
[105] S.S. Md Sani, W.H. Han, M.A. Bujang, H.J. Ding, K.L. Ng, and M.A. Amir Shariffuddin, Evaluation of creatine kinase and liver enzymes in identification of severe dengue. BMC Infect Dis 17 (2017) 505.
[106] M.F. Wu, S.T. Chen, and S.L. Hsieh, Distinct regulation of dengue virus-induced inflammasome activation in human macrophage subsets. J Biomed Sci 20 (2013) 36.
[107] S. Aguirre, P. Luthra, M.T. Sanchez-Aparicio, A.M. Maestre, J. Patel, F. Lamothe, A.C. Fredericks, S. Tripathi, T. Zhu, J. Pintado-Silva, L.G. Webb, D. Bernal-Rubio, A. Solovyov, B. Greenbaum, V. Simon, C.F. Basler, L.C.F. Mulder, A. García-Sastre, and A. Fernandez-Sesma, Dengue virus NS2B protein targets cGAS for degradation and prevents mitochondrial DNA sensing during infection. Nature Microbiology 2 (2017) 17037.
[108] M. Bhattacharya, D. Bhowmik, Y. Tian, H. He, F. Zhu, and Q. Yin, The Dengue virus protease NS2B3 cleaves cyclic GMP-AMP synthase to suppress cGAS activation. Journal of Biological Chemistry 299 (2023) 102986.
[109] S.-Y. Pu, R. Wouters, S. Schor, J. Rozenski, R. Barouch-Bentov, L.I. Prugar, C.M. O'Brien, J.M. Brannan, J.M. Dye, P. Herdewijn, S. De Jonghe, and S. Einav, Optimization of Isothiazolo[4,3-b]pyridine-Based Inhibitors of Cyclin G Associated Kinase (GAK) with Broad-Spectrum Antiviral Activity. J Med Chem 61 (2018) 6178-6192.
[110] R. Wouters, S.Y. Pu, M. Froeyen, E. Lescrinier, S. Einav, P. Herdewijn, and S. De Jonghe, Cyclin G-associated kinase (GAK) affinity and antiviral activity studies of a series of 3-C-substituted isothiazolo[4,3-b]pyridines. Eur J Med Chem 163 (2019) 256-265.
[111] C.F. Marinho, E.L. Azeredo, A. Torrentes-Carvalho, A. Marins-Dos-Santos, C.F. Kubelka, L.J. de Souza, R.V. Cunha, and L.M. de-Oliveira-Pinto, Down-regulation of complement receptors on the surface of host monocyte even as in vitro complement pathway blocking interferes in dengue infection. PLoS One 9 (2014) e102014.
[112] M. Butler, N. Chotiwan, C.D. Brewster, J.E. DiLisio, D.F. Ackart, B.K. Podell, R.J. Basaraba, R. Perera, S.L. Quackenbush, and J. Rovnak, Cyclin-Dependent Kinases 8 and 19 Regulate Host Cell Metabolism during Dengue Virus Serotype 2 Infection. Viruses 12 (2020).
[113] W.L. Wu, L.J. Ho, D.M. Chang, C.H. Chen, and J.H. Lai, Triggering of DC migration by dengue virus stimulation of COX-2-dependent signaling cascades in vitro highlights the significance of these cascades beyond inflammation. Eur J Immunol 39 (2009) 3413-22.

[114] G. Li, T. Feng, W. Pan, X. Shi, and J. Dai, DEAD-box RNA helicase DDX3X inhibits DENV replication via regulating type one interferon pathway. Biochem Biophys Res Commun 456 (2015) 327-32.
[115] M.L. Vetter, M.A. Rodgers, M.P. Patricelli, and P.L. Yang, Chemoproteomic profiling identifies changes in DNA-PK as markers of early dengue virus infection. ACS Chem Biol 7 (2012) 2019-26.
[116] A. Labeau, E. Simon-Loriere, M.L. Hafirassou, L. Bonnet-Madin, S. Tessier, A. Zamborlini, T. Dupré, N. Seta, O. Schwartz, M.L. Chaix, C. Delaugerre, A. Amara, and L. Meertens, A Genome-Wide CRISPR-Cas9 Screen Identifies the Dolichol-Phosphate Mannose Synthase Complex as a Host Dependency Factor for Dengue Virus Infection. J Virol 94 (2020).
[117] J.L. Smith, D.A. Stein, D. Shum, M.A. Fischer, C. Radu, B. Bhinder, H. Djaballah, J.A. Nelson, K. Früh, and A.J. Hirsch, Inhibition of dengue virus replication by a class of small-molecule compounds that antagonize dopamine receptor d4 and downstream mitogen-activated protein kinase signaling. J Virol 88 (2014) 5533-42.
[118] M.M. Gonzalez Lopez Ledesma, G. Costa Navarro, H.M. Pallares, A. Paletta, F. De Maio, N.G. Iglesias, L. Gebhard, S. Oviedo Rouco, D.S. Ojeda, L. de Borba, M. Giraldo, R. Rajsbaum, A. Ceballos, N.J. Krogan, P.S. Shah, and A.V. Gamarnik, Dengue virus NS5 degrades ERC1 during infection to antagonize NF-kB activation. Proc Natl Acad Sci U S A 120 (2023) e2220005120.
[119] H.A. Rothan, Y. Zhong, M.A. Sanborn, T.C. Teoh, J. Ruan, R. Yusof, J. Hang, M.J. Henderson, and S. Fang, Small molecule grp94 inhibitors block dengue and Zika virus replication. Antiviral Res 171 (2019) 104590.
[120] R.J. Lin, H.L. Chien, S.Y. Lin, B.L. Chang, H.P. Yu, W.C. Tang, and Y.L. Lin, MCPIP1 ribonuclease exhibits broad-spectrum antiviral effects through viral RNA binding and degradation. Nucleic Acids Res 41 (2013) 3314-26.
[121] P. Bagchi, K. Speckhart, A. Kennedy, A.W. Tai, and B. Tsai, A specific EMC subunit supports Dengue virus infection by promoting virus membrane fusion essential for cytosolic genome delivery. PLoS Pathog 18 (2022) e1010717.
[122] T.H. Chang, S.R. Chen, C.Y. Yu, Y.S. Lin, Y.S. Chen, T. Kubota, M. Matsuoka, and Y.L. Lin, Dengue virus serotype 2 blocks extracellular signal-regulated kinase and nuclear factor-κB activation to downregulate cytokine production. PLoS One 7 (2012) e41635.
[123] C.S. Villas-Bôas, T.M. Conceição, J. Ramírez, A.B. Santoro, A.T. Da Poian, and M. Montero-Lomelí, Dengue virus-induced regulation of the host cell translational machinery. Braz J Med Biol Res 42 (2009) 1020-6.
[124] X. Chen, J. Xia, Q. Zhao, Y. Wang, J. Liu, L. Feng, J. He, and P. Zhang, Eukaryotic initiation factor 4AI interacts with NS4A of Dengue virus and plays an antiviral role. Biochem Biophys Res Commun 461 (2015) 148-53.
[125] J.E. Fraser, C. Wang, K.W. Chan, S.G. Vasudevan, and D.A. Jans, Novel dengue virus inhibitor 4-HPR activates ATF4 independent of protein kinase R-like Endoplasmic Reticulum Kinase and elevates levels of eIF2α phosphorylation in virus infected cells. Antiviral Res 130 (2016) 1-6.

[126] S.M. Rawlinson, M.J. Pryor, P.J. Wright, and D.A. Jans, CRM1-mediated nuclear export of dengue virus RNA polymerase NS5 modulates interleukin-8 induction and virus production. J Biol Chem 284 (2009) 15589-97.
[127] N.S. Heaton, R. Perera, K.L. Berger, S. Khadka, D.J. Lacount, R.J. Kuhn, and G. Randall, Dengue virus nonstructural protein 3 redistributes fatty acid synthase to sites of viral replication and increases cellular fatty acid synthesis. Proc Natl Acad Sci U S A 107 (2010) 17345-50.
[128] M. Cortese, A. Kumar, P. Matula, L. Kaderali, P. Scaturro, H. Erfle, E.G. Acosta, S. Buehler, A. Ruggieri, L. Chatel-Chaix, K. Rohr, and R. Bartenschlager, Reciprocal Effects of Fibroblast Growth Factor Receptor Signaling on Dengue Virus Replication and Virion Production. Cell Reports 27 (2019) 2579-2592.e6.
[129] K. Bidet, D. Dadlani, and M.A. Garcia-Blanco, G3BP1, G3BP2 and CAPRIN1 are required for translation of interferon stimulated mRNAs and are targeted by a dengue virus non-coding RNA. PLoS Pathog 10 (2014) e1004242.
[130] L.K. Silva, R.E. Blanton, A.R. Parrado, P.S. Melo, V.G. Morato, E.A. Reis, J.P. Dias, J.M. Castro, P.F. Vasconcelos, K.A. Goddard, M.L. Barreto, M.G. Reis, and M.G. Teixeira, Dengue hemorrhagic fever is associated with polymorphisms in JAK1. Eur J Hum Genet 18 (2010) 1221-7.
[131] C.L. Chen, C.F. Lin, S.W. Wan, L.S. Wei, M.C. Chen, T.M. Yeh, H.S. Liu, R. Anderson, and Y.S. Lin, Anti-dengue virus nonstructural protein 1 antibodies cause NO-mediated endothelial cell apoptosis via ceramide-regulated glycogen synthase kinase-3β and NF-κB activation. J Immunol 191 (2013) 1744-52.
[132] A.M. Cuartas-López, and J.C. Gallego-Gómez, Glycogen synthase kinase 3ß participates in late stages of Dengue virus-2 infection. Mem Inst Oswaldo Cruz 115 (2020) e190357.
[133] H. Puerta-Guardo, S.B. Biering, F.T.G. de Sousa, J. Shu, D.R. Glasner, J. Li, S.F. Blanc, P.R. Beatty, and E. Harris, Flavivirus NS1 Triggers Tissue-Specific Disassembly of Intercellular Junctions Leading to Barrier Dysfunction and Vascular Leak in a GSK-3β-Dependent Manner. Pathogens 11 (2022).
[134] C. Gandikota, F. Mohammed, L. Gandhi, D. Maisnam, U. Mattam, D. Rathore, A. Chatterjee, K. Mallick, A. Billoria, V.S.V. Prasad, N.B.V. Sepuri, and M. Venkataramana, Mitochondrial Import of Dengue Virus NS3 Protease and Cleavage of GrpEL1, a Cochaperone of Mitochondrial Hsp70. J Virol 94 (2020).
[135] T.T. Tsai, C.L. Chen, C.C. Tsai, and C.F. Lin, Targeting heat shock factor 1 as an antiviral strategy against dengue virus replication in vitro and in vivo. Antiviral Res 145 (2017) 44-53.
[136] D.N. Fusco, H. Pratt, S. Kandilas, S.S. Cheon, W. Lin, D.A. Cronkite, M. Basavappa, K.L. Jeffrey, A. Anselmo, R. Sadreyev, C. Yapp, X. Shi, J.F. O'Sullivan, R.E. Gerszten, T. Tomaru, S. Yoshino, T. Satoh, and R.T. Chung, HELZ2 Is an IFN Effector Mediating Suppression of Dengue Virus. Front Microbiol 8 (2017) 240.
[137] C.K. Tseng, C.K. Lin, Y.H. Wu, Y.H. Chen, W.C. Chen, K.C. Young, and J.C. Lee, Human heme oxygenase 1 is a potential host cell factor against dengue virus replication. Sci Rep 6 (2016) 32176.

[138] J.M. Chen, Y.C. Fan, J.W. Lin, Y.Y. Chen, W.L. Hsu, and S.S. Chiou, Bovine Lactoferrin Inhibits Dengue Virus Infectivity by Interacting with Heparan Sulfate, Low-Density Lipoprotein Receptor, and DC-SIGN. Int J Mol Sci 18 (2017).
[139] L.W. Chu, C.J. Yang, K.J. Peng, P.L. Chen, S.J. Wang, and Y.H. Ping, TIM-1 As a Signal Receptor Triggers Dengue Virus-Induced Autophagy. Int J Mol Sci 20 (2019).
[140] S. Friedrich, S. Engelmann, T. Schmidt, G. Szczepankiewicz, S. Bergs, U.G. Liebert, B.M. Kümmerer, R.P. Golbik, and S.E. Behrens, The Host Factor AUF1 p45 Supports Flavivirus Propagation by Triggering the RNA Switch Required for Viral Genome Cyclization. J Virol 92 (2018).
[141] K.P. Mishra, Shweta, D. Diwaker, and L. Ganju, Dengue virus infection induces upregulation of hn RNP-H and PDIA3 for its multiplication in the host cell. Virus Res 163 (2012) 573-9.
[142] J.E. Brunetti, L.A. Scolaro, and V. Castilla, The heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a host factor required for dengue virus and Junín virus multiplication. Virus Res 203 (2015) 84-91.
[143] R. Soto-Acosta, P. Bautista-Carbajal, M. Cervantes-Salazar, A.H. Angel-Ambrocio, and R.M. Del Angel, DENV up-regulates the HMG-CoA reductase activity through the impairment of AMPK phosphorylation: A potential antiviral target. PLoS Pathog 13 (2017) e1006257.
[144] Y.S. Padwad, K.P. Mishra, M. Jain, S. Chanda, and L. Ganju, Dengue virus infection activates cellular chaperone Hsp70 in THP-1 cells: downregulation of Hsp70 by siRNA revealed decreased viral replication. Viral Immunol 23 (2010) 557-65.
[145] T. Chareonsirisuthigul, S. Kalayanarooj, and S. Ubol, Dengue virus (DENV) antibody-dependent enhancement of infection upregulates the production of anti-inflammatory cytokines, but suppresses anti-DENV free radical and pro-inflammatory cytokine production, in THP-1 cells. J Gen Virol 88 (2007) 365-375.
[146] D.A. Álvarez-Díaz, A.A. Gutiérrez-Díaz, E. Orozco-García, A. Puerta-González, C.I. Bermúdez-Santana, and J.C. Gallego-Gómez, Dengue virus potentially promotes migratory responses on endothelial cells by enhancing pro-migratory soluble factors and miRNAs. Virus Res 259 (2019) 68-76.
[147] S. Ubol, T. Chareonsirisuthigul, J. Kasisith, and C. Klungthong, Clinical isolates of dengue virus with distinctive susceptibility to nitric oxide radical induce differential gene responses in THP-1 cells. Virology 376 (2008) 290-6.
[148] Y.S. Kao, L.C. Wang, P.C. Chang, H.M. Lin, Y.S. Lin, C.Y. Yu, C.C. Chen, C.F. Lin, T.M. Yeh, S.W. Wan, J.R. Wang, T.S. Ho, C.C. Chu, B.C. Zhang, and C.P. Chang, Negative regulation of type I interferon signaling by integrin-linked kinase permits dengue virus replication. PLoS Pathog 19 (2023) e1011241.
[149] Y. Li, J. Xie, S. Wu, J. Xia, P. Zhang, C. Liu, P. Zhang, and X. Huang, Protein kinase regulated by dsRNA downregulates the interferon production in dengue virus- and dsRNA-stimulated human lung epithelial cells. PLoS One 8 (2013) e55108.
[150] R.C. Gomila, G.W. Martin, and L. Gehrke, NF90 binds the dengue virus RNA 3' terminus and is a positive regulator of dengue virus replication. PLoS One 6 (2011) e16687.

[151] D. Yamane, H. Feng, E.E. Rivera-Serrano, S.R. Selitsky, A. Hirai-Yuki, A. Das, K.L. McKnight, I. Misumi, L. Hensley, W. Lovell, O. González-López, R. Suzuki, M. Matsuda, H. Nakanishi, T. Ohto-Nakanishi, T. Hishiki, E. Wauthier, T. Oikawa, K. Morita, L.M. Reid, P. Sethupathy, M. Kohara, J.K. Whitmire, and S.M. Lemon, Basal expression of interferon regulatory factor 1 drives intrinsic hepatocyte resistance to multiple RNA viruses. Nat Microbiol 4 (2019) 1096-1104.
[152] G.P. Sreekanth, A. Chuncharunee, B. Cheunsuchon, S. Noisakran, P.T. Yenchitsomanus, and T. Limjindaporn, JNK1/2 inhibitor reduces dengue virus-induced liver injury. Antiviral Res 141 (2017) 7-18.
[153] M. Escalera-Cueto, I. Medina-Martínez, R.M. del Angel, J. Berumen-Campos, A.L. Gutiérrez-Escolano, and M. Yocupicio-Monroy, Let-7c overexpression inhibits dengue virus replication in human hepatoma Huh-7 cells. Virus Research 196 (2015) 105-112.
[154] B.A. Hackett, and S. Cherry, Flavivirus internalization is regulated by a size-dependent endocytic pathway. Proc Natl Acad Sci U S A 115 (2018) 4246-4251.
[155] Y.C. Lai, Y.C. Chuang, C.P. Chang, Y.S. Lin, G.C. Perng, H.C. Wu, S.L. Hsieh, and T.M. Yeh, Minocycline suppresses dengue virus replication by down-regulation of macrophage migration inhibitory factor-induced autophagy. Antiviral Res 155 (2018) 28-38.
[156] S. Wu, L. He, Y. Li, T. Wang, L. Feng, L. Jiang, P. Zhang, and X. Huang, miR-146a facilitates replication of dengue virus by dampening interferon induction by targeting TRAF6. J Infect 67 (2013) 329-41.
[157] R. Mishra, A. Lahon, and A.C. Banerjea, Dengue Virus Degrades USP33-ATF3 Axis via Extracellular Vesicles to Activate Human Microglial Cells. J Immunol 205 (2020) 1787-1798.
[158] S. Kanokudom, T. Vilaivan, N. Wikan, C. Thepparit, D.R. Smith, and W. Assavalapsakul, miR-21 promotes dengue virus serotype 2 replication in HepG2 cells. Antiviral Res 142 (2017) 169-177.
[159] N. Wu, N. Gao, D. Fan, J. Wei, J. Zhang, and J. An, miR-223 inhibits dengue virus replication by negatively regulating the microtubule-destabilizing protein STMN1 in EAhy926 cells. Microbes Infect 16 (2014) 911-22.
[160] X. Zhu, Z. He, Y. Hu, W. Wen, C. Lin, J. Yu, J. Pan, R. Li, H. Deng, S. Liao, J. Yuan, J. Wu, J. Li, and M. Li, MicroRNA-30e* suppresses dengue virus replication by promoting NF-κB-dependent IFN production. PLoS Negl Trop Dis 8 (2014) e3088.
[161] J.L. Smith, S. Jeng, S.K. McWeeney, and A.J. Hirsch, A MicroRNA Screen Identifies the Wnt Signaling Pathway as a Regulator of the Interferon Response during Flavivirus Infection. J Virol 91 (2017).
[162] N. Ahmed, N. Ahmed, and J.P. Pezacki, miR-383 Regulates Hepatic Lipid Homeostasis and Response to Dengue Virus Infection. ACS Infect Dis 8 (2022) 928-941.
[163] J.C. Castrillón-Betancur, and S. Urcuqui-Inchima, Overexpression of miR-484 and miR-744 in Vero cells alters Dengue virus replication. Mem Inst Oswaldo Cruz 112 (2017) 281-291.

[164] J.A. Castillo, J.C. Castrillón, M. Diosa-Toro, J.G. Betancur, G. St Laurent, 3rd, J.M. Smit, and S. Urcuqui-Inchima, Complex interaction between dengue virus replication and expression of miRNA-133a. BMC Infect Dis 16 (2016) 29.
[165] Y.C. Su, Y.F. Huang, Y.W. Wu, H.F. Chen, Y.H. Wu, C.C. Hsu, Y.C. Hsu, and J.C. Lee, MicroRNA-155 inhibits dengue virus replication by inducing heme oxygenase-1-mediated antiviral interferon responses. Faseb j 34 (2020) 7283-7294.
[166] Y. Nie, D. Deng, L. Mou, Q. Long, J. Chen, and J. Wu, Dengue Virus 2 NS2B Targets MAVS and IKKε to Evade the Antiviral Innate Immune Response. J Microbiol Biotechnol 33 (2023) 600-606.
[167] G.P. Sreekanth, A. Chuncharunee, A. Sirimontaporn, J. Panaampon, C. Srisawat, A. Morchang, S. Malakar, P. Thuwajit, S. Kooptiwut, A. Suttitheptumrong, P. Songprakhon, S. Noisakran, P.T. Yenchitsomanus, and T. Limjindaporn, Role of ERK1/2 signaling in dengue virus-induced liver injury. Virus Res 188 (2014) 15-26.
[168] I. Ceballos-Olvera, S. Chávez-Salinas, F. Medina, J.E. Ludert, and R.M. del Angel, JNK phosphorylation, induced during dengue virus infection, is important for viral infection and requires the presence of cholesterol. Virology 396 (2010) 30-6.
[169] C.C. Carter, F.D. Mast, J.P. Olivier, N.M. Bourgeois, A. Kaushansky, and J.D. Aitchison, Dengue activates mTORC2 signaling to counteract apoptosis and maximize viral replication. Frontiers in Cellular and Infection Microbiology 12 (2022).
[170] M. Sinha, U. Chakraborty, A. Kool, M. Chakravarti, S. Das, S. Ghosh, L. Thakur, A. Khuranna, D. Nayak, B. Basu, S. Kar, R. Ray, and S. Das, In-vitro antiviral action of Eupatorium perfoliatum against dengue virus infection: Modulation of mTOR signaling and autophagy. J Ethnopharmacol 282 (2022) 114627.
[171] W. Kong, J. Mao, Y. Yang, J. Yuan, J. Chen, Y. Luo, T. Lai, and L. Zuo, Mechanisms of mTOR and Autophagy in Human Endothelial Cell Infected with Dengue Virus-2. Viral Immunol 33 (2020) 61-70.
[172] D. Olagnier, F.E. Scholte, C. Chiang, I.C. Albulescu, C. Nichols, Z. He, R. Lin, E.J. Snijder, M.J. van Hemert, and J. Hiscott, Inhibition of dengue and chikungunya virus infections by RIG-I-mediated type I interferon-independent stimulation of the innate antiviral response. J Virol 88 (2014) 4180-94.
[173] Y.L. Lin, C.C. Liu, J.I. Chuang, H.Y. Lei, T.M. Yeh, Y.S. Lin, Y.H. Huang, and H.S. Liu, Involvement of oxidative stress, NF-IL-6, and RANTES expression in dengue-2-virus-infected human liver cells. Virology 276 (2000) 114-26.
[174] Y.I. Angleró-Rodríguez, P. Pantoja, and C.A. Sariol, Dengue virus subverts the interferon induction pathway via NS2B/3 protease-IκB kinase epsilon interaction. Clin Vaccine Immunol 21 (2014) 29-38.
[175] L.J. Ho, L.F. Hung, C.Y. Weng, W.L. Wu, P. Chou, Y.L. Lin, D.M. Chang, T.Y. Tai, and J.H. Lai, Dengue virus type 2 antagonizes IFN-alpha but not IFN-gamma antiviral effect via down-regulating Tyk2-STAT signaling in the human dendritic cell. J Immunol 174 (2005) 8163-72.
[176] Y.L. Cheng, Y.S. Lin, C.L. Chen, T.T. Tsai, C.C. Tsai, Y.W. Wu, Y.D. Ou, Y.Y. Chu, J.M. Wang, C.Y. Yu, and C.F. Lin, Activation of Nrf2 by the dengue virus

causes an increase in CLEC5A, which enhances TNF-α production by mononuclear phagocytes. Sci Rep 6 (2016) 32000.
[177] J. Saini, U. Thapa, B. Bandyopadhyay, S. Vrati, and A. Banerjee, Knockdown of NEAT1 restricts dengue virus replication by augmenting interferon alpha-inducible protein 27 via the RIG-I pathway. J Gen Virol 104 (2023).
[178] Y.M. Useche, M. Ribeiro-Alves, B.N. Restrepo, D.M. Salgado, C.F. Narváez, O. Campo, E. Avendaño, C. Martínez, J.C. Chacon-Duque, and G. Bedoya, Single-Nucleotide Polymorphisms in NOD1, RIPK2, MICB, PLCE1, TNF, and IKBKE Genes Associated with Symptomatic Dengue in Children from Colombia. Viral Immunol 31 (2018) 613-623.
[179] A.S. Puschnik, C.D. Marceau, Y.S. Ooi, K. Majzoub, N. Rinis, J.N. Contessa, and J.E. Carette, A Small-Molecule Oligosaccharyltransferase Inhibitor with Pan-flaviviral Activity. Cell Rep 21 (2017) 3032-3039.
[180] G.P. Sreekanth, A. Chuncharunee, A. Sirimontaporn, J. Panaampon, S. Noisakran, P.T. Yenchitsomanus, and T. Limjindaporn, SB203580 Modulates p38 MAPK Signaling and Dengue Virus-Induced Liver Injury by Reducing MAPKAPK2, HSP27, and ATF2 Phosphorylation. PLoS One 11 (2016) e0149486.
[181] A. Nagila, J. Netsawang, A. Suttitheptumrong, A. Morchang, S. Khunchai, C. Srisawat, C. Puttikhunt, S. Noisakran, P.T. Yenchitsomanus, and T. Limjindaporn, Inhibition of p38MAPK and CD137 signaling reduce dengue virus-induced TNF-α secretion and apoptosis. Virol J 10 (2013) 105.
[182] A. Barbachano-Guerrero, T.P. Endy, and C.A. King, Dengue virus non-structural protein 1 activates the p38 MAPK pathway to decrease barrier integrity in primary human endothelial cells. J Gen Virol 101 (2020) 484-496.
[183] H.W. Shyu, Y.Y. Lin, L.C. Chen, Y.F. Wang, T.M. Yeh, S.J. Su, W.C. Cheng, C.Y. Chen, K.H. Lin, and M.C. Chou, The dengue virus envelope protein induced PAI-1 gene expression via MEK/ERK pathways. Thromb Haemost 104 (2010) 1219-27.
[184] J. You, S. Hou, N. Malik-Soni, Z. Xu, A. Kumar, R.A. Rachubinski, L. Frappier, and T.C. Hobman, Flavivirus Infection Impairs Peroxisome Biogenesis and Early Antiviral Signaling. J Virol 89 (2015) 12349-61.
[185] C.J. Lee, C.L. Liao, and Y.L. Lin, Flavivirus activates phosphatidylinositol 3-kinase signaling to block caspase-dependent apoptotic cell death at the early stage of virus infection. J Virol 79 (2005) 8388-99.
[186] H.H. Chen, C.C. Chen, Y.S. Lin, P.C. Chang, Z.Y. Lu, C.F. Lin, C.L. Chen, and C.P. Chang, AR-12 suppresses dengue virus replication by down-regulation of PI3K/AKT and GRP78. Antiviral Res 142 (2017) 158-168.
[187] A. Lahon, R.P. Arya, and A.C. Banerjea, Dengue Virus Dysregulates Master Transcription Factors and PI3K/AKT/mTOR Signaling Pathway in Megakaryocytes. Front Cell Infect Microbiol 11 (2021) 715208.
[188] W. Noppakunmongkolchai, T. Poyomtip, T. Jittawuttipoka, N. Luplertlop, A. Sakuntabhai, S. Chimnaronk, S. Jirawatnotai, and R. Tohtong, Inhibition of protein kinase C promotes dengue virus replication. Virol J 13 (2016) 35.
[189] T.J. Shen, C.L. Chen, T.T. Tsai, M.K. Jhan, C.H. Bai, Y.C. Yen, C.W. Tsai, C.Y. Lee, P.C. Tseng, C.Y. Yu, and C.F. Lin, Hyperglycemia exacerbates dengue

virus infection by facilitating poly(A)-binding protein-mediated viral translation. JCI Insight 7 (2022).
[190] O. Choksupmanee, W. Tangkijthavorn, K. Hodge, K. Trisakulwattana, W. Phornsiricharoenphant, V. Narkthong, S. Tulakarnwong, C. Ngamphiw, S. Tongsima, and S. Chimnaronk, Specific Interaction of DDX6 with an RNA Hairpin in the 3' UTR of the Dengue Virus Genome Mediates G(1) Phase Arrest. J Virol 95 (2021) e0051021.
[191] C.K. Lin, C.K. Tseng, Y.H. Wu, C.C. Liaw, C.Y. Lin, C.H. Huang, Y.H. Chen, and J.C. Lee, Cyclooxygenase-2 facilitates dengue virus replication and serves as a potential target for developing antiviral agents. Sci Rep 7 (2017) 44701.
[192] J.J. Chu, and P.L. Yang, c-Src protein kinase inhibitors block assembly and maturation of dengue virus. Proc Natl Acad Sci U S A 104 (2007) 3520-5.
[193] D. Diwaker, K.P. Mishra, L. Ganju, and S.B. Singh, Protein disulfide isomerase mediates dengue virus entry in association with lipid rafts. Viral Immunol 28 (2015) 153-60.
[194] S.W. Wan, C.F. Lin, Y.T. Lu, H.Y. Lei, R. Anderson, and Y.S. Lin, Endothelial cell surface expression of protein disulfide isomerase activates β1 and β3 integrins and facilitates dengue virus infection. J Cell Biochem 113 (2012) 1681-91.
[195] D. Diwaker, K.P. Mishra, and L. Ganju, Effect of modulation of unfolded protein response pathway on dengue virus infection. Acta Biochim Biophys Sin (Shanghai) 47 (2015) 960-8.
[196] L.L. Li, S.T. Hu, S.H. Wang, H.H. Lee, Y.T. Wang, and Y.H. Ping, Positive transcription elongation factor b (P-TEFb) contributes to dengue virus-stimulated induction of interleukin-8 (IL-8). Cell Microbiol 12 (2010) 1589-603.
[197] J. Wongtrakul, T. Thongtan, S. Pannengpetch, N. Wikan, D. Kantamala, B. Kumrapich, W. Suwan, and D.R. Smith, Phosphoproteomic analysis of dengue virus infected U937 cells and identification of pyruvate kinase M2 as a differentially phosphorylated phosphoprotein. Sci Rep 10 (2020) 14493.
[198] W.C. Tang, R.J. Lin, C.L. Liao, and Y.L. Lin, Rab18 facilitates dengue virus infection by targeting fatty acid synthase to sites of viral replication. J Virol 88 (2014) 6793-804.
[199] A.M. Airo, M.D. Urbanowski, J. Lopez-Orozco, J.H. You, T.D. Skene-Arnold, C. Holmes, V. Yamshchikov, N. Malik-Soni, L. Frappier, and T.C. Hobman, Expression of flavivirus capsids enhance the cellular environment for viral replication by activating Akt-signalling pathways. Virology 516 (2018) 147-157.
[200] B. Liu, T.T. Gao, X.Y. Fu, Z.H. Xu, H. Ren, P. Zhao, Z.T. Qi, and Z.L. Qin, PTEN Lipid Phosphatase Activity Enhances Dengue Virus Production through Akt/FoxO1/Maf1 Signaling. Virol Sin 36 (2021) 412-423.
[201] N.J. Lennemann, and C.B. Coyne, Dengue and Zika viruses subvert reticulophagy by NS2B3-mediated cleavage of FAM134B. Autophagy 13 (2017) 322-332.
[202] A.M. Cuartas-López, C.E. Hernández-Cuellar, and J.C. Gallego-Gómez, Disentangling the role of PI3K/Akt, Rho GTPase and the actin cytoskeleton on dengue virus infection. Virus Res 256 (2018) 153-165.
[203] S.M. Casseb, D.B. Simith, K.F. Melo, M.H. Mendonça, A.C. Santos, V.L. Carvalho, A.C. Cruz, and P.F. Vasconcelos, Drosha, DGCR8, and Dicer mRNAs

are down-regulated in human cells infected with dengue virus 4, and play a role in viral pathogenesis. Genet Mol Res 15 (2016).
[204] X. Huang, Y. Yue, D. Li, Y. Zhao, L. Qiu, J. Chen, Y. Pan, J. Xi, X. Wang, Q. Sun, and Q. Li, Antibody-dependent enhancement of dengue virus infection inhibits RLR-mediated Type-I IFN-independent signalling through upregulation of cellular autophagy. Sci Rep 6 (2016) 22303.
[205] J.K. Sprokholt, T.M. Kaptein, J.L. van Hamme, R.J. Overmars, S.I. Gringhuis, and T.B.H. Geijtenbeek, RIG-I-like Receptor Triggering by Dengue Virus Drives Dendritic Cell Immune Activation and T(H)1 Differentiation. J Immunol 198 (2017) 4764-4771.
[206] D. Diwaker, K.P. Mishra, L. Ganju, and S.B. Singh, Rhodiola inhibits dengue virus multiplication by inducing innate immune response genes RIG-I, MDA5 and ISG in human monocytes. Arch Virol 159 (2014) 1975-86.
[207] A.M. Nasirudeen, H.H. Wong, P. Thien, S. Xu, K.P. Lam, and D.X. Liu, RIG-I, MDA5 and TLR3 synergistically play an important role in restriction of dengue virus infection. PLoS Negl Trop Dis 5 (2011) e926.
[208] T.H. Chang, C.L. Liao, and Y.L. Lin, Flavivirus induces interferon-beta gene expression through a pathway involving RIG-I-dependent IRF-3 and PI3K-dependent NF-kappaB activation. Microbes Infect 8 (2006) 157-71.
[209] Y. Wang, X. Chen, J. Xie, S. Zhou, Y. Huang, Y.P. Li, X. Li, C. Liu, J. He, and P. Zhang, RNA Helicase A Is an Important Host Factor Involved in Dengue Virus Replication. J Virol 93 (2019).
[210] Jill M. Perreira, Aaron M. Aker, G. Savidis, Christopher R. Chin, William M. McDougall, Jocelyn M. Portmann, P. Meraner, Miles C. Smith, M. Rahman, Richard E. Baker, A. Gauthier, M. Franti, and Abraham L. Brass, RNASEK Is a V-ATPase-Associated Factor Required for Endocytosis and the Replication of Rhinovirus, Influenza A Virus, and Dengue Virus. Cell Reports 12 (2015) 850-863.
[211] S. Lei, Y.P. Tian, W.D. Xiao, S. Li, X.C. Rao, J.L. Zhang, J. Yang, X.M. Hu, and W. Chen, ROCK is involved in vimentin phosphorylation and rearrangement induced by dengue virus. Cell Biochem Biophys 67 (2013) 1333-42.
[212] M.G. Anupriya, S. Singh, N.V. Hulyalkar, and E. Sreekumar, Sphingolipid signaling modulates trans-endothelial cell permeability in dengue virus infected HMEC-1 cells. Prostaglandins & Other Lipid Mediators 136 (2018) 44-54.
[213] B. Shue, A.I. Chiramel, B. Cerikan, T.H. To, S. Frölich, S.M. Pederson, E.N. Kirby, N.S. Eyre, R.F.W. Bartenschlager, S.M. Best, and M.R. Beard, Genome-Wide CRISPR Screen Identifies RACK1 as a Critical Host Factor for Flavivirus Replication. J Virol 95 (2021) e0059621.
[214] R. Zhang, J.J. Miner, M.J. Gorman, K. Rausch, H. Ramage, J.P. White, A. Zuiani, P. Zhang, E. Fernandez, Q. Zhang, K.A. Dowd, T.C. Pierson, S. Cherry, and M.S. Diamond, A CRISPR screen defines a signal peptide processing pathway required by flaviviruses. Nature 535 (2016) 164-8.
[215] J.N. Clarke, L.K. Davies, J.K. Calvert, B.L. Gliddon, W.H.A. Shujari, A.L. Aloia, K.J. Helbig, M.R. Beard, S.M. Pitson, and J.M. Carr, Reduction in sphingosine kinase 1 influences the susceptibility to dengue virus infection by altering antiviral responses. J Gen Virol 97 (2016) 95-109.

