Chikungunya virus time course infection of human macrophages reveals intracellular signaling pathways relevant to repurposed therapeutics

Madison Gray1, Israel Guerrero-Arguero1,2, Antonio Solis-Leal1,2, Richard A. Robison1, Bradford K. Berges1 and Brett E. Pickett1

1 Microbiology and Molecular Biology, Brigham Young University, Provo, Utah, United States of America
2 Population Health and Host-pathogen Interactions Programs, Texas Biomedical Research Institute, San Antonio, Texas, United States of America

ABSTRACT

Background: Chikungunya virus (CHIKV) is a mosquito-borne pathogen, within the Alphavirus genus of the Togaviridae family, that causes ~1.1 million human infections annually. CHIKV uses Aedes albopictus and Aedes aegypti mosquitoes as insect vectors. Human infections can develop arthralgia and myalgia, which results in debilitating pain for weeks, months, and even years after acute infection. No therapeutic treatments or vaccines currently exist for many alphaviruses, including CHIKV. Targeting the phagocytosis of CHIKV by macrophages after mosquito transmission plays an important role in early productive viral infection in humans, and could reduce viral replication and/or symptoms.

Methods: To better characterize the transcriptional response of macrophages during early infection, we generated RNA-sequencing data from a CHIKV-infected human macrophage cell line at eight or 24 hours post-infection (hpi), together with mock-infected controls. We then calculated differential gene expression, enriched functional annotations, modulated intracellular signaling pathways, and predicted therapeutic drugs from these sequencing data.

Results: We observed 234 pathways were significantly affected 24 hpi, resulting in six potential pharmaceutical treatments to modulate the affected pathways. A subset of significant pathways at 24 hpi includes AGE-RAGE, Fc epsilon RI, Chronic myeloid leukemia, Fc gamma R-mediated phagocytosis, and Ras signaling. We found that the MAPK1 and MAPK3 proteins are shared among this subset of pathways and that Telmisartan and Dasatinib are strong candidates for repurposed small molecule therapeutics that target human processes. The results of our analysis can be further characterized in the wet lab to contribute to the development of host-based prophylactics and therapeutics.

Subjects Bioinformatics, Computational Biology, Molecular Biology, Virology, Infectious Diseases
Keywords Chikungunya virus, Transcriptomics, Signaling pathways, Drug repurposing, Macrophage, Virology

How to cite this article Gray M, Guerrero-Arguero I, Solis-Leal A, Robison RA, Berges BK, Pickett BE. 2022. Chikungunya virus time course infection of human macrophages reveals intracellular signaling pathways relevant to repurposed therapeutics. PeerJ 10:e13090 DOI 10.7717/peerj.13090

Copyright 2022 Gray et al.
Distributed under Creative Commons CC-BY 4.0
INTRODUCTION

Chikungunya virus (CHIKV) infects approximately 1.1 million people per year in over 100 countries worldwide (Goupil & Mores, 2016), with over one billion people at-risk of becoming infected with the virus due to the presence of the required mosquito vector in tropical and subtropical climates (Nasci, 2014; Nsoesie et al., 2016; Goupil & Mores, 2016). CHIKV infections were primarily observed in India prior to 2015 when it emerged in the Western Hemisphere, which may be at least partially attributable to the role of Aedes albopictus mosquitoes as active transmission vectors in addition to Aedes aegypti, which had been considered the primary vector (Pagès et al., 2009; Delatte et al., 2010). CHIKV is an arthrogenic alphavirus, which can cause chronic rheumatoid arthritis-like symptoms in the peripheral joints of some patients, likely caused by viral presence in the joint tissue (Nakaya et al., 2012; Lounibos & Kramer, 2016). The name of the virus itself means “that which bends up”, referring to the painful posture of those who develop chronic sequelae after acute infection. Currently, no cure, treatment, or vaccine exists for most alphaviruses, including CHIKV.

The host immune response, which includes the rapid recruitment of macrophages, is initiated shortly after the virus infects a human via a mosquito vector (Haist et al., 2017). CHIKV initially invades stromal cells bypassing their intracellular defense mechanisms to induce apoptosis (Srivastava et al., 2020). Cellular apoptosis is advantageous to the virus which is ensconced within the produced apoptotic blebs that get consumed by phagocytic cells including macrophages (Srivastava et al., 2020). Within the monocytes, the virus is stabilized and begins to replicate preceding the acute phase of infection (Sourisseau et al., 2007; Wikan et al., 2012; Srivastava et al., 2020). Infected macrophages then operate as transporters for the virus, collecting in peripheral tissues, and serving as a possible mechanism for the severe joint pain associated with CHIKV infection (Her et al., 2010; Srivastava et al., 2020). Chronic CHIKV-infections still have detectable amounts of infected macrophage cells in their synovial tissue (Her et al., 2010). This process enables the virus to go through multiple rounds of infection in macrophages and other cells, which contributes to the onset of symptoms and pathogenesis. As such, maximizing the antiviral response in the macrophage to reduce virion production and/or escape during acute infection could lead to a host-based therapeutic strategy with smaller risks of the emergence of resistance in the future—especially when compared to antiviral strategies that strictly target viral proteins.

The aims of the current study are two-fold: (1) to gain a better understanding of the intracellular transcriptional response to CHIKV infection in human macrophages at 8 and 24 hpi, and (2) to apply that mechanistic knowledge to predict existing drugs that could be repurposed as either prophylactic or therapeutic treatments to minimize viral replication and spread from macrophages. Specifically, we calculated statistically significant differentially expressed genes (DEGs), functional gene annotations, and intracellular signaling pathways to improve our understanding of how macrophages respond to CHIKV infection. We then mined this information to predict existing
therapeutic treatments that could be repurposed to reverse key host-pathogen interactions
to reduce virus replication, infection, and pathogenesis. To our knowledge, this is the first
RNA-seq experiment involving CHIKV infection of cultured human macrophages.
The results obtained from this study contribute to ongoing efforts to develop effective
prophylactics and/or therapeutic treatments.

**MATERIALS AND METHODS**

**Cell infection**

Macrophages were differentiated from the U937* human monocyte cell line and infected
with CHIKV as described previously (*Guerrero-Arguero et al.*, 2020). Briefly, monocytes
were propagated with fetal bovine serum (FBS), Penicillin/Streptomycin, L-glutamine,
and HEPES buffer. The cells were then cultured in T-75 culture flasks at 37 °C before
being transferred to six-well tissue culture plates. Cells were then induced to become
adherent macrophages by exposing them to phorbol 12-mystrate 13-acetate (PMA) and
incubating in 3 mL of RPMI 1640 complete media at 37 °C for 24 h. PMA-differentiated
U937 monocyte-derived macrophages were then transferred to 12-well tissue culture
plates prior to infection with the CHIKV-LR strain at 0.1 multiplicity of infection (MOI)
and incubated at 37 °C with 5% CO₂ for 2 h. The virus-containing media was then
removed prior to washing the cells three times with PBS and adding fresh media prior
to incubation. Mock-infected cells were incubated using the same protocol and reagents,
and only lacked the presence of virus.

**RNA extraction and RNA-sequencing**

Biological replicates, in duplicate, of CHIKV-infected and mock-infected macrophages
were harvested at eight and 24 h post-infection (hpi) by removing the supernatant and
washing with PBS. RNA was extracted from all duplicate samples using Trizol reagent
according to the manufacturer’s protocol as performed previously (*Guerrero-Arguero
et al.*, 2020). A NanoDrop instrument was then used to quantify the concentration of
RNA as approximately 1.8 ng/µL for each sample. Reverse transcription of the RNA into
cDNA was then performed prior to generating Nextera XT sequencing libraries and
samples were barcoded for multiplexing. The concentration of post-capture libraries for
the sample pools ranged from 0.02 to 0.3 ng/µL. An Illumina NovaSeq instrument was
then used to produce paired-end 150 bp reads for each sample for downstream
analysis. An average of ~40 million reads were collected from the control samples, with
~11 million collected from the eight hpi samples and ~188 million collected from the
24 hpi samples.