[216] W.H. Al-Shujairi, J.N. Clarke, L.T. Davies, M.R. Pitman, J.K. Calvert, A.L. Aloia, S.M. Pitson, and J.M. Carr, In vitro and in vivo roles of sphingosine kinase 2 during dengue virus infection. J Gen Virol 100 (2019) 629-641.
[217] A. Morchang, R.C.H. Lee, P.-t. Yenchitsomanus, G.P. Sreekanth, S. Noisakran, J.J.H. Chu, and T. Limjindaporn, RNAi screen reveals a role of SPHK2 in dengue virus–mediated apoptosis in hepatic cell lines. PLOS ONE 12 (2017) e0188121.
[218] J.M. Carr, T. Kua, J.N. Clarke, J.K. Calvert, J.R. Zebol, M.R. Beard, and S.M. Pitson, Reduced sphingosine kinase 1 activity in dengue virus type-2 infected cells can be mediated by the 3' untranslated region of dengue virus type-2 RNA. J Gen Virol 94 (2013) 2437-2448.
[219] R. Kumar, T. Agrawal, N.A. Khan, Y. Nakayama, and G.R. Medigeshi, Identification and characterization of the role of c-terminal Src kinase in dengue virus replication. Sci Rep 6 (2016) 30490.
[220] P. Vincetti, F. Caporuscio, S. Kaptein, A. Gioiello, V. Mancino, Y. Suzuki, N. Yamamoto, E. Crespan, A. Lossani, G. Maga, G. Rastelli, D. Castagnolo, J. Neyts, P. Leyssen, G. Costantino, and M. Radi, Discovery of Multitarget Antivirals Acting on Both the Dengue Virus NS5-NS3 Interaction and the Host Src/Fyn Kinases. J Med Chem 58 (2015) 4964-4975.
[221] J. Ashour, M. Laurent-Rolle, P.Y. Shi, and A. García-Sastre, NS5 of dengue virus mediates STAT2 binding and degradation. J Virol 83 (2009) 5408-18.
[222] M. Mazzon, M. Jones, A. Davidson, B. Chain, and M. Jacobs, Dengue virus NS5 inhibits interferon-alpha signaling by blocking signal transducer and activator of transcription 2 phosphorylation. J Infect Dis 200 (2009) 1261-70.
[223] S. Srivastava, N. Chaudhary, A. Ojha, P. Guchhait, and A.K. Patel, Signal transducer and activator of transcription 3 (STAT3) acts as a proviral factor for dengue virus propagation. Virus Res 300 (2021) 198436.
[224] P.K. Kakumani, G.R. Medigeshi, I. Kaur, P. Malhotra, S.K. Mukherjee, and R.K. Bhatnagar, Role of human GRP75 in miRNA mediated regulation of dengue virus replication. Gene 586 (2016) 7-11.
[225] J. Zhao, R. Li, Y. Li, J. Chen, F. Feng, and C. Sun, Broadly Antiviral Activities of TAP1 through Activating the TBK1-IRF3-Mediated Type I Interferon Production. Int J Mol Sci 22 (2021).
[226] P. Zhang, S. Wu, L. Li, Z. Liang, Y. Li, L. Feng, and X. Huang, Adjuvant PIKA protects hepatoma cells from dengue virus infection by promoting a TBK-1-dependent innate immune response. Arch Virol 158 (2013) 829-38.
[227] N.A. Dalrymple, V. Cimica, and E.R. Mackow, Dengue Virus NS Proteins Inhibit RIG-I/MAVS Signaling by Blocking TBK1/IRF3 Phosphorylation: Dengue Virus Serotype 1 NS4A Is a Unique Interferon-Regulating Virulence Determinant. mBio 6 (2015) e00553-15.
[228] T.M. Yeh, S.H. Liu, K.C. Lin, C. Kuo, S.Y. Kuo, T.Y. Huang, Y.R. Yen, R.K. Wen, L.C. Chen, and T.F. Fu, Dengue virus enhances thrombomodulin and ICAM-1 expression through the macrophage migration inhibitory factor induction of the MAPK and PI3K signaling pathways. PLoS One 8 (2013) e55018.
[229] J.F. Arboleda, G.J. Fernandez, and S. Urcuqui-Inchima, Vitamin D-mediated attenuation of miR-155 in human macrophages infected with dengue virus: Implications for the cytokine response. Infect Genet Evol 69 (2019) 12-21.

[230] D.J. Udawatte, D.M. Lang, J.R. Currier, C.L. Medin, and A.L. Rothman, Dengue virus downregulates TNFR1- and TLR3-stimulated NF-κB activation by targeting RIPK1. Frontiers in Cellular and Infection Microbiology 12 (2022).
[231] N. Wu, X. Gou, P. Hu, Y. Chen, J. Ji, Y. Wang, and L. Zuo, Mechanism of autophagy induced by activation of the AMPK/ERK/mTOR signaling pathway after TRIM22-mediated DENV-2 infection of HUVECs. Virol J 19 (2022) 228.
[232] G. Manokaran, E. Finol, C. Wang, J. Gunaratne, J. Bahl, E.Z. Ong, H.C. Tan, O.M. Sessions, A.M. Ward, D.J. Gubler, E. Harris, M.A. Garcia-Blanco, and E.E. Ooi, Dengue subgenomic RNA binds TRIM25 to inhibit interferon expression for epidemiological fitness. Science 350 (2015) 217-21.
[233] M. Long, H. Wang, X. Ning, F. Jia, L. Zhang, Y. Pan, J. Chen, X. Wang, K. Feng, X. Cao, Y. Liu, and Q. Sun, Functional analysis of differentially expressed long non-coding RNAs in DENV-3 infection and antibody-dependent enhancement of viral infection. Virus Res 319 (2022) 198883.
[234] B. Liu, N.L. Li, J. Wang, P.-Y. Shi, T. Wang, M.A. Miller, and K. Li, Overlapping and Distinct Molecular Determinants Dictating the Antiviral Activities of TRIM56 against Flaviviruses and Coronavirus. Journal of Virology 88 (2014) 13821-13835.
[235] T. Bagga, N.K. Tulsian, Y.K. Mok, R.M. Kini, and J. Sivaraman, Mapping of molecular interactions between human E3 ligase TRIM69 and Dengue virus NS3 protease using hydrogen-deuterium exchange mass spectrometry. Cell Mol Life Sci 79 (2022) 233.
[236] K. Wang, C. Zou, X. Wang, C. Huang, T. Feng, W. Pan, Q. Wu, P. Wang, and J. Dai, Interferon-stimulated TRIM69 interrupts dengue virus replication by ubiquitinating viral nonstructural protein 3. PLoS Pathog 14 (2018) e1007287.
[237] K. Tabata, M. Arimoto, M. Arakawa, A. Nara, K. Saito, H. Omori, A. Arai, T. Ishikawa, E. Konishi, R. Suzuki, Y. Matsuura, and E. Morita, Unique Requirement for ESCRT Factors in Flavivirus Particle Formation on the Endoplasmic Reticulum. Cell Rep 16 (2016) 2339-47.
[238] J.B. Callaway, S.A. Smith, K.P. McKinnon, A.M. de Silva, J.E. Crowe, Jr., and J.P. Ting, Spleen Tyrosine Kinase (Syk) Mediates IL-1β Induction by Primary Human Monocytes during Antibody-enhanced Dengue Virus Infection. J Biol Chem 290 (2015) 17306-20.
[239] M.J. Clark, C. Miduturu, A.G. Schmidt, X. Zhu, J.D. Pitts, J. Wang, S. Potisopon, J. Zhang, A. Wojciechowski, J.J. Hann Chu, N.S. Gray, and P.L. Yang, GNF-2 Inhibits Dengue Virus by Targeting Abl Kinases and the Viral E Protein. Cell Chem Biol 23 (2016) 443-52.
[240] M. de Wispelaere, A.J. LaCroix, and P.L. Yang, The small molecules AZD0530 and dasatinib inhibit dengue virus RNA replication via Fyn kinase. J Virol 87 (2013) 7367-81.
[241] Y.T. Tsai, Y.H. Chen, D.M. Chang, P.C. Chen, and J.H. Lai, Janus kinase/signal transducer and activator of transcription 3 signaling pathway is crucial in chemokine production from hepatocytes infected by dengue virus. Exp Biol Med (Maywood) 236 (2011) 1156-65.

[242] P. Wang, L. Yang, G. Cheng, G. Yang, Z. Xu, F. You, Q. Sun, R. Lin, E. Fikrig, and R.E. Sutton, UBXN1 interferes with Rig-I-like receptor-mediated antiviral immune response by targeting MAVS. Cell Rep 3 (2013) 1057-70.
[243] J.H. Lai, D.W. Wu, C.H. Wu, L.F. Hung, C.Y. Huang, S.M. Ka, A. Chen, Z.F. Chang, and L.J. Ho, Mitochondrial CMPK2 mediates immunomodulatory and antiviral activities through IFN-dependent and IFN-independent pathways. iScience 24 (2021) 102498.
[244] H. Ye, X. Duan, M. Yao, L. Kang, Y. Li, S. Li, B. Li, and L. Chen, USP18 Mediates Interferon Resistance of Dengue Virus Infection. Front Microbiol 12 (2021) 682380.
[245] S. Singh, M.G. Anupriya, A. Modak, and E. Sreekumar, Dengue virus or NS1 protein induces trans-endothelial cell permeability associated with VE-Cadherin and RhoA phosphorylation in HMEC-1 cells preventable by Angiopoietin-1. J Gen Virol 99 (2018) 1658-1670.
[246] R.C. Seet, A.W. Chow, A.M. Quek, Y.H. Chan, and E.C. Lim, Relationship between circulating vascular endothelial growth factor and its soluble receptors in adults with dengue virus infection: a case-control study. Int J Infect Dis 13 (2009) e248-53.
[247] Y. Wan, W. Wu, Y. Wan, L. Li, J. Zhang, X. Chen, S. Liu, and X. Yao, Brivanib alaninate inhibited dengue virus proliferation through VEGFR2/AMPK pathway. Pharmacol Res 170 (2021) 105721.
[248] C.S. Teo, and J.J. Chu, Cellular vimentin regulates construction of dengue virus replication complexes through interaction with NS4A protein. J Virol 88 (2014) 1897-913.
[249] V. Huerta, P. Toledo, N. Fleitas, A. Martín, D. Pupo, A. Yero, M. Sarría, A. Sánchez, V. Besada, Y. Ramos, G. Márquez, O. Guirola, and G. Chinea, Receptor-activated human α2-macroglobulin interacts with the envelope protein of dengue virus and protects virions from temperature-induced inactivation through multivalent binding. J Gen Virol 95 (2014) 2668-2676.
[250] R. Roskoski, Jr., Properties of FDA-approved small molecule protein kinase inhibitors: A 2023 update. Pharmacol Res 187 (2023) 106552.
[251] H. Kaplon, S. Crescioli, A. Chenoweth, J. Visweswaraiah, and J.M. Reichert, Antibodies to watch in 2023. mAbs 15 (2023) 2153410.
[252] T.T.X. Huynh, T.X. Pham, G.H. Lee, J.B. Lee, S.G. Lee, D. Tark, Y.S. Lim, and S.B. Hwang, Amuvatinib Blocks SARS-CoV-2 Infection at the Entry Step of the Viral Life Cycle. Microbiol Spectr 11 (2023) e0510522.
[253] E. Bekerman, G. Neveu, A. Shulla, J. Brannan, S.Y. Pu, S. Wang, F. Xiao, R. Barouch-Bentov, R.R. Bakken, R. Mateo, J. Govero, C.M. Nagamine, M.S. Diamond, S. De Jonghe, P. Herdewijn, J.M. Dye, G. Randall, and S. Einav, Anticancer kinase inhibitors impair intracellular viral trafficking and exert broad-spectrum antiviral effects. J Clin Invest 127 (2017) 1338-1352.
[254] W.C. Chen, Y. Simanjuntak, L.W. Chu, Y.H. Ping, Y.L. Lee, Y.L. Lin, and W.S. Li, Benzenesulfonamide Derivatives as Calcium/Calmodulin-Dependent Protein Kinase Inhibitors and Antiviral Agents against Dengue and Zika Virus Infections. J Med Chem 63 (2020) 1313-1327.

[255] A. Duran, N. Valero, J. Mosquera, E. Fuenmayor, and M. Alvarez-Mon, Gefitinib and pyrrolidine dithiocarbamate decrease viral replication and cytokine production in dengue virus infected human monocyte cultures. Life Sci 191 (2017) 180-185.
[256] T. Limjindaporn, J. Panaampon, S. Malakar, S. Noisakran, and P.T. Yenchitsomanus, Tyrosine kinase/phosphatase inhibitors decrease dengue virus production in HepG2 cells. Biochem Biophys Res Commun 483 (2017) 58-63.
[257] H.J. Valencia, M.C.A.M. de Aguiar, M.A. Costa, D.C. Mendonça, E.V. Reis, N.E.C. Arias, B.P. Drumond, and C.A. Bonjardim, Evaluation of kinase inhibitors as potential therapeutics for flavivirus infections. Archives of Virology 166 (2021) 1433-1438.
[258] L.C. de Oliveira, A.M. Ribeiro, J.D. Albarnaz, A.A. Torres, L.F.Z. Guimarães, A.K. Pinto, S. Parker, K. Doronin, J.D. Brien, M.R. Buller, and C.A. Bonjardim, The small molecule AZD6244 inhibits dengue virus replication in vitro and protects against lethal challenge in a mouse model. Arch Virol 165 (2020) 671-681.
[259] A. Anwar, T. Hosoya, K.M. Leong, H. Onogi, Y. Okuno, T. Hiramatsu, H. Koyama, M. Suzuki, M. Hagiwara, and M.A. Garcia-Blanco, The kinase inhibitor SFV785 dislocates dengue virus envelope protein from the replication complex and blocks virus assembly. PLoS One 6 (2011) e23246.
[260] A. Morchang, U. Yasamut, J. Netsawang, S. Noisakran, W. Wongwiwat, P. Songprakhon, C. Srisawat, C. Puttikhunt, W. Kasinrerk, P. Malasit, P.-t. Yenchitsomanus, and T. Limjindaporn, Cell death gene expression profile: Role of RIPK2 in dengue virus-mediated apoptosis. Virus Research 156 (2011) 25-34.
[261] E. Branche, W.W. Tang, K.M. Viramontes, M.P. Young, N. Sheets, Y. Joo, A.T. Nguyen, and S. Shresta, Synergism between the tyrosine kinase inhibitor sunitinib and Anti-TNF antibody protects against lethal dengue infection. Antiviral Res 158 (2018) 1-7.
[262] S. Wati, S.M. Rawlinson, R.A. Ivanov, L. Dorstyn, M.R. Beard, D.A. Jans, S.M. Pitson, C.J. Burrell, P. Li, and J.M. Carr, Tumour necrosis factor alpha (TNF-alpha) stimulation of cells with established dengue virus type 2 infection induces cell death that is accompanied by a reduced ability of TNF-alpha to activate nuclear factor kappaB and reduced sphingosine kinase-1 activity. J Gen Virol 92 (2011) 807-18.
[263] A.L. Aloia, J.K. Calvert, J.N. Clarke, L.T. Davies, K.J. Helbig, S.M. Pitson, and J.M. Carr, Investigation of sphingosine kinase 1 in interferon responses during dengue virus infection. Clin Transl Immunology 6 (2017) e151.
[264] M. Cortese, K. Mulder, L. Chatel-Chaix, P. Scaturro, B. Cerikan, A. Plaszczyca, U. Haselmann, M. Bartenschlager, C.J. Neufeldt, and R. Bartenschlager, Determinants in Nonstructural Protein 4A of Dengue Virus Required for RNA Replication and Replication Organelle Biogenesis. J Virol 95 (2021) e0131021.
[265] C.D. Marceau, A.S. Puschnik, K. Majzoub, Y.S. Ooi, S.M. Brewer, G. Fuchs, K. Swaminathan, M.A. Mata, J.E. Elias, P. Sarnow, and J.E. Carette, Genetic dissection of Flaviviridae host factors through genome-scale CRISPR screens. Nature 535 (2016) 159-63.
[266] G. Savidis, W.M. McDougall, P. Meraner, J.M. Perreira, J.M. Portmann, G. Trincucci, S.P. John, A.M. Aker, N. Renzette, D.R. Robbins, Z. Guo, S. Green,

T.F. Kowalik, and A.L. Brass, Identification of Zika Virus and Dengue Virus Dependency Factors using Functional Genomics. Cell Rep 16 (2016) 232-246.
[267] M.L. Hafirassou, L. Meertens, C. Umaña-Diaz, A. Labeau, O. Dejarnac, L. Bonnet-Madin, B.M. Kümmerer, C. Delaugerre, P. Roingeard, P.O. Vidalain, and A. Amara, A Global Interactome Map of the Dengue Virus NS1 Identifies Virus Restriction and Dependency Host Factors. Cell Rep 21 (2017) 3900-3913.
[268] M. Long, Y. Pan, J. Chen, F. Jia, H. Wang, D. Li, K. Feng, L. Yan, X. Wang, X. Ning, L. Qiu, J. Zhang, and Q. Sun, Sweeping analysis of transcript profile in dengue virus serotype 3 infection and antibody-dependent enhancement of infection. Virulence 12 (2021) 2764-2776.
[269] T. Anastassiadis, S.W. Deacon, K. Devarajan, H. Ma, and J.R. Peterson, Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. Nat Biotechnol 29 (2011) 1039-45.
[270] T.S. Gujral, L. Peshkin, and M.W. Kirschner, Exploiting polypharmacology for drug target deconvolution. Proc Natl Acad Sci U S A 111 (2014) 5048-53.
[271] N. Arang, H.S. Kain, E.K. Glennon, T. Bello, D.R. Dudgeon, E.N.F. Walter, T.S. Gujral, and A. Kaushansky, Identifying host regulators and inhibitors of liver stage malaria infection using kinase activity profiles. Nature Communications 8 (2017).
[272] S. Dankwa, M.M. Dols, L. Wei, E.K.K. Glennon, H.S. Kain, A. Kaushansky, and J.D. Smith, Exploiting polypharmacology to dissect host kinases and kinase inhibitors that modulate endothelial barrier integrity. Cell Chem Biol 28 (2021) 1679-1692.e4.
[273] L. Wei, S. Dankwa, K. Vijayan, J.D. Smith, and A. Kaushansky, Temporally resolved kinase regulatory networks control endothelial barrier integrity. bioRxiv (2022) 2022.09.19.508598.
[274] F.D. Mast, A.T. Navare, A.M. van der Sloot, J. Coulombe-Huntington, M.P. Rout, N.S. Baliga, A. Kaushansky, B.T. Chait, A. Aderem, C.M. Rice, A. Sali, M. Tyers, and J.D. Aitchison, Crippling life support for SARS-CoV-2 and other viruses through synthetic lethality. J Cell Biol 219 (2020).
[275] M. Kciuk, A. Gielecińska, S. Mujwar, M. Mojzych, and R. Kontek, Cyclin-Dependent Kinase Synthetic Lethality Partners in DNA Damage Response. Int J Mol Sci 23 (2022).
[276] C.J. Lord, and A. Ashworth, PARP inhibitors: Synthetic lethality in the clinic. Science 355 (2017) 1152-1158.
[277] X. Yang, M.B.M. Quam, T. Zhang, and S. Sang, Global burden for dengue and the evolving pattern in the past 30 years. J Travel Med 28 (2021).
[278] B. Troost, and J.M. Smit, Recent advances in antiviral drug development towards dengue virus. Curr Opin Virol 43 (2020) 9-21.
[279] V. Tricou, N.N. Minh, T.P. Van, S.J. Lee, J. Farrar, B. Wills, H.T. Tran, and C.P. Simmons, A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. PLoS Negl Trop Dis 4 (2010) e785.
[280] J. Whitehorn, C.V.V. Nguyen, L.P. Khanh, D.T.H. Kien, N.T.H. Quyen, N.T.T. Tran, N.T. Hang, N.T. Truong, L.T. Hue Tai, N.T. Cam Huong, V.T. Nhon, T. Van Tram, J. Farrar, M. Wolbers, C.P. Simmons, and B. Wills, Lovastatin for the

Treatment of Adult Patients With Dengue: A Randomized, Double-Blind, Placebo-Controlled Trial. Clin Infect Dis 62 (2016) 468-476.
[281] J.G. Low, C. Sung, L. Wijaya, Y. Wei, A.P.S. Rathore, S. Watanabe, B.H. Tan, L. Toh, L.T. Chua, Y. Hou, A. Chow, S. Howe, W.K. Chan, K.H. Tan, J.S. Chung, B.P. Cherng, D.C. Lye, P.A. Tambayah, L.C. Ng, J. Connolly, M.L. Hibberd, Y.S. Leo, Y.B. Cheung, E.E. Ooi, and S.G. Vasudevan, Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. Lancet Infect Dis 14 (2014) 706-715.
[282] N. Kumar, S. Sharma, R. Kumar, B.N. Tripathi, S. Barua, H. Ly, and B.T. Rouse, Host-Directed Antiviral Therapy. Clinical Microbiology Reviews 33 (2020) 10.1128/cmr.00168-19.
[283] H.J. Valencia, M. de Aguiar, M.A. Costa, D.C. Mendonça, E.V. Reis, N.E.C. Arias, B.P. Drumond, and C.A. Bonjardim, Evaluation of kinase inhibitors as potential therapeutics for flavivirus infections. Arch Virol 166 (2021) 1433-1438.
[284] G.P. Sreekanth, P.T. Yenchitsomanus, and T. Limjindaporn, Role of mitogen-activated protein kinase signaling in the pathogenesis of dengue virus infection. Cell Signal 48 (2018) 64-68.
[285] Y.S. Tian, Y. Zhou, T. Takagi, M. Kameoka, and N. Kawashita, Dengue Virus and Its Inhibitors: A Brief Review. Chem Pharm Bull (Tokyo) 66 (2018) 191-206.
[286] S.Y. Pu, F. Xiao, S. Schor, E. Bekerman, F. Zanini, R. Barouch-Bentov, C.M. Nagamine, and S. Einav, Feasibility and biological rationale of repurposing sunitinib and erlotinib for dengue treatment. Antiviral Res 155 (2018) 67-75.
[287] C.R. Lambeth, L.J. White, R.E. Johnston, and A.M. de Silva, Flow cytometry-based assay for titrating dengue virus. J Clin Microbiol 43 (2005) 3267-72.
[288] J.M. Silva, and M. McMahon, The fastest Western in town: a contemporary twist on the classic Western blot analysis. J Vis Exp (2014) e51149.
[289] P.V. Hornbeck, B. Zhang, B. Murray, J.M. Kornhauser, V. Latham, and E. Skrzypek, PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. Nucleic Acids Res 43 (2015) D512-20.
[290] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, N. Amin, B. Schwikowski, and T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13 (2003) 2498-504.
[291] P.Y. Chia, T.L. Thein, S.W.X. Ong, D.C. Lye, and Y.S. Leo, Severe dengue and liver involvement: an overview and review of the literature. Expert Rev Anti Infect Ther 18 (2020) 181-189.
[292] G.P. Sreekanth, A. Chuncharunee, P.T. Yenchitsomanus, and T. Limjindaporn, Crocetin Improves Dengue Virus-Induced Liver Injury. Viruses 12 (2020).
[293] K. Salokas, X. Liu, T. Öhman, I. Chowdhury, L. Gawriyski, S. Keskitalo, and M. Varjosalo, Physical and functional interactome atlas of human receptor tyrosine kinases. EMBO reports 23 (2022).
[294] Y. Liu, L. Chen, W. Liu, D. Li, J. Zeng, Q. Tang, Y. Zhang, F. Luan, and N. Zeng, Cepharanthine Suppresses Herpes Simplex Virus Type 1 Replication Through the Downregulation of the PI3K/Akt and p38 MAPK Signaling Pathways. Frontiers in Microbiology 12 (2021).

[295] M.A. Rahman, F.M. Shorobi, M.N. Uddin, S. Saha, and M.A. Hossain, Quercetin attenuates viral infections by interacting with target proteins and linked genes in chemicobiological models. In Silico Pharmacol 10 (2022) 17.
[296] R. Trenker, and N. Jura, Receptor tyrosine kinase activation: From the ligand perspective. Current Opinion in Cell Biology 63 (2020) 174-185.
[297] Z. Lateef, and L.M. Wise, Exploitation of receptor tyrosine kinases by viral-encoded growth factors. Growth Factors 36 (2018) 118-140.
[298] S.N. Pattanakitsakul, K. Rungrojcharoenkit, R. Kanlaya, S. Sinchaikul, S. Noisakran, S.T. Chen, P. Malasit, and V. Thongboonkerd, Proteomic analysis of host responses in HepG2 cells during dengue virus infection. J Proteome Res 6 (2007) 4592-600.
[299] M. Miao, F. Yu, D. Wang, Y. Tong, L. Yang, J. Xu, Y. Qiu, X. Zhou, and X. Zhao, Proteomics Profiling of Host Cell Response via Protein Expression and Phosphorylation upon Dengue Virus Infection. Virol Sin 34 (2019) 549-562.
[300] R. Kanlaya, S.N. Pattanakitsakul, S. Sinchaikul, S.T. Chen, and V. Thongboonkerd, The ubiquitin-proteasome pathway is important for dengue virus infection in primary human endothelial cells. J Proteome Res 9 (2010) 4960-71.
[301] M.S.J. McDermott, A. Canonici, L. Ivers, B.C. Browne, S.F. Madden, N.A. O'Brien, J. Crown, and N. O'Donovan, Dual inhibition of IGF1R and ER enhances response to trastuzumab in HER2 positive breast cancer cells. Int J Oncol 50 (2017) 2221-2228.
[302] A.K. Chakraborty, C. Zerillo, and M.P. DiGiovanna, In vitro and in vivo studies of the combination of IGF1R inhibitor figitumumab (CP-751,871) with HER2 inhibitors trastuzumab and neratinib. Breast Cancer Research and Treatment 152 (2015) 533-544.
[303] T. Okamoto, T. Suzuki, S. Kusakabe, M. Tokunaga, J. Hirano, Y. Miyata, and Y. Matsuura, Regulation of Apoptosis during Flavivirus Infection. Viruses 9 (2017).
[304] J.J.H. Chu, and P.L. Yang, Pharmacological C-Abl Kinase Inhibitors as Potential Anti-Viral Molecules for Dengue Virus. International Journal of Infectious Diseases 12 (2008) e297.
[305] L. He, S.Y. Wu, T.L. Wang, P. Zhang, and X. Huang, [Induction of VEGF in human monocytes by DENV infection and the regulatory mechanism]. Bing Du Xue Bao 28 (2012) 652-7.
[306] W.C. Chen, M. Hossen, W. Liu, C.H. Yen, C.H. Huang, Y.C. Hsu, and J.C. Lee, Grape Seed Proanthocyanidins Inhibit Replication of the Dengue Virus by Targeting NF-kB and MAPK-Mediated Cyclooxygenase-2 Expression. Viruses 15 (2023).
[307] J.P.R. Pelletier, and F. Mukhtar, Chapter 16 - Passive Monoclonal and Polyclonal Antibody Therapies. in: R.W. Maitta, (Ed.), Immunologic Concepts in Transfusion Medicine, Elsevier, 2020, pp. 251-348.
[308] K. Hwang, J.H. Yoon, J.H. Lee, and S. Lee, Recent Advances in Monoclonal Antibody Therapy for Colorectal Cancers. Biomedicines 9 (2021).
[309] J. Reimand, R. Isserlin, V. Voisin, M. Kucera, C. Tannus-Lopes, A. Rostamianfar, L. Wadi, M. Meyer, J. Wong, C. Xu, D. Merico, and G.D. Bader, Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap. Nat Protoc 14 (2019) 482-517.

[310] P. Bhatt, S.P. Sabeena, M. Varma, and G. Arunkumar, Current Understanding of the Pathogenesis of Dengue Virus Infection. Current Microbiology 78 (2021) 17-32.
[311] A. Pandey, S.L. Ding, Q.-M. Qin, R. Gupta, G. Gomez, F. Lin, X. Feng, L. Fachini da Costa, S.P. Chaki, M. Katepalli, E.D. Case, E.J. van Schaik, T. Sidiq, O. Khalaf, A. Arenas, K.S. Kobayashi, J.E. Samuel, G.M. Rivera, R.C. Alaniz, S.-H. Sze, X. Qian, W.J. Brown, A. Rice-Ficht, W.K. Russell, T.A. Ficht, and P. de Figueiredo, Global Reprogramming of Host Kinase Signaling in Response to Fungal Infection. Cell Host & Microbe 21 (2017) 637-649.e6.
[312] A. Virzì, A.A. Roca Suarez, T.F. Baumert, and J. Lupberger, Rewiring Host Signaling: Hepatitis C Virus in Liver Pathogenesis. Cold Spring Harb Perspect Med 10 (2020).
[313] P. Wei, W.W. Wong, J.S. Park, E.E. Corcoran, S.G. Peisajovich, J.J. Onuffer, A. Weiss, and W.A. Lim, Bacterial virulence proteins as tools to rewire kinase pathways in yeast and immune cells. Nature 488 (2012) 384-8.
[314] Z. Lim, N.K.B. Mohd-Ismail, E. Png, C.W. Sze, Q. Lin, W. Hong, S.G. Lim, Y.J. Tan, and J. Gunaratne, Phosphoproteomics Unravel HBV Triggered Rewiring of Host Phosphosignaling Events. Int J Mol Sci 23 (2022).
[315] M.V. Kuleshov, Z. Xie, A.B.K. London, J. Yang, John E. Evangelista, A. Lachmann, I. Shu, D. Torre, and A. Ma'ayan, KEA3: improved kinase enrichment analysis via data integration. Nucleic Acids Research 49 (2021) W304-W316.
[316] P. Casado, J.C. Rodriguez-Prados, S.C. Cosulich, S. Guichard, B. Vanhaesebroeck, S. Joel, and P.R. Cutillas, Kinase-substrate enrichment analysis provides insights into the heterogeneity of signaling pathway activation in leukemia cells. Sci Signal 6 (2013) rs6.
[317] V. Vella, G. Giamas, and A. Ditsiou, Diving into the dark kinome: lessons learned from LMTK3. Cancer Gene Therapy 29 (2022) 1077-1079.
[318] M.K. Poh, G. Shui, X. Xie, P.Y. Shi, M.R. Wenk, and F. Gu, U18666A, an intra-cellular cholesterol transport inhibitor, inhibits dengue virus entry and replication. Antiviral Res 93 (2012) 191-8.
[319] H. Fahimi, M. Mohammadipour, H. Haddad Kashani, F. Parvini, and M. Sadeghizadeh, Dengue viruses and promising envelope protein domain III-based vaccines. Appl Microbiol Biotechnol 102 (2018) 2977-2996.
[320] H. Kuruppu, W. Wickramanayake, C. Jeewandara, D. Peranantharajah, H.S. Colambage, L. Perera, L. Gomes, A. Wijewickrama, G.S. Ogg, and G.N. Malavige, Adipokine levels and their association with clinical disease severity in patients with dengue. medRxiv (2023).
[321] S. Sivasubramanian, S. Mohandas, V. Gopalan, V. Vimal Raj, K. Govindan, P. Varadarajan, K. Kaveri, and K.M. Ramkumar, The utility of inflammatory and endothelial factors in the prognosis of severe dengue. Immunobiology 227 (2022) 152289.
[322] S. Ramón-García, C. Ng, H. Anderson, J.D. Chao, X. Zheng, T. Pfeifer, Y. Av-Gay, M. Roberge, and C.J. Thompson, Synergistic drug combinations for tuberculosis therapy identified by a novel high-throughput screen. Antimicrob Agents Chemother 55 (2011) 3861-9.

[323] M. Rogosnitzky, P. Okediji, and I. Koman, Cepharanthine: a review of the antiviral potential of a Japanese-approved alopecia drug in COVID-19. Pharmacol Rep 72 (2020) 1509-1516.
[324] P. Phumesin, J. Panaampon, R. Kariya, T. Limjindaporn, P.T. Yenchitsomanus, and S. Okada, Cepharanthine inhibits dengue virus production and cytokine secretion. Virus Res 325 (2023) 199030.
[325] Y.B. Zhou, Y.F. Wang, Y. Zhang, L.Y. Zheng, X.A. Yang, N. Wang, J.H. Jiang, F. Ma, D.T. Yin, C.Y. Sun, and Q.D. Wang, In vitro activity of cepharanthine hydrochloride against clinical wild-type and lamivudine-resistant hepatitis B virus isolates. Eur J Pharmacol 683 (2012) 10-5.
[326] S. Gu, W.Y. Fu, A.K.Y. Fu, E.P.S. Tong, F.C.F. Ip, X. Huang, and N.Y. Ip, Identification of new EphA4 inhibitors by virtual screening of FDA-approved drugs. Sci Rep 8 (2018) 7377.
[327] P.A. Tsao, J.P. Estes, J.J. Griggs, D.C. Smith, and M.E.V. Caram, Cardiovascular and Metabolic Toxicity of Abiraterone in Castration-resistant Prostate Cancer: Post-marketing Experience. Clin Genitourin Cancer 17 (2019) e592-e601.
[328] T. McGovern, J. McNamee, S. Marcus, and J. Kashani, When Too Much Is Enough: Pediatric Cyproheptadine Overdose with Confirmatory Level. Clin Pract Cases Emerg Med 1 (2017) 205-207.
[329] S. Cirmi, A. El Abd, L. Letinier, M. Navarra, and F. Salvo, Cardiovascular Toxicity of Tyrosine Kinase Inhibitors Used in Chronic Myeloid Leukemia: An Analysis of the FDA Adverse Event Reporting System Database (FAERS). Cancers (Basel) 12 (2020).
[330] M. Viganò, M. La Milia, M.V. Grassini, N. Pugliese, M. De Giorgio, and S. Fagiuoli, Hepatotoxicity of Small Molecule Protein Kinase Inhibitors for Cancer. Cancers (Basel) 15 (2023).
[331] L.P. Osborn, and P.R. Cohen, Afatinib-Associated Cutaneous Toxicity: A Correlation of Severe Skin Reaction with Dramatic Tumor Response in a Woman with Exon 19 Deletion Positive Non-Small-Cell Lung Cancer. Cureus 8 (2016) e763.
[332] M.M. Alasmari, A Review of Margetuximab-Based Therapies in Patients with HER2-Positive Metastatic Breast Cancer. Cancers (Basel) 15 (2022).
[333] X. Wang, A. Huang, Y. Lu, S. Gao, W. Hu, and H. Cheng, Drug-induced liver injury associated with dacomitinib: A case report. Front Oncol 12 (2022) 979462.
[334] Z. Guo, Y. Ding, M. Wang, J. Liu, Q. Zhai, and Q. Du, Safety of trastuzumab deruxtecan: A meta-analysis and pharmacovigilance study. J Clin Pharm Ther 47 (2022) 1837-1844.
[335] J. Gligorov, X. Pivot, B. Ataseven, M. De Laurentiis, K.H. Jung, A. Manikhas, H. Abdel Azim, K. Gupta, A. Alexandrou, L. Herraez-Baranda, N. Tosti, and E. Restuccia, Safety and efficacy of adjuvant subcutaneous trastuzumab in human epidermal growth factor receptor 2-positive early breast cancer: Final results of the SafeHER study. Breast 64 (2022) 151-158.
[336] B. Moy, and P.E. Goss, Lapatinib-associated toxicity and practical management recommendations. Oncologist 12 (2007) 756-65.

[337] H. Wang, H. Cao, and Z. Guo, Efficacy, toxicity and prognostic factors of pyrotinib-involved neoadjuvant therapy in HER2-positive breast cancer: A retrospective study. Oncol Lett 26 (2023) 314.
[338] V.F. Borges, C. Ferrario, N. Aucoin, C. Falkson, Q. Khan, I. Krop, S. Welch, A. Conlin, J. Chaves, P.L. Bedard, M. Chamberlain, T. Gray, A. Vo, and E. Hamilton, Tucatinib Combined With Ado-Trastuzumab Emtansine in Advanced ERBB2/HER2-Positive Metastatic Breast Cancer: A Phase 1b Clinical Trial. JAMA Oncol 4 (2018) 1214-1220.
[339] K. Montazeri, and J. Bellmunt, Erdafitinib for the treatment of metastatic bladder cancer. Expert Review of Clinical Pharmacology 13 (2020) 1-6.
[340] S.U. Gandhy, S.J. Casak, S.L. Mushti, J. Cheng, S. Subramaniam, H. Zhao, M. Zhao, Y. Bi, G. Liu, J. Fan, O. Adeniyi, R. Charlab, D. Kufrin, M.D. Thompson, K. Jarrell, D. Auth, S.J. Lemery, R. Pazdur, P.G. Kluetz, and L.A. Fashoyin-Aje, FDA Approval Summary: Futibatinib for Unresectable Advanced or Metastatic, Chemotherapy Refractory Intrahepatic Cholangiocarcinoma with FGFR2 Fusions or Other Rearrangements. Clin Cancer Res (2023).
[341] C. Kang, Infigratinib: First Approval. Drugs 81 (2021) 1355-1360.
[342] N.I.o.D.a.D.a.K. Diseases, Pemigatinib, 2022.
[343] S.K. Krishnamoorthy, V. Relias, S. Sebastian, V. Jayaraman, and M.W. Saif, Management of regorafenib-related toxicities: a review. Therap Adv Gastroenterol 8 (2015) 285-97.
[344] R.B. Costa, R.L.B. Costa, S.M. Talamantes, J.B. Kaplan, M.A. Bhave, A. Rademaker, C. Miller, B.A. Carneiro, D. Mahalingam, and Y.K. Chae, Systematic review and meta-analysis of selected toxicities of approved ALK inhibitors in metastatic non-small cell lung cancer. Oncotarget 9 (2018) 22137-22146.
[345] S.M. Couch, Teprotumumab (Tepezza) for Thyroid Eye Disease. Mo Med 119 (2022) 36-41.
[346] M.A. Pruis, G.D.M. Veerman, H.C. Hassing, D.A.C. Lanser, M.S. Paats, R.H.N. van Schaik, R.H.J. Mathijssen, O. Manintveld, and A.C. Dingemans, Cardiac Toxicity of Alectinib in Patients With ALK+ Lung Cancer: Outcomes of Cardio-Oncology Follow-Up. JACC CardioOncol 5 (2023) 102-113.
[347] M. Schmidinger, and R. Danesi, Management of Adverse Events Associated with Cabozantinib Therapy in Renal Cell Carcinoma. Oncologist 23 (2018) 306-315.
[348] M.D. Aydemirli, E. Kapiteijn, K.R.M. Ferrier, P.B. Ottevanger, T.P. Links, A.N.A. van der Horst-Schrivers, K.E. Broekman, R.H.H. Groenwold, and J. Zwaveling, Effectiveness and toxicity of lenvatinib in refractory thyroid cancer: Dutch real-life data. European Journal of Endocrinology 182 (2020) 131-138.
[349] F. Griesinger, G. Curigliano, M. Thomas, V. Subbiah, C.S. Baik, D.S.W. Tan, D.H. Lee, D. Misch, E. Garralda, D.W. Kim, A.J. van der Wekken, J.F. Gainor, L. Paz-Ares, S.V. Liu, G.P. Kalemkerian, Y. Houvras, D.W. Bowles, A.S. Mansfield, J.J. Lin, V. Smoljanovic, A. Rahman, S. Kong, A. Zalutskaya, M. Louie-Gao, A.L. Boral, and J. Mazières, Safety and efficacy of pralsetinib in RET fusion-positive non-small-cell lung cancer including as first-line therapy: update from the ARROW trial. Ann Oncol 33 (2022) 1168-1178.
[350] L.J. Wirth, E. Sherman, B. Robinson, B. Solomon, H. Kang, J. Lorch, F. Worden, M. Brose, J. Patel, S. Leboulleux, Y. Godbert, F. Barlesi, J.C. Morris, T.K.

Owonikoko, D.S.W. Tan, O. Gautschi, J. Weiss, C. de la Fouchardière, M.E. Burkard, J. Laskin, M.H. Taylor, M. Kroiss, J. Medioni, J.W. Goldman, T.M. Bauer, B. Levy, V.W. Zhu, N. Lakhani, V. Moreno, K. Ebata, M. Nguyen, D. Heirich, E.Y. Zhu, X. Huang, L. Yang, J. Kherani, S.M. Rothenberg, A. Drilon, V. Subbiah, M.H. Shah, and M.E. Cabanillas, Efficacy of Selpercatinib in RET-Altered Thyroid Cancers. N Engl J Med 383 (2020) 825-835.
[351] S. Sehdev, Sunitinib toxicity management - a practical approach. Can Urol Assoc J 10 (2016) S248-s251.
[352] H.Q. Doan, M.I. Hu, J. Goldstein, S.A. Piha-Paul, V. Subbiah, and A.B. Patel, Vandetanib photoinduced cutaneous toxicities. Cutis 103 (2019) E24-e29.
[353] M.A.S. Khan, A. Al Mosabbir, E. Raheem, A. Ahmed, R.R. Rouf, M. Hasan, F.B. Alam, N. Hannan, S. Yesmin, R. Amin, N. Ahsan, S. Anwar, S. Afroza, and M.S. Hossain, Clinical spectrum and predictors of severity of dengue among children in 2019 outbreak: a multicenter hospital-based study in Bangladesh. BMC Pediatrics 21 (2021) 478.
[354] A.M. Lue, M.-A.E.H. Richards-Dawson, G.M. Gordon-Strachan, S.M. Kodilinye, J.A.T. Dunkley-Thompson, T.D. James-Powell, C.A. Pryce, C.D.s. Mears, J.J. Anzinger, K. Webster-Kerr, and C.D.C. Christie, Severity and Outcomes of Dengue in Hospitalized Jamaican Children in 2018–2019 During an Epidemic Surge in the Americas. Frontiers in Medicine 9 (2022).
[355] S.N. HAMMOND, A. BALMASEDA, L. PÉREZ, Y. TELLEZ, S.I. SABORÍO, J.C. MERCADO, E. VIDEA, Y. RODRIGUEZ, M.A. PÉREZ, R. CUADRA, S. SOLANO, J. ROCHA, W. IDIAQUEZ, A. GONZALEZ, and E. HARRIS, DIFFERENCES IN DENGUE SEVERITY IN INFANTS, CHILDREN, AND ADULTS IN A 3-YEAR HOSPITAL-BASED STUDY IN NICARAGUA. The American Journal of Tropical Medicine and Hygiene Am J Trop Med Hyg 73 (2005) 1063-1070.
[356] C.K. Bodinayake, A.D. Nagahawatte, V. Devasiri, N.J. Dahanayake, G.B. Wijayaratne, N.P. Weerasinghe, M. Premamali, T. Sheng, B.P. Nicholson, H.A. Ubeysekera, R.M. Kurukulasooriya, A.D. de Silva, T. Østbye, C.W. Woods, and L.G. Tillekeratne, Outcomes among children and adults at risk of severe dengue in Sri Lanka: Opportunity for outpatient case management in countries with high disease burden. PLoS Negl Trop Dis 15 (2021) e0010091.
[357] M.T. Alera, A. Srikiatkhachorn, J.M. Velasco, I.A. Tac-An, C.B. Lago, H.E. Clapham, S. Fernandez, J.W. Levy, B. Thaisomboonsuk, C. Klungthong, L.R. Macareo, A. Nisalak, L. Hermann, D. Villa, and I.-K. Yoon, Incidence of Dengue Virus Infection in Adults and Children in a Prospective Longitudinal Cohort in the Philippines. PLOS Neglected Tropical Diseases 10 (2016) e0004337.
[358] G. Paz-Bailey, L. Sánchez-González, B. Torres-Velasquez, E.S. Jones, J. Perez-Padilla, T.M. Sharp, O. Lorenzi, M. Delorey, J.L. Munoz-Jordan, K.M. Tomashek, S.H. Waterman, L.I. Alvarado, and V. Rivera-Amill, Predominance of Severe Plasma Leakage in Pediatric Patients With Severe Dengue in Puerto Rico. The Journal of Infectious Diseases 226 (2022) 1949-1958.
[359] L. Qiu, S.E. Patterson, L.F. Bonnac, and R.J. Geraghty, Nucleobases and corresponding nucleosides display potent antiviral activities against dengue virus

possibly through viral lethal mutagenesis. PLoS Negl Trop Dis 12 (2018) e0006421.
[360] R.J. Geraghty, M.T. Aliota, and L.F. Bonnac, Broad-Spectrum Antiviral Strategies and Nucleoside Analogues. Viruses 13 (2021).
[361] M.E.H. Kayesh, and K. Tsukiyama-Kohara, Mammalian animal models for dengue virus infection: a recent overview. Arch Virol 167 (2022) 31-44.
[362] A.B. Byrne, A.G. García, J.M. Brahamian, A. Mauri, A. Ferretti, F.P. Polack, and L.B. Talarico, A murine model of dengue virus infection in suckling C57BL/6 and BALB/c mice. Animal Model Exp Med 4 (2021) 16-26.

APPENDIX

The following contents are publications that I co-authored in supplement to my dissertation research for which I have received journal permission to include here.

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Dengue activates mTORC2 signaling to counteract apoptosis and maximize viral replication

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The mechanistic target of rapamycin (mTOR) functions in two distinct complexes: mTORC1, and mTORC2. mTORC1 has been implicated in the pathogenesis of flaviviruses including dengue, where it contributes to the establishment of a pro-viral autophagic state. Activation of mTORC2 occurs upon infection with some viruses, but its functional role in viral pathogenesis remains poorly understood. In this study, we explore the consequences of a physical protein-protein interaction between dengue non-structural protein 5 (NS5) and host cell mTOR proteins during infection. Using shRNA to differentially target mTORC1 and mTORC2 complexes, we show that mTORC2 is required for optimal dengue replication. Furthermore, we show that mTORC2 is activated during viral replication, and that mTORC2 counteracts virus-induced apoptosis, promoting the survival of infected cells. This work reveals a novel mechanism by which the dengue flavivirus can promote cell survival to maximize viral replication.

KEYWORDS

mechanistic target of rapamycin (mTOR), mechanistic target of rapamycin complex 2 (mTORC2), apoptosis, non-structural protein 5, flavivirus, pathogenesis, viral replication, dengue virus (DENV)

Introduction

Mechanistic target of rapamycin (mTOR) is a ubiquitous, essential serine/threonine kinase that functions in several key aspects of cell biology [reviewed in (Laplante and Sabatini, 2009; Battaglioni et al., 2022; Simcox and Lamming, 2022)]. mTOR exerts its actions as a component of two distinct complexes, mTORC1 and mTORC2. mTORC1 is composed of mTOR, raptor, and mLST8, with PRAS40 and deptor also being present in some cell types (Simcox and Lamming, 2022). mTORC1 functions as a master regulator of anabolic/catabolic homeostasis. In conditions of nutritional abundance, mTOR phosphorylates p70 ribosomal protein S6K (S6K) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), leading to increased protein synthesis. In conditions of nutrient scarcity mTORC1 is inactivated, stimulating autophagy, which allows for the recycling and turnover of cellular organelles and protein complexes (Laplante and Sabatini, 2009).

mTORC2 is composed of mTOR, rictor, mLST8, and SIN1 (Laplante and Sabatini, 2009; Battaglioni et al., 2022; Simcox and Lamming, 2022). mTORC2 has distinct roles from mTORC1 in cellular physiology, but these roles are less well understood than those of mTORC1. mTORC2 promotes cell survival and proliferation through phosphorylation of AKT at ser473 (Laplante and Sabatini, 2012). It is also known to play a role in the maintenance of the actin cytoskeleton, and when inactivated results in morphological abnormalities in some cell lines (Jacinto et al., 2004; Sarbassov et al., 2004). It has been suggested that mTORC2 may modulate translational machinery due to its association with ribosomes; however, the ramifications of this interaction are not well understood (Zinzalla et al., 2011). Although mTORC1 is the canonical regulator of autophagy, mTORC2 has been implicated in the regulation of specific autophagic processes such as chaperone-mediated autophagy and mTORC1-independent autophagy (Arias et al., 2015; Lampada et al., 2017).

Given the important role of mTORC1 in regulating cellular metabolism, it is not surprising that several viruses have evolved mechanisms to modulate mTORC1 signaling (Buchkovich et al., 2008; Le Sage et al., 2016). Numerous viruses have been shown to manipulate mTORC1 activity during infection; some viruses activate mTORC1 to maintain cellular anabolic machinery, whereas others suppress mTORC1 activity to favor cap-independent viral protein synthesis (Buchkovich et al., 2008; Le Sage et al., 2016). Accordingly, mTOR is actively being explored as a potential host based anti-viral therapeutic target (Maiese, 2020).

In the case of dengue, virus-induced modulation of mTORC1 has been suspected due to the importance of autophagy in dengue infection (Lee et al., 2008; Heaton and Randall, 2010; Lee et al., 2013; Metz et al., 2015), although this interaction of the virus with mTORC1 has not been comprehensively investigated. mTORC signaling is also dysregulated in dengue infection of megakaryocytes (Lahon et al., 2021). The importance of mTORC1 in dengue replication is supported by studies demonstrating increased viral replication in the response to pharmacologic mTOR inhibition and a recent study implicating mTORC1 in the dengue-induced activation of lipophagy (Mateo et al., 2013; Jordan and Randall, 2017). In contrast to mTORC1, potential roles for mTORC2 in virus-host interaction are comparatively poorly understood. Activation of mTORC2 has been documented in human cytomegalovirus, West Nile and influenza infection (Kudchodkar et al., 2006; Shives et al., 2014; Kuss-Duerkop et al., 2017), but the functional role of mTORC2 in these infections remains unknown. Furthermore, to our knowledge no role for mTORC2 has been described in dengue infection.

Here, we describe a role for mTORC2 in promoting cell survival during dengue infection. We find that the dengue non-structural protein 5 (NS5) interacts with mTORC1 and mTORC2 complexes, and that dengue infection leads to the activation of mTORC2 signaling. We report that inactivation of mTORC2 signaling leads to a decrease in viral replication and an increase in virus-induced apoptosis and cell death. These findings suggest a mechanism by which dengue counteracts apoptosis to maintain cell survival and maximize viral replication.

Results

Dengue NS5 protein interacts with mTORC1 and mTORC2

Quantitative proteomics [I-DIRT; Isotopic Differentiation of Interactions as Random or Targeted (Tackett et al., 2005)], designed to identify *bona fide* dengue-host protein-protein interactions, defined a high-confidence protein interaction network including a predicted interaction between the dengue NS5 protein and mTOR (Carpp et al., 2014). To validate and further study this interaction, we performed co-immunoprecipitation experiments using exogenously expressed NS5. GFP-tagged NS5 or GFP alone was transfected into 293FT cells, leading to modest expression of the fusion protein in the cytosol with nuclear accumulation (Supplemental Figure 1), as has been previously reported (Kumar et al., 2013). Protein complexes were then affinity purified using GFP-specific nanobodies (Fridy et al., 2014). Subsequent western blotting of affinity purified NS5 complexes revealed mTOR, raptor, and rictor proteins, demonstrating that NS5 interacts with both mTORC1 and mTORC2 (Figure 1A).

We also assayed for the NS5-mTORC interaction in dengue-infected HepG2 hepatoma cells (Figure 1B). HepG2 hepatoma cells were used due to the hepatotropic nature of dengue, and the extensive previous research of dengue with this cell line, including its replication, and its roles in modulating host processes such as

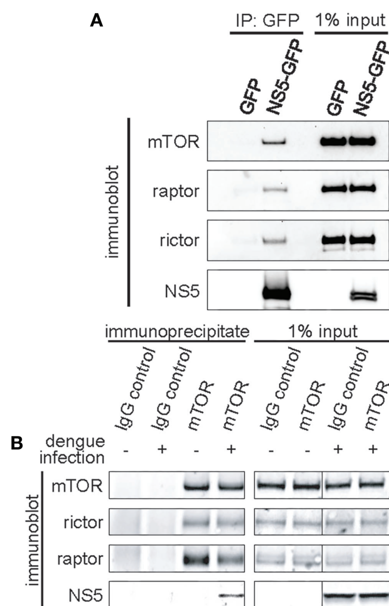

FIGURE 1
Dengue NS5 interacts with mTORC1 and mTORC2. **(A)**, Immunoprecipitation of NS5-GFP fusion protein identifies mTORC1 and mTORC2 interacting proteins. 293FT cells were transfected with expression plasmids encoding GFP or NS5-GFP fusion protein, and NS5-GFP was affinity captured from the cell lysate using anti-GFP nanobodies. 50% of the eluates or 1% of the input lysate were then assayed by western blot analysis with the indicated antibodies. **(B)**, mTOR interacts with NS5 during dengue infection. HepG2 cells were infected with dengue virus, serotype 2, at an MOI of 4. mTOR was immunoenriched from the lysate using anti-mTOR antibodies. Separately, nonspecific IgG was used as an immunoprecipitation control. 50% of the eluates or 1% of the input lysate were then assayed by western blot analysis with the indicated antibodies.

apoptosis, autophagy and ER stress (Suksanpaisan et al., 2007; Thepparit et al., 2013; Carpp et al., 2014; Jordan and Randall, 2017). mTOR was immunopurified and the eluate probed for NS5. As a control, we also probed for rictor and raptor (Figure 1B). As expected, NS5 was immunopurified in the mTOR pulldown in dengue-infected cells, but absent in the IgG immunoprecipitation control. Intriguingly, less raptor protein associated mTOR in dengue-infected cells, whereas rictor was not impacted (Figure 1B). These experiments confirm the previous I-DIRT results (Tackett et al., 2005) and establish the sufficiency of NS5 alone for interacting with mTORC1 and mTORC2, i.e. not requiring the presence of other viral proteins, such as NS3, to initiate and/or stabilize the interaction.

mTORC2 is activated during dengue replication and is required for efficient viral replication

To investigate the impact of NS5-mTOR interactions on dengue viral infection, and to explore the role of NS5 interacting with mTORC1 and mTORC2, the expression of each complex was silenced using lentivirus-delivered shRNA targeting mTOR, raptor (a component of mTORC1) or rictor (a component of mTORC2) or, as a control, a nonspecific scrambled oligo-sequence (Sarbassov et al., 2005). Transduction of the corresponding shRNA resulted in substantially decreased protein abundance of mTOR, Raptor or Rictor (Figure 2A). To assess whether raptor and rictor knockdown disrupted the signaling activity of mTORC1 and mTORC2, we examined the phosphorylation status of well-characterized downstream targets (p70 S6K thr389 for mTORC1 and AKT ser473 for mTORC2) (Burnett et al., 1998; Sarbassov et al., 2005). Knockdown of raptor led to diminished mTORC1 signaling as evidenced by decreased S6K thr389 phosphorylation, and knockdown of rictor led to diminished mTORC2 signaling as evidenced by decreased AKT ser473 phosphorylation (Figure 2A). We observed that depletion of mTORC1 led to a modest reciprocal activation of mTORC2 activity, consistent with prior reports suggesting that mTORC1 represses mTORC2 signaling (Julien et al., 2010) (Figure 2A). The converse did not appear to be the case, as depletion of mTORC2 did not lead to increased phosphorylation of S6K by mTORC1 (Figure 2A). Furthermore, mTOR knockdown diminished levels of raptor and rictor, but knockdown of neither raptor nor rictor influenced levels of each other (Figure 2A).

We next assessed the effect of reduced mTOR, raptor (mTORC1), or rictor (mTORC2) protein levels on dengue serotype 2 (DENV2) replication by infecting respective knockdown cells and quantifying the amount of infectious virus released (Figure 2B). Interestingly, rictor knockdown led to a substantial decrease in the amount of viral replication, while raptor knockdown led to a small but significant increase in replication. No significant effect on viral replication was observed when mTOR protein was knocked down (Figure 2B).

Having observed a decrease in viral replication with mTORC2 inactivation, we next asked whether dengue infection affected the activity of mTORC2 (Figure 2C). HepG2 cells were infected with DENV-2 and lysates were probed for the phosphorylation status of the downstream targets AKT and S6K by western blot. We observed an increase in mTORC2-specific phosphorylation at AKT ser473 in infected cells compared to the mock treatment, with the ratio of ser473 phosphorylated AKT to total AKT significantly increased in infected cells (Figure 2C, $p < 0.001$). In contrast, AKT ser473 phosphorylation was not increased when cells were treated with UV-inactivated virus (Figure 2D).

To assess the breadth of AKT activation resulting from dengue infection, Huh7 cells were separately infected with three isolates of DENV-2 (MON601, K0049 and IQT2913) or an isolate of DENV-3 (H87), or DENV-4 (H241). In all cases, we observed an increase in the phosphorylation of AKT ser473 (Figure 2E). The subtle variation in degree of AKT

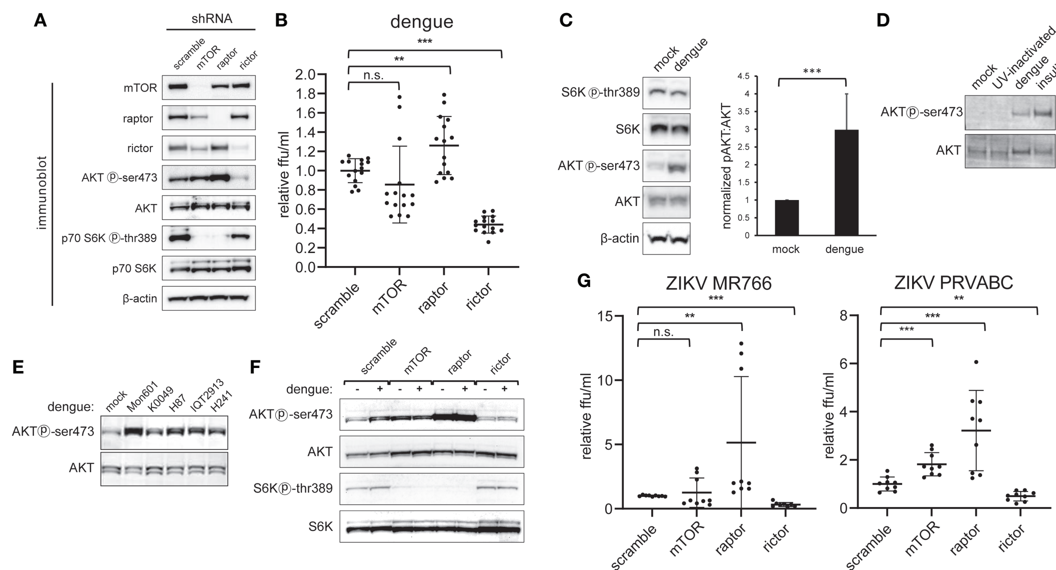

FIGURE 2

Dengue infection activates mTORC2 signaling and is required for maximal viral replication. *A*, Selective inactivation of mTORC1 and mTORC2 in HepG2 cells. Cells were transduced with lentivirus encoding shRNA directed against mTOR protein, Raptor, or Rictor, or with a non-specific scramble sequence, and then selected with puromycin. Lysates were analyzed by western blot with the indicated antibodies. *B*, mTORC2 inactivation diminishes dengue replication. Cells were transduced with lentiviral shRNA vectors as in **(A)** and were then infected with dengue MON601 at a MOI of 1. 24 h later cell culture supernatants were collected and titrated on Vero cells. The data represent scramble-normalized values from 4 independent experiments. **(C)**, mTORC2 is activated during dengue infection. HepG2 cells were infected with dengue at MOI of 4. Cells were collected at 36 h post-infection and analyzed by western blot with the indicated antibodies. The bar graph shows the ratio of phospho-AKT to total AKT band intensity averaged from 7 independent experiments. **(D)**, Competent dengue virus infection is required for AKT activation. HepG2 cells were infected with dengue at a MOI of 1, or with UV-inactivated virus. Mock and insulin treatment were used as a negative and positive control for AKT activation, respectively. Lysates were analyzed by western blot with the indicated antibodies. **(E)**, mTORC2 activation is conserved across dengue serotypes. Experiments were performed as in **(C)** using Huh7 cells infected with DENV-2 MON601, DENV-2 K0049, DENV-3 H87, DENV-2 IQT2913, or DENV-4 H241. **(F)**, Rictor knockdown abrogates mTORC2 activation by dengue. Cells were transduced with shRNA encoding lentivirus as in **(A)** and were infected with dengue at MOI of 4. Cells were collected at 36 hpi and were analyzed by western blot with the indicated antibodies. **(G)** mTORC2 abrogation negatively impacts Zika virus (ZIKV) replication. Experiments were performed as in **(B)** but with ZIKV MR788 and ZIKV PRVABC. Error bars are one standard deviation. p values are derived from 2-tailed Student's t test; n.s., not significant; ** denotes $p < 0.01$, and *** denotes $p < 0.001$.

phosphorylation may correspond to variable infectivity across the isolates or strain specific differences in the degree of activation; nonetheless, all strains activated AKT.