**Statistical power analysis**

The Scotty tool was used to confirm the sequencing depth and number of samples were
above what was necessary to achieve sufficient statistical power (*Busby et al.*, 2013).
RNA-seq data preprocessing

The fastq files containing the RNA-sequencing data were subjected to analysis by the Snakemake-based Automated Reproducible MOdular Workflow for Preprocessing and Differential Analysis of RNA-seq Data (ARMOR) software. Specifically, the fastq files, associated metadata, and a configuration file for each dataset were used as input to the ARMOR workflow (Orjuela et al., 2019). This workflow, which uses the python-based Snakemake workflow language (Köster & Rahmann, 2018), performs the following steps: trimming reads with TrimGalore (Krueger et al., 2021), performing quality control with FastQC (s-andrews, 2021), mapping and quantifying reads to the human GRCh38 transcriptome with Salmon (Patro et al., 2017), generating DEG lists with edgeR (Robinson, McCarthy & Smyth, 2010), performing GO enrichment with Camera (Wu & Smyth, 2012), and identifying significant splice variants from the detected transcripts with DRIMseq (Nowicka & Robinson, 2016).

Signaling pathway enrichment

The DEGs from ARMOR were then used as input to the signaling pathway impact analysis (SPIA) algorithm to identify intracellular signaling pathways that were significantly enriched with DEGs (Tarca et al., 2009). SPIA implements a bootstrapping approach to generate a null distribution for DEGs in each pathway, then uses the null distribution to calculate the significance threshold. Over 1,500 public signaling pathways from the KEGG, Reactome, NCI, BioCarta, and Panther databases were used to test for enrichment. The regulatory patterns from these analyses were then cross-referenced to the Open Targets (opentargets.org) database to identify known drug targets that were present in each of the significant pathways, and which could be repurposed as host-based therapeutics for CHIKV.

Drug repurposing and ranking

To increase the number of high-confidence potential therapeutics, a ranking strategy was implemented. For this study, a focus on small molecules was justified to facilitate ease of therapeutic distribution in geographical regions lacking adequate cold-storage resources. To simplify the multiple characteristics that were considered in the ranking of the results, a numerical score that represents a variety of metrics was assigned to each result. Contributing factors to this score include but are not exclusive to: whether the drug has been through clinical trials or has obtained US Food and Drug Administration (FDA) approval, if the drug had been approved and tested in multiple studies, the toxicity of the drug (LD$_{50}$ values and/or recommended diet restrictions, manufacturer safety statements, requirements of administration, and long-term impacts or risks that are included in drug consumption), prior publications or studies discussing the drug’s effectiveness against CHIKV, and whether any complications were indicated during trials—either during or after FDA approval.
RESULTS

Power analysis
We began by confirming that our experimental design included appropriate numbers of reads and biological replicates to achieve sufficient statistical power. Using two biological replicates and at least 10 million reads, our analysis showed that ~35% of genes could be detected at a log₂ fold-change of ≥1.6 with a p-value of <0.05. We observed the replicate dispersion among mock-infected samples was 0.89, while that for the 24 hpi samples was 0.44. In addition, hierarchical clustering grouped the mock-infected samples together and the 24 hpi samples together. We calculated that ~55% of genes could be detected with a log₂ fold-change of >3, which is acceptable. We calculated the measurement bias, the percentage of genes measured with at least 95% of the maximum power to be ~94%, which is also within the acceptable range.

Differential expression and gene ontology enrichment shows lag in immune response
We first quantified the intracellular transcriptional response of human macrophages to CHIKV infection by calculating the log₂ fold-change and false discovery rate (FDR) adjusted p-values for DEGs. We constructed these comparisons to compare the samples from CHIKV-infected macrophages at 8 and 24 hpi to the mock-infected cells. We plotted the relationship between all detected genes and retained those with a FDR p-value smaller than 0.05. Interestingly, we found no genes that surpassed our significance threshold of 0.05 for the eight hpi comparison, so this comparison was ignored in subsequent analyses. This may be at least partially due to the lower number of reads generated for the eight hpi samples. In contrast, we observed 9,670 genes that had significant differential expression at 24 hpi (Supplemental File 1), with a majority of DEGs being downregulated during early infection (Fig. 1). A subset of the most significant genes that were impacted by virus infection at 24 h post-infection included those that code for extracellular mucins (MUC3A, MUC12, MUC5B), an immunoglobulin gene (IGFN1), a nucleosome assembly protein (NAP1L1), and Ras protein activator (RASA4) as a Calcium signaling component.

We performed a Gene Ontology (GO) enrichment analysis on the DEG list to identify the cellular component, molecular mechanism, and/or biological process annotations that are assigned to each DEG result from the CHIKV-infected macrophages. The 24 hpi GO analysis identified eight statistically significant GO terms (Supplemental File 2). Significant GO terms include metal ion binding, nucleic acid binding, cell adhesion and extracellular matrix organization.

Signaling pathway enrichment reveals intracellular stress
We next applied a robust signaling pathway enrichment algorithm to identify a total of 234 signaling pathways that were significantly affected at 24 hpi (Supplemental File 3). Seven significant pathways that were positively or negatively affected during the early stages of CHIKV infection in macrophages seemed especially relevant (Table 1). Collections of
interacting proteins with the most significant corrected $p$-values also included Olfactory Transduction (Fig. 2), AGE-RAGE (Fig. 3), and Stimuli-Sensing Channels (Fig. 4). Our observation of the AGE-RAGE pathway is expected as it contains inflammatory proteins including interleukins, NF-κB, and JAK-STAT that regulate the acute inflammatory response and the innate immune system (Chen et al., 2018). The Olfactory Transduction pathway consists of many proteins that contribute to the regulation of Calcium signaling, presumably via Calmodulin. We also observed pathways involving Wnt signaling, extracellular matrix receptors, Insulin-like growth factor 1, Calcineurin, adherens junctions, and HIF-1-alpha (hypoxia) signaling.

Table 1 Top seven intracellular signaling pathways impacted by CHIKV in monocyte-derived human macrophages at 24 hpi.

| Name of pathway                                      | pSize $^*$ | NDE $^**$ | pGFWER $^+$ | Status $^{++}$ | Source database            |
|------------------------------------------------------|------------|-----------|-------------|----------------|---------------------------|
| Stimuli-sensing channels                             | 100        | 76        | 2.71E−04    | Activated      | Reactome                  |
| Wnt signaling pathway                                | 219        | 139       | 2.94E−04    | Activated      | Panther                   |
| ECM-receptor interaction                             | 79         | 58        | 3.31E−04    | Inhibited      | KEGG                      |
| IGF1 pathway                                         | 30         | 22        | 0.000975    | Activated      | NCI                       |
| Role of Calcineurin-dependent NFAT signaling in lymphocytes | 36         | 26        | 0.001006    | Activated      | NCI                       |
| Stabilization and expansion of the E-cadherin adherens junction | 42         | 30        | 0.001025    | Activated      | NCI                       |
| HIF-1-alpha transcription factor network             | 64         | 44        | 0.001294    | Activated      | NCI                       |

Notes:

$^*$ Number of genes assigned to the pathway.

$^**$ Quantity of DE genes found in each pathway.