Abrogation of the increased AKT ser473 phosphorylation was observed in cells depleted for rictor, but not mTOR or raptor. As in Figure 2A, knockdown of mTOR protein or raptor led to increased mTORC2 activity, which was not further increased by dengue infection (Figure 2F). These experiments demonstrate that mTORC2 is required for dengue-induced AKT ser473 phosphorylation.

To evaluate if mTORC2's role in dengue virus replication is conserved in other flaviviruses, we infected HepG2 cells depleted for mTOR, raptor, or rictor with two separate isolates of the Zika virus (ZIKV MR766 and PRVABC59). In each case, increased viral replication was observed in cells depleted for raptor while decreased viral replication was observed in cells depleted for rictor (Figure 2G). These data suggest that the role of mTORC2 on infection is conserved in the related flavivirus, Zika virus.

mTORC2 inhibition does not affect cellular morphology, growth rates or dengue-induced LC3-II accumulation

The increase in mTORC2 activity during dengue infection and the requirement for mTORC2 activity for maximal viral replication led us to investigate potential physiological functions of mTORC2 that are exploited by the virus. Because mTOR has also been shown to play a role in the maintenance of cell morphology by modulating ion channel activity and altering dynamics of the actin cytoskeleton (Jacinto et al., 2004; Sarbassov et al., 2004), we examined the morphology of mTORC2-depleted cells and the distribution of their actin cytoskeletons by microscopy. Cells treated with shRNA targeting rictor showed normal cellular morphology, normal cell spreading and a morphologically normal actin cytoskeleton (Figure 3A), suggesting that mTORC2 inactivation was not causing derangements in cell architecture in this setting.

mTORC2 has been shown to play a role in regulating cell proliferation (Oh and Jacinto, 2011), so we considered the possibility that our findings could be explained by altered cell growth rates. We assessed cell growth rates in knockdown cells by measuring cell counts and CFSE dilution. No significant differences in growth rates were observed upon comparison of mTORC2 knockdown cells with control cells (Figures 3B, C).

Although autophagy is canonically regulated by mTORC1, we also considered the possibility that inactivation of mTORC2 suppresses dengue replication by altering autophagy through indirect regulatory effects on mTORC1. While the effect that dengue has on the movement of cargo through the canonical autophagy pathway is unclear, it is well known that dengue induces the accumulation of autophagosomes, characterized by accumulation of lipidated LC3 protein (LC3-II) (Lee et al., 2008; Heaton and Randall, 2010; Metz et al., 2015; Jordan and Randall, 2017). To assess effects of mTORC2 inhibition on the pro-autophagic activity of dengue, we measured LC3-II isoform levels in dengue-infected knockdown cells and observed similar degrees of dengue-induced LC3-II accumulation (Figure 3D), suggesting that mTORC2 inactivation does not block the effects of dengue on autophagy.

mTORC2 inactivation causes increased dengue-mediated apoptosis and cell death

mTORC2 could function as a pro-survival factor in infected cells, given the known role for mTORC2 in regulating cell survival (Oh and Jacinto, 2011; Lamming et al., 2014; Zou et al., 2016). Rictor knockdown or scramble control cells were infected with dengue for 24 or 36 h and, as a positive control for apoptosis, treated the cells with staurosporine. We then asked whether cell death, or apoptosis specifically, was altered in the context of mTORC2 inhibition by measuring activated caspase 3 expression and cell viability within infected and uninfected cells using flow cytometry. At 24 hours post-infection (hpi), we observed a similar frequency of infected cells when comparing control cells to rictor or raptor knockdown cells, arguing against an early block in viral replication in mTORC2-deficient cells. However, at 36 hpi, rictor knockdown was associated with a significant decrease in the proportion of infected cells (Figures 4A, B). In contrast, raptor knockdown cells exhibited a non-significant increase in infection. Rictor knockdown cells also produced less infectious virus at 24 and

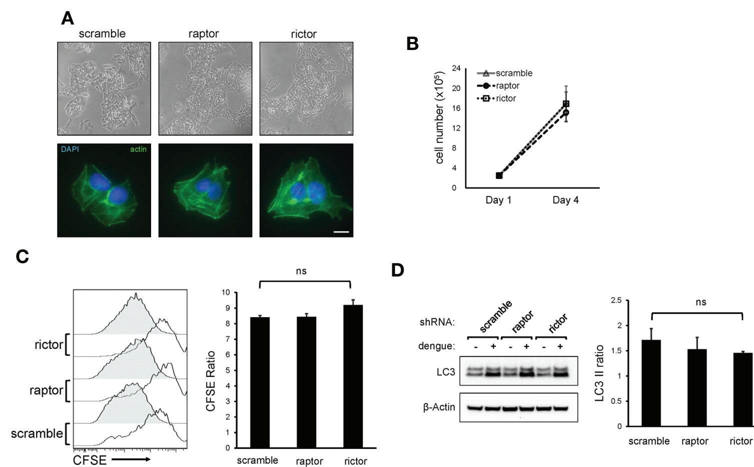

FIGURE 3
mTORC2 inactivation does not alter HepG2 cell morphology, cytoskeletal architecture, growth rate, or LC3 lipidation in response to dengue infection. **(A)**, HepG2 cells were transduced with lentiviral vectors encoding shRNA directed against mTOR protein, Raptor or Rictor, or with a non-specific scramble sequence and were selected with puromycin. Cells were imaged by light microscopy (top panels) or stained with fluorophore-labeled phalloidin and imaged with fluorescence microscopy (bottom panels). Bar = 100 µm **(B)**, Cells were transduced with shRNA lentiviral vectors as in **(A)** and were plated at equal densities. Cells were then counted using an automated cell counter 4 days later. Data represent cell counts from 4 separate cultures. **(C)**, Cells were transduced with shRNA lentiviral vectors as in **(A)** and were plated at equal densities. Cells were then loaded with CFSE and analyzed by flow cytometry 24 h (open histograms) and 96 h (shaded histograms) later. Bar graph represents the ratio of CFSE mean fluorescence intensity between the 24 and 96 h timepoint, averaged from 4 separate cultures. **(D)**, mTORC2 inhibition does not alter dengue-mediate LC3-II lipidation. Cells were transduced with shRNA lentiviral vectors as in **(A)** and were infected with dengue at MOI 4. Cells were harvested at 36 h post-infection and lysates were analyzed by western blot with the indicated antibodies. The bar graph shows the ratio of LC3-II band intensity between dengue infected and mock treated cells from 3 independent experiments. Error bars are one standard deviation. p values are derived from 2-tailed Student's t test; n.s., not significant.

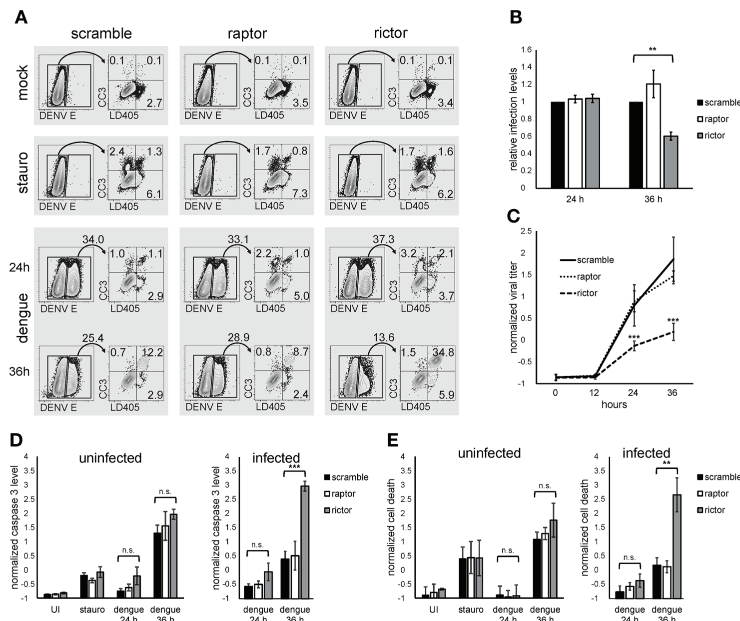

FIGURE 4
mTORC2 inhibition leads to increased dengue-induced apoptosis and cell death. **(A)**, HepG2 cells were infected with dengue at MOI of 4 for 24 or 36 h, mock treated, or treated with 5 μM staurosporine overnight. Cells were then collected and stained with a cell-impermeable amine reactive dye (LD405), followed by fixation, permeabilization, and staining with anti-activated caspase 3 and anti-flavivirus E protein antibodies. Infected (E-positive) cells were gated and activated caspase 3 (CC3) and LD405 expression were analyzed within that population. Numbers represent population frequencies in each gate/quadrant. **(B)**, Cells were treated as in **(A)**, and the percent of infected (E-positive) cells was calculated. The bar graph shows the average values for 3 independent experiments, each normalized to the scramble condition. **(C)**, Cells were treated as in **(A)**, and the amount of infectious virus present in the supernatant was quantified at the indicated timepoints. The bar graph represents Z-normalized average values from 3 independent experiments. **(D)**, Cells were treated as in **(A)**, and the percent of infected cells positive for caspase 3 was calculated. The bar graph represents *Z*-normalized values from 3 independent experiments. **(E)**, Cells were treated as in **(A)**, and the percent of infected cells positive for LD405 was calculated. The bar graph represents *Z*-normalized values from 3 independent experiments. p values are derived from 2-tailed Student's t test; n.s., not significant; ** denotes $p < 0.01$, and *** denotes $p < 0.001$.

36 hpi, whereas raptor knockdown cells produced virus at levels indistinguishable from that produced by the scramble control cells (Figure 4C). Assessment of activated caspase 3 expression and cell viability at 24 h revealed a small increase in apoptosis and cell death in rictor-deficient cells, which were not statistically significant (Figures 4A, D, E). However, by 36 hpi, a marked increase in apoptosis and cell death was observed in infected rictor knockdown cells, both of which were highly significant (4*A*, *D*, and *E*, $p < 0.001$ for activated caspase 3 level, $p < 0.01$ for cell viability). Interestingly, in contrast to what we observed in dengue infected cells, staurosporine treatment increased cell death and apoptosis at similar levels in the scramble control, rictor, and raptor knockdown cells, and there were no statistically significant differences in the uninfected populations (Figures 4A, D, E). This suggests that the increased apoptosis observed in dengue-infected cells was specific for virus-induced apoptosis in the rictor knockdown cells and not a general property of all pro-apoptosis stimuli. Thus, dengue-infected cells harbor a specific sensitivity to mTORC2 inhibition.

Discussion

In this study, we describe a role for host mTORC2 in dengue replication, demonstrating that dengue can interact with cellular mTOR signaling, activating mTORC2 and, as a result, promoting cell survival and efficient viral replication. Specifically, the data we present here suggest that mTORC2 plays a role in supporting viral production by counteracting virus-induced apoptosis of the host cell. The induction of apoptosis in dengue infection has been well-described, occurring in several cell types both *in vitro* and *in vivo*, including endothelial cells, dendritic cells, and hepatocytes (Limonta et al., 2007; Torrentes-Carvalho et al., 2009; Martins Sde et al., 2012; Lin et al., 2014). The finding of apoptotic cells in human autopsy specimens from severe dengue cases has led to speculation that apoptosis contributes to pathogenesis in these cases (Limonta et al., 2007). While apoptosis may contribute to tissue damage and pathogenesis from the host perspective, it is also an important mechanism for host control of viral infection, triggering cell death before infectious progeny can be released

(Orzalli and Kagan, 2017) and shaping the subsequent immune response.

Since uncontrolled apoptosis would be detrimental to viral replication, it is not surprising that dengue has evolved a mechanism to attenuate the induction of apoptosis in infected cells. In the present study, we found that disrupting mTORC2 signaling in infected cells leads to an increased frequency of apoptosis in cell death, and the induction of apoptosis corresponds to a decrease in the release of viral progeny from infected cells. Interestingly, the susceptibility of mTORC2-deficient cells to apoptosis was specific for dengue infection in our experiments, as neither baseline apoptosis nor apoptosis in response to staurosporine treatment increased over control upon depletion of mTORC2. Furthermore, we found that dengue infection triggers the activation of mTORC2, which likely represents a viral adaptation to maintain cell survival during infection. While this strategy has not been described in other viral infections, it has been reported as mechanism for cancer cell survival and metastasis in several malignancies (Kim et al., 2017). The finding that mTORC2 is a regulator of cell death during dengue infection opens the possibility of host targeted interventions, including synthetic lethal strategies, that serve to tune apoptotic responses in infected cells to limit pathogenesis or viral spread and/or to amplify protective immune responses (Mast et al., 2020).

Dengue NS5 protein binds to mTOR, suggesting that the viral protein modulates mTOR signaling during infection. NS5 appears to bind both mTORC1 and mTORC2, evidenced by the co-immunoprecipitation of raptor and rictor with NS5 (Carpp et al., 2014). Our data are consistent with a model where this binding event stabilizes the mTORC2 complex, amplifies signaling through Akt, boosts cell survival, and facilitates optimal viral propagation (Figure 5). The detailed molecular consequences of the NS5-mTOR interaction remain to be investigated. One possibility is that NS5 binds to mTORC2 and directly facilitates activation of the complex or acts as an adaptor protein to stabilize interactions with downstream targets of the complex. Given that there is crosstalk between mTORC1 and mTORC2 signaling (Tyakht et al., 2014), it is also possible that interaction of NS5 with mTORC1 could activate mTORC2, either *via* de-repression of mTORC2 or through alterations in mTOR protein stoichiometry.

The findings we present here also highlight the role of proteomic approaches in understanding virus-host interactions. Much of the recent focus in dengue research has utilized high-throughput genetic screens employing approaches such as RNAi and CRISPR (Sessions et al., 2009; Marceau et al., 2016; Savidis et al., 2016). While these genetic screens have identified host dependency factors, they rely on measuring viral gene expression or cell survival as the readout for infection resistance, and they are likely biased toward host factors involved in the early stages of infection such as viral entry and gene expression as opposed to host factors needed for late infection events such as viral release, maturation, and host cell survival. For this reason, it is perhaps not surprising that components of the autophagy machinery and mTOR signaling

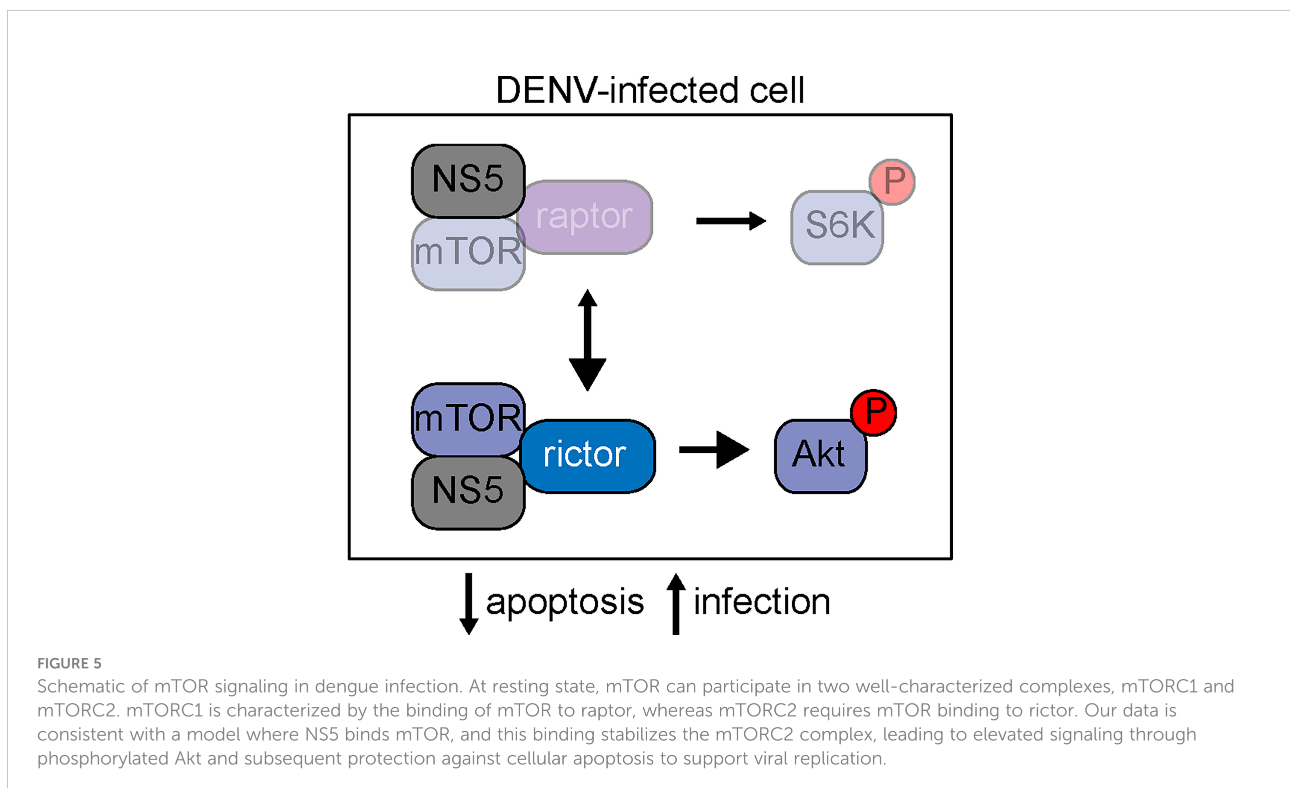

FIGURE 5
Schematic of mTOR signaling in dengue infection. At resting state, mTOR can participate in two well-characterized complexes, mTORC1 and mTORC2. mTORC1 is characterized by the binding of mTOR to raptor, whereas mTORC2 requires mTOR binding to rictor. Our data is consistent with a model where NS5 binds mTOR, and this binding stabilizes the mTORC2 complex, leading to elevated signaling through phosphorylated Akt and subsequent protection against cellular apoptosis to support viral replication.

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have not been consistently identified in these screens, despite the roles of these host factors reported here and by others (Mateo et al., 2013; Jordan and Randall, 2017). Moreover, genetic screens stop short of identifying specific molecular interactions between virus and host that mediate regulatory processes, which can be elucidated when interactions between viral and host proteins are interrogated *via* proteomics. Nonetheless, it is notable that rictor inhibition was associated with decreased relative infection in 2 RNAi libraries previously reported, although that finding did not meet the authors' criteria for further validation (Savidis et al., 2016). In contrast to genetic screens, proteomics methods can identify host interactions at all stages of the viral life cycle, and subsequent validation can identify important host interactions. When combined with quantitative approaches to distinguish high-probability interactors from non-specific binding, proteomics approaches can identify host factors with high validity (Carpp et al., 2014).

The observation that mTORC2 is required for efficient dengue replication raises the possibility of mTOR as a target for host-directed antiviral therapeutic development. mTOR is a highly "druggable" target, and intense interest in the mTOR pathways in oncology and neuropathology fields has spurred the development of multiple new small molecule inhibitors with high specificity for mTOR (Xie et al., 2016; Zou et al., 2016). In the solid organ transplant infectious disease field, data have begun to emerge that certain mTOR inhibitors (used as anti-organ rejection medications) may have anti-viral properties, reducing the risk of some viral reactivation syndromes (Pascual et al., 2016). In the case of dengue *per se*, candidate compounds would likely need to have mTORC2 specificity, since mTORC1 inhibition appears to enhance viral replication. While most newer generation mTOR inhibitors have dual mTORC1/C2 specificity, a recently developed compound CID613034 has been described that specifically inhibits mTORC2, demonstrating the feasibility of specific mTORC2 inhibition (Benavides-Serrato et al., 2017). Since multiple biochemical steps occur between mTORC2 assembly and the resultant anti-apoptotic outcome, components of the mTORC2 signaling cascade might also provide useful targets for host-based interventions.

The role of mTORC2 signaling in the pathogenesis of other viral infections remains to be determined; however, there is evidence that other viruses including West Nile, influenza and human cytomegalovirus may also stimulate mTORC2 signaling (Kudchodkar et al., 2006; Shives et al., 2014; Kuss-Duerkop et al., 2017). Whether mTORC2 is important for viral replication and serves as an anti-apoptotic mechanism in those viruses remains to be determined, but it is possible that mTORC2 modulation is a common mechanism used by several viruses to counteract the host's programmed cell death response. If that is the case, host-directed therapeutic interventions targeting mTORC2 could be active against multiple viral pathogens.

Materials and methods

Antibodies and reagents

The following antibodies were obtained from Cell Signaling Technology (Danvers, MA) and used at 1:1000 for western blotting: Raptor clone 24C10, Rictor clone D16H9, phospho-Rictor thr1135 clone D30A6, mTOR 7C10, AKT clone 11E7, phospho-AKT ser473 clone D9E (used at 1:2000), p70 S6K clone 49D7, phospho-p70 S6K thr389 clone 108D2, LC3A/B clone D3U4C, and B-actin clone 13E5. The dengue 2 E protein antibody PA5-32246 was used at 1:10,000 for western blotting (Thermo-Fisher Scientific, Waltham, MA). Secondary detection for western blotting used anti-rabbit HRP antibody diluted 1:10,000 (Amersham ECL, GE Healthcare, Chicago, IL). Polyclonal affinity purified NS3 and NS5 proteins were produced as previously described (Carpp et al., 2014). The pan-flavivirus E antibody 4G2 was prepared from hybridoma supernatants and purified by protein A/G chromatography. For flow cytometry experiments, 4G2 was conjugated to FITC according to the manufacturer's instructions (FluoroTag kit, Sigma-Aldrich, St. Louis, MO). For immunoprecipitation, the dimerized GFP nanobody construct LaG-16–G4S–LaG-2 (green lobster; gift from Michael Rout), mTOR 7C10, and Normal Rabbit IgG (2729S; Cell Signaling Technology, Danvers, MA), were prepared as previously described (Fridy et al., 2014). For microscopy, phalloidin-Alexafluor 488 was obtained from Thermo-Fisher scientific and used according to their instructions. Staurosporine was obtained from Millipore Sigma (Burlington, MA) and was used at 5 μM concentration.

Plasmids

The lentiviral shRNA plasmids pLKO.1 scramble, Raptor_2, mTOR_2, and Rictor_2 were gifts from David Sabatini (Addgene plasmids 1864, 1858, 1856, and 1854). pMD2.G and psPAX2 were gifts from Didier Trono (Addgene plasmids 12259 and 12260). Dengue NS5-GFP fusion construct was generated by inserting the dengue New Guinea C NS5 coding sequence from pDVW601 (Pryor et al., 2001) into pACGFP1-N1 (Clontech) as previously described (Carpp et al., 2014).

Cell culture

HEK293FT, HepG2, Huh7 and Vero cells were obtained from the ATCC (Manassas, VA) and cultured at 37°C in 5% CO_2, in

medium composed of MEM supplemented with 10% FBS, 1× non-essential amino acids, 50 units/mL of penicillin and 50 μg/mL of streptomycin. Medium was replenished frequently during experiments to avoid signaling changes caused by nutrient or growth factor depletion. The *Aedes albopictus* derived cell line C6/36 was propagated at 28°C in 5% CO_2 in medium composed of MEM supplemented with 10% FBS, 1× non-essential amino acids, 50 units/mL of penicillin and 50 μg/mL of streptomycin. The B lymphocyte hybridoma cell line D1-4G2-4-15 was obtained from the ATCC (Manassas, VA), and was maintained in ATCC Hybri-Care Medium supplemented with 10% FBS and 1.5 g/L sodium bicarbonate.

Transfections

HEK293FT cells were plated to 70-80% density in antibiotic free medium. Transfection complexes were prepared by mixing plasmid DNA with polyethyleneimine (PEI Max 40k, Polysciences Inc., Warrington, PA) in a 1:4 mass ratio. After 15 min incubation at room temperature DNA complexes were added dropwise to the cell cultures.

Affinity capture

To prepare affinity-capture beads, the dimerized GFP nanobody LaG-16–G4S–LaG-2 (green lobster), mTOR IgG, and non-specific IgG were covalently linked to magnetic beads (Dynabeads M-270 epoxy Thermo Fisher Scientific) as previously described (Cristea and Chait, 2011). Briefly, 5 μg of nanobody were used per 1 mg of Dynabeads, with conjugations carried out in 0.1 M sodium phosphate buffer and 1 M ammonium sulfate, with an 18- to 20-h incubation at 30°C. Beads were then washed sequentially with 0.1 M sodium phosphate buffer, 100 mM glycine pH 2.5, 10 mM Tris-HCl pH 8.8, 100 mM triethylamine, 1× PBS (4 times), PBS + 0.5% Triton X-100, and 1× PBS. For affinity capture experiments, cells were harvested 48 h after transfection, or 24 h post DENV infection. Cells were washed with ice cold PBS and then lysed with 1% Triton X-100, 0.5% sodium deoxycholate, 110 mM potassium acetate pH 7.5, 20 mM HEPES, 2 mM $MgCl_2$, 25 mM NaCl, and 1× protease/phosphatase inhibitor cocktail (Cell Signaling Technology). Lysates were clarified by centrifugation for 10 min at 13,000 × *g* at 4°C. Affinity capture beads were immediately added to the clarified lysate and incubated for 10 min at room temperature with rotation. Beads were then washed 3× with lysis buffer, and bound protein complexes eluted with 1.1× LDS sample buffer for 10 min at 70°C. For SDS-PAGE and western blot analysis, 10× reducing agent (Thermo-Fisher Scientific) was added and samples were heated for an additional 10 min at 70°C. SDS-PAGE and western blot analysis were performed as described below. Gel staining was performed using Sypro Ruby fluorescent gel stain (Thermo-Fisher Scientific) according to the manufacturer's instructions.

shRNA mediated gene silencing

To generate lentiviral vector stocks, shRNA constructs were cotransfected with pMD2.G and psPAX2 into HEK 293T cells. Supernatants were harvested, passed through 0.45 μm filters, layered on 20% sucrose cushions, and centrifuged at 100,000 × *g* for 4 h at 4°C. Lentiviral pellets were resuspended in OptiMEM and stored at -80°C until use. For lentiviral transductions, viral stocks were diluted to the desired concentration with OptiMEM and 0.8 μg/mL polybrene and added to cells. At 48 h post-transduction, cells containing stably integrated constructs were selected using 2 μg/mL puromycin. Experiments were performed on cell lines that were maintained and passaged for no more than 3 weeks before discarding and establishing fresh cell lines.

Virus and infections

DENV-2 MON601, a molecular clone of DENV-2 New Guinea strain C[46]; DENV-2 K0049, a clone of a southeast Asian isolate; DENV-3 H87, a clone of a Philippines isolate from 1956; DENV-2 IQT2913, a clone of a Peru isolate from 1996; DENV-4 H241, a clone of a Philippines isolate from 1956; ZIKV MR766, a clone of a Ugandan ZIKV isolate from 1947; and ZIKV PRVABC, a clone of a Puerto Rican ZIKV isolate from 2015, were generated by transfection of *in vitro*-transcribed RNA into Vero cells, followed by no more than 5 passages in C6/36 cells. Virus was propagated by interchangeably infecting 80% confluent C6/36 or Vero monolayers with low-passage stock virus at a MOI of 0.01, and harvesting infectious supernatants 5-7 days post-infection. Infectious supernatants were cleared of cellular debris by centrifugation and filtration through 0.2μm PVFD membrane then stored at -80°C until use. Virus stocks and experimental infectious supernatants were titrated using a flow cytometry approach which has been described elsewhere (Lambeth et al., 2005). Briefly, serially diluted virus stocks were used to infect Vero cells in a multi-well plate. Cells were harvested 20-24 h post-infection, fixed and permeabilized, and stained with 4G2-FITC. The percentage of infected cells was then used to calculate the number of fluorescence forming units (FFU) per milliliter of inoculum. To generate inactivated virus, the virus stock was irradiated by ultraviolet light for one hour at room temperature prior to infection. For experimental infections, virus was diluted in OptiMEM to the desired MOI and incubated on cells for 90 min at 37°C. Virus was then

removed, the cells washed, and complete growth medium added. To inactivate virus, virus was exposed to UV light for 1 h in a 6-well plate in a biosafety cabinet.

Western blot analysis

Cells were placed on ice and washed with ice-cold PBS. Cells were then collected and lysed with Triton X-100 lysis buffer (1% Triton X-100, 120 mM NaCl, 1 mM EDTA, 40 mM HEPES pH 7.4, and 1× protease and phosphatase inhibitor cocktail (Cell Signaling Technology)). To prepare whole cell extracts for the immunoprecipitation load control, SDS lysis buffer (2% SDS, 50 mM Tris pH 7.4, 5% glycerol, 5 mM EDTA, 1 mM NaF, 1 mM DTT, and 1× phosphatase & protease inhibitor cocktail) was used instead of Triton X-100 lysis buffer. Protein concentration was determined using BCA assay and a BSA standard curve, and equivalent amounts of protein were mixed with 4× LDS sample buffer and 10× reducing agent (Thermo-Fisher Scientific), followed by denaturation at 70°C for 10 min. Proteins were then resolved on 4-12% Bis-Tris gels (for lower molecular weight proteins) or 3-8% Tris-Acetate gels (for higher molecular weight proteins) (NuPage, Thermo-Fisher Scientific) and run in MOPS or Tris-Acetate running buffer respectively. Proteins were transferred to PVDF membrane and blocked in 5% milk/TBS-T for 1-2 h. Primary antibodies were diluted in 5% BSA/TBS-T, and incubated overnight at 4°C. Membranes were washed and incubated with anti-rabbit HRP antibody for 1-2 h at room temperature. Membranes were then washed with TBS-T, exposed to chemiluminescent substrate, and imaged using a digital CCD platform (Fluorchem E, Protein Simple, San Jose, CA). Band densitometry was performed using ImageJ software.

Fluorescence microscopy

Cells were fixed with 4% paraformaldehyde/PBS for 15 min at room temperature, permeabilized with 0.1% Triton X-100/PBS for 10 min at room temperature and blocked with 5% normal goat serum in 0.05% Tween-20/PBS. Cells were then stained with phalloidin-Alexafluor 488 (Thermo Fisher Scientific) for 1 h at room temperature and mounted using medium containing DAPI.