$^+$ Bonferroni-corrected $p$-value for each pathway.

$^{++}$ The predicted direction of pathway modulation.
Figure 2  Protein-protein interaction network for the Olfactory Transduction pathway at 24 hpi with CHIKV. Nodes in orange are members of the pathway that are significantly downregulated. Nodes in purple are members of the pathway that are significantly upregulated. Nodes in cyan are unaffected members of the pathway.
In an effort to identify DEGs that were shared across multiple pathways, we compared the members of multiple significant signaling pathways. We found that a subset of the modulated pathways shared DEGs including MAPK3 and MAPK1.

Drug repurposing analysis identifies potential therapeutics for CHIKV
We cross-referenced all proteins in each significant signaling pathway against the opentargets.org database to identify known human drug targets within each significant signaling pathway and the associated existing small molecules that bind to each drug target in each pathway. We anticipated that this approach would identify drug targets that play a key role in fundamental viral processes, which would reduce virus replication and/or disease when targeted by a drug. As expected from the copious number of significant pathways, we predicted 136 pharmaceutical drugs from the 24 hpi pathways (Table 2). We then ranked these potential therapeutics with a novel toxicity ranking algorithm (Table 3). Briefly, this algorithm generates a single value that represents toxicity, safety, clinical trial status, and past repurposing efforts in virology. The top drugs predicted by our
work included Telmisartan, Sunitinib, Etanercept, Vorinostat, Dasatinib, and Regorafenib as potential therapeutic drugs that could be repurposed to reduce signs, symptoms, and/or pathogenesis associated with infection by Chikungunya virus (Table 4).

**DISCUSSION**

The purpose of this study was to identify genes, functional annotations, and intracellular signaling pathways at 24 hpi that improve our mechanistic understanding of CHIKV infection of macrophages to aid in the prediction of prophylactics and/or treatments. To our knowledge, this is the first study to generate RNA-sequencing data from CHIKV-infected human macrophages. These cells play a key role in both the early stages of systemic spread in the human host, as well as the chronic sequelae after acute infection. Our results were also used to predict repurposed therapeutic drugs that could potentially be used as antivirals to combat CHIKV infection, replication, and/or pathogenesis in human macrophages. Throughout this study, we found that CHIKV-infected human macrophages modulate various biological pathways that generate intracellular and immune-related signals that likely contribute to the characteristic symptoms of fever, polyarthritis, and/or chronic pain.
| Drug name     | ECM-receptor interaction | Influenza A infection | Salmonella transduction | Fc epsilon RI signaling pathway | Pathogenic *Escherichia coli* infection | Renal cell carcinoma | Chronic myeloid leukemia | Fc gamma R-mediated phagocytosis | AGE-RAGE signaling pathway in diabetic complications | Ras signaling pathway |
|---------------|--------------------------|----------------------|-------------------------|-------------------------------|---------------------------------------|-------------------|--------------------------|---------------------------------|-------------------------------------------------|---------------------|
| ALPELISIB     | X⁴                       | X⁴                   | X⁴                      | X⁴                            | X⁴                                    | X⁴                | X⁴                       | X⁴                              | X⁴                                              | X⁴                  |
| IDELALISIB    | X⁴                       | X⁴                   | X⁴                      | X⁴                            | X⁴                                    | X⁴                | X⁴                       | X⁴                              | X⁴                                              | X⁴                  |
| REGORAFENIB   | X⁴                       | X⁴                   |                         | X⁴                            | X⁴                                    | X⁴                | X⁴                       | X⁴                              | X⁴                                              | X⁴                  |
| MIDOUSTAURIN  | X⁴                       |                      |                         | X⁴                            | X⁴                                    | X⁴                | X⁴                       | X⁴                              | X⁴                                              | X⁴                  |
| DASATINIB     |                         |                      |                         | X⁴                            |                                       | X⁴                | X⁴                       | X⁴                              | X⁴                                              | X⁴                  |
| CANAKINUMAB   | X⁴                       | X⁴                   |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| LUSPATERCEPT  |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| ABLIERCEPT    |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| RANIBIZUMAB   |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| BENZIODARONE  |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| ETANERCEPT    | X⁴                       |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| INFliximab    |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Collagenase   |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Clostridium   |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Histolyticum  |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| CONBERCEPT    |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| TOFACITINIB   | X⁴                       |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| IMATINIB      |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| NETARSUDIL    | X⁴                       |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| NILOTINIB     |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| DIPYRIDAMOLE  |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Entrectinib   |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Interferon Alfa-2B |          |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Interferon Beta-1A |         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Interferon Beta-1B |           |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Panobinostat  |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Peginterferon Alfa-2A |         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Peginterferon Alfa-2B |         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Peginterferon Beta-1A |       |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Pentoxyfylline |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Romidepsin    |                         |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |
| Rontalizumab  | X⁴                       |                      |                         |                               |                                       |                   |                          |                                 | X⁴                                              |                     |

**Table 2** Therapeutics that significantly modulate the same pathways found in human macrophages infected with CHIKV at 24 hpi.

Gray et al. (2022), PeerJ, DOI 10.7717/peerj.13090
| Drug name       | ECM-receptor interaction | Influenza A infection | Salmonella infection | Olfactory transduction | Fc epsilon RI signaling pathway | Pathogenic Escherichia coli infection | Renal cell carcinoma | Chronic myeloid leukemia | Fc gamma R-mediated phagocytosis | AGE-RAGE signaling pathway in diabetic complications | Ras signaling pathway |
|-----------------|--------------------------|-----------------------|----------------------|------------------------|-------------------------------|--------------------------------------|----------------------|--------------------------|---------------------------------|--------------------------------|--------------------------|
| SIFALIMUMAB     | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| SUNITINIB       | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| USTEKINUMAB     | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| VORINOSTAT      |                         |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| ABEMACICLIB     | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| DOCETAXEL       | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| PACLITAXEL      | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| COLCHICINE      | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| VEDOLIZUMAB     | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| LEVETIRACETAM   | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| OCRIPLASMIN     | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| VINCristINE     |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| HYDROXYCHLOROQUINE |                      |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| NINTEDANIB      | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| ROXADUSTAT      |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| AMISULPRIDE     |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| VALSARTAN       |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| VINFLUNINE      |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| ZOLEDRONIC ACID |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| LENVATINIB      |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| LITHIUM CARBONATE |                      |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| NATALIZUMAB     | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| SORAFENIB       |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| TIROFIBAN       | X¹                        |                       |                      |                        |                               |                                      |                      |                          |                                 |                                |                          |
| VINORELBINE     |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| ALENDRONIC ACID |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| DAPRODUSTAT     |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| IMIQUIMOD       |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| LOSARTAN        |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| PAZOPANIB       |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| ANLOTINIB       |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| AXITINIB        |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| CD24FC          |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |
| ERDAFitinib     |                              |                       |                      |                        |                               |                                      |                      |                          |                                 |                                | X¹                       |

(Continued)
| Drug name               | ECM-receptor interaction | Influenza A infection | Salmonella infection | Olfactory transduction | Fc epsilon RI signaling pathway | Pathogenic *Escherichia coli* infection | Renal cell carcinoma | Chronic myeloid leukemia | Fc gamma R-mediated phagocytosis | AGE-RAGE signaling pathway in diabetic complications | Ras signaling pathway |
|------------------------|--------------------------|----------------------|---------------------|------------------------|---------------------------------|----------------------------------------|---------------------|--------------------------|------------------------------------|-------------------------------------------------|---------------------|
| ERIBULIN               | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| INSULIN DETEMIR        | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| INSULIN GLARGINE       | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| IXABEPILONE            | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| TELMISARTAN            | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| VARESPLADIB METHYL     | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| VINBLASTINE            | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| BARICITINIB            | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| CABAZITAXEL            | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| CABOZANTINIB           | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| ERLOTINIB              | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| GEFITINIB              | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| IBANDRONIC ACID        | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| IBRUTINIB              | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| INSULIN GLULISINE      | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| INSULIN LISPRO         | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| INSULIN PORK           | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| INSULIN SUSP ISOPHANE  | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| BEEF                   | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| PONATINIB              | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| RISEDRONIC ACID        | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| TRASTUZUMAB EMTANSINE  | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
| VANDETANIB             | X                       |                      |                     |                        |                                 |                                        |                     |                          |                                    |                                                 |                     |
Table 3  Predicted drug rankings by toxicity.

| Drug name     | Type       | Clinically tested | Withdrawn** | Virus application³ | FDA approved | Chikungunya studies** | Toxicity (Grayscale) | Grade |
|---------------|------------|-------------------|-------------|--------------------|--------------|------------------------|----------------------|-------|
| TELMISARTAN   | Small molecule | 4                  | Yes         | Yes                | Yes          | Yes                    | 7                    | 4     |
| SUNITINIB     | Small molecule | 4                  | Yes         | Yes                | Yes          | Yes                    | 7                    | 4     |
| ETANERCEPT    | Biotech: Protein base | 4 | Yes         | Yes                | Yes          | Yes                    | 6                    | 4     |
| VORINOSTAT    | Small molecule | 3                  | Yes         | Yes                | Yes          | Yes                    | 6                    | 4     |
| DASATINIB     | Small molecule | 4                  | Yes         | Yes                | Yes          | Yes                    | 5                    | 4     |
| REGORAFENIB   | Small molecule | 4                  | Yes         | Yes                | Yes          | Yes                    | 2                    | 4     |
| CANAKINUMAB   | Biotech: Protein base | 3 | Yes         | Yes                | Yes          | Yes                    | 5                    | 3     |
| DIPYRIDAMOLE  | Small molecule | 4                  | Yes         | Yes                | Yes          | Yes                    | 8                    | 3     |
| PENTOXIFYLLINE| Small molecule | 4                  | Yes         | Yes                | Yes          | Yes                    | 7                    | 3     |
| INFLIXIMAB    | Biotech: Protein base | 4 | Yes         | Yes                | Yes          | Yes                    | 7                    | 3     |
| ROMIDEPSIN    | Small molecule | 3                  | Yes         | Yes                | Yes          | Yes                    | 3                    | 3     |
| LUSPATERCEPT  | Biotech: Protein base | 3 | Yes         | Yes                | Yes          | Yes                    | 8                    | 3     |
| ENTRECTINIB   | Small molecule | 1                  | Yes         | Yes                | Yes          | Yes                    | 6                    | 2     |
| MIDOSTAURIN   | Small molecule | 2                  | Yes         | Yes                | Yes          | Yes                    | 5                    | 2     |
| ALPELISIB     | Small molecule | 2                  | Yes         | Yes                | Yes          | Yes                    | 4                    | 2     |
| PANOBINOSTAT  | Small molecule | 3                  | Yes         | Yes                | Yes          | Yes                    | 2                    | 2     |
| IDELALISIB    | Small molecule | 3                  | Yes         | Yes                | Yes          | Yes                    | 2                    | 2     |
| BENZIODARONE  | Small molecule | X                  | Yes         | Yes                | Yes          | Yes                    | 1                    | −9    |

Grade Scale

1 unacceptable (<4)  2 poor (5–20)  3 acceptable (21–35)  4 exceptional (36−)

Notes:

* Clinical trial phase for drug’s original indication.
** Drug was withdrawn from market.
³ Drug has been involved in previous antiviral studies.
** Prior publications discussing the drug’s effectiveness against CHIKV.
*** Toxicity of the drug determined by FD₅₀ values and/or recommended diet restrictions, manufacturer safety statements, requirements of administration, and long-term impacts or risks that are included in drug consumption, all of these elements were evaluated by an assigned numerical value under the Grayscale (1–10 scale, where 1 is the most toxic).

Table 4  Top six ranked therapeutics for CHIKV.

| Drug          | Type       | Clinically tested | Used with viruses | FDA approved | Chikungunya studies | Toxicity (Grayscale) | Grade |
|---------------|------------|-------------------|-------------------|--------------|---------------------|----------------------|-------|
| TELMISARTAN   | Small molecule | 4                  | Yes               | Yes          | Yes                 | 7                    | 4     |
| SUNITINIB     | Small molecule | 4                  | Yes               | Yes          | Yes                 | 7                    | 4     |
| ETANERCEPT    | Biotech: Protein base | 4 | Yes         | Yes                | Yes          | Yes                    | 6                    | 4     |
| VORINOSTAT    | Small molecule | 3                  | Yes               | Yes          | Yes                 | 6                    | 4     |
| DASATINIB     | Small molecule | 4                  | Yes               | Yes          | Yes                 | 5                    | 4     |
| REGORAFENIB   | Small molecule | 4                  | Yes               | Yes          | Yes                 | 2                    | 4     |

Gray et al. (2022), PeerJ, DOI 10.7717/peerj.13090
Interestingly, the lack of significant DEGs at eight hpi suggests at least two possible explanations: (1) a potential lag in the intracellular macrophage response to CHIKV infection, or (2) the selected MOI produced an infected macrophage population that was too small to confidently detect differential expression. Since CHIKV has a positive-sense genome, we do not expect this lagging response to be caused by a delay in viral genome replication. Rather, we believe that this observation is more likely due to the macrophages not initiating a response until after a sufficient number of CHIKV proteins have been produced in the cell. Prior studies have observed approximately $10^6$ plaque-forming units per mL and $10^6$ viral RNA copies per mL produced from cells infected at 0.1 MOI at 24 hpi in the same human monocyte-derived macrophage cell line (*Guerrero-Arguero et al.*, 2020). Future experiments could provide additional insight into this hypothesis.

While this study focuses on the response of human macrophages infected with Chikungunya virus, prior CHIKV studies have independently validated a subset of our results. A mechanistic analysis of mouse samples identified various differentially expressed genes that are also present in our results, namely MFSD1, and FAM49B (*Nakaya et al.*, 2012). Given that this prior study and ours were performed in different cell types, it is likely that these shared genes represent similar antiviral responses across multiple cell types.

Although minimal prior work has directly measured gene expression in CHIKV-infected macrophages, our observation of a larger number of DEGs being downregulated is somewhat expected given the primary immune functions performed by these host cells. Studies in other cell types have reported multiple cellular functions being inhibited by CHIKV infection including immune response (*Selvamani, Mishra & Singh*, 2014; *Sharma, Balakathiresan & Maheshwari*, 2015; *Dhenni et al.*, 2021).

Prior studies examined the effect that CHIKV infection had on human skin fibroblast (HSF) cells, which showed several significant modulated signaling pathways at 24 hpi, particularly the “Wnt signaling”, “Stimuli-sensing channels”, and “ECM-receptor interaction” pathways (*Komiya & Habas*, 2008; *Parashar et al.*, 2018; *Roy, Byrareddy & Reid*, 2020; *Landers et al.*, 2021; *Ghildiyal & Gabrani*, 2021). These common pathways partially validate the relevance of our findings and may represent shared intracellular functions that contribute to the infected state of cells. The Olfactory Transduction pathway, which affects Calcium signaling, has been observed in prior work with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Vesicular Stomatitis Virus (*Mishra, Byrareddy & Nayak*, 2020; *Krishnamoorthy et al.*, 2021). A previous study has shown that Calcium modulation can affect CHIKV replication in macrophages (*Sanjai Kumar et al.*, 2021; *Melton et al.*, 2002). The AGE-RAGE pathway, which contains JAK-STAT components, interleukins, and other proteins has not, to our knowledge, been directly reported in prior Chikungunya studies. Our findings are relevant given prior work showing the CHIKV nsP2 protein inhibits JAK-STAT signaling to regulate aspects of the inflammatory response (*Fros et al.*, 2010), which contributes to many of the primary symptoms of CHIKV (*Lu et al.*, 2015; *Elgner et al.*, 2016).