Flow cytometry

For viability analysis, cells were trypsinized, washed with PBS, and stained with a cell impermeable amine reactive dye (LIVE/DEAD Violet A.K.A. LD405, ThermoFisher Scientific) according to the manufacturer's instructions. Cells were fixed and permeabilized using the BD Cytofix/Cytoperm kit according to the manufacturer's instructions (BD Biosciences). Permeabilized cells were stained with fluorophore-conjugated antibodies as indicated in the text. Cells were analyzed on a BD LSR-II cytometer, and data were analyzed using FlowJo software.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

CC, FM, JO, NB, AK, and JA have made substantial, direct, and intellectual contributions to the work, and have approved it for publication. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.979996/full#supplementary-material

References

Arias, E., Koga, H., Diaz, A., Mocholi, E., Patel, B., and Cuervo, A. M. (2015). Lysosomal mTORC2/PHLPP1/Akt regulate chaperone-mediated autophagy. *Mol. Cell* 59, 270–284. doi: 10.1016/j.molcel.2015.05.030

Battaglioni, S., Benjamin, D., Walchli, M., Maier, T., and Hall, M. N. (2022). mTOR substrate phosphorylation in growth control. *Cell* 185, 1814–1836. doi: 10.1016/j.cell.2022.04.013

Benavides-Serrato, A., Lee, J., Holmes, B., Landon, K. A., Bashir, T., Jung, M. E., et al. (2017). Specific blockade of rictor-mTOR association inhibits mTORC2 activity and is cytotoxic in glioblastoma. *PLoS One* 12, e0176599. doi: 10.1371/journal.pone.0176599

Buchkovich, N. J., Yu, Y., Zampieri, C. A., and Alwine, J. C. (2008). The TORrid affairs of viruses: Effects of mammalian DNA viruses on the PI3K-Akt-mTOR signalling pathway. *Nat. Rev. Microbiol.* 6, 266–275. doi: 10.1038/nrmicro1855

Burnett, P. E., Barrow, R. K., Cohen, N. A., Snyder, S. H., and Sabatini, D. M. (1998). RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proc. Natl. Acad. Sci. U.S.A.* 95, 1432–1437. doi: 10.1073/pnas.95.4.1432

Carpp, L. N., Rogers, R. S., Moritz, R. L., and Aitchison, J. D. (2014). Quantitative proteomic analysis of host-virus interactions reveals a role for golgi brefeldin a resistance factor 1 (GBF1) in dengue infection. *Mol. Cell Proteomics* 13, 2836–2854. doi: 10.1074/mcp.M114.038984

Cristea, I. M., and Chait, B. T. (2011). Conjugation of magnetic beads for immunopurification of protein complexes. *Cold Spring Harb. Protoc.* 2011, pdbprot5610. doi: 10.1101/pdb.prot5610

Fridy, P. C., Li, Y., Keegan, S., Thompson, M. K., Nudelman, I., Scheid, J. F., et al. (2014). A robust pipeline for rapid production of versatile nanobody repertoires. *Nat. Methods* 11, 1253–1260. doi: 10.1038/nmeth.3170

Heaton, N. S., and Randall, G. (2010). Dengue virus-induced autophagy regulates lipid metabolism. *Cell Host Microbe* 8, 422–432. doi: 10.1016/j.chom.2010.10.006

Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Ruegg, M. A., Hall, A., et al. (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat. Cell Biol.* 6, 1122–1128. doi: 10.1038/ncb1183

Jordan, T. X., and Randall, G. (2017). Dengue virus activates the AMP kinase-mTOR axis to stimulate a proviral lipophagy. *J. Virol.* 91(11):e02020-16. doi: 10.1128/JVI.02020-16

Julien, L. A., Carriere, A., Moreau, J., and Roux, P. P. (2010). mTORC1-activated S6K1 phosphorylates rictor on threonine 1135 and regulates mTORC2 signaling. *Mol. Cell Biol.* 30, 908–921. doi: 10.1128/MCB.00601-09

Kim, L. C., Cook, R. S., and Chen, J. (2017). mTORC1 and mTORC2 in cancer and the tumor microenvironment. *Oncogene* 36, 2191–2201. doi: 10.1038/onc.2016.363

Kudchodkar, S. B., Yu, Y., Maguire, T. G., and Alwine, J. C. (2006). Human cytomegalovirus infection alters the substrate specificities and rapamycin sensitivities of raptor- and rictor-containing complexes. *Proc. Natl. Acad. Sci. U.S.A.* 103, 14182–14187. doi: 10.1073/pnas.0605825103

Kumar, A., Buhler, S., Selisko, B., Davidson, A., Mulder, K., Canard, B., et al. (2013). Nuclear localization of dengue virus nonstructural protein 5 does not strictly correlate with efficient viral RNA replication and inhibition of type I interferon signaling. *J. Virol.* 87, 4545–4557. doi: 10.1128/JVI.03083-12

Kuss-Duerkop, S. K., Wang, J., Mena, I., White, K., Metreveli, G., Sakthivel, R., et al. (2017). Influenza virus differentially activates mTORC1 and mTORC2 signaling to maximize late stage replication. *PloS Pathog.* 13, e1006635. doi: 10.1371/journal.ppat.1006635

Lahon, A., Arya, R. P., and Banerjea, A. C. (2021). Dengue virus dysregulates master transcription factors and PI3K/AKT/mTOR signaling pathway in megakaryocytes. *Front. Cell Infect. Microbiol.* 11, 715208. doi: 10.3389/fcimb.2021.715208

Lambeth, C. R., White, L. J., Johnston, R. E., and de Silva, A. M. (2005). Flow cytometry-based assay for titrating dengue virus. *J. Clin. Microbiol.* 43, 3267–3272. doi: 10.1128/JCM.43.7.3267-3272.2005

Lamming, D. W., Demirkan, G., Boylan, J. M., Mihaylova, M. M., Peng, T., Ferreira, J., et al. (2014). Hepatic signaling by the mechanistic target of rapamycin complex 2 (mTORC2). *FASEB J.* 28, 300–315. doi: 10.1096/fj.13-237743

Lampada, A., O'Prey, J., Szabadkai, G., Ryan, K. M., Hochhauser, D., and Salomoni, P. (2017). mTORC1-independent autophagy regulates receptor tyrosine kinase phosphorylation in colorectal cancer cells *via* an mTORC2-mediated mechanism. *Cell Death Differ.* 24, 1045–1062. doi: 10.1038/cdd.2017.41

Laplante, M., and Sabatini, D. M. (2009). mTOR signaling at a glance. *J. Cell Sci.* 122, 3589–3594. doi: 10.1242/jcs.051011

Laplante, M., and Sabatini, D. M. (2012). mTOR signaling in growth control and disease. *Cell* 149, 274–293. doi: 10.1016/j.cell.2012.03.017

Lee, Y. R., Hu, H. Y., Kuo, S. H., Lei, H. Y., Lin, Y. S., Yeh, T. M., et al. (2013). Dengue virus infection induces autophagy: an *in vivo* study. *J. BioMed. Sci.* 20, 65. doi: 10.1186/1423-0127-20-65

Lee, Y. R., Lei, H. Y., Liu, M. T., Wang, J. R., Chen, S. H., Jiang-Shieh, Y. F., et al. (2008). Autophagic machinery activated by dengue virus enhances virus replication. *Virology* 374, 240–248. doi: 10.1016/j.virol.2008.02.016

Le Sage, V., Cinti, A., Amorim, R., and Mouland, A. J. (2016). Adapting the stress response: Viral subversion of the mTOR signaling pathway. *Viruses* 8 (6), 152. doi: 10.3390/v8060152

Limonta, D., Capo, V., Torres, G., Perez, A. B., and Guzman, M. G. (2007). Apoptosis in tissues from fatal dengue shock syndrome. *J. Clin. Virol.* 40, 50–54. doi: 10.1016/j.jcv.2007.04.024

Lin, J. C., Lin, S. C., Chen, W. Y., Yen, Y. T., Lai, C. W., Tao, M. H., et al. (2014). Dengue viral protease interaction with NF-kappaB inhibitor alpha/beta results in endothelial cell apoptosis and hemorrhage development. *J. Immunol.* 193, 1258–1267. doi: 10.4049/jimmunol.1302675

Maiese, K. (2020). The mechanistic target of rapamycin (mTOR): Novel considerations as an antiviral treatment. *Curr. Neurovasc. Res.* 17, 332–337. doi: 10.2174/1567202617666200425205122

Marceau, C. D., Puschnik, A. S., Majzoub, K., Ooi, Y. S., Brewer, S. M., Fuchs, G., et al. (2016). Genetic dissection of flaviviridae host factors through genome-scale CRISPR screens. *Nature* 535, 159–163. doi: 10.1038/nature18631

Martins Sde, T., Silveira, G. F., Alves, L. R., Duarte dos Santos, C. N., and Bordignon, J. (2012). Dendritic cell apoptosis and the pathogenesis of dengue. *Viruses* 4, 2736–2753. doi: 10.3390/v4112736

Mast, F. D., Navare, A. T., van der Sloot, A. M., Coulombe-Huntington, J., Rout, M. P., Baliga, N. S., et al. (2020). Crippling life support for SARS-CoV-2 and other viruses through synthetic lethality. *J. Cell Biol.* 219(10), e202006159. doi: 10.1083/jcb.202006159

Mateo, R., Nagamine, C. M., Spagnolo, J., Mendez, E., Rahe, M., Gale, M.Jr., et al. (2013). Inhibition of cellular autophagy deranges dengue virion maturation. *J. Virol.* 87, 1312–1321. doi: 10.1128/JVI.02177-12

Metz, P., Chiramel, A., Chatel-Chaix, L., Alvisi, G., Bankhead, P., Mora-Rodriguez, R., et al. (2015). Dengue virus inhibition of autophagic flux and dependency of viral replication on proteasomal degradation of the autophagy receptor p62. *J. Virol.* 89, 8026–8041. doi: 10.1128/JVI.00787-15

Oh, W. J., and Jacinto, E. (2011). mTOR complex 2 signaling and functions. *Cell Cycle* 10, 2305–2316. doi: 10.4161/cc.10.14.16586

Orzalli, M. H., and Kagan, J. C. (2017). Apoptosis and necroptosis as host defense strategies to prevent viral infection. *Trends Cell Biol.* 27, 800–809. doi: 10.1016/j.tcb.2017.05.007

Pascual, J., Royuela, A., Fernandez, A. M., Herrero, I., Delgado, J. F., Sole, A., et al. (2016). Role of mTOR inhibitors for the control of viral infection in solid organ transplant recipients. *Transpl. Infect. Dis.* 18, 819–831. doi: 10.1111/tid.12601

Pryor, M. J., Carr, J. M., Hocking, H., Davidson, A. D., Li, P., and Wright, P. J. (2001). Replication of dengue virus type 2 in human monocyte-derived macrophages: comparisons of isolates and recombinant viruses with substitutions at amino acid 390 in the envelope glycoprotein. *Am. J. Trop. Med. Hyg.* 65, 427–434. doi: 10.4269/ajtmh.2001.65.427

Sarbassov, D. D., Ali, S. M., Kim, D. H., Guertin, D. A., Latek, R. R., Erdjument-Bromage, H., et al. (2004). Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr. Biol.* 14, 1296–1302. doi: 10.1016/j.cub.2004.06.054

Sarbassov, D. D., Guertin, D. A., Ali, S. M., and Sabatini, D. M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307, 1098–1101. doi: 10.1126/science.1106148

Savidis, G., McDougall, W. M., Meraner, P., Perreira, J. M., Portmann, J. M., Trincucci, G., et al. (2016). Identification of zika virus and dengue virus dependency factors using functional genomics. *Cell Rep.* 16, 232–246. doi: 10.1016/j.celrep.2016.06.028

Sessions, O. M., Barrows, N. J., Souza-Neto, J. A., Robinson, T. J., Hershey, C. L., Rodgers, M. A., et al. (2009). Discovery of insect and human dengue virus host factors. *Nature* 458, 1047–1050. doi: 10.1038/nature07967

Shives, K. D., Beatman, E. L., Chamanian, M., O'Brien, C., Hobson-Peters, J., and Beckham, J. D. (2014). West Nile virus-induced activation of mammalian target of rapamycin complex 1 supports viral growth and viral protein expression. *J. Virol.* 88, 9458–9471. doi: 10.1128/JVI.01323-14

Simcox, J., and Lamming, D. W. (2022). The central moTOR of metabolism. *Dev. Cell* 57, 691–706. doi: 10.1016/j.devcel.2022.02.024

Suksanpaisan, L., Cabrera-Hernandez, A., and Smith, D. R. (2007). Infection of human primary hepatocytes with dengue virus serotype 2. *J. Med. Virol.* 79, 300–307. doi: 10.1002/jmv.20798

Tackett, A. J., DeGrasse, J. A., Sekedat, M. D., Oeffinger, M., Rout, M. P., and Chait, B. T. (2005). I-DIRT, a general method for distinguishing between specific and nonspecific protein interactions. *J. Proteome Res.* 4, 1752–1756. doi: 10.1021/pr050225e

Thepparit, C., Khakpoor, A., Khongwichit, S., Wikan, N., Fongsaran, C., Chingsuwanrote, P., et al. (2013). Dengue 2 infection of HepG2 liver cells results in endoplasmic reticulum stress and induction of multiple pathways of cell death. *BMC Res. Notes* 6, 372. doi: 10.1186/1756-0500-6-372

Torrentes-Carvalho, A., Azeredo, E. L., Reis, S. R., Miranda, A. S., Gandini, M., Barbosa, L. S., et al. (2009). Dengue-2 infection and the induction of apoptosis in human primary monocytes. *Mem. Inst. Oswaldo Cruz* 104, 1091–1099. doi: 10.1590/S0074-02762009000800005s

Tyakht, A. V., Ilina, E. N., Alexeev, D. G., Ischenko, D. S., Gorbachev, A. Y., Semashko, T. A., et al. (2014). RNA-Seq gene expression profiling of HepG2 cells: The influence of experimental factors and comparison with liver tissue. *BMC Genomics* 15, 1108. doi: 10.1186/1471-2164-15-1108

Xie, J., Wang, X., and Proud, C. G. (2016). *mTOR inhibitors in cancer therapy*. 5, 2078. doi: 10.12688/f1000research.9207.1

Zinzalla, V., Stracka, D., Oppliger, W., and Hall, M. N. (2011). Activation of mTORC2 by association with the ribosome. *Cell* 144, 757–768. doi: 10.1016/j.cell.2011.02.014

Zou, Z., Chen, J., Yang, J., and Bai, X. (2016). Targeted inhibition of Rictor/mTORC2 in cancer treatment: A new era after rapamycin. *Curr. Cancer Drug Targets* 16, 288–304. doi: 10.2174/1568009616666151113120830

CHEMICAL REVIEWS

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Host-targeted Interventions as an Exciting Opportunity to Combat Malaria

Kamalakannan Vijayan, Ling Wei, Elizabeth K. K. Glennon, Christa Mattocks, Natasha Bourgeois, Bart Staker, and Alexis Kaushansky*

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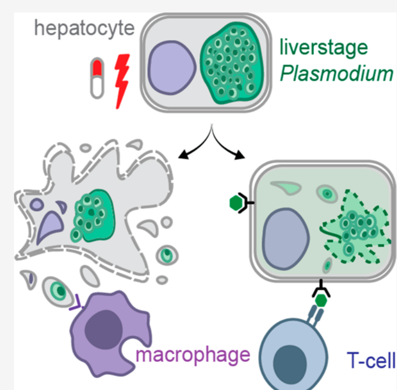
ABSTRACT: Terminal and benign diseases alike in adults, children, pregnant women, and others are successfully treated by pharmacological inhibitors that target human enzymes. Despite extensive global efforts to fight malaria, the disease continues to be a massive worldwide health burden, and new interventional strategies are needed. Current drugs and vector control strategies have contributed to the reduction in malaria deaths over the past 10 years, but progress toward eradication has waned in recent years. Resistance to antimalarial drugs is a substantial and growing problem. Moreover, targeting dormant forms of the malaria parasite *Plasmodium vivax* is only possible with two approved drugs, which are both contraindicated for individuals with glucose-6-phosphate dehydrogenase deficiency and in pregnant women. *Plasmodium* parasites are obligate intracellular parasites and thus have specific and absolute requirements of their hosts. Growing evidence has described these host necessities, paving the way for opportunities to pharmacologically target host factors to eliminate *Plasmodium* infection. Here, we describe progress in malaria research and adjacent fields and discuss key challenges that remain in implementing host-directed therapy against malaria.



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1. INTRODUCTION

Malaria is a severe life-threatening disease caused by parasites of the genus *Plasmodium*. In 2019, there were an estimated 409 000 deaths caused by malaria and 229 million cases worldwide.[1] Children under the age of five are the most vulnerable to malaria and accounted for 67% of the deaths reported in 2019.[1] Of the 172 *Plasmodium* species, six (*Plasmodium malariae, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale curtisi, Plasmodium ovale wallikeri*, and *Plasmodium knowlesi)* can infect humans.[1,2] *P. falciparum* and *P. vivax* pose the biggest threat to humankind. *P. falciparum* is the primary cause of malaria disease in Africa, accounting for 99.7% cases in this region, 71% of cases in the WHO eastern Mediterranean region, and 65% of cases in the WHO Western Pacific region.[3] *P. vivax* is widespread in South East Asia, making up 47% of cases in India and 75% of cases in the WHO America region.[3] *P. vivax* malaria is far less common in Africa because the population is largely Duffy antigen negative.[4] Individuals lacking the duffy receptor, a common polymorphism in individuals of African descent, are largely resistant to *P. vivax* malaria. This association between epidemiology and genetics illustrates the first key component required for effective host-targeted therapy: permutations in the host that do not cause adverse health effects can lead to dramatically reduced susceptibility to parasite infection.

1.1. The Malaria Parasite Life Cycle: A Journey of Diverse Host Interactions

Malaria is transmitted by the bite of female *Anopheles* mosquitoes infected with *Plasmodium* species. During a blood meal, infected mosquitoes inject sporozoites, the infective, motile stage of *Plasmodium* spp., into the skin. After transmission, the parasite must engage multiple human cell types and tissues, navigating innate immune responses along the way. *Plasmodium* sporozoites exit the dermis by traversing into a blood vessel.[5,6] In the blood vessel, sporozoites are carried to the liver, where they traverse the sinusoidal endothelium and infect hepatocytes.[7] Within the hepatocyte, parasites dedifferentiate into uninucleate trophozoite forms prior to undergoing a dramatic, asymptomatic expansion during their schizont stage.[8] For *P. falciparum*, this development begins within 2−3 days of liver infection and completes after ∼7 days; the amount of time spent in the liver varies across species. *P. vivax* and *P. ovale* also have dormant forms, called hypnozoites, which can emerge from the liver weeks, months, or years after the initial infection, leading to relapse. These dormant forms are particularly challenging to target pharmacologically, and new interventions against them are needed.[9,10]

Once development within the liver is complete, parasites exit the hepatocyte as packages of merozoites that bud off from the cell (called merosomes) and re-enter the circulation and invade red blood cells (reviewed in refs 11, 12). Within the erythrocyte, intracellular parasites modify the cell membrane by exporting a series of proteins and reorganizing the host with various structures including Maurer's clefts,[13] Schüffner's dots,[13] and knobs.[14] These structures, along with the expression of parasite proteins on the erythrocyte surface, allow infected erythrocytes to cytoadhere to various endothelia throughout the body; this cytoadhesion is associated with pathogenesis.[13,14] Parasites express a single member of the ∼60 gene *Pf*EMP1 family at a given time, and each member of the family is associated with a specific mode of erythrocyte binding.[15] For instance, parasites that express the *Pf*EMP1 var2CSA are localized to the placenta and associated with placental malaria, whereas parasites that express DC8 and DC13 *Pf*EMP1 bind endothelial protein C receptor on brain endothelial cells and are associated with cerebral malaria.[16] Importantly, the expression of specific *var* genes in the field can be used to predict symptoms in infected individuals.[17]

During asexual replication in erythrocytes, some parasites undergo epigenetic[18] and then transcriptional reprogramming[19−21] and differentiate into sexual gametocytes. Gametocyte maturation is thought to occur in the bone marrow.[22] Once mature gametocytes have developed they are thought to exit the marrow and re-enter the bloodstream.[23] Upon mosquito bite, these sexual forms are taken up by the mosquito to complete sexual replication and begin the new infection cycle.

1.2. Current Status of Malaria Intervention: Efforts toward Eradication

Many effective antimalarials are currently used in the clinic. This includes artemisinin-based combination therapies (ACTs), chloroquine phosphate, sulfadoxine/pyrimethamine, mefloquine, primaquine phosphate, halofantrine, and quinine, each of which can eliminate symptomatic infection and facilitate prophylaxis against malaria (reviewed in ref 24). Currently, the standard of treatment varies worldwide. These differences are dependent on local availability, current status of drug resistant parasites, and a multitude of political and economic factors. This complex picture of malaria chemotherapy illustrates that the need for antimalarial drugs varies across the world and can vary over time as parasites with different phenotypes and drug sensitivity spread. Importantly, we do not have a strong arsenal of antiparasitic drugs to pull from in the event of robust spread of resistance to artemisinin-based regimens. Although there are efforts to develop new drugs that target drug-resistant parasites, increased prevalence of multidrug resistant parasites has left the world, especially countries with limited capacity to control malaria, highly susceptible to increasing malaria-caused deaths.

Movement of humans from one area to another has spread drug resistant parasites.[25] Further, population mobility may amplify the heterogeneity of resistance when diverse parasite genotypes cohabitate a population and mate in the mosquito host.[25] As climate changes, the distribution of *Anopheles* mosquitoes, and subsequent malaria transmission, is also shifting.[26] It has been suggested that low transmission, which may occur in newly introduced malaria settings, is associated with an increase in disease severity and an extension of the transmission window, a pattern which could lead to the generation of resistant parasites.[27] Further, in high transmission settings where coinfection of mixed parasite strains in the same host are very common,[27] parasites have the opportunity to generate additional phenotypes, as mating between genetically dissimilar parasites has been shown to

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preferentially generate mixed-genotype offspring.[28] While the full ramifications of altering parasite distribution remain unclear, it is reasonable to predict that diversity of parasite genotypes may produce strains with varying capacity to withstand drug pressure. Treatment in these settings removes the drug-sensitive competitors, thereby resulting in increased growth and transmission of resistant parasites. The emergence of resistance to almost all the drugs in the antimalarial armamentarium, and fear of developing resistance to the new antimalarials, has highlighted the urgency of developing alternate interventional strategies.

Multiple new antimalarials and combination therapies are in clinical development that have efficacy against drug-resistant parasites with the potential to be used as single-dose regimens.[29,30] The ambitious efforts by Medicines for Malaria Venture (MMV) and others in the field to eliminate malaria also include approaches that could target several stages of parasite life cycle. Yet, major hurdles to this work exist. MMV recently released a target candidate profile (TCP) that suggests several classes of antimalarials are needed to support a robust malaria eradication campaign. Specifically, the candidate drugs targeting asexual blood stage (TCP-1), antirelapse/hypnozoites (TCP-3), liver schizonts (TCP-4), and transmission blocking (TCP-5 and 6) are essential for driving down numbers of malaria cases worldwide.[9] Importantly, a major hurdle to malaria eradication is the *P. vivax* hypnozoite, as highlighted in TCP-3. Hypnozoites can remain in the liver for weeks, months, or even years and later reactivate, leading to relapsing and symptomatic blood stage infection.[31] It has been hypothesized that as much of 50% of *vivax* transmission events are a result of hypnozoite relapse.[32] Unfortunately, there are extremely limited pharmacological options for the elimination of hypnozoites, and the use of these drugs is hampered by severe toxicity in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency.[33] Fortunately, there is evidence to support the hypothesis that altering the hepatocyte environment is a potent antimalarial strategy.

2. RATIONALE FOR HOST-DIRECTED THERAPY FOR MALARIA

Host-directed therapy (HDT) aims to exploit the dependence of any obligate pathogen upon their host. Importantly, the key determinant of success for HDT is the same for any other drug: an appropriate therapeutic index must be achieved by targeting malignant cells (in this case, infected cells) while keeping healthy cells intact. While it has been speculated that this is more difficult to achieve in the context of targeting host enzymes than pathogen enzymes (reviewed in ref 34), in actuality, the contributions to a therapeutic index are often not fully captured by this reductionist thinking. While anti-infectives are often marketed as "specific" to a nonhuman target, the reality is that all drugs exhibit polypharmacology: the propensity to have many targets.[35] In the case of anti-infectives that target factors specific to the pathogen, off-target proteins can still be targeted in the host, either in the cells of interest or in other cell types. Therefore, the effective therapeutic index is not as simple as it is often reported, for instance, by comparing the IC_{50} for a compound against the pathogen-specific and human-specific enzymes. Because HDT, by definition targets host factors, it is critical that the host factors targeted are not essential for uninfected cells. While at first glance, this may seem difficult to achieve, the reality is that ~90% of the human genome is nonessential in adults,[36−38] and small molecules and biologics that target the host are regularly used in the clinic to treat ailments as benign as acne and as malignant as melanoma.[39] These host-targeting therapeutics are used safely in a huge portion of the human population, including children, pregnant women, and those with pre-existing conditions. In 2017 alone, ~87% of the drugs approved for use in the clinic by the Food and Drug Administration had a described target of a human molecule. Thus, targeting nonessential human molecules can be safe and efficient, and these molecules are available over the counter and prescribed by pediatricians and geriatric specialists alike.[36−38]

While resistance could, at least conceptually, be selected against an HDT, it would likely require that the parasite redirect its infection strategy to compensate for the blocked host factor. It is reasonable to think that this would occur at a lower frequency than a single point mutation that stops a parasite-targeted drug from binding its target. HDTs could take multiple forms: stopping infection, dampening pathogenesis, or enhancing naturally occurring immunity to malaria. HDT-based approaches have been used clinically for multiple diseases (discussed in the following sections). In the context of infectious diseases, these therapies can be used either as standalone drugs or in combination with pathogen-directed anti-infectives. Given the infancy of this approach in the malaria field, we discuss orthologous approaches in other systems that provide proof-of-concept studies for targeting malaria.

3. HDTS AS ANTIMALARIAL INTERVENTIONS

Plasmodium, as an obligate intracellular parasite, must exploit and subvert host functions for survival and growth. Understanding host−pathogen interactions that govern *Plasmodium* survival within its host could inform the identification of HDTs. While the application of HDTs in malaria is still in the exploratory realm, other fields have begun to explore HDTs in the clinic, in some cases, with success. In this section, we describe some examples and draw parallels to opportunities within the malaria field.

3.1. The Liver Stage of Malaria Is an Important Target

In this review, we focus on the liver stage (LS) of *Plasmodium* infection. While the host factors that regulate interactions with other key cell types and tissues are different, the concepts that underlie HDTs design are consistent. Host interactions and potential targets for intervention in the erythrocyte,[24,40−44] blood−brain barrier,[41] and mosquito stages[41] have been reviewed elsewhere. We focus on *Plasmodium* LS infection as targeting this stage is critical: success would eliminate all initial and relapsing disease and subsequent transmission. As LS is brief and asymptomatic, utilizing a host-targeting drug could be envisioned for mass drug administration in areas specifically with high intensity seasonal transmission or as a prophylactic traveler's medicine. It could also be utilized for the treatment of relapsing malaria in *P. vivax* endemic areas. However, it is unlikely to be used for postexposure prophylaxis for *P. falciparum* because diagnosis occurs after the liver stage is complete.

Following the release of sporozoites into the dermis by a bite of a female *Anopheles* mosquito, motile parasites enter a blood vessel and are carried through the bloodstream to the liver. There, sporozoites cross the sinusoidal endothelium, traversing through Kupffer cells or liver endothelial sinusoidal cells.[45] Once they reach the parenchyma, parasites continue to

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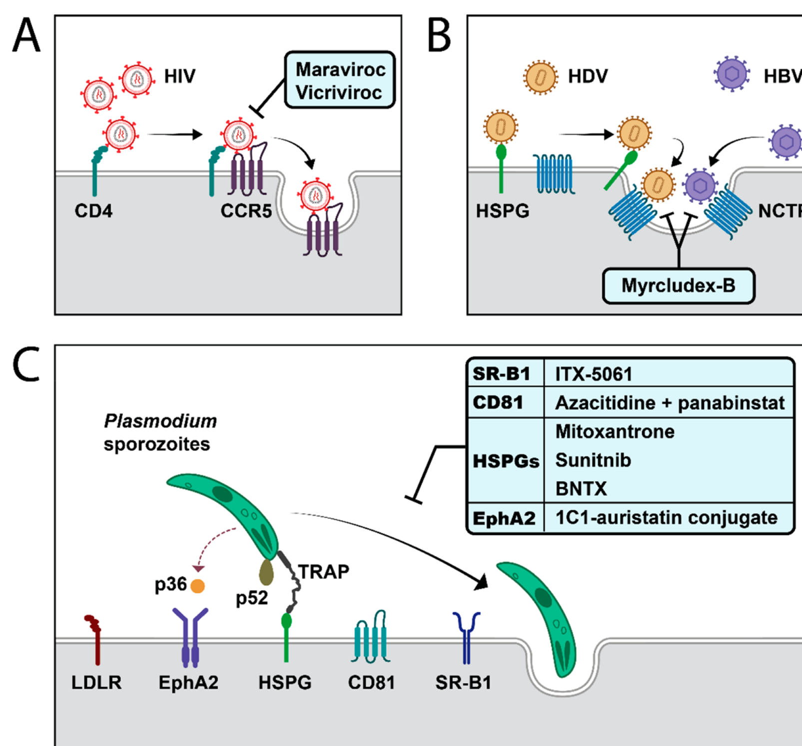

Figure 1. Entry inhibitors as potential HDTs. (A) HIV entry is mediated by the interaction of viral envelope protein with receptor CD4 and coreceptor CCR5. Entry encompasses three steps: CD4 binding, coreceptor binding, and plasma membrane fusion. Maraviroc binds to the transmembrane region of CCR5, thereby inducing conformational changes that block binding by the viral envelope protein that could block these interactions and subsequent entry. (B) HDV entry is first mediated by an attachment step, resulting from viral interaction with HSPGs. Later, a specific interaction with sodium taurocholate (NTCP) cotransporting polypeptide facilitates entry. In contrast, HBV directly binds to NTCP. Myrcludex-B binds NTCP and inactivates its receptor function for both HBV and HDV. (C) *Plasmodium* sporozoites have been demonstrated to rely on CD81, HSPG, SR-B1, EphA2, and LDL-R in a species-specific manner. Here in this review, we suggest some of the known inhibitors (in the light-blue boxes).

traverse hepatocytes,[5] at least in part by forming transient vacuoles that enable them to tunnel through cells.[46] After several rounds of traversal the sporozoite selects a hepatocyte for invasion, forming a parasitophorous vacuole membrane (PVM), derived from the hepatocyte plasma membrane, to establish the intracellular niche. Within the PVM, liver-resident parasites undergo asexual schizogony to form tens of thousands of merozoites and egress from the infected liver cell. Merozoites escape into the hepatic vein, infect erythrocytes, and asexually replicate in circulation, leading to population expansion and the clinical symptoms of malaria (reviewed in refs 11, 12).

Although it is the blood-stage infection that causes clinical disease, the LS represents a vulnerable bottleneck for therapeutic interventions to prevent malaria. The LS also plays a key role in *P. vivax* infection and transmission. Unlike *P. falciparum*, *P. vivax* has multiple paths through the liver. Upon initial infection, *P. vivax* sporozoites can form rapidly dividing schizonts that multiply tens of thousands of times over the course of ~8 days, leading to blood stage infection. A second population of parasites does not initially undergo rapid division; this population of LSs has been termed "hypnozoites".[47] Hypnozoites can reactivate to complete LS development and symptomatic blood-stage infection weeks, months, or years later, yet are not susceptible to most antimalarials. Currently, the only drugs capable of targeting hypnozoites are from the 8-aminoquinoline family, and their use is restricted due to toxicity in individuals with some G6PD polymorphisms.[48] This limitation creates a hurdle for the implementation of mass drug administration campaigns that have been proposed and implemented as a route to eradication in high transmission settings.[49] Even within populations where 8-aminoquinolines are not contraindicated, side effects often limit the use of these drugs. Side effects of these drugs include neutropenia, gastrointestinal tract disturbance, and methemoglobinemia.[50]

Plasmodium LS parasites rely heavily on the host infrastructure to survive, grow, and develop. Host-targeted interventions provide an alternative to existing antimalarials by providing multiple opportunities to combat both dormant and developing parasites by targeting one or more of the following processes: (i) entry, (ii) development, and (iii) exit. Here, we provide examples of HDT use in the context of disease and review recent advances in our understanding of interactions between the parasite and its hepatocyte host with an eye toward key points of intervention for the design or repurposing of host-targeted therapies.

4. ENTRY FACTORS AS HOST-DIRECTED THERAPIES

4.1. Evidence for Host-directed Therapy against Viral Entry

Maraviroc was developed as an HDT against drug-resistant human immunodeficiency virus (HIV) (reviewed in ref 51) (Figure 1A). Entry of HIV-1 is facilitated by the presence of the CCR5 chemokine receptor.[52,53] Antagonists of CCR5, which dampen its coreceptor function, abrogate viral entry.[54]

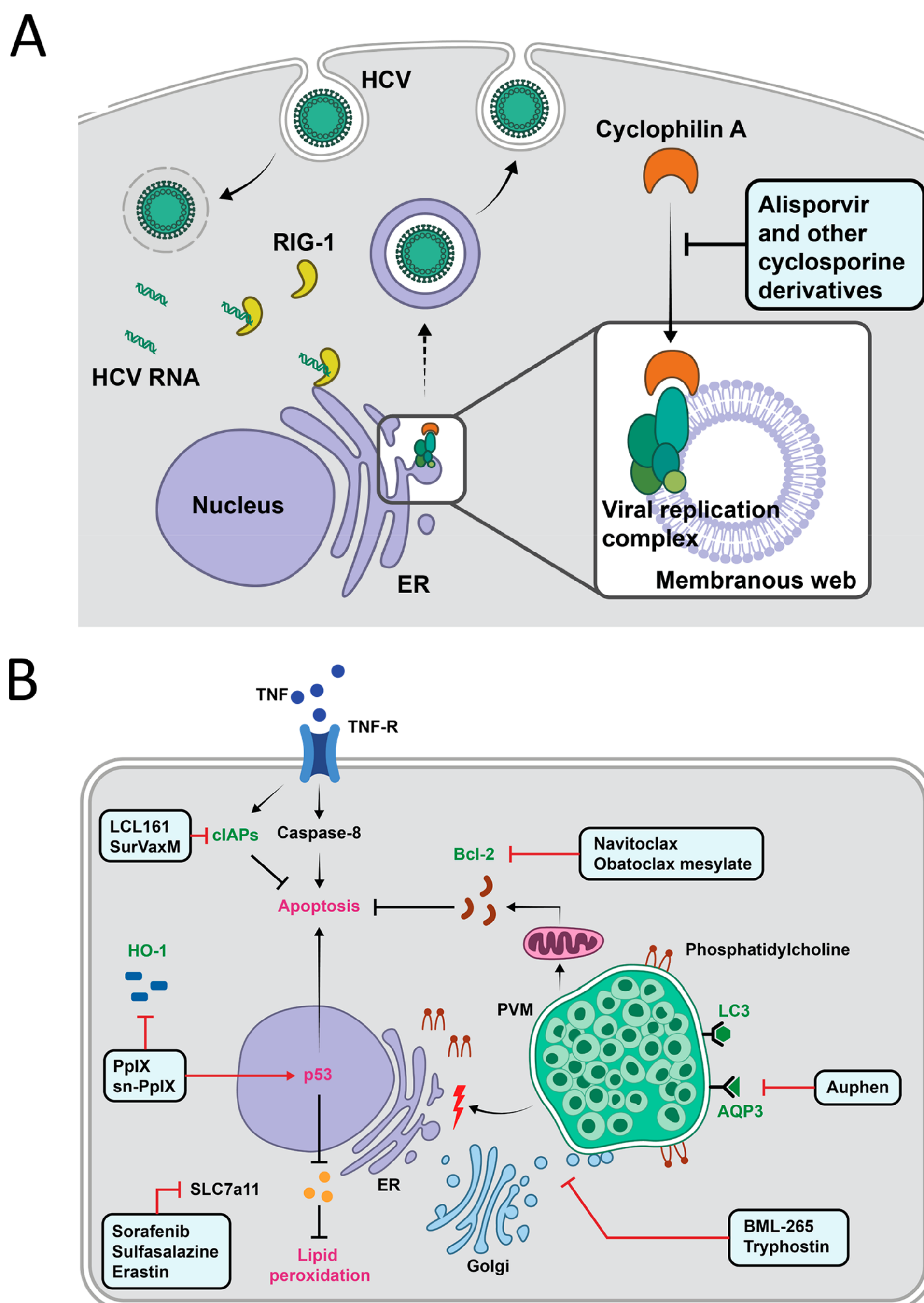

Figure 2. Targeting development factors as HDTs. (A) Upon entry of HCV into the cell, viral RNA is translated at the endoplasmic reticulum (ER) and a membranous replication factory, termed a membranous web, is formed. Cyclophilin A (CypA) binds to the viral replication complex on the membranous web and aids HCV replication. By blocking the interaction between CypA and the viral replication complex, cyclophilin inhibitors (for example, alisporivir) abrogate HCV replication. (B) Once inside, the hepatocyte *Plasmodium* interacts with a myriad of host factors and rewires host processes to evade defense and to establish its niche. During the parasite development, several mechanisms and pathways that impair the host's ability to defend against the parasite become activated. Drugs that activate host defense pathways or that block inhibitory pathways offer new therapeutic approaches. Host factors or processes depicted in green positively regulate infection. while those shown in pink negatively regulates infection. The boxes with red arrows indicate potential host-targeting drugs that enhance host pathways to defend against parasite. The boxes with red inhibitory arrows indicate the drugs that prevent the inhibitory pathways from impairing host defense. These drugs represent potential host-targeted interventions that could enhance host defense and eliminate the pathogens.

Maraviroc (Selzentry/Celsentri; Pfizer) targets CCR5 and was approved as a HIV drug in 2007 (reviewed in ref 51). As expected for an HDT, resistance to Maraviroc is low despite the fact that it is typically prescribed to individuals who have failed first line therapies, often due to the virus developing drug resistance (reviewed in ref 51).

Another example of an entry inhibitor that has been used in the clinic is myrcludex B. This peptide binds to the cell surface molecule sodium taurocholate cotransporting polypeptide (NTCP), which is required for productive infection by Hepatitis B and D viruses (HBV and HDV, respectively)[55,56] (Figure 1B). In combination with polyethylene glycol-

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conjugated (pegylated) interferon α2a (PegIFNα2a), myrcludex B exhibits synergistic antiviral effect on HDV and HBV.[57] Myrcludex B received orphan drug status from the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA), and additional clinical trials are in progress.[34] Together, myrcludex B and Maraviroc provide proof-of-concept that targeting pathogen entry is an effective strategy for HDT against infectious diseases.

4.2. Hepatocyte Entry Factors as Potential Prophylactic Targets against LS Malaria

Hepatocytes are highly heterogeneous and exhibit differential susceptibility to sporozoite entry.[58] Importantly, some hepatocytes, while entirely viable, are highly refractory to sporozoite infection.[58,59] Recent evidence suggests that entry is a complex process, and sporozoites rely on different mechanisms of entry in different situations (reviewed in refs 60, 61) (Figure 1C). Specifically, cluster of differentiation 81 (CD81) is critical for entry of *P. yoelii* and *P. falciparum* sporozoites but not *P. berghei* or *P. vivax* sporozoites.[62] Further, recombinant CD81 failed to block the sporozoites entry, suggesting that the CD81 could act as a coreceptor or that there exists an alternate invasion strategy in the absence of CD81.[61,62] In contrast, host SR-B1 identified from a RNAi screen on lipoprotein pathway was found to be essential for *P. berghei* and *P. vivax* but not for *P. falciparum* and *P. yoelii*.[63,64] SR-B1 plays a putative role in organizing CD81 and regulates L-FABP, a host liver fatty acid binding protein essential for LS development.[65,66] While these host molecules have been described to regulate sporozoite invasion, they also play an important role in host lipid transport and physiologically crucial functions in cells.[61] Erythropoietin-producing hepatocellular carcinoma A2 receptor (EphA2) also plays a critical role in *P. yoelii* and *P. falciparum* infection.[67] Interestingly, EphA2 has been implicated in a range of different infection processes including hepatitis C virus,[68] Kaposi's sarcoma-associated herpesvirus, HIV,[68] and chlamydia infection.[69] EphA2 receptor is overexpressed in a subpopulation of cells within tumors of several cancer types and has been recognized as a potential therapeutic target (reviewed in ref 70). Antibody drug conjugates generated by MedImmune, combining EphA2 antibody, 1C1, and microtubule-disrupting auristatin drug have been tested in the clinic[71] (reviewed in ref 72). It is tempting to speculate that robustly targeting this receptor could work as a broad anti-infective, yet in some settings it appears to not regulate sporozoite entry.[73] The host heparan sulfate proteoglycans (HSPGs), which modulate the actions of many extracellular ligands, provide an environmental signal that modulates the behavior of *Plasmodium* sporozoites.[74] Specifically, sporozoites prefer to migrate through cells with low levels of sulfated HSPGs, whereas contact with highly sulfated HSPGs containing cells, such as liver hepatocytes, triggers productive invasion.[74] Although HSPGs seem to be a critical host factor for hepatocyte invasion by *Plasmodium*, their role in the critical functions of the liver may limit their potential as targets for antimalarial intervention.

Whether host entry receptors are robust targets for HDT remains an interesting topic for further exploration. On one hand, it has been predicted that elimination of even a portion of hypnozoites would play a major role in driving eradication efforts.[75] In contrast, invasion happens quickly after mosquito bite, so intervention would need to happen rapidly, and pharmaceutical agents would likely need to be present at high levels prior to infection. Together, these features create a logistical hurdle, particularly in resource-poor areas. A final point to consider is that the wide range of entry mechanisms may lead to a scenario where one treatment regimen does not block all entry.[64] Given these limitations, HDTs that block entry would likely be of most utility for travelers to *P. vivax*-endemic areas, with the aim of eliminating the initiation of LS infection that ultimately leads to relapse.

5. HDTS TARGETING THE GROWTH AND DEVELOPMENT OF INTRACELLULAR PATHOGENS

5.1. Evidence for Host-directed Therapy against HCV Replication

Targeting host factors that are critical for viral replication has contributed clinically to HCV treatment. Chaperone cyclophilin A (CypA) is a ubiquitously distributed immunophilin family protein and is essential for the assembly of HCV replicase complex[76,77] (Figure 2A). CypA interacts with domain II of NS5A of HCV and stimulates RNA binding in an isomerase-dependent manner.[78] The small molecule cyclosporine A binds and sequesters CypA from NS5A containing HCV replicase complex and inhibits HCV replication.[76] While the immunosuppressive nature of CypA limits its use, Alisporivir, a nonimmunosuppressive cyclosporine A derivative, has been developed to inhibit cyclophilin and block the replication of multiple HCV genotypes in vitro and in the clinic.[79,80] In phase III trials, Alisporivir exhibited high barrier to the development of drug-resistant viruses, suggesting this may be a useful interventional strategy for drug resistant HCV.[76,77] Importantly, several flaviviruses (dengue virus, Japanese encephalitis virus, yellow fever virus), HIV,[81] SARS viruses,[82] cytomegalovirus (herpes),[83] and influenza A virus[79,80] have been demonstrated to rely on CypA for replication. Given this, it is tempting to speculate that CypA inhibitors could act as potential broad-spectrum host-targeted antivirals (reviewed in ref 34). For example, Alisporivir has recently been used in patients with, or at risk of, severe manifestations of SARS-CoV-2 infection.[82] This example illustrates an important opportunity for HDT: that multiple infections have overlapping requirements from their host cells, and thus might be targeted with the same HDT. Developing drugs that target multiple infections with a single regimen has advantages in manufacturing, deployment, and opportunity for rapid implementation during an outbreak or pandemic.

5.2. Potential Host-targeted Interventions against *Plasmodium* LS Growth and Development

After hepatocyte invasion, *Plasmodium* parasites replicate exponentially enclosed within the PVM. Within this intracellular niche, the parasite replicates tens of thousands of times over the course of 2−10 days, ultimately generating red blood cell infectious forms. The PVM is derived from the host plasma membrane and then modified by both host and parasite proteins and lipids. For instance, parasite early transcribed membrane proteins (eTRAMP) UIS3 and UIS4 are exported to the PVM after sporozoite entry.[84] In addition to the transport of parasites proteins, there is growing evidence that host vesicles and proteins are trafficked to the PVM (Figure 2B). For example, host aquoporin-3, typically associated with transport of glycerol and water, is not generally present at measurable levels in hepatocytes but is selectively induced during *Plasmodium* infection and trafficked to the PVM during LS development.[85,86] Genetic depletion of aquoproin-3 by

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RNAi or chemical disruption with AQP3 inhibitor Auphen significantly reduces *P. berghei* and *P. vivax* LS and blood stage infection.[85,86]

A growing body of work has characterized the cellular biological features that contribute to the growth and development of the *Plasmodium* LS parasite, yet little is understood about the nondividing hypnozoite. As a result, few strategies are available to target this source of relapse, as highlighted by MMV TCP-3.[9] New evidence suggests that targeting AQP3 with the small molecule inhibitor Auphen eliminates hypnozoites and schizonts alike,[86] providing proof-of-concept that targeting critical host factors might provide a tool for eliminating the source of reactivating parasites, along with several other stages of the malaria life cycle.

6. HOST METABOLISM AS HDT

Hepatocytes that harbor LS parasites are distinct from their uninfected neighbors. Most obvious is the rapid growth of the size of the infected cell. In the uninfected liver, the size of individual hepatocytes is tightly regulated (reviewed in ref 87) and even follows a day-night cycle.[88] It is interesting to speculate how this tight regulation might be exploited by the parasite, which always enters the liver in the evenings after the *Anopheles* blood meal and has a circadian rhythm of its own.[89] While the molecular details of this process remain unknown, the regulation of hepatocyte size is overcome by the intracellular parasite. The *Plasmodium* LS divides extraordinarily rapidly, requiring lipids to support its growth. Parasite de novo synthesis of fatty acids is required to complete development, at least for rodent-infecting species of malaria, but parasites can complete a majority of LS development without parasite-derived fatty acids.[90,91] Specifically, FabB/F^{-} parasites, which are deficient in parasite de novo fatty acid biosynthesis, develop normally for the first 40 h of *P. yoelii* and *P. berghei* LS development.[90,91] The developing LS parasite relies on host lipids such as L-FAB,[65] LDL,[92] and phosphatidylcholine[93] for development. In contrast, *Plasmodium* does not rely on lipid droplets for its development;[93] it has been hypothesized that vesicle-mediated trafficking facilitates host lipid acquisition by the parasite,[94] as there is growing evidence that host vesicular trafficking is heavily redirected toward the parasite. Specifically, coat protein I (COPI) coated retrograde vesicles,[94] late endosomes,[95,96] autophagosomes,[97] and lysosomes[98] are trafficked to the PVM and are thought to fuse with the PVM during LS development.[97] We have recently hypothesized that this trafficking is directed by the relocalization of the host microtubule organizing center to the parasite periphery during LS development.[99] Future studies will elucidate if small molecules that alter lipid content or the scavenging ability of infected hepatocytes are effective HDTs against LS parasites.

Availability of nutrients appears to regulate *Plasmodium* growth on the whole animal level as well as the molecular level. A high fat diet (HFD) reduces parasite load in livers of C57BI/6 mice due to an increase in reactive oxygen species production.[100] Interestingly, a high fat diet may have contributed to the relative malaria resistance of the Fulani ethnic groups in Africa as compared to other ethnic groups with which they live in sympatric fashion.[100] In contrast, mice on a restrictive diet are protected from experimental cerebral malaria.[101] This study found that the *Plasmodium* parasites sense the host diet through the KIN kinase and, in turn, adjust their multiplication rate. Importantly, each of these studies opens the possibility that infection by, and severity of, malaria may be altered by dietary means.

Even beyond dietary alterations, targeting metabolic processes to modulate *Plasmodium* infection may not require extensive de novo drug development. Multiple processes that are associated with type II diabetes and liver disease, including lipid homeostasis, endoplasmic reticulum (ER) stress, sequestration of host vesicles, and the inhibition of AMP-activated protein kinase (AMPK) have also been tied to LS infection (reviewed in ref 41) (Figure 2B). For example, ER stress, which restores cellular homeostasis, is activated in the context of type II diabetes,[102] fatty liver disease,[103] and viral hepatitis,[103] also promotes LS development.[103] Type II diabetes, fatty liver disease, and viral hepatitis are all the focus of drug development campaigns and include a myriad of drugs already in the clinic. As one example, clinically approved AMPK agonists, salicylate and metformin, used to treat type II diabetes, reduces LS burden.[104,105]

7. OXIDATIVE STRESS AND CELL DEATH REGULATORS AS HDT

7.1. Targeting Oxidative Stress in the Clinic

One of the hallmarks of cancer is an increase in oxidative stress as a result of deregulated cellular energetics.[106] Moderate levels of oxidative stress due to reactive oxygen species (ROS) are essential for cell survival, proliferation, and metastasis. Uncontrolled, ROS can play a role in promoting multiple forms of cell death (reviewed in ref 107). Harnessing oxidative stress with the aim to enhance ROS production for selective killing of cancer cells has become a major strategy of chemotherapy.[108] An increasing number of therapeutic strategies are being developed to combat cancer by either decreasing ROS and depriving tumors of fuel or elevating ROS levels to overwhelm the redox adaptation in the tumor (reviewed in ref 109).

One class of pharmacological ROS-inducing small molecules aims to selectively induce a form of cell death in malignant cells called ferroptosis. Ferroptosis is a nonapoptotic mechanism of cell death to which only a subset of cells is sensitive. It can be induced by the small molecule Erastin[110] and is dependent on ROS, lipid peroxidation, and iron.[111] Ferroptosis can play a role in immunity, as T cells deficient for GPX4, a key regulator of ferroptosis, have a defect in maintaining homeostatic balance.[112] A range of drug-resistant tumors are sensitive to the inhibition of cystine−glutamate antiporter (xCT), while nearly all nontransformed, uninfected tissues are recalcitrant to this inhibition.[113−115] An extensive array of small molecule inhibitors to xCT have been developed and are in a wide range of clinical trials. For example, sorafenib has contributed to overcoming resistance hepatocellular carcinoma,[116] advanced renal cell carcinoma (reviewed in ref 117), and thyroid carcinoma (reviewed in ref 117) and is currently approved by EMA and the FDA for clinical use. Another small molecule, Temozolomide (TMZ), that operates in an xCT/SLC7a11-dependent manner, is currently being used as treatment for malignant gliomas as an adjuvant therapy alongside radiotherapy.[118] With the ongoing efforts in xCT/SLC7a11-targeted drug development, promising signals are indeed beginning to emerge.

7.1.1. Targeting Oxidative Stress during *Plasmodium* Liver Infection.

We and others have contributed to an understanding of the mechanism behind ROS sensitivity in

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Plasmodium-infected hepatocyte, paving the way for possible intervention. Chemical inhibitors targeting the SLC7a11-GPX4 pathway selectively elevate lipid peroxides in infected cells, which localize within the parasite and lead to the elimination of LS parasites.[119] The tumor suppressor P53 has been previously demonstrated to block SLC7a11,[111] and in the context of liver infection curtails *Plasmodium* development through the generation of lipid peroxides.[120] Importantly, the relevance of p53 signaling to malaria infection does not appear to be restricted to the laboratory. A systematic longitudinal pediatric study to identify immune signatures that are associated with control of malaria fever and parasitemia has revealed that p53 upregulation in monocytes attenuates malaria-induced inflammation and predicts protection from fever.[121]

Plasmodium-infected cells exhibit elevated ROS and lipid peroxidation levels and susceptibility to chemotherapeutic agents that increase ROS generation (i.e., Erasin, sorafenib, etc.).[119] Interestingly, lipid peroxides appear largely localized to the parasite, suggesting that targeting this pathway may be specific to infection and not impact surrounding cells.[119] Heme oxygenase 1 (HO-1), a stress-responsive enzyme that serves to dampen inflammation, is elevated in the *Plasmodium* infected liver and its abrogation results in the resolution of LS infection.[122] These findings are consistent with the model that *Plasmodium* infection induces an inflammatory state in the liver that must be counteracted by hepatocyte-intrinsic and surrounding immune cells for parasite survival. Given the active drug development efforts that target ROS regulatory elements and our rapidly evolving knowledge of other pathogens that may also be sensitized to ROS levels (reviewed in ref 123), targeting ROS and/or lipid peroxidation, holds promise as a generalized anti-infective strategy.

7.2. Targeting Cell Death Stress in the Clinic: The Specialized Case of Oncogene Addiction

Cancers are characterized by six hallmarks: (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) activating invasion and metastasis, (4) enabling replicative immortality, (5) inducing angiogenesis, and (6) resisting cell death.[106] While some of the phenotypic hallmarks associated with these changes are not directly applicable to the study of infectious diseases, there are parallels: infected cells may operate independently of external stimuli that attack the infected cell (akin to evading growth suppressors) and must resist cell death. Given this, we and others[124] have hypothesized that insights amassed by the oncology field may shed light on the properties of infected cells. Taken a step further, the overlapping features of transformed cells and infected cells may provide opportunities to combat infection.

While cancers vary in their propensity to resist treatment, the concept of oncogene addiction has been proposed as a way to delineate and exploit particular susceptibilities of transformed cells. Specifically, it has been shown that by characterizing the levels of BH3 domain proteins within a tumor, it can be predicted on which pro-apoptotic BH3 domain proteins that tumor relies.[125] While it is true that a hallmark of cancer is the resistance from cell death, it has been demonstrated that in this case, transformed cells are actually sensitized to cell death when it is induced by the blocking of specific antiapoptotic B-cell lymphoma 2 (Bcl-2) family members (reviewed in ref 126). Put another way, these cells are "addicted" to the activity of a member of the Bcl-2 family.

This conceptual construct creates a useful framework for viewing infected cells: pathogens evolve immune evasion strategies to resist cell death in many settings, and this capacity leaves the infected cells addicted to specific antideath/pro-survival factors. The goal, then, of developing host-targeted interventions must be to identify those factors upon which the infected cells rely and exploit them to eliminate infected (addicted) cells, while leaving uninfected (unaddicted) cells intact.

7.2.1. Targeting Infected Cell Death *Plasmodium* Liver Infection. Host cell death signaling serves both to limit infection by pathogens and to promote pathogenesis. Left unregulated, innate sensors can induce cell death mechanisms to protect the host by destroying the replicative niche of intracellular pathogens and the pathogen itself.[124,127] To survive, intracellular pathogens must passively or actively avoid host cell death. LS infected cells have several mechanisms by which to do this. First, hepatocytes traversed by *P. berghei* are thought to release hepatocyte growth factor (HGF), which promotes mesenchymal−epithelial transition factor (MET) signaling and renders neighbor hepatocytes susceptible to infection by inhibiting apoptosis for *P. berghei* infected hepatocytes.[5] Yet, this mechanism does not appear to be conserved to *P. yoelii* or *P. falciparum* in some settings.[128] Interestingly, *Plasmodium*-infected hepatocytes resist induction of apoptosis by extrinsic stimuli such as Fas and phosphoinositide 3-kinases inhibition[127] but are highly susceptible to inhibition of the Bcl-2 family that initiates intrinsic apoptosis.[120] The cellular inhibitor of apoptosis protein (cIAP) is upregulated in infected hepatocytes and genetic or pharmacological targeting of cIAP using clinical-stage antagonists preferentially kills infected cells via activation of the extrinsic apoptotic pathway, suggesting that cIAPs may, like the Bcl-2 family, represent a point of addiction for the infected cells.[124] Collectively, these studies demonstrate the sensitivity of parasite-infected host cells to certain host cell death pathways and underscore the promise of targeting cell death machinery to halt *Plasmodium* progression. A detailed understanding of the biology behind their roles in *Plasmodium* infection may inform strategies to intervene at the host−parasite interface.

8. EXPLOITING SYNTHETIC LETHALITY

Synthetic lethality occurs between two genes when a loss-of-function mutation in either gene individually has little impact on cell viability, but the loss-of-function of both genes results in cell death.[129] We and others have recently proposed that this feature of biological networks presents an opportunity to target infected cells in the context of viral infections;[130] the idea is easily transferred to the context of parasitic infections as well. Put simply, the idea is that the state of infection in the cell renders a subset of proteins nonfunctional, and this creates susceptibilities that are specific for infected cells over uninfected cells. The loss of function in infected cells could be due to protein binding with a parasite protein or due to a transcriptional or translation change in the level of the protein as a response to infection. These changes on their own must not be lethal to the host cell, as this would lead to the demise of the pathogen as well. However, when combined with the loss-of-function of synthetic lethal partners within the host cell, the infected cell would not survive. Importantly, loss-of-function of the partner, on its own, also would not lead to cellular demise. This feature gives rise to the opportunity to

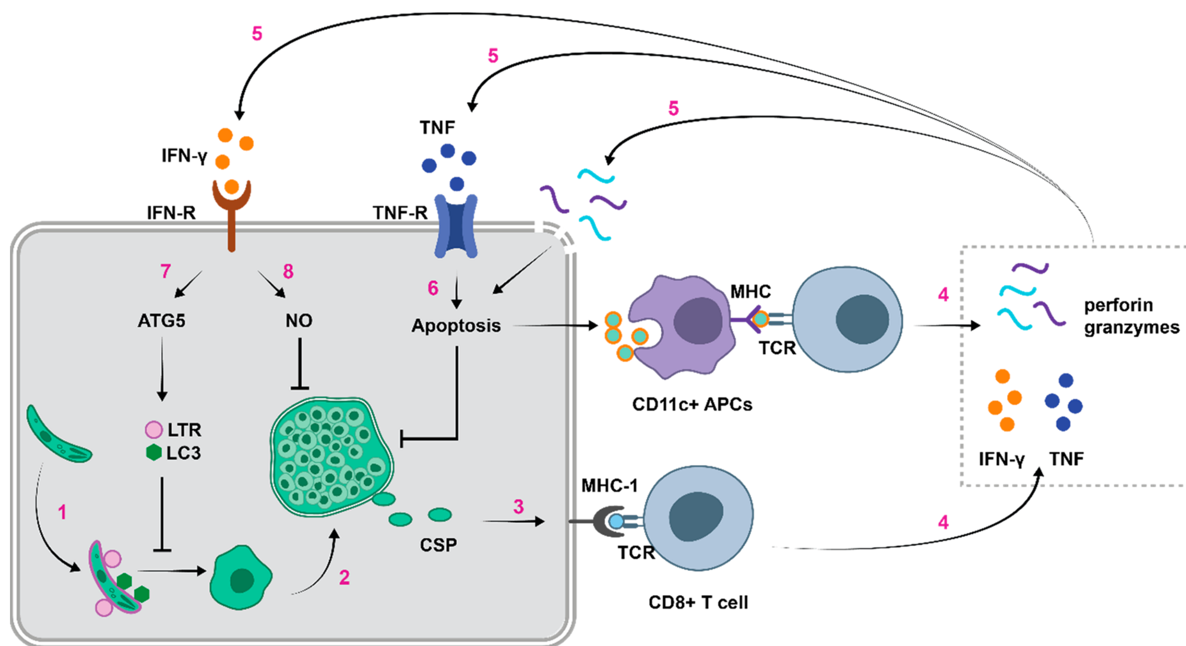

Figure 3. HDTs that boost immune response during LS. Schematic representation of the immune activation events during LS *Plasmodium* infection. Following invasion [1] into hepatocytes, sporozoites mature into LS form [2], forming a parasitophorous vacuole membrane (PVM). Infected hepatocytes prime CD8+ T cells in following ways, (i) hepatocytes sense the parasite, then process and present the parasite antigen to CD8+ cell via MHC class-I receptor [3]. (ii) CD11c+ APCs in the liver-draining lymph nodes acquires *Plasmodium* from infected hepatocytes, possibly following apoptosis of infected hepatocyte, and prime CD8+ T cells [4]. Following activation, CD8+ T cells induce cytokines IFN-γ and TNF and the expression of perforin and granzyme [5] to trigger extrinsic cell death pathways in infected hepatocytes [6]. Additionally, IFN-γ restricts LS development either via LC3-associated phagocytosis, promoting the fusion of lysosomes with LS compartment [7] or by increased NO production [8].

target a host protein and, in doing so, generate a robust therapeutic index where infected, but not uninfected, cells are eliminated at a range of drug concentrations.

9. THE INTERPLAY BETWEEN HOST−PATHOGEN INTERACTIONS AND IMMUNITY

9.1. Immune Modulators in the Clinic

Until the approval of direct-acting antiviral (DAA) based therapies for chronic hepatitis C in 2015, interferon (IFN) therapy was the primarily treatment for patients infected with HCV and HBV.[131] It is still practiced in countries that are unable to afford the high-cost DAA treatment.[131] Several recombinant IFNs including IFN alfacon-1, a recombinant and synthetic type I IFN, recombinant IFNα2a (Roferon-A) and IFNα2b (Intron A), PegIFNα2a (Pegasys), and PegIFNα2b (PegIntron) have been licensed (reviewed in ref 34). Major limitations of IFN-based therapy are the numerous side effects, which include fever, fatigue, depression, and exacerbated autoimmunity (reviewed in ref 34). Despite these shortcomings, Pegasys has certain advantages over the DAA lamivudine including the lack of drug resistance, a finite treatment course (usually 24−48 weeks), and the induction of a durable elimination of viral load (reviewed in ref 34).

IFN therapy has also played a crucial role in HIV-1 treatment.[132] A major challenge to the successful treatment of HIV infection is the persistence of latent reservoir CD4+ T-cells.[133] One of the strategies employed to overcome this challenge is reactivation of latent HIV-1, thereby allowing the immune effector mechanisms to identify and eliminate the infected cells.[133] FDA-approved retinoic acid derivative, acitretin stimulates RIG-I pathway in the host cell. Following activation, RIG-1 triggers the transcription of the pro-viral genome and senses HIV RNA, thereby mounting an IFN response that results in the apoptosis of HIV-infected cells.[134] Owing to a plethora of therapeutic applications, IFN remains a tool to tune the host immune system that could confer protection against multiple infections and coinfections alike.

9.1.1. Tuning the Innate Response for Optimal Immunity against LS Infection. In 1967, it was demonstrated that the administration of whole rodent-infecting *Plasmodium* sporozoites could elicit protection in vaccinated animals;[135] this approach was extended to humans in 1973.[136] Since then, iterations and refinements on this approach have been heavily tested as vaccine candidates. Today, to generate a *Pf*SPZ-based vaccine, parasites are attenuated by irradiation, genetic manipulation, or chemical inhibition (reviewed in ref 137). Parasites are then administered via mosquito bite[138] or by intravenous administration of cryo-preserved whole sporozoites.[139] While these vaccines are highly effective in controlled human malaria infection settings,[138] the efficacy of these vaccine candidates diminishes when administered to malaria-exposed individuals.[140] These findings provide two important insights: first, that sterilizing protection is possible using whole parasite vaccines, and second, that immune responses may need to be tuned in order to provide robust protection in the most relevant settings. Molecules that tune immune responses are widely available in the clinic and thus represent an opportunity for drug repurposing for HDT against malaria. Moreover, because individuals in malaria endemic areas are exposed to many infections throughout their lifetimes without sterilizing immunity, HDTs could serve to adjuvant these naturally occurring infections into providing vaccine-level protection.

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Until recently, the host was considered relatively immunologically passive during the LS of *Plasmodium* due to the lack of symptomatic infection during this stage. Recent evidence provides proof-of-concept that using HDTs to target LS infection can result in immunity against subsequent challenge. A recent study has demonstrated that cIAP1 is upregulated in infected hepatocytes.[124] When cIAPs are blocked, LS parasites fail to survive in the liver but induce potent immunity to subsequent challenge,[124] similar to what is observed by using other means of attenuating LS parasites. It will be important to test if this is specific for IAP inhibitors or if many HDTs elicit this pattern of inducing immunity.

Infected hepatocytes "sense" LS parasites and activate a type I IFN response, which recruits inflammatory lymphocytes to the site of infection.[141,142] The primed lymphocytes, in particular natural killer cells, limit the parasite load upon reinfection. Multiple immune cell subsets (NK and NKT cells) produce IFN-γ in response to type I IFN signaling by *Plasmodium*-infected hepatocytes.[143,144] Although capable of reducing the parasite load during secondary infection, the innate type I IFN response is not only insufficient to sterilize infection but also drives a dysfunctional T cell program, thereby minimizing protection against subsequent infection.[145] One possibility for HDT would be to minimize the induction of these nonfunctional T cells in order to boost protection afforded by whole parasites.

CD8+ T cells are a critical component of the protective response to liver *Plasmodium* infection (reviewed in ref 146), thus their biology represents another potential point for antimalaria HDT. CD8+ T cells are either primed by (1) CD11c+ APCs that acquire sporozoite antigen at the site of inoculation or from infected hepatocytes[147] or (2) by direct presentation of *Plasmodium* antigen by infected hepatocytes via an MHC class I receptor.[148] These processes are critical for protective immunity against subsequent challenge by sporozoites (reviewed in ref 146). CD8+ T cells are endowed with multiple effector pathways to eliminate LS infection (Figure 3). Direct effector pathways include the release of perforin and granzymes,[148] and indirect effector mechanisms include the production of IFN-γ and TNF.[149−151] IFN-γ has been demonstrated to confer protection either by (i) inducing LC3 associated phagocytosis in hepatocytes[152] or (ii) increasing expression of inducible nitric oxide synthetase, which results in increased production of nitric oxide,[148] and (iii) increased expression of MHC class I, which enhances the recognition of antigens by memory CD8+ T cells.[153]

Attenuated whole-parasite vaccines elicit potent protective immunity in animals and humans by targeting CD8+ T cells and converting them into liver resident T cells (Trm).[154] However, the efficacy of these vaccines is hindered by the failure to produce protective CD8+ T cell response using a single antigen[155] as well as practical complications in using whole-parasite for multiple antigen response as it requires a cumbersome amount of parasite to achieve protective immunity in the field (reviewed in ref 146). Recent efforts have been made to overcome this hurdle by "priming and trapping" T cells using viral vectored malaria antigens.[156] This method initially primes circulating T cells and then traps them in the liver during the boosting phase with liver-tropic viral antigen delivery systems.[155] While the specific host regulators that facilitate this process remain largely unexplored, their discovery could lead to HDT that boost liver-targeted CD8 immunity.