Our subsequent analysis of DEGs and the associated signaling pathways identified potential therapeutic drugs that could be repurposed for CHIKV. Prior studies have...
reported progress on developing therapeutics that directly target viral proteins (Khan et al., 2010; Scuotto et al., 2017; Ho et al., 2018). In contrast, our drug repurposing analysis evaluated existing therapeutics that target human proteins that participate in key signaling pathways. A benefit of this approach is the reduced likelihood of the virus becoming resistant to such treatment since human genes mutate exponentially slower and are involved in key processes that play a role in virus replication.

Our approach to prioritizing drugs incorporated a novel toxicity metric to facilitate ranking of existing therapeutic drugs. Small molecules have been an area of active research for their anti-viral properties, and were purposefully targeted in our analysis due to their general chemical stability and minimal need for refrigeration during transport and/or storage. This is particularly important given that many CHIKV infections occur in geographical regions that may not have consistent access to cold-storage resources. Six of our drug predictions have already been found to reduce the effects of Chikungunya virus in cells including telmisartan, sunitinib, etanercept, vorinostat, and dasatinib (Bekerman et al., 2017; Dye, Brannan & Geneva Foundation Tacoma United States, 2017; Broeckel et al., 2019; Haese, Powers & Streblow, 2020) (Zaid et al., 2011; Roberts et al., 2015; Haridas & Haridas, 2019; “U18666A inhibits classical swine fever virus replication through interference with intracellular cholesterol trafficking,” 2019; Tripathi et al., 2020; De et al., 2021; Liang et al., 2019).

Vorinostat, trade name Zolina, is used to treat Cutaneous T-cell Lymphoma (CTCL) as a histone deacetylase inhibitor. Recently Vorinostat has performed well as an antiviral therapy for HIV and West Nile Virus (Lu et al., 2015; Wichit et al., 2017). Vorinostat enhances the antiviral qualities of U18666A, which is a multivesicular body (MVB) inhibitor that hinders the release of cholesterol from lysosomes. This effect has been observed to inhibit CHIKV replication in human skin fibroblasts (Lu et al., 2015; Elgner et al., 2016; Wichit et al., 2017; Ghildiyal & Gabrani, 2021).

Telmisartan, an angiotensin II receptor antagonist commonly known as Micardis or Pritor, was ranked the highest in our ranked results of repurposed drug candidates (PubChem, 2021). Recently, Telmisartan has risen in popularity from severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) clinical testing (Duarte et al., 2020; Rothlin et al., 2020). This drug has been shown to regulate glucose and lipid metabolism. Further testing is needed to determine whether any possible anti-inflammatory response effect is observed. A prior in silico drug repurposing study showed that Telisartan was predicted to have a docking affinity of $-9.3 \pm 0.1$ kcal/mol to the CHIKV nsP2 protein (Montes-Grajales et al., 2020). Binding to a viral protein would be an added benefit as a potential therapeutic, particularly when combined with Novobiocin to inhibit nsP2 protease activity which causes the eventual dampening of the antiviral response (Montes-Grajales et al., 2020; Tripathi et al., 2020; Battisti, Urban & Langer, 2021).

Sunitinib, or Sutent, is a receptor tyrosine kinase inhibitor that affects protein translation, and is currently used to treat renal cancer (Bekerman et al., 2017). The testing of Sunitinib with Dengue virus showed reduced viral load in the serum and tissue (Pu et al., 2018). Sunitinib combined with Erlotinib can modify the inflammatory cytokine responses in models of dengue virus infection (Pu et al., 2018), which could also be relevant to
CHIKV (Bekerman et al., 2017; Dye, Brannan & Geneva Foundation Tacoma United States, 2017; Pu et al., 2018). Sunitinib is currently being investigated in conjunction with oncolytic virotherapy (Kottke et al., 2010).

Dasatinib is a tyrosine kinase inhibitor that is commonly sold by the name Sprycel and is used for treating leukemia. This compound has shown promise as a prophylactic treatment for patients who are at-risk for HIV infection (Salgado et al., 2020). Results from the “Src Family Kinase Inhibitors Block Translation of Alphavirus Subgenomic mRNAs” demonstrated Dasatinib’s ability to directly affect viral RNA synthesis and decrease CHIKV RNA association with polysomes, indicating the importance of Src Family Kinase (SFK) activity in virus replication (Broeckel et al., 2019). Dasatinib has been found to block CHIKV replication and reduces infection-induced apoptosis in cells (Bekerman et al., 2017; Dye, Brannan & Geneva Foundation Tacoma United States, 2017; Haese, Powers & Streblow, 2020; Broeckel et al., 2019).

A multi-kinase inhibitor, Regorafenib (Stivarga), is used for the treatment of colorectal cancer and advanced gastrointestinal complications. It has a high level of toxicity and is only used after other treatments such as chemotherapy have been exhausted. This drug has anti-angiogenic properties and was shown to inhibit virus replication when administered prophylactically (Roberts et al., 2015). Sorafenib and Regorafenib can be used interchangeably, only differing in a single fluorine atom in their structures (Goel et al., 2018). A prior study confirmed that Sorafenib-treated cells suppress CHIKV replication (Roberts et al., 2015).

A variety of systemic clinical signs and symptoms are associated with CHIKV which are not limited to headache, myalgia, arthralgia, retro-orbital pain (Leo et al., 2009; Tan et al., 2018). Rash and other mucocutaneous signs, such as centrofacial hyperpigmentation, have also been observed and used as retrospective diagnosis (Mattar et al., 2015; Singal, 2017; Panigrahi, Chakraborty & Sil, 2021). Additional experiments will be needed to determine whether minimizing the infection and antiviral response in macrophages affects such systemic signs.

Past work has documented that patients infected with CHIKV can develop side effects or adverse reactions while undergoing long-term pharmaceutical treatments (Kim et al., 2016; Sil et al., 2021). We acknowledge that therapeutics can have unanticipated side effects when used in vivo and expect that subsequent validation experiments will be needed to show whether this is the case for the therapeutics we identified in this study.

Although this study primarily focused on predicting small molecule therapeutic treatments, an exception was made for Etanercept, a biotech fusion protein, because of its anti-inflammatory properties and acceptable safety profile ("Etanercept"; Jazwinski et al., 2011). Etanercept (Enbrel) manages a variety of inflammatory conditions including rheumatoid arthritis. Chikungunya virus has previously been characterized as having significant DEG overlap with rheumatoid arthritis (Nakaya et al., 2012; Suhrbier, 2019; Bautista-Vargas, Puerta-Sarmiento & Cañas, 2020). Although it is not recommended to treat patients who have pre-existing rheumatoid arthritis and are infected with the Chikungunya virus, otherwise-healthy patients could potentially benefit from receiving Etanercept treatments (Zaid et al., 2011; Haridas & Haridas, 2019). Future experiments
will be required to determine the most effective dosage in human cells and to test the ability of these drugs to protect macrophages against CHIKV infection.

**CONCLUSIONS**

Our analysis found zero significant DEGs at eight hpi in human monocyte-derived macrophages, but 9,676 genes, and eight GO terms in the macrophages at 24 hpi. We identified 234 biological pathways that were significantly altered in human monocyte-derived macrophages during CHIKV infection that represented AGE-RAGE, innate immunity, cell cycle-related pathways, and Calcium signaling. Our drug repurposing analysis predicted that a combination of Telmisartan, Sunitinib, and/or Dasatinib could be used as a potential therapeutic for CHIKV infection since together they modulate multiple intracellular host and viral processes in macrophages at 24 hpi. We expect that this study will contribute to ongoing efforts to develop effective prophylactic and/or therapeutic treatments for CHIKV.

**ACKNOWLEDGEMENTS**

We are grateful to Dr. Jonathan Miner at Washington University School of Medicine in St. Louis for providing the La Reunion strain of CHIKV. We also thank the Office of Research Computing at Brigham Young University for access to the campus high-performance computing infrastructure.

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Funding**

This work was supported by startup funds provided by Brigham Young University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**

The following grant information was disclosed by the authors: Brigham Young University.

**Competing Interests**

Brett E. Pickett is an Academic Editor for PeerJ.

**Author Contributions**

- Madison Gray performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Israel Guerrero-Arguero conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Antonio Solis-Leal conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Richard A. Robison conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Bradford K. Berges conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Brett E. Pickett conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:
The differentially expressed genes, Gene Ontology enrichments, intracellular signaling pathways and the multiple hypothesis-corrected p-values are available in the Supplemental Files.
The data is available at NCBI GEO: GSE182287.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13090#supplemental-information.

REFERENCES
Battisti V, Urban E, Langer T. 2021. Antivirals against the Chikungunya virus. Viruses 13(7):1307 DOI 10.3390/v13071307.

Bautista-Vargas M, Puerta-Sarmiento G, Cañas CA. 2020. Characteristics of Chikungunya virus infection in patients with established rheumatoid arthritis. Clinical Rheumatology 39(12):3639–3642 DOI 10.1007/s10067-020-05198-x.

Bekerman E, Neveu G, Shulla A, Brannan J, Pu S-Y, Wang S, Xiao F, Barouch-Bentov R, Bakken RR, Mateo R, Govero J, Nagamine CM, Diamond MS, De Jonghe S, Herdewijn P, Dye JM, Randall G, Einav S. 2017. Anticancer kinase inhibitors impair intracellular viral trafficking and exert broad-spectrum antiviral effects. The Journal of Clinical Investigation 127(4):1338–1352 DOI 10.1172/JCI89857.

Broeckel R, Sarkar S, May NA, Totonchy J, Kreklywich CN, Smith P, Graves I, DeFilippis VR, Heise MT, Morrison TE, Moorman N, Streblow DN. 2019. Src family kinase inhibitors block translation of alphavirus subgenomic mRNAs. Antimicrobial Agents and Chemotherapy 63(4):114 DOI 10.1128/AAC.02325-18.

Busby MA, Stewart C, Miller CA, Grzeda KR, Marth GT. 2013. Scotty: a web tool for designing RNA-Seq experiments to measure differential gene expression. Bioinformatics 29(5):656–657 DOI 10.1093/bioinformatics/btt015.

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. 2018. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9:7204–7218 DOI 10.18632/oncotarget.23208.

De S, Mamidi P, Ghosh S, Keshry SS, Mahish C, Pani SS, Laha E, Ray A, Datey A, Chatterjee S, Singh S, Mukherjee T, Khamaru S, Chattopadhyay S, Subudhi BB, Chattopadhyay S. 2021. Telmisartan restricts Chikungunya virus infection in vitro and in vivo through the AT1/PPAR-γ/MAPKs pathways. Antimicrobial Agents and Chemotherapy 66(1):e0148921 DOI 10.1128/AAC.01489-21.

Delatte H, Desvars A, Bouétard A, Bord S, Gimonneau G, Vourc’h G, Fontenille D. 2010. Blood-feeding behavior of Aedes albopictus, a vector of Chikungunya on La Réunion. Vector Borne and Zoonotic Diseases 10:249–258 DOI 10.1089/vbz.2009.0026.
Dhenni R, Yohan B, Alisjahbana B, Lucanus A, Riswari SF, Megawati D, Haryanto S, Gampamole D, Hayati RF, Sari K, Witari NPD, Myint KSA, Sasmono RT. 2021. Comparative cytokine profiling identifies common and unique serum cytokine responses in acute chikungunya and dengue virus infection. BMC Infectious Diseases 21:639 DOI 10.1186/s12879-021-06339-6.

Duarte M, Pelorosso F, Nicolosi L, Victoria Salgado M, Vetulli H, Aquieri A, Azzato F, Basconcel M, Castro M, Coyle J, Davolos I, Esparza E, Criado IF, Gregori R, Mastrodonato P, Rubio MC, Sarquis S, Wahlmann F, Rothlin RP. 2020. Telmisartan for treatment vid-19 patiof Coents: an open randomized clinical trial-a preliminary report. medRxiv. Available at https://www.medrxiv.org/content/10.1101/2020.08.04.20167205v2.

Dye J, Brannan J, Geneva Foundation Tacoma United States. 2017. Selective AAK1 and GAK inhibitors for combating dengue and other emerging viral infections. Tacoma United States: Geneva Foundation.

Elgner F, Ren H, Medvedev R, Ploen D, Himmelsbach K, Boller K, Hildt E. 2016. The intracellular cholesterol transport inhibitor U18666A inhibits the exosome-dependent release of mature hepatitis C virus. Journal of Virology 90:11181–11196 DOI 10.1128/JVI.01053.

Fros JJ, Liu WJ, Prow NA, Geertsema C, Ligtenberg M, Vanlandingham DL, Schnettler E, Vlak JM, Suhrbier A, Khromykh AA, Pijlman GP. 2010. Chikungunya virus nonstructural protein 2 inhibits type I/II interferon-stimulated JAK-STAT signaling. Journal of Virology 84:10877–10887 DOI 10.1128/JVI.00949-10.

Ghildiyal R, Gabrani R. 2021. Computational approach to decipher cellular interactors and drug targets during co-infection of SARS-CoV-2, Dengue, and Chikungunya virus. Virusdisease 32:1–10 DOI 10.1007/s13337-021-00665-8.

Goel G, Wang F, Bank T, Malnassy G, Qiu W. 2018. Figure 2 Chemical structure of Sorafenib (A) and Regorafenib (B). Available at https://www.researchgate.net/figure/Chemical-structure-of-Sorafenib-A-and-Regorafenib-B-Note-The-only-structural_fig1_323574672 (accessed 31 July 2021).

Goupil BA, Mores CN. 2016. A review of Chikungunya Virus-induced Arthralgia: clinical manifestations, therapeutics, and pathogenesis. The Open Rheumatology Journal 10:129–140 DOI 10.2174/1874312901610010129.

Guerrero-Arguero I, Hoj TR, Tass ES, Berges BK, Robison RA. 2020. A comparison of Chikungunya virus infection, progression, and cytokine profiles in human PMA-differentiated U937 and murine RAW264.7 monocyte derived macrophages. PLOS ONE 15:e0230328 DOI 10.1371/journal.pone.0230328.

Haese N, Powers J, Streblow DN. 2020. Small molecule inhibitors targeting chikungunya virus. Current Topics in Microbiology and Immunology 435:107–139 DOI 10.1007/82_2020_195.

Haist KC, Burack KS, Davenport BJ, Morrison TE. 2017. Inflammatory monocytes mediate control of acute alphavirus infection in mice. PLOS Pathogens 13:e1006748 DOI 10.1371/journal.ppat.1006748.