10. THE IMPORTANCE OF CONSIDERING NETWORK REWIRING FOR THE DESIGN OF HDT

In the previous sections, we have considered a collection of scenarios by which *Plasmodium*-infected cells exhibit altered sensitivity to drugs and other external stimuli, providing opportunities for HDTs against *Plasmodium* infection with a suitable therapeutic index when compared to uninfected cells. We have also examined how host immune responses to naturally occurring *Plasmodium* infection, or deliberate infection with attenuated or otherwise killed *Plasmodium* sporozoites, could be tuned with host-targeted therapeutics to promote immunity. In many of these instances, knowledge of the biology of the targets can come into play, informing therapy. Yet, the biology of an infected cell, whether infected with a parasite that will ultimately lead to symptomatic infection or one that will ultimately die and elicit an immune response, is fundamentally unique. The *Plasmodium* parasite adds a genome of over 5000 genes to the infected cell, many of which are transcribed and translated to produce proteins, which may interact with the infected cell. Moreover, because *Plasmodium* sporozoites select only a subset of hepatocytes,[58] infected cells may begin with a unique biology that is typically not described in experiments that describe the behavior of collections of cells. Indeed, we have recently demonstrated that *Plasmodium*-infected hepatocytes exhibit unique properties in the phospho-signaling cascade that modulates RPS6 phosphorylation, despite the canonical nature of this pathway and its conservation from yeast to humans.[157] Thus, to fully understand the most susceptible targets in infected cells, as well as the consequences of altering those targets, it is important to understand, globally, how infected cells vary from uninfected cells. Fortunately, a wide variety of different transcriptomic,[85,141,142,158,159] proteomic,[159] and metabolomic[93] data sets have been collected to elucidate the features of the host response to infection. These resources can be further mined for unique properties of infected cells and will hopefully lead to new host-based intervention strategies in the years and decades to come.

11. MODEL-INFORMED DRUG DEVELOPMENT FOR MALARIA THERAPEUTICS

Large-scale biological and chemical data, electronic health records, and clinical trial reports have rapidly accumulated in recent decades. Many of these data are standardized and accessible.[160] Meanwhile, advances in computational methods facilitate efficient integration and processing of complex and high-dimensional data. Together, these advances provide enhanced opportunities for in silico approaches to drug development. Specifically, these approaches can facilitate the integration of large data sets from diverse sources, scaling up quantitative representations of drug space, improved accuracy of predicting druggable targets, and efficient mechanistic assessment of drug's pharmacological effects.[161] In addition, in silico drug discovery is able to generate testable hypotheses, reveal novel mechanisms of action between drugs and targets, and provide insight into potential side effects.[162] Because the battery of data available on drugs is largely on host-targeted drugs, these approaches are highly adaptable to the identification of novel host-targeted drugs and may lag when they are applied to identify parasite-targeted drugs.

The most commonly used in silico approaches for drug discovery include signature matching, pathway-based or

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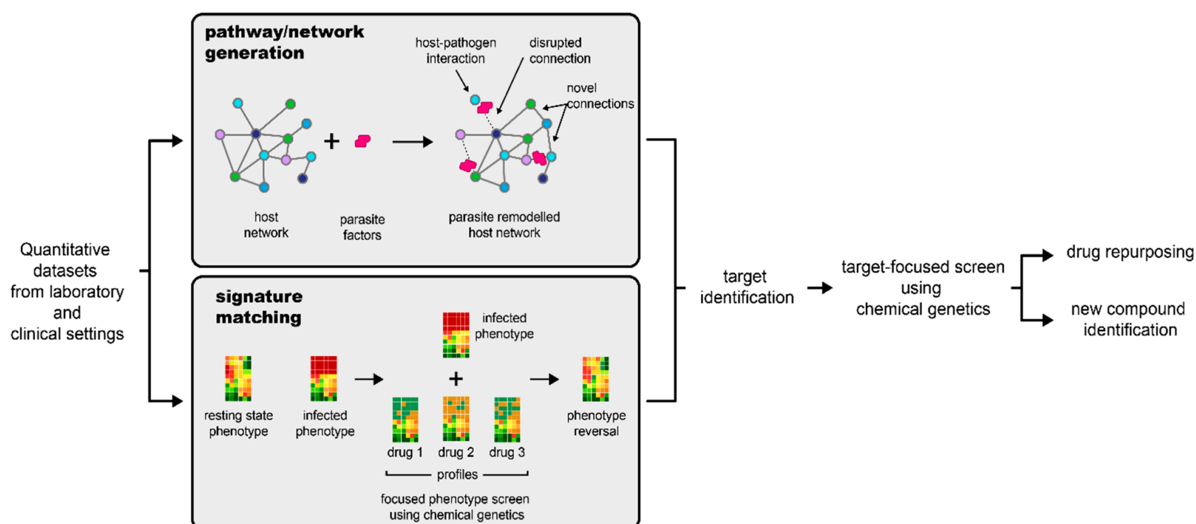

Figure 4. Model-informed drug development strategies. Quantitative multiomics data sets collected from laboratory or clinical settings can be processed and integrated by computational tools. Facilitated by in silico analyzing and modeling methods, essential host regulators or regulatory components that control *Plasmodium* development can be identified. This information can lead to the discovery of drugs with therapeutic efficacy.

network generation, and machine learning (Figure 4). Signature matching is based on monitoring phenotypic change in infected cells; this can be done through monitoring collections of transcripts or proteins. A negative correlation between the drug-treated phenotype and characteristics in a diseased phenotype suggests that the drug may be able to reverse the signature of the disease. This would lead to the hypothesis that the drug might drive cells from a diseased state back to a healthy state. This signature reversion principle has been successfully applied in a wide range of therapeutic areas including cancer, cardiovascular diseases, metabolic disorders, and inflammation-associated diseases.[163−166]

Network-based methods aim to capture the complex relationships between different scales of information from resting and diseased states and to facilitate the identification of drugs that best reverse network patterns associated with a disease.[167] The major advantage of a network-based strategy is, if a target protein associated with the disease is not druggable, exploring molecules upstream or downstream of the target can provide opportunities for further drug discovery applications. In addition, network-based methods are able to identify pathways or network modules shared by different disease states, including multiple infections, which enables the development of drugs that have pharmacological actions on multiple pathogens or facilitating drug repurposing from well-studied diseases.[168,169]

Machine learning is now routinely applied to drug development. Machine learning algorithms, including linear or logistic regression, random forest, support vector machines, and deep neural networks, have been successfully implemented in research settings to identify drug−target interactions and druggable targets for a range of diseases[170,171] (reviewed in ref 172). Machine learning is a feature-based approach that can be incorporated into other above-mentioned methods or used to interpret chemical screens, as is the case in kinase regression. We have recently applied this approach to identify kinase and kinase inhibitor regulators of *Plasmodium* liver infection.[173] In kinase regression, drug specificities for ∼300 kinases of interest are used in combination with a small screen of kinase inhibitors to identify key kinases and kinase inhibitors that regulate a phenotype, in this case, infection.

Taken together, by systematically exploring drug and/or target space, in silico drug discovery allows the identification of novel druggable targets, identifying previously unknown mechanisms of action for existing drugs, and can aid in the design of new drugs with higher efficacy. While HDT for malaria remains a nascent area, the accumulation of rich biological and chemical information around host targets and rapidly advancing computational tools could bring HDT for malaria to fruition.

12. CONCLUSIONS AND NEXT STEPS

Targeting the host presents multiple opportunities for eliminating malaria infection and could even boost immunity to subsequent infection. Such an approach is less likely to engender resistance than conventional antiparasitic therapies and could be facilitated rapidly by repurposing existing drugs. Toward this goal, recent progress has identified host regulators of LS malaria that could serve as targets for HDT against *Plasmodium* infection. Moreover, an increasing collection of global data sets that provide initial insights into how host regulatory systems are systematically altered in the context of infection could provide the first step to identify host-targeted drugs that could shift a cell that is hospitable to infection to one that clears infection and induces immunity. Whether HDTs against malaria are best used in isolation or in conjunction with existing antiparasitic drugs remains to be explored. Exploiting the untapped resource of host-targeting drugs, many of which are already approved for use in healthy individuals, could provide an important tool in the fight toward malaria eradication.

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ABBREVIATIONS

ACTs = artemisinin-based combination therapies
MMV = Medicines for Malaria Venture
TCP = target candidate profile
G6PD = glucose-6-phosphate dehydrogenase
HDT = host-directed therapy
PVM = parasitophorous vacuole membrane
HIV = human immunodeficiency virus
NTCP = sodium-taurocholate
PegIFNα2a = polyethylene glycol-conjugated interferon α2a
EMA = European Medicines Agency
FDA = Food and Drug Administration
HBV = hepatitis B virus
HDV = hepatitis D virus
HCV = hepatitis C virus
CD81 = cluster of differentiation 81
EphA2 = erythropoietin-producing hepatocellular carcinoma A2 receptor
HSPGs = heparan sulfate proteoglycans
CypA = cyclophilin A
ER = endoplasmic reticulum
eTRAMP = early transcribed membrane proteins
UIS = upregulated in sporozoites
AQP-3 = aquoporin-3
LS = liver-stage
COPI = coat protein I
HFD = high fat diet
AMPK = AMP-activated protein kinase
ROS = reactive oxygen species

xCT = cystine−glutamate antiporter
TMZ = temozolomide
HO-1 = heme oxygenase 1
Bcl-2 = B-cell lymphoma 2
HGF = hepatocyte growth factor
MET = mesenchymal−epithelial transition factor
cIAP = cellular inhibitor of apoptosis protein
DAA = direct acting antiviral
IFN = interferon

REFERENCES

(1) *World Malaria Report 2020: 20 Years of Global Progress and Challenges*; World Health Organization: Geneva, 2020; https://www.who.int/publications/i/item/9789240015791.

(2) Ansari, H. R.; Templeton, T. J.; Subudhi, A. K.; Ramaprasad, A.; Tang, J.; Lu, F.; Naeem, R.; Hashish, Y.; Oguike, M. C.; Benavente, E. D.; et al. Genome-Scale Comparison of Expanded Gene Families in *Plasmodium Ovale Wallikeri* and *Plasmodium Ovale Curtisi* with *Plasmodium Malariae* and with Other *Plasmodium* Species. *Int. J. Parasitol.* **2016**, *46*, 685.

(3) *World Malaria Report 2019*; World Health Organization: Geneva, 2019; https://www.who.int/publications/i/item/9789241565721.

(4) Miller, L. H.; Mason, S. J.; Clyde, D. F.; McGinniss, M. H. The Resistance Factor to *Plasmodium Vivax* in Blacks. The Duffy-Blood-Group Genotype, Fyfy. *N. Engl. J. Med.* **1976**, *295*, 302.

(5) Mota, M. M.; Pradel, G.; Vanderberg, J. P.; Hafalla, J. C.; Frevert, U.; Nussenzweig, R. S.; Nussenzweig, V.; Rodriguez, A. Migration of *Plasmodium* Sporozoites through Cells before Infection. *Science* **2001**, *291*, 141.

(6) Hopp, C. S.; Chiou, K.; Ragheb, D. R.; Salman, A. M.; Khan, S. M.; Liu, A. J.; Sinnis, P. Longitudinal Analysis of *Plasmodium* Sporozoite Motility in the Dermis Reveals Component of Blood Vessel Recognition. *eLife* **2015**, *4*, e07789.

(7) Amino, R.; Giovannini, D.; Thiberge, S.; Gueirard, P.; Boisson, B.; Dubremetz, J. F.; Prevost, M. C.; Ishino, T.; Yuda, M.; Menard, R. Host Cell Traversal Is Important for Progression of the Malaria Parasite through the Dermis to the Liver. *Cell Host Microbe* **2008**, *3*, 88.

(8) Sturm, A.; Amino, R.; van de Sand, C.; Regen, T.; Retzlaff, S.; Rennenberg, A.; Krueger, A.; Pollok, J. M.; Menard, R.; Heussler, V. T. Manipulation of Host Hepatocytes by the Malaria Parasite for Delivery into Liver Sinusoids. *Science* **2006**, *313*, 1287.

(9) Burrows, J. N.; Duparc, S.; Gutteridge, W. E.; Hooft van Huijsduijnen, R.; Kaszubska, W.; Macintyre, F.; Mazzuri, S.; Mohrle, J. J.; Wells, T. N. C. New Developments in Anti-Malarial Target Candidate and Product Profiles. *Malar. J.* **2017**, *16*, 26.

(10) Lu, K. Y.; Derbyshire, E. R. Tafenoquine: A Step toward Malaria Elimination. *Biochemistry* **2020**, *59*, 911.

(11) Kappe, S. H.; Kaiser, K.; Matuschewski, K. The *Plasmodium* Sporozoite Journey: A Rite of Passage. *Trends Parasitol.* **2003**, *19*, 135.

(12) Heussler, V.; Sturm, A.; Langsley, G. Regulation of Host Cell Survival by Intracellular *Plasmodium* and *Theileria* Parasites. *Parasitology* **2006**, *132*, S49.

(13) Mundwiler-Pachlatko, E.; Beck, H. P. Maurer's Clefts, the Enigma of *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 19987.

(14) Kilejian, A. Characterization of a Protein Correlated with the Production of Knob-Like Protrusions on Membranes of Erythrocytes Infected with *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 4650.

(15) Smith, J. D.; Chitnis, C. E.; Craig, A. G.; Roberts, D. J.; Hudson-Taylor, D. E.; Peterson, D. S.; Pinches, R.; Newbold, C. I.; Miller, L. H. Switches in Expression of *Plasmodium falciparum* Var Genes Correlate with Changes in Antigenic and Cytoadherent Phenotypes of Infected Erythrocytes. *Cell* **1995**, *82*, 101.

(16) Turner, L.; Lavstsen, T.; Berger, S. S.; Wang, C. W.; Petersen, J. E.; Avril, M.; Brazier, A. J.; Freeth, J.; Jespersen, J. S.; Nielsen, M. A.; et al. Severe Malaria Is Associated with Parasite Binding to Endothelial Protein C Receptor. *Nature* **2013**, *498*, 502.

(17) Bernabeu, M.; Danziger, S. A.; Avril, M.; Vaz, M.; Babar, P. H.; Brazier, A. J.; Herricks, T.; Maki, J. N.; Pereira, L.; Mascarenhas, A.; et al. Severe Adult Malaria Is Associated with Specific Pfemp1 Adhesion Types and High Parasite Biomass. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E3270.

(18) Kyes, S.; Horrocks, P.; Newbold, C. Antigenic Variation at the Infected Red Cell Surface in Malaria. *Annu. Rev. Microbiol.* **2001**, *55*, 673.

(19) Kafsack, B. F.; Rovira-Graells, N.; Clark, T. G.; Bancells, C.; Crowley, V. M.; Campino, S. G.; Williams, A. E.; Drought, L. G.; Kwiatkowski, D. P.; Baker, D. A.; et al. A Transcriptional Switch Underlies Commitment to Sexual Development in Malaria Parasites. *Nature* **2014**, *507*, 248.

(20) Poran, A.; Notzel, C.; Aly, O.; Mencia-Trinchant, N.; Harris, C. T.; Guzman, M. L.; Hassane, D. C.; Elemento, O.; Kafsack, B. F. C. Single-Cell Rna Sequencing Reveals a Signature of Sexual Commitment in Malaria Parasites. *Nature* **2017**, *551*, 95.

(21) Kent, R. S.; Modrzynska, K. K.; Cameron, R.; Philip, N.; Billker, O.; Waters, A. P. Inducible Developmental Reprogramming Redefines Commitment to Sexual Development in the Malaria Parasite *Plasmodium Berghei*. *Nat. Microbiol.* **2018**, *3*, 1206.

(22) Smalley, M. E.; Abdalla, S.; Brown, J. The Distribution of *Plasmodium falciparum* in the Peripheral Blood and Bone Marrow of Gambian Children. *Trans. R. Soc. Trop. Med. Hyg.* **1981**, *75*, 103.

(23) Joice, R.; Nilsson, S. K.; Montgomery, J.; Dankwa, S.; Egan, E.; Morahan, B.; Seydel, K. B.; Bertuccini, L.; Alano, P.; Williamson, K. C.; et al. *Plasmodium falciparum* Transmission Stages Accumulate in the Human Bone Marrow. *Sci. Transl. Med.* **2014**, *6*, 244re5.

(24) Cowman, A. F.; Healer, J.; Marapana, D.; Marsh, K. Malaria: Biology and Disease. *Cell* **2016**, *167*, 610.

(25) Rajagopalan, P. K.; Jambulingam, P.; Sabesan, S.; Krishnamoorthy, K.; Rajendran, S.; Gunasekaran, K.; Kumar, N. P.; Prothero, R. M. Population Movement and Malaria Persistence in Rameswaram Island. *Soc. Sci. Med.* **1986**, *22*, 879.

(26) Paaijmans, K. P.; Blanford, S.; Bell, A. S.; Blanford, J. I.; Read, A. F.; Thomas, M. B. Influence of Climate on Malaria Transmission Depends on Daily Temperature Variation. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 15135.

(27) Bushman, M.; Antia, R.; Udhayakumar, V.; de Roode, J. C. Within-Host Competition Can Delay Evolution of Drug Resistance in Malaria. *PLoS Biol.* **2018**, *16*, No. e2005712.

(28) Li, X.; Kumar, S.; McDew-White, M.; Haile, M.; Cheeseman, I. H.; Emrich, S.; Button-Simons, K.; Nosten, F.; Kappe, S. H. I.; Ferdig, M. T.; et al. Genetic Mapping of Fitness Determinants across the Malaria Parasite *Plasmodium falciparum* Life Cycle. *PLoS Genet.* **2019**, *15*, No. e1008453.

(29) Shanks, G. D.; Edstein, M. D.; Jacobus, D. Evolution from Double to Triple-Antimalarial Drug Combinations. *Trans. R. Soc. Trop. Med. Hyg.* **2015**, *109*, 182.

(30) Ashley, E. A.; Phyo, A. P. Drugs in Development for Malaria. *Drugs* **2018**, *78*, 861.

(31) White, N. J. Determinants of Relapse Periodicity in *Plasmodium vivax* Malaria. *Malar. J.* **2011**, *10*, 297.

(32) White, M. T.; Karl, S.; Battle, K. E.; Hay, S. I.; Mueller, I.; Ghani, A. C. Modelling the Contribution of the Hypnozoite Reservoir to *Plasmodium vivax* Transmission. *eLife* **2014**, *3*, e04692.

(33) Beutler, E.; Dern, R. J.; Flanagan, C. L.; Alving, A. S. The Hemolytic Effect of Primaquine. Vii. Biochemical Studies of Drug-Sensitive Erythrocytes. *J. Lab. Clin. Med.* **1955**, *45*, 286.

(34) Kaufmann, S. H. E.; Dorhoi, A.; Hotchkiss, R. S.; Bartenschlager, R. Host-Directed Therapies for Bacterial and Viral Infections. *Nat. Rev. Drug Discovery* **2018**, *17*, 35.

(35) Hopkins, A. L. Network Pharmacology: The Next Paradigm in Drug Discovery. *Nat. Chem. Biol.* **2008**, *4*, 682.

(36) Wang, T.; Birsoy, K.; Hughes, N. W.; Krupczak, K. M.; Post, Y.; Wei, J. J.; Lander, E. S.; Sabatini, D. M. Identification and

https://doi.org/10.1021/acs.chemrev.1c00062
Chem. Rev. 2021, 121, 10452−10468

Characterization of Essential Genes in the Human Genome. *Science* **2015**, *350*, 1096.
(37) Blomen, V. A.; Majek, P.; Jae, L. T.; Bigenzahn, J. W.; Nieuwenhuis, J.; Staring, J.; Sacco, R.; van Diemen, F. R.; Olk, N.; Stukalov, A.; et al. Gene Essentiality and Synthetic Lethality in Haploid Human Cells. *Science* **2015**, *350*, 1092.
(38) Hart, T.; Chandrashekhar, M.; Aregger, M.; Steinhart, Z.; Brown, K. R.; MacLeod, G.; Mis, M.; Zimmermann, M.; Fradet-Turcotte, A.; Sun, S.; et al. High-Resolution Crispr Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities. *Cell* **2015**, *163*, 1515.
(39) Haq, R.; Fisher, D. E. Targeting Melanoma by Small Molecules: Challenges Ahead. *Pigm. Cell Melanoma Res.* **2013**, *26*, 464.
(40) Lim, C.; Dankwa, S.; Paul, A. S.; Duraisingh, M. T. Host Cell Tropism and Adaptation of Blood-Stage Malaria Parasites: Challenges for Malaria Elimination. *Cold Spring Harbor Perspect. Med.* **2017**, *7*, a025494.
(41) Glennon, E. K. K.; Dankwa, S.; Smith, J. D.; Kaushansky, A. Opportunities for Host-Targeted Therapies for Malaria. *Trends Parasitol.* **2018**, *34*, 843.
(42) Scully, E. J.; Kanjee, U.; Duraisingh, M. T. Molecular Interactions Governing Host-Specificity of Blood Stage Malaria Parasites. *Curr. Opin. Microbiol.* **2017**, *40*, 21.
(43) Boulet, C.; Doerig, C. D.; Carvalho, T. G. Manipulating Eryptosis of Human Red Blood Cells: A Novel Antimalarial Strategy? *Front. Cell. Infect. Microbiol.* **2018**, *8*, 419.
(44) Carvalho, T. G.; Morahan, B.; John von Freyend, S.; Boeuf, P.; Grau, G.; Garcia-Bustos, J.; Doerig, C. The Ins and Outs of Phosphosignalling in *Plasmodium*: Parasite Regulation and Host Cell Manipulation. *Mol. Biochem. Parasitol.* **2016**, *208*, 2.
(45) Frevert, U.; Usynin, I.; Baer, K.; Klotz, C. Nomadic or Sessile: Can Kupffer Cells Function as Portals for Malaria Sporozoites to the Liver? *Cell. Microbiol.* **2006**, *8*, 1537.
(46) Risco-Castillo, V.; Topcu, S.; Marinach, C.; Manzoni, G.; Bigorgne, A. E.; Briquet, S.; Baudin, X.; Lebrun, M.; Dubremetz, J. F.; Silvie, O. Malaria Sporozoites Traverse Host Cells within Transient Vacuoles. *Cell Host Microbe* **2015**, *18*, 593.
(47) Krotoski, W. A.; Krotoski, D. M.; Garnham, P. C.; Bray, R. S.; Killick-Kendrick, R.; Draper, C. C.; Targett, G. A.; Guy, M. W. Relapses in Primate Malaria: Discovery of Two Populations of Exoerythrocytic Stages. Preliminary Note. *Br. Med. J.* **1980**, *280*, 153.
(48) Beutler, E. The Hemolytic Effect of Primaquine and Related Compounds: A Review. *Blood* **1959**, *14*, 103.
(49) Newby, G.; Hwang, J.; Koita, K.; Chen, I.; Greenwood, B.; von Seidlein, L.; Shanks, G. D.; Slutsker, L.; Kachur, S. P.; Wegbreit, J.; et al. Review of Mass Drug Administration for Malaria and Its Operational Challenges. *Am. J. Trop. Med. Hyg.* **2015**, *93*, 125.
(50) Hill, D. R.; Baird, J. K.; Parise, M. E.; Lewis, L. S.; Ryan, E. T.; Magill, A. J. Primaquine: Report from Cdc Expert Meeting on Malaria Chemoprophylaxis I. *Am. J. Trop. Med. Hyg.* **2006**, *75*, 402.
(51) Woollard, S. M.; Kanmogne, G. D. Maraviroc: A Review of Its Use in Hiv Infection and Beyond. *Drug Des., Dev. Ther.* **2015**, *9*, 5447.
(52) Lopalco, L. Ccr5: From Natural Resistance to a New Anti-Hiv Strategy. *Viruses* **2010**, *2*, 574.
(53) Melikyan, G. B. Hiv Entry: A Game of Hide-and-Fuse? *Curr. Opin. Virol.* **2014**, *4*, 1.
(54) Watson, C.; Jenkinson, S.; Kazmierski, W.; Kenakin, T. The Ccr5 Receptor-Based Mechanism of Action of 873140, a Potent Allosteric Noncompetitive Hiv Entry Inhibitor. *Mol. Pharmacol.* **2005**, *67*, 1268.
(55) Ni, Y.; Lempp, F. A.; Mehrle, S.; Nkongolo, S.; Kaufman, C.; Falth, M.; Stindt, J.; Koniger, C.; Nassal, M.; Kubitz, R.; et al. Hepatitis B and D Viruses Exploit Sodium Taurocholate Co-Transporting Polypeptide for Species-Specific Entry into Hepatocytes. *Gastroenterology* **2014**, *146*, 1070.
(56) Yan, H.; Zhong, G.; Xu, G.; He, W.; Jing, Z.; Gao, Z.; Huang, Y.; Qi, Y.; Peng, B.; Wang, H.; et al. Sodium Taurocholate Cotransporting Polypeptide Is a Functional Receptor for Human Hepatitis B and D Virus. *eLife* **2012**, *1*, No. e00049.
(57) Bogomolov, P.; Alexandrov, A.; Voronkova, N.; Macievich, M.; Kokina, K.; Petrachenkova, M.; Lehr, T.; Lempp, F. A.; Wedemeyer, H.; Haag, M.; et al. Treatment of Chronic Hepatitis D with the Entry Inhibitor Myrcludex B: First Results of a Phase Ib/Iia Study. *J. Hepatol.* **2016**, *65*, 490.
(58) Yang, A. S. P.; van Waardenburg, Y. M.; van de Vegte-Bolmer, M.; van Gemert, G.-J. A.; Graumans, W.; de Wilt, J. H. W.; Sauerwein, R. W. Zonal Human Hepatocytes Are Differentially Permissive to *Plasmodium falciparum* Malaria Parasites. *EMBO J.* **2021**, *40*, 106583.
(59) Kaushansky, A.; Austin, L. S.; Mikolajczak, S. A.; Lo, F. Y.; Miller, J. L.; Douglass, A. N.; Arang, N.; Vaughan, A. M.; Gardner, M. J.; Kappe, S. H. I. Susceptibility to *Plasmodium yoelii* Preerythrocytic Infection in Balb/C Substrains Is Determined at the Point of Hepatocyte Invasion. *Infect. Immun.* **2015**, *83*, 39.
(60) Vaughan, A. M.; Kappe, S. H. I. Malaria Parasite Liver Infection and Exoerythrocytic Biology. *Cold Spring Harbor Perspect. Med.* **2017**, *7*, a025486.
(61) Prudencio, M.; Mota, M. M. Targeting Host Factors to Circumvent Anti-Malarial Drug Resistance. *Curr. Pharm. Des.* **2013**, *19*, 290.
(62) Silvie, O.; Greco, C.; Franetich, J. F.; Dubart-Kupperschmitt, A.; Hannoun, L.; van Gemert, G. J.; Sauerwein, R. W.; Levy, S.; Boucheix, C.; Rubinstein, E.; et al. Expression of Human Cd81 Differently Affects Host Cell Susceptibility to Malaria Sporozoites Depending on the *Plasmodium* Species. *Cell. Microbiol.* **2006**, *8*, 1134.
(63) Rodrigues, C. D.; Hannus, M.; Prudencio, M.; Martin, C.; Goncalves, L. A.; Portugal, S.; Epiphanio, S.; Akinc, A.; Hadwiger, P.; Jahn-Hofmann, K.; et al. Host Scavenger Receptor Sr-BI Plays a Dual Role in the Establishment of Malaria Parasite Liver Infection. *Cell Host Microbe* **2008**, *4*, 271.
(64) Manzoni, G.; Marinach, C.; Topcu, S.; Briquet, S.; Grand, M.; Tolle, M.; Gransagne, M.; Lescar, J.; Andolina, C.; Franetich, J. F.; et al. *Plasmodium* P36 Determines Host Cell Receptor Usage During Sporozoite Invasion. *eLife* **2017**, *9*, e25903.
(65) Mikolajczak, S. A.; Jacobs-Lorena, V.; MacKellar, D. C.; Camargo, N.; Kappe, S. H. L-Fabp Is a Critical Host Factor for Successful Malaria Liver Stage Development. *Int. J. Parasitol.* **2007**, *37*, 483.
(66) Yalaoui, S.; Huby, T.; Franetich, J. F.; Gego, A.; Rametti, A.; Moreau, M.; Collet, X.; Siau, A.; van Gemert, G. J.; Sauerwein, R. W.; et al. Scavenger Receptor Bi Boosts Hepatocyte Permissiveness to *Plasmodium* Infection. *Cell Host Microbe* **2008**, *4*, 283.
(67) Kaushansky, A.; Douglass, A. N.; Arang, N.; Vigdorovich, V.; Dambrauskas, N.; Kain, H. S.; Austin, L. S.; Sather, D. N.; Kappe, S. H. Malaria Parasites Target the Hepatocyte Receptor Epha2 for Successful Host Infection. *Science* **2015**, *350*, 1089.
(68) Blumenthal, M. J.; Schutz, C.; Meintjes, G.; Mohamed, Z.; Mendelson, M.; Ambler, J. M.; Whitby, D.; Mackelprang, R. D.; Carse, S.; Katz, A. A.; et al. Epha2 Sequence Variants Are Associated with Susceptibility to Kaposi's Sarcoma-Associated Herpesvirus Infection and Kaposi's Sarcoma Prevalence in Hiv-Infected Patients. *Cancer Epidemiol.* **2018**, *56*, 133.
(69) Subbarayal, P.; Karunakaran, K.; Winkler, A. C.; Rother, M.; Gonzalez, E.; Meyer, T. F.; Rudel, T. Ephrina2 Receptor (Epha2) Is an Invasion and Intracellular Signaling Receptor for Chlamydia Trachomatis. *PLoS Pathog.* **2015**, *11*, No. e1004846.
(70) Ireton, R. C.; Chen, J. Epha2 Receptor Tyrosine Kinase as a Promising Target for Cancer Therapeutics. *Curr. Cancer Drug Targets* **2005**, *5*, 149.
(71) Annunziata, C. M.; Kohn, E. C.; LoRusso, P.; Houston, N. D.; Coleman, R. L.; Buzoianu, M.; Robbie, G.; Lechleider, R. Phase 1, Open-Label Study of Medi-547 in Patients with Relapsed or Refractory Solid Tumors. *Invest. New Drugs* **2013**, *31*, 77.
(72) Janes, P. W.; Vail, M. E.; Gan, H. K.; Scott, A. M. Antibody Targeting of Eph Receptors in Cancer. *Pharmaceuticals* **2020**, *13*, 88.
(73) Langlois, A. C.; Marinach, C.; Manzoni, G.; Silvie, O. *Plasmodium* Sporozoites Can Invade Hepatocytic Cells Independently of the Ephrin Receptor A2. *PLoS One* **2018**, *13*, No. e0200032.