Haridas VM, Haridas K. 2019. Managing chikungunya arthritis using etanercept. Internet Journal of Rheumatology and Clinical Immunology 7(1):CS6 DOI 10.15305/ijrci/v7i1/307.

Her Z, Malleret B, Chan M, Ong EKS, Wong S-C, Kwek DJC, Tolou H, Lin RTP, Tambyah PA, Rênia L, Ng LFP. 2010. Active infection of human blood monocytes by Chikungunya virus triggers an innate immune response. The Journal of Immunology 184(10):5903–5913 DOI 10.4049/jimmunol.0904181.
Ho Y-J, Liu F-C, Yeh C-T, Yang CM, Lin C-C, Lin T-Y, Hsieh P-S, Hu M-K, Gong Z, Lu J-W. 2018. Micafungin is a novel anti-viral agent of chikungunya virus through multiple mechanisms. Antiviral Research 159(Suppl. 1):134–142 DOI 10.1016/j.antiviral.2018.10.005.

Jazwinski AB, Jezsik J, Ardoin SP, McCallum RM, Tillmann HL. 2011. Etanercept treatment to enable successful hepatitis C virus clearance in a patient with rheumatoid arthritis. Gastroenterology & Hepatology 7:772–774.

Khan M, Santhosh SR, Tiwari M, Lakshmana Rao PV, Parida M. 2010. Assessment of in vitro prophylactic and therapeutic efficacious of chloroquine against Chikungunya virus in vero cells. Journal of Medical Virology 82(5):817–824 DOI 10.1002/jmv.21663.

Kim DK, Lee SW, Nam HS, Jeon DS, Park NR, Nam YH, Lee SK, Baek YH, Han SY, Lee SW. 2016. A case of sorafenib-induced DRESS syndrome in hepatocelluar carcinoma. The Korean Journal of Gastroenterology = Taehan Sohwaig Hakhoe chi 67(6):337–340 DOI 10.4166/kjg.2016.67.6.337.

Komiya Y, Habas R. 2008. Wnt signal transduction pathways. Organogenesis 4(2):68–75 DOI 10.4161/org.4.2.5851.

Kottke T, Hall G, Pulido J, Diaz RM, Thompson J, Chong H, Selby P, Coffey M, Pandha H, Chester J, Melcher A, Harrington K, Vile R. 2010. Antiangiogenic cancer therapy combined with oncolytic virotherapy leads to regression of established tumors in mice. The Journal of Clinical Investigation 120(5):1551–1560 DOI 10.1172/JCI41431.

Krishnamoorthy P, Raj AS, Roy S, Kumar NS, Kumar H. 2021. Comparative transcriptome analysis of SARS-CoV, MERS-CoV, and SARS-CoV-2 to identify potential pathways for drug repurposing. Computers in Biology and Medicine 128(3):104123 DOI 10.1016/j.compbiomed.2020.104123.

Krueger F, James F, Ewels P, Afyounian E, Schuster-Boeckler B. 2021. FelixKrueger/TrimGalore: v0.6.7 - DOI via Zenodo. Zenodo. Available at https://github.com/FelixKrueger/TrimGalore/releases.

Köster J, Rahmann S. 2018. Snakemake—a scalable bioinformatics workflow engine. Bioinformatics 34(20):3600 DOI 10.1093/bioinformatics/bty350.

Landers VD, Wilkey DW, Merchant ML, Mitchell TC, Sokoloski KJ. 2021. The alphaviral capsid protein inhibits IRAK1-dependent TLR Signaling. Viruses 13(3):377 DOI 10.3390/v13030377.

Leo YS, Chow ALP, Lin L, Lin L, Ng LC. 2009. Chikungunya outbreak, Singapore, 2008. Emerging Infectious Diseases 15(5):836–837 DOI 10.3201/eid1505.081390.

Liang XD, Zhang YN, Liu CC, Chen J, Park NH, Baloch AS, Zhou B. 2019. U18666A inhibits classical swine fever virus replication through interference with intracellular cholesterol trafficking. Veterinary Microbiology 238:108436 DOI 10.1016/j.vetmic.2019.108436.

Loufivos LP, Kramer LD. 2016. Invasiveness of Aedes aegypti and Aedes albopictus and vectorial capacity for Chikungunya virus. The Journal of Infectious Diseases 214(suppl 5):S453–S458 DOI 10.1093/infdis/jiw285.

Lu F, Liang Q, Abi-Mosleh L, Das A, De Brabander JK, Goldstein JL, Brown MS. 2015. Identification of NPC1 as the target of U18666A, an inhibitor of lysosomal cholesterol export and Ebola infection. eLife 4:e12177 DOI 10.7554/eLife.12177.

Mattar S, Miranda J, Pinzon H, Tique V, Bolanos A, Aponte J, Arrieta G, Gonzalez M, Barrios K, Contreras H, Alvarez J, Aleman A. 2015. Outbreak of Chikungunya virus in the north Caribbean area of Colombia: clinical presentation and phylogenetic analysis. Journal of Infection in Developing Countries 9(10):1126–1132 DOI 10.3855/jidc.6670.
Melton JV, Ewart GD, Weir RC, Board PG, Lee E, Gage PW. 2002. Alphavirus 6K proteins form ion channels. *The Journal of Biological Chemistry* **277**(49):46923–46931 DOI 10.1074/jbc.M207847200.

Mishra AR, Byrareddy SN, Nayak D. 2020. IFN-I independent antiviral immune response to vesicular stomatitis virus challenge in mouse brain. *Vaccines* **8**(2):326 DOI 10.3390/vaccines8020326.

Montes-Grajales D, Puerta-Guardo H, Espinosa DA, Harris E, Caicedo-Torres W, Olivero-Verbel J, Martinez-Romero E. 2020. In silico drug repurposing for the identification of potential candidate molecules against arboviruses infection. *Antiviral Research* **173**:104668 DOI 10.1016/j.antiviral.2019.104668.

Nakaya HI, Gardner J, Poo Y-S, Major L, Pulendran B, Suhrbier A. 2012. Gene profiling of Chikungunya virus arthritis in a mouse model reveals significant overlap with rheumatoid arthritis. *Arthritis and Rheumatism* **64**(11):3553–3563 DOI 10.1002/art.34631.

Nasci RS. 2014. Movement of chikungunya virus into the Western hemisphere. *Emerging Infectious Diseases* **20**(8):1394–1395 DOI 10.3201/eid2008.140333.

Nowicka M, Robinson MD. 2016. DRIMSeq: a Dirichlet-multinomial framework for multivariate count outcomes in genomics. *F1000Research* **5**:1356 DOI 10.12688/f1000research.8900.2.

Nsoesie EO, Kraemer MU, Golding N, Pigott DM, Brady OJ, Moyes CL, Johansson MA, Gething PW, Velayudhan R, Khan K, Hay SI, Brownstein JS. 2016. Global distribution and environmental suitability for chikungunya virus, 1952 to 2015. *Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin* **21**:1560–7917 DOI 10.2807/1560-7917.ES.2016.21.20.30234.

Orjuela S, Huang R, Hembach KM, Robinson MD, Soneson C. 2019. ARMOR: an automated reproducible dural workflow for preprocessing and differential analysis of NA-seq data. *G3* **9**:2089–2096 DOI 10.1534/g3.119.400185.

Pagès F, Peyrefitte CN, Mve MT, Jarjaval F, Brisse S, Iteman I, Gravier P, Tolou H, Nkoghe D, Grandadam M. 2009. Aedes albopictus mosquito: the main vector of the 2007 Chikungunya outbreak in Gabon. *PLOS ONE* **4**:e4691 DOI 10.1371/journal.pone.0004691.

Panigrahi A, Chakraborty S, Sil A. 2021. Chik sign in chikungunya fever. *Infection* **49**:1075–1076 DOI 10.1007/s15010-020-01472-x.

Parashar D, Paingankar MS, More A, Patil P, Amdekar S. 2018. Altered microRNA expression signature in Chikungunya-infected mammalian fibroblast cells. *Virus Genes* **54**:502–513 DOI 10.1007/s11262-018-1578-8.

Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. 2017. Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods* **14**(4):417–419 DOI 10.1038/nmeth.4197.

Pu S-Y, Xiao F, Schor S, Bekerman E, Zanini F, Barouch-Bentov R, Nagamine CM, Einav S. 2018. Feasibility and biological rationale of repurposing sunitinib and erlotinib for dengue treatment. *Antiviral Research* **155**(2):67–75 DOI 10.1016/j.antiviral.2018.05.001.

PubChem. 2021. Telmisartan. Available at https://pubchem.ncbi.nlm.nih.gov/compound/65999 (accessed 31 July 2021).

Roberts JL, Tavallai M, Nourbakhsh A, Fidanza A, Cruz-Luna T, Smith E, Siembida P, Plamondon P, Cycon KA, Doern CD, Booth L, Dent P. 2015. GRP78/Dna K is a target for Nexavar/Stivarga/Votrient in the treatment of human malignancies, viral infections and bacterial diseases. *Journal of Cellular Physiology* **230**:2552–2578 DOI 10.1002/jcp.25014.
Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**(1):139–140 DOI 10.1093/bioinformatics/btp616.

Rothlin RP, Vetulli HM, Duarte M, Pelorosso FG. 2020. Telmisartan as tentative angiotensin receptor blocker therapeutic for COVID-19. *Drug Development Research* **81**(7):768–770 DOI 10.1002/ddr.21679.

Roy E, Byrareddy SN, Reid SP. 2020. Role of microRNAs in bone pathology during chikungunya virus infection. *Viruses* **12**(11):1207 DOI 10.3390/v12111207.

s-andrews. 2021. GitHub-s-andrews/FastQC: a quality control analysis tool for high throughput sequencing data. Available at https://github.com/s-andrews/FastQC (accessed 23 November 2021).

Salgado M, Martinez-Picado J, Gálvez C, Rodríguez-Mora S, Rivaya B, Urrea V, Mateos E, Alcamí J, Coiras M. 2020. Dasatinib protects humanized mice from acute HIV-1 infection. *Biochemical Pharmacology* **174**:113625 DOI 10.1016/j.bcp.2019.113625.

Sanjai Kumar P, Nayak TK, Mahish C, Sahoo SS, Radhakrishnan A, De S, Datey A, Sahu RP, Goswami C, Chattopadhyay S, Chattopadhyay S. 2021. Inhibition of transient receptor potential vanilloid 1 (TRPV1) channel regulates chikungunya virus infection in macrophages. *Archives of Virology* **166**:139–155 DOI 10.1007/s00705-020-04852-8.

Scuotto M, Abdelnabi R, Collarile S, Schiraldi C, Delang L, Massa A, Ferla S, Brancale A, Leyssen P, Neys J, Filosa R. 2017. Discovery of novel multi-target indole-based derivatives as potent and selective inhibitors of chikungunya virus replication. *Bioorganic & Medicinal Chemistry* **25**:327–337 DOI 10.1016/j.bmc.2016.10.037.

Selvamani SP, Mishra R, Singh SK. 2014. Chikungunya virus exploits miR-146a to regulate NF-κB pathway in human synovial fibroblasts. *PLOS ONE* **9**(e103624) DOI 10.1371/journal.pone.0103624.

Sharma A, Balakathiresan NS, Maheshwari RK. 2015. Chikungunya virus infection alters expression of microRNAs involved in cellular proliferation, immune response and apoptosis. *Intervirology* **58**:332–341 DOI 10.1159/000441309.

Singal A. 2017. Chikungunya and skin: current perspective. *Indian Dermatology Online Journal* **8**(5):307–309 DOI 10.4103/idoj.IDOJ_93_17.

Sourisseau M, Schilte C, Casartelli N, Trouillet C, Guivel-Benhassine F, Rudnicka D, Sol-Foulon N, Le Roux K, Prevost M-C, Fsihi H, Frenkkel M-P, Blanchet F, Afonso PV, Ceccaldi P-E, Ozden S, Gessain A, Schuffenecker I, Verhasselt B, Zamborlini A, Saib A, Rey FA, Arenzana-Seisdedos F, Després P, Michault A, Albert ML, Schwartz O. 2007. Characterization of reemerging chikungunya virus. *PLOS Pathogens* **3**(6):e89 DOI 10.1371/journal.ppat.0030089.

Srivastava P, Kumar A, Hasan A, Mehta D, Kumar R, Sharma C, Sunil S. 2020. Disease resolution in Chikungunya—what decides the outcome? *Frontiers in Immunology* **11**:420 DOI 10.3389/fimmu.2020.00695.

Suhrbier A. 2019. Rheumatic manifestations of chikungunya: emerging concepts and interventions. *Nature Reviews Rheumatology* **15**(10):597–611 DOI 10.1038/s41584-019-0276-9.

Tan Y, Pickett BE, Srivastava S, Gresh L, Balmaseda A, Amedeo P, Hu L, Puri V, Fedorova NB, Halpin RA, LaPoinite MP, Cone MR, Heberlein-Larson L, Kramer LD, Ciota AT, Gordon A,
Shabman RS, Das SR, Harris E. 2018. Differing epidemiological dynamics of Chikungunya virus in the Americas during the 2014–2015 epidemic. *PLOS Neglected Tropical Diseases* 12(7):e0006670 DOI 10.1371/journal.pntd.0006670.

Tarca AL, Draghici S, Khatri P, Hassan SS, Mittal P, Kim J-S, Kim CJ, Kusanovic JP, Romero R. 2009. A novel signaling pathway impact analysis. *Bioinformatics* 25(1):75–82 DOI 10.1093/bioinformatics/btn577.

Tripathi PK, Soni A, Singh Yadav SP, Kumar A, Gaurav N, Raghavendhar S, Sharma P, Sunil S, Ashish, Jayaram B, Patel AK. 2020. Evaluation of novobiocin and telmisartan for anti-CHIKV activity. *Virology* 548:250–260 DOI 10.1016/j.virol.2020.05.010.

Wichit S, Hamel R, Bernard E, Talignani L, Diop F, Ferraris P, Liegeois F, Ekchariyawat P, Luplertlop N, Surasombatpattana P, Thomas F, Merits A, Choumet V, Roques P, Yssel H, Briant L, Missé D. 2017. Imipramine inhibits chikungunya virus replication in human skin fibroblasts through interference with intracellular cholesterol trafficking. *Scientific Reports* 7:3145 DOI 10.1038/s41598-017-03316-5.

Wikan N, Sakoonwatanyoo P, Ubol S, Yoksan S, Smith DR. 2012. Chikungunya virus infection of cell lines: analysis of the East, Central and South African Lineage. *PLOS ONE* 7:e31102 DOI 10.1371/journal.pone.0031102.

Wu D, Smyth GK. 2012. Camera: a competitive gene set test accounting for inter-gene correlation. *Nucleic Acids Research* 40(17):e133 DOI 10.1093/nar/gks461.

Zaid A, Rulli NE, Rolph MS, Suhrbier A, Mahalingam S. 2011. Disease exacerbation by etanercept in a mouse model of alphaviral arthritis and myositis. *Arthritis and Rheumatism* 63(2):488–491 DOI 10.1002/art.30112.