(74) Frevert, U.; Sinnis, P.; Cerami, C.; Shreffler, W.; Takacs, B.; Nussenzweig, V. Malaria Circumsporozoite Protein Binds to Heparan Sulfate Proteoglycans Associated with the Surface Membrane of Hepatocytes. *J. Exp. Med.* **1993**, *177*, 1287.
(75) White, M. T.; Walker, P.; Karl, S.; Hetzel, M. W.; Freeman, T.; Waltmann, A.; Laman, M.; Robinson, L. J.; Ghani, A.; Mueller, I. Mathematical Modelling of the Impact of Expanding Levels of Malaria Control Interventions on *Plasmodium vivax*. *Nat. Commun.* **2018**, *9*, 3300.
(76) Watashi, K.; Hijikata, M.; Hosaka, M.; Yamaji, M.; Shimotohno, K. Cyclosporin a Suppresses Replication of Hepatitis C Virus Genome in Cultured Hepatocytes. *Hepatology* **2003**, *38*, 1282.
(77) Yang, F.; Robotham, J. M.; Nelson, H. B.; Irsigler, A.; Kenworthy, R.; Tang, H. Cyclophilin a Is an Essential Cofactor for Hepatitis C Virus Infection and the Principal Mediator of Cyclosporine Resistance in Vitro. *J. Virol.* **2008**, *82*, 5269.
(78) Kaul, A.; Stauffer, S.; Berger, C.; Pertel, T.; Schmitt, J.; Kallis, S.; Zayas Lopez, M.; Lohmann, V.; Luban, J.; Bartenschlager, R. Essential Role of Cyclophilin a for Hepatitis C Virus Replication and Virus Production and Possible Link to Polyprotein Cleavage Kinetics. *PLoS Pathog.* **2009**, *5*, No. e1000546.
(79) Flisiak, R.; Feinman, S. V.; Jablkowski, M.; Horban, A.; Kryczka, W.; Pawlowska, M.; Heathcote, J. E.; Mazzella, G.; Vandelli, C.; Nicolas-Metral, V.; et al. The Cyclophilin Inhibitor Debio 025 Combined with Peg Ifnalpha2a Significantly Reduces Viral Load in Treatment-Naive Hepatitis C Patients. *Hepatology* **2009**, *49*, 1460.
(80) Hopkins, S.; DiMassimo, B.; Rusnak, P.; Heuman, D.; Lalezari, J.; Sluder, A.; Scorneaux, B.; Mosier, S.; Kowalczyk, P.; Ribeill, Y.; et al. The Cyclophilin Inhibitor Scy-635 Suppresses Viral Replication and Induces Endogenous Interferons in Patients with Chronic Hcv Genotype 1 Infection. *J. Hepatol.* **2012**, *57*, 47.
(81) Gamble, T. R.; Vajdos, F. F.; Yoo, S.; Worthylake, D. K.; Houseweart, M.; Sundquist, W. I.; Hill, C. P. Crystal Structure of Human Cyclophilin a Bound to the Amino-Terminal Domain of Hiv-1 Capsid. *Cell* **1996**, *87*, 1285.
(82) Pfefferle, S.; Schopf, J.; Kogl, M.; Friedel, C. C.; Muller, M. A.; Carbajo-Lozoya, J.; Stellberger, T.; von Dall'Armi, E.; Herzog, P.; Kallies, S.; et al. The Sars-Coronavirus-Host Interactome: Identification of Cyclophilins as Target for Pan-Coronavirus Inhibitors. *PLoS Pathog.* **2011**, *7*, No. e1002331.
(83) Meyers-Elliott, R. H.; Chitjian, P. A.; Billups, C. B. Effects of Cyclosporine a on Clinical and Immunological Parameters in Herpes Simplex Keratitis. *Invest. Ophthalmol. Vis. Sci.* **1987**, *28*, 1170.
(84) MacKellar, D. C.; Vaughan, A. M.; Aly, A. S.; DeLeon, S.; Kappe, S. H. A Systematic Analysis of the Early Transcribed Membrane Protein Family Throughout the Life Cycle of *Plasmodium yoelii*. *Cell. Microbiol.* **2011**, *13*, 1755.
(85) Posfai, D.; Sylvester, K.; Reddy, A.; Ganley, J. G.; Wirth, J.; Cullen, Q. E.; Dave, T.; Kato, N.; Dave, S. S.; Derbyshire, E. R. *Plasmodium* Parasite Exploits Host Aquaporin-3 During Liver Stage Malaria Infection. *PLoS Pathog.* **2018**, *14*, No. e1007057.
(86) Posfai, D.; Maher, S. P.; Roesch, C.; Vantaux, A.; Sylvester, K.; Peneau, J.; Popovici, J.; Kyle, D. E.; Witkowski, B.; Derbyshire, E. R. *Plasmodium vivax* Liver and Blood Stages Recruit the Druggable Host Membrane Channel Aquaporin-3. *Cell Chem. Biol.* **2020**, *27*, 719.
(87) Ramboer, E.; De Craene, B.; De Kock, J.; Vanhaecke, T.; Berx, G.; Rogiers, V.; Vinken, M. Strategies for Immortalization of Primary Hepatocytes. *J. Hepatol.* **2014**, *61*, 925.
(88) Sinturel, F.; Gerber, A.; Mauvoisin, D.; Wang, J.; Gatfield, D.; Stubblefield, J. J.; Green, C. B.; Gachon, F.; Schibler, U. Diurnal Oscillations in Liver Mass and Cell Size Accompany Ribosome Assembly Cycles. *Cell* **2017**, *169*, 651.
(89) Rijo-Ferreira, F.; Acosta-Rodriguez, V. A.; Abel, J. H.; Kornblum, I.; Bento, I.; Kilaru, G.; Klerman, E. B.; Mota, M. M.; Takahashi, J. S. The Malaria Parasite Has an Intrinsic Clock. *Science* **2020**, *368*, 746.
(90) Vaughan, A. M.; O'Neill, M. T.; Tarun, A. S.; Camargo, N.; Phuong, T. M.; Aly, A. S.; Cowman, A. F.; Kappe, S. H. Type Ii Fatty Acid Synthesis Is Essential Only for Malaria Parasite Late Liver Stage Development. *Cell. Microbiol.* **2009**, *11*, 506.
(91) van Schaijk, B. C.; Kumar, T. R.; Vos, M. W.; Richman, A.; van Gemert, G. J.; Li, T.; Eappen, A. G.; Williamson, K. C.; Morahan, B. J.; Fishbaugher, M.; et al. Type Ii Fatty Acid Biosynthesis Is Essential for *Plasmodium falciparum* Sporozoite Development in the Midgut of Anopheles Mosquitoes. *Eukaryotic Cell* **2014**, *13*, 550.
(92) Labaied, M.; Jayabalasingham, B.; Bano, N.; Cha, S. J.; Sandoval, J.; Guan, G.; Coppens, I. *Plasmodium* Salvages Cholesterol Internalized by Ldl and Synthesized De Novo in the Liver. *Cell. Microbiol.* **2011**, *13*, 569.
(93) Itoe, M. A.; Sampaio, J. L.; Cabal, G. G.; Real, E.; Zuzarte-Luis, V.; March, S.; Bhatia, S. N.; Frischknecht, F.; Thiele, C.; Shevchenko, A.; et al. Host Cell Phosphatidylcholine Is a Key Mediator of Malaria Parasite Survival During Liver Stage Infection. *Cell Host Microbe* **2014**, *16*, 778.
(94) Raphemot, R.; Toro-Moreno, M.; Lu, K. Y.; Posfai, D.; Derbyshire, E. R. Discovery of Druggable Host Factors Critical to *Plasmodium* Liver-Stage Infection. *Cell Chem. Biol.* **2019**, *26*, 1253.
(95) Petersen, W.; Stenzel, W.; Silvie, O.; Blanz, J.; Saftig, P.; Matuschewski, K.; Ingmundson, A. Sequestration of Cholesterol within the Host Late Endocytic Pathway Restricts Liver-Stage *Plasmodium* Development. *Mol. Biol. Cell* **2017**, *28*, 726.
(96) Lopes da Silva, M.; Thieleke-Matos, C.; Cabrita-Santos, L.; Ramalho, J. S.; Wavre-Shapton, S. T.; Futter, C. E.; Barral, D. C.; Seabra, M. C. The Host Endocytic Pathway Is Essential for *Plasmodium berghei* Late Liver Stage Development. *Traffic* **2012**, *13*, 1351.
(97) Real, E.; Rodrigues, L.; Cabal, G. G.; Enguita, F. J.; Mancio-Silva, L.; Mello-Vieira, J.; Beatty, W.; Vera, I. M.; Zuzarte-Luis, V.; Figueira, T. N.; et al. *Plasmodium* Uis3 Sequesters Host LC3 to Avoid Elimination by Autophagy in Hepatocytes. *Nat. Microbiol.* **2018**, *3*, 17.
(98) Niklaus, L.; Agop-Nersesian, C.; Schmuckli-Maurer, J.; Wacker, R.; Grunig, V.; Heussler, V. T. Deciphering Host Lysosome-Mediated Elimination of *Plasmodium berghei* Liver Stage Parasites. *Sci. Rep.* **2019**, *9*, 7967.
(99) Vijayan, K.; Arang, N.; Wei, L.; Morrison, R.; Geiger, R.; Parks, K. R.; Lewis, A. J.; Mast, F. D.; Douglass, A. N.; Kain, H. S. et al. A Genome-Wide Crispr-Cas9 Screen Identifies Host Factors Essential for Optimal *Plasmodium* Liver Stage Development. *bioRxiv* **2020** DOI: 10.1101/2020.08.31.275867.
(100) Zuzarte-Luis, V.; Mello-Vieira, J.; Marreiros, I. M.; Liehl, P.; Chora, A. F.; Carret, C. K.; Carvalho, T.; Mota, M. M. Dietary Alterations Modulate Susceptibility to *Plasmodium* Infection. *Nat. Microbiol* **2017**, *2*, 1600.
(101) Mancio-Silva, L.; Slavic, K.; Grilo Ruivo, M. T.; Grosso, A. R.; Modrzynska, K. K.; Vera, I. M.; Sales-Dias, J.; Gomes, A. R.; MacPherson, C. R.; Crozet, P.; et al. Nutrient Sensing Modulates Malaria Parasite Virulence. *Nature* **2017**, *547*, 213.
(102) Cnop, M.; Foufelle, F.; Velloso, L. A. Endoplasmic Reticulum Stress, Obesity and Diabetes. *Trends Mol. Med.* **2012**, *18*, 59.
(103) Malhi, H.; Kaufman, R. J. Endoplasmic Reticulum Stress in Liver Disease. *J. Hepatol.* **2011**, *54*, 795.
(104) Ruivo, M. T. G.; Vera, I. M.; Sales-Dias, J.; Meireles, P.; Gural, N.; Bhatia, S. N.; Mota, M. M.; Mancio-Silva, L. Host Ampk Is a Modulator of *Plasmodium* Liver Infection. *Cell Rep.* **2016**, *16*, 2539.
(105) Medina Vera, I.; Grilo Ruivo, M. T.; Lemos Rocha, L. F.; Marques, S.; Bhatia, S. N.; Mota, M. M.; Mancio-Silva, L. Targeting Liver Stage Malaria with Metformin. *JCI Insight* **2019**, *4*, 127441.
(106) Hanahan, D.; Weinberg, R. A. Hallmarks of Cancer: The Next Generation. *Cell* **2011**, *144*, 646.
(107) Moloney, J. N.; Cotter, T. G. Ros Signalling in the Biology of Cancer. *Semin. Cell Dev. Biol.* **2018**, *80*, 50.
(108) Postovit, L.; Widmann, C.; Huang, P.; Gibson, S. B. Harnessing Oxidative Stress as an Innovative Target for Cancer Therapy. *Oxid. Med. Cell. Longevity* **2018**, *2018*, 6135739.
(109) Perillo, B.; Di Donato, M.; Pezone, A.; Di Zazzo, E.; Giovannelli, P.; Galasso, G.; Castoria, G.; Migliaccio, A. Ros in

Cancer Therapy: The Bright Side of the Moon. *Exp. Mol. Med.* **2020**, *52*, 192.
(110) Dixon, S. J.; Patel, D. N.; Welsch, M.; Skouta, R.; Lee, E. D.; Hayano, M.; Thomas, A. G.; Gleason, C. E.; Tatonetti, N. P.; Slusher, B. S.; Stockwell, B. R.; et al. Pharmacological Inhibition of Cystine-Glutamate Exchange Induces Endoplasmic Reticulum Stress and Ferroptosis. *eLife* **2014**, *3*, No. e02523.
(111) Dixon, S. J.; Lemberg, K. M.; Lamprecht, M. R.; Skouta, R.; Zaitsev, E. M.; Gleason, C. E.; Patel, D. N.; Bauer, A. J.; Cantley, A. M.; Yang, W. S.; et al. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. *Cell* **2012**, *149*, 1060.
(112) Matsushita, M.; Freigang, S.; Schneider, C.; Conrad, M.; Bornkamm, G. W.; Kopf, M. T Cell Lipid Peroxidation Induces Ferroptosis and Prevents Immunity to Infection. *J. Exp. Med.* **2015**, *212*, 555.
(113) Dai, L.; Cao, Y.; Chen, Y.; Kaleeba, J. A.; Zabaleta, J.; Qin, Z. Genomic Analysis of Xct-Mediated Regulatory Network: Identification of Novel Targets against Aids-Associated Lymphoma. *Oncotarget* **2015**, *6*, 12710.
(114) Huang, Y.; Dai, Z.; Barbacioru, C.; Sadee, W. Cystine-Glutamate Transporter Slc7a11 in Cancer Chemosensitivity and Chemoresistance. *Cancer Res.* **2005**, *65*, 7446.
(115) Zhai, B.; Hu, F.; Yan, H.; Zhao, D.; Jin, X.; Fang, T.; Pan, S.; Sun, X.; Xu, L. Bufalin Reverses Resistance to Sorafenib by Inhibiting Akt Activation in Hepatocellular Carcinoma: The Role of Endoplasmic Reticulum Stress. *PLoS One* **2015**, *10*, No. e0138485.
(116) Connell, L. C.; Harding, J. J.; Abou-Alfa, G. K. Advanced Hepatocellular Cancer: The Current State of Future Research. *Curr. Treat. Options Oncol.* **2016**, *17*, 43.
(117) Corrado, A.; Ferrari, S. M.; Politti, U.; Mazzi, V.; Miccoli, M.; Materazzi, G.; Antonelli, A.; Ulisse, S.; Fallahi, P.; Miccoli, P. Aggressive Thyroid Cancer: Targeted Therapy with Sorafenib. *Minerva Endocrinol.* **2017**, *42*, 64.
(118) Sehm, T.; Rauh, M.; Wiendieck, K.; Buchfelder, M.; Eyupoglu, I. Y.; Savaskan, N. E. Temozolomide Toxicity Operates in a Xct/Slc7a11 Dependent Manner and Is Fostered by Ferroptosis. *Oncotarget* **2016**, *7*, 74630.
(119) Kain, H. S.; Glennon, E. K. K.; Vijayan, K.; Arang, N.; Douglass, A. N.; Fortin, C. L.; Zuck, M.; Lewis, A. J.; Whiteside, S. L.; Dudgeon, D. R. Liver Stage Malaria Infection Is Controlled by Host Regulators of Lipid Peroxidation. *Cell Death Differ.* **2020**, *27*, 44.
(120) Kaushansky, A.; Ye, A. S.; Austin, L. S.; Mikolajczak, S. A.; Vaughan, A. M.; Camargo, N.; Metzger, P. G.; Douglass, A. N.; MacBeath, G.; Kappe, S. H. Suppression of Host P53 Is Critical for *Plasmodium* Liver-Stage Infection. *Cell Rep.* **2013**, *3*, 630.
(121) Tran, T. M.; Guha, R.; Portugal, S.; Skinner, J.; Ongoiba, A.; Bhardwaj, J.; Jones, M.; Moebius, J.; Venepally, P.; Doumbo, S.; et al. A Molecular Signature in Blood Reveals a Role for P53 in Regulating Malaria-Induced Inflammation. *Immunity* **2019**, *51*, 750.
(122) Epiphanio, S.; Mikolajczak, S. A.; Goncalves, L. A.; Pamplona, A.; Portugal, S.; Albuquerque, S.; Goldberg, M.; Rebelo, S.; Anderson, D. G.; Akinc, A.; et al. Heme Oxygenase-1 Is an Anti-Inflammatory Host Factor That Promotes Murine *Plasmodium* Liver Infection. *Cell Host Microbe* **2008**, *3*, 331.
(123) Agop-Nersesian, C.; Niklaus, L.; Wacker, R.; Theo Heussler, V. Host Cell Cytosolic Immune Response During *Plasmodium* Liver Stage Development. *FEMS Microbiol. Rev.* **2018**, *42*, 324.
(124) Ebert, G.; Lopaticki, S.; O'Neill, M. T.; Steel, R. W. J.; Doerflinger, M.; Rajasekaran, P.; Yang, A. S. P.; Erickson, S.; Ioannidis, L.; Arandjelovic, P.; et al. Targeting the Extrinsic Pathway of Hepatocyte Apoptosis Promotes Clearance of *Plasmodium* Liver Infection. *Cell Rep.* **2020**, *30*, 4343.
(125) Vo, T. T.; Letai, A. Bh3-Only Proteins and Their Effects on Cancer. *Adv. Exp. Med. Biol.* **2010**, *687*, 49.
(126) Thomas, S.; Quinn, B. A.; Das, S. K.; Dash, R.; Emdad, L.; Dasgupta, S.; Wang, X. Y.; Dent, P.; Reed, J. C.; Pellecchia, M.; et al. Targeting the Bcl-2 Family for Cancer Therapy. *Expert Opin. Ther. Targets* **2013**, *17*, 61.
(127) van de Sand, C.; Horstmann, S.; Schmidt, A.; Sturm, A.; Bolte, S.; Krueger, A.; Lutgehetmann, M.; Pollok, J. M.; Libert, C.; Heussler, V. T. The Liver Stage of *Plasmodium berghei* Inhibits Host Cell Apoptosis. *Mol. Microbiol.* **2005**, *58*, 731.
(128) Kaushansky, A.; Kappe, S. H. The Crucial Role of Hepatocyte Growth Factor Receptor During Liver-Stage Infection Is Not Conserved among *Plasmodium* Species. *Nat. Med.* **2011**, *17*, 1180.
(129) Dobzhansky, T. Genetics of Natural Populations; Recombination and Variability in Populations of Drosophila *Pseudoobscura*. *Genetics* **1946**, *31*, 269.
(130) Mast, F. D.; Navare, A. T.; van der Sloot, A. M.; Coulombe-Huntington, J.; Rout, M. P.; Baliga, N. S.; Kaushansky, A.; Chait, B. T.; Aderem, A.; Rice, C. M.; et al. Crippling Life Support for Sars-Cov-2 and Other Viruses through Synthetic Lethality. *J. Cell Biol.* **2020**, *219*, e202006159.
(131) Heim, M. H. 25 Years of Interferon-Based Treatment of Chronic Hepatitis C: An Epoch Coming to an End. *Nat. Rev. Immunol.* **2013**, *13*, 535.
(132) Poli, G.; Orenstein, J. M.; Kinter, A.; Folks, T. M.; Fauci, A. S. Interferon-Alpha but Not Azt Suppresses Hiv Expression in Chronically Infected Cell Lines. *Science* **1989**, *244*, 575.
(133) Cillo, A. R.; Mellors, J. W. Which Therapeutic Strategy Will Achieve a Cure for Hiv-1? *Curr. Opin. Virol.* **2016**, *18*, 14.
(134) Li, P.; Kaiser, P.; Lampiris, H. W.; Kim, P.; Yukl, S. A.; Havlir, D. V.; Greene, W. C.; Wong, J. K. Stimulating the Rig-I Pathway to Kill Cells in the Latent Hiv Reservoir Following Viral Reactivation. *Nat. Med.* **2016**, *22*, 807.
(135) Nussenzweig, R. S.; Vanderberg, J.; Most, H.; Orton, C. Protective Immunity Produced by the Injection of X-Irradiated Sporozoites of *Plasmodium berghei*. *Nature* **1967**, *216*, 160.
(136) Clyde, D. F.; Most, H.; McCarthy, V. C.; Vanderberg, J. P. Immunization of Man against Sporozite-Induced *falciparum* Malaria. *Am. J. Med. Sci.* **1973**, *266*, 169.
(137) Zaidi, I.; Diallo, H.; Conteh, S.; Robbins, Y.; Kolasny, J.; Orr-Gonzalez, S.; Carter, D.; Butler, B.; Lambert, L.; Brickley, E.; et al. Gammadelta T Cells Are Required for the Induction of Sterile Immunity During Irradiated Sporozoite Vaccinations. *J. Immunol.* **2017**, *199*, 3781.
(138) Roestenberg, M.; McCall, M.; Hopman, J.; Wiersma, J.; Luty, A. J.; van Gemert, G. J.; van de Vegte-Bolmer, M.; van Schaijk, B.; Teelen, K.; Arens, T.; et al. Protection against a Malaria Challenge by Sporozoite Inoculation. *N. Engl. J. Med.* **2009**, *361*, 468.
(139) Roestenberg, M.; Teirlinck, A. C.; McCall, M. B.; Teelen, K.; Makamdop, K. N.; Wiersma, J.; Arens, T.; Beckers, P.; van Gemert, G.; van de Vegte-Bolmer, M.; et al. Long-Term Protection against Malaria after Experimental Sporozoite Inoculation: An Open-Label Follow-up Study. *Lancet* **2011**, *377*, 1770.
(140) Sissoko, M. S.; Healy, S. A.; Katile, A.; Omaswa, F.; Zaidi, I.; Gabriel, E. E.; Kamate, B.; Samake, Y.; Guindo, M. A.; Dolo, A.; et al. Safety and Efficacy of Pfspz Vaccine against *Plasmodium falciparum* Via Direct Venous Inoculation in Healthy Malaria-Exposed Adults in Mali: A Randomised, Double-Blind Phase 1 Trial. *Lancet Infect. Dis.* **2017**, *17*, 498.
(141) Miller, J. L.; Sack, B. K.; Baldwin, M.; Vaughan, A. M.; Kappe, S. H. I. Interferon-Mediated Innate Immune Responses against Malaria Parasite Liver Stages. *Cell Rep.* **2014**, *7*, 436.
(142) Liehl, P.; Zuzarte-Luís, V.; Chan, J.; Zillinger, T.; Baptista, F.; Carapau, D.; Konert, M.; Hanson, K. K.; Carret, C.; Lassnig, C.; et al. Host-Cell Sensors for *Plasmodium* Activate Innate Immunity against Liver-Stage Infection. *Nat. Med.* **2014**, *20*, 47.
(143) Pied, S.; Roland, J.; Louise, A.; Voegtle, D.; Soulard, V.; Mazier, D.; Cazenave, P.-A. Liver Cd4-Cd8- Nk1.1+ Tcr$\alpha\beta$ Intermediate Cells Increase During Experimental Malaria Infection and Are Able to Exhibit Inhibitory Activity against the Parasite Liver Stage in Vitro. *J. Immunol.* **2000**, *164*, 1463.
(144) Roland, J.; Soulard, V.; Sellier, C.; Drapier, A.-M.; Di Santo, J. P.; Cazenave, P.-A.; Pied, S. Nk Cell Responses to *Plasmodium* Infection and Control of Intrahepatic Parasite Development. *J. Immunol.* **2006**, *177*, 1229.

(145) Minkah, N. K.; Wilder, B. K.; Sheikh, A. A.; Martinson, T.; Wegmair, L.; Vaughan, A. M.; Kappe, S. H. I. Innate Immunity Limits Protective Adaptive Immune Responses against Pre-Erythrocytic Malaria Parasites. *Nat. Commun.* **2019**, *10*, 3950.
(146) Kurup, S. P.; Butler, N. S.; Harty, J. T. T Cell-Mediated Immunity to Malaria. *Nat. Rev. Immunol.* **2019**, *19*, 457.
(147) Kurup, S. P.; Anthony, S. M.; Hancox, L. S.; Vijay, R.; Pewe, L. L.; Moioffer, S. J.; Sompallae, R.; Janse, C. J.; Khan, S. M.; Harty, J. T. Monocyte-Derived Cd11c(+) Cells Acquire *Plasmodium* from Hepatocytes to Prime Cd8 T Cell Immunity to Liver-Stage Malaria. *Cell Host Microbe* **2019**, *25*, 565.
(148) Sun, P.; Schwenk, R.; White, K.; Stoute, J. A.; Cohen, J.; Ballou, W. R.; Voss, G.; Kester, K. E.; Heppner, D. G.; Krzych, U. Protective Immunity Induced with Malaria Vaccine, Rts,S, Is Linked to *Plasmodium falciparum* Circumsporozoite Protein-Specific Cd4+ and Cd8+ T Cells Producing Ifn-Gamma. *J. Immunol.* **2003**, *171*, 6961.
(149) Jobe, O.; Donofrio, G.; Sun, G.; Liepinsh, D.; Schwenk, R.; Krzych, U. Immunization with Radiation-Attenuated *Plasmodium berghei* Sporozoites Induces Liver Ccd8alpha+Dc That Activate Cd8+T Cells against Liver-Stage Malaria. *PLoS One* **2009**, *4*, No. e5075.
(150) Nganou-Makamdop, K.; van Gemert, G. J.; Arens, T.; Hermsen, C. C.; Sauerwein, R. W. Long Term Protection after Immunization with P. *berghei* Sporozoites Correlates with Sustained Ifnγ Responses of Hepatic Cd8+ Memory T Cells. *PLoS One* **2012**, *7*, No. e36508.
(151) Clark, I. A.; Hunt, N. H.; Butcher, G. A.; Cowden, W. B. Inhibition of Murine Malaria (*Plasmodium chabaudi*) in Vivo by Recombinant Interferon-Gamma or Tumor Necrosis Factor, and Its Enhancement by Butylated Hydroxyanisole. *J. Immunol.* **1987**, *139*, 3493.
(152) Boonhok, R.; Rachaphaew, N.; Duangmanee, A.; Chobson, P.; Pattaradilokrat, S.; Utaisincharoen, P.; Sattabongkot, J.; Ponpuak, M. Lap-Like Process as an Immune Mechanism Downstream of Ifn-Γ in Control of the Human Malaria *Plasmodium vivax* Liver Stage. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E3519.
(153) Seguin, M. C.; Klotz, F. W.; Schneider, I.; Weir, J. P.; Goodbary, M.; Slayter, M.; Raney, J. J.; Aniagolu, J. U.; Green, S. J. Induction of Nitric Oxide Synthase Protects against Malaria in Mice Exposed to Irradiated *Plasmodium berghei* Infected Mosquitoes: Involvement of Interferon Gamma and Cd8+ T Cells. *J. Exp. Med.* **1994**, *180*, 353.
(154) Epstein, J. E.; Tewari, K.; Lyke, K. E.; Sim, B. K.; Billingsley, P. F.; Laurens, M. B.; Gunasekera, A.; Chakravarty, S.; James, E. R.; Sedegah, M.; et al. Live Attenuated Malaria Vaccine Designed to Protect through Hepatic Cd8(+) T Cell Immunity. *Science* **2011**, *334*, 475.
(155) Doll, K. L.; Pewe, L. L.; Kurup, S. P.; Harty, J. T. Discriminating Protective from Nonprotective *Plasmodium*-Specific Cd8+ T Cell Responses. *J. Immunol.* **2016**, *196*, 4253.
(156) Ewer, K. J.; Sierra-Davidson, K.; Salman, A. M.; Illingworth, J. J.; Draper, S. J.; Biswas, S.; Hill, A. V. Progress with Viral Vectored Malaria Vaccines: A Multi-Stage Approach Involving "Unnatural Immunity. *Vaccine* **2015**, *33*, 7444.
(157) Glennon, E. K. K.; Austin, L. S.; Arang, N.; Kain, H. S.; Mast, F. D.; Vijayan, K.; Aitchison, J. D.; Kappe, S. H. I.; Kaushansky, A. Alterations in Phosphorylation of Hepatocyte Ribosomal Protein S6 Control *Plasmodium* Liver Stage Infection. *Cell Rep.* **2019**, *26*, 3391.
(158) Albuquerque, S. S.; Carret, C.; Grosso, A. R.; Tarun, A. S.; Peng, X.; Kappe, S. H.; Prudêncio, M.; Mota, M. M. Host Cell Transcriptional Profiling During Malaria Liver Stage Infection Reveals a Coordinated and Sequential Set of Biological Events. *BMC Genomics* **2009**, *10*, 270.
(159) Tarun, A. S.; Peng, X.; Dumpit, R. F.; Ogata, Y.; Silva-Rivera, H.; Camargo, N.; Daly, T. M.; Bergman, L. W.; Kappe, S. H. A Combined Transcriptome and Proteome Survey of Malaria Parasite Liver Stages. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 305.
(160) Margolis, R.; Derr, L.; Dunn, M.; Huerta, M.; Larkin, J.; Sheehan, J.; Guyer, M.; Green, E. D. The National Institutes of Health's Big Data to Knowledge (Bd2k) Initiative: Capitalizing on Biomedical Big Data. *J. Am. Med. Inform. Assoc.* **2014**, *21*, 957.
(161) Pushpakom, S.; Iorio, F.; Eyers, P. A.; Escott, K. J.; Hopper, S.; Wells, A.; Doig, A.; Guilliams, T.; Latimer, J.; McNamee, C.; et al. Drug Repurposing: Progress, Challenges and Recommendations. *Nat. Rev. Drug Discovery* **2019**, *18*, 41.
(162) Hodos, R. A.; Kidd, B. A.; Shameer, K.; Readhead, B. P.; Dudley, J. T. In Silico Methods for Drug Repurposing and Pharmacology. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2016**, *8*, 186.
(163) Wagner, A.; Cohen, N.; Kelder, T.; Amit, U.; Liebman, E.; Steinberg, D. M.; Radonjic, M.; Ruppin, E. Drugs That Reverse Disease Transcriptomic Signatures Are More Effective in a Mouse Model of Dyslipidemia. *Mol. Syst. Biol.* **2015**, *11*, 791.
(164) Hsieh, Y. Y.; Chou, C. J.; Lo, H. L.; Yang, P. M. Repositioning of a Cyclin-Dependent Kinase Inhibitor Gw8510 as a Ribonucleotide Reductase M2 Inhibitor to Treat Human Colorectal Cancer. *Cell Death Discovery* **2016**, *2*, 16027.
(165) Choi, W. T.; Duggineni, S.; Xu, Y.; Huang, Z.; An, J. Drug Discovery Research Targeting the Cxc Chemokine Receptor 4 (CXCR4). *J. Med. Chem.* **2012**, *55*, 977.
(166) Malcomson, B.; Wilson, H.; Veglia, E.; Thillaiyampalam, G.; Barsden, R.; Donegan, S.; El Banna, A.; Elborn, J. S.; Ennis, M.; Kelly, C.; et al. Connectivity Mapping (Sscmap) to Predict A20-Inducing Drugs and Their Antiinflammatory Action in Cystic Fibrosis. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E3725.
(167) Lotfi Shahreza, M.; Ghadiri, N.; Mousavi, S. R.; Varshosaz, J.; Green, J. R. A Review of Network-Based Approaches to Drug Repositioning. *Briefings Bioinf.* **2018**, *19*, 878.
(168) Emig, D.; Ivliev, A.; Pustovalova, O.; Lancashire, L.; Bureeva, S.; Nikolsky, Y.; Bessarabova, M. Drug Target Prediction and Repositioning Using an Integrated Network-Based Approach. *PLoS One* **2013**, *8*, No. e60618.
(169) Cheng, F.; Liu, C.; Jiang, J.; Lu, W.; Li, W.; Liu, G.; Zhou, W.; Huang, J.; Tang, Y. Prediction of Drug-Target Interactions and Drug Repositioning Via Network-Based Inference. *PLoS Comput. Biol.* **2012**, *8*, No. e1002503.
(170) Wang, Y. B.; You, Z. H.; Yang, S.; Yi, H. C.; Chen, Z. H.; Zheng, K. A Deep Learning-Based Method for Drug-Target Interaction Prediction Based on Long Short-Term Memory Neural Network. *BMC Med. Inf. Decis. Making* **2020**, *20*, 49.
(171) Bock, J. R.; Gough, D. A. Virtual Screen for Ligands of Orphan G Protein-Coupled Receptors. *J. Chem. Inf. Model.* **2005**, *45*, 1402.
(172) Vamathevan, J.; Clark, D.; Czodrowski, P.; Dunham, I.; Ferran, E.; Lee, G.; Li, B.; Madabhushi, A.; Shah, P.; Spitzer, M.; et al. Applications of Machine Learning in Drug Discovery and Development. *Nat. Rev. Drug Discovery* **2019**, *18*, 463.
(173) Arang, N.; Kain, H. S.; Glennon, E. K.; Bello, T.; Dudgeon, D. R.; Walter, E. N. F.; Gujral, T. S.; Kaushansky, A. Identifying Host Regulators and Inhibitors of Liver Stage Malaria Infection Using Kinase Activity Profiles. *Nat. Commun.* **2017**, *8*, 1232.

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Host-targeted Interventions as an Exciting Opportunity to Combat Malaria

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