Published in final edited form as:

Nat Microbiol. 2024 February ; 9(2): 322–335. doi:10.1038/s41564-023-01586-6.

Mechanistic insights into bone remodelling dysregulation by human viral pathogens

Camila C. S. Caetano[1], **Tamiris Azamor**[1], **Nikki M. Meyer**[1], **Chineme Onwubueke**[1,2], **Cassandra M. Calabrese**[3], **Leonard H. Calabrese**[3], **Anabelle Visperas**[4], **Nicolas S. Piuzzi**[4], **M. Elaine Husni**[3], **Suan-Sin Foo**[1,2,5], **Weiqiang Chen**[1,2,5]

[1]Infection Biology Program, Global Center for Pathogen Research and Human Health, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA.

[2]Cleveland Clinic Lerner College of Medicine, Case Western Reserve University, Cleveland, OH, USA.

[3]Department of Rheumatic and Immunologic Diseases, Cleveland Clinic, Cleveland, OH, USA.

[4]Department of Orthopedic Surgery, Cleveland Clinic, Cleveland, OH, USA.

[5]These authors contributed equally: Suan-Sin Foo, Weiqiang Chen.

Abstract

Bone-related diseases (osteopathologies) associated with human virus infections have increased around the globe. Recent findings have highlighted the intricate interplay between viral infection, the host immune system and the bone remodelling process. Viral infections can disrupt bone homeostasis, contributing to conditions such as arthritis and soft tissue calcifications. Osteopathologies can occur after arbovirus infections such as chikungunya virus, dengue virus and Zika virus, as well as respiratory viruses, such as severe acute respiratory syndrome coronavirus 2 and enteroviruses such as Coxsackievirus B. Here we explore how human viruses dysregulate bone homeostasis, detailing viral factors, molecular mechanisms, host immune response changes and bone remodelling that ultimately result in osteopathologies. We highlight model systems and technologies to advance mechanistic understanding of viral-mediated bone alterations. Finally, we propose potential prophylactic and therapeutic strategies, introduce ‘osteovirology’ as a research field highlighting the underestimated roles of viruses in bone-related diseases, and discuss research avenues for further investigation.

Correspondence should be addressed to Suan-Sin Foo or Weiqiang Chen. foos@ccf.org; chenw3@ccf.org.

Author contributions

W.C. and S.-S.F. contributed to the conceptualization of the manuscript. C.C.S.C., T.A., N.M.M., S.-S.F. and W.C. contributed to writing and revision of the manuscript. C.O., C.M.C., L.H.C., A.V., N.S.P. and M.E.H. contributed with additional text, ideas, edits and approval of the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Microbiology* thanks Victoria Baxter and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Approximately 15% of total body mass consists of skeletal bones, providing crucial structural integrity and support for soft tissues. The intricate interplay between bone metabolism and immunology, termed osteoimmunology[1], is vital for maintaining bone health. Dysregulation of osteoimmunology can lead to inflammatory bone loss or soft tissue calcifications, mimicking bone formation[2] (Box 1 and Figs. 1 and 2). Beyond spontaneous autoimmunity, bone cancers and age-associated degeneration, microbial infections can trigger bone diseases and tissue calcifications, giving rise to the emerging field of osteomicrobiology[3].

The coronavirus disease 2019 (COVID-19) pandemic has brought attention to virus research. Although the pathogenesis of COVID-19 is still being unravelled, emerging evidence suggests an association with arthritis[4–6]. Even before the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), viruses such as chikungunya (CHIKV) and dengue (DENV) have long been associated with arthritis and bone loss[7]. Additionally, Zika virus (ZIKV) and Coxsackievirus B (CVB) have been associated with abnormal soft-tissue calcifications[8,9]. In this Perspective, we delve into the host–virus mechanisms underlying CHIKV, DENV and SARS-CoV-2 infections that result in viral arthritis, as well as ZIKV and CVB that induce pathological soft-tissue calcifications. Potential technologies for virus-induced bone loss and calcifications are discussed (Box 2), presenting innovative approaches to address these osteopathologies. We propose the term ‘osteovirology’ to emphasize the underappreciated roles of viruses in mediating pathological bone loss and soft-tissue calcifications.

Virus-mediated bone loss and arthritis

Pathogenic bone loss and arthritis are conditions characterized by the destruction of bone tissue and joint inflammation, often resulting in pain, limited mobility and decreased quality of life (Box 1). Several clinically relevant RNA viruses (CHIKV, DENV and SARS-CoV-2) have been reported to be involved in osteoclast (OC)-mediated bone disease. Here, we discuss and speculate on potential mechanisms of virus-induced arthritis and related bone pathology. These mechanisms shed light on how viruses can disrupt the delicate balance of bone remodelling and immune response, leading to a better understanding of the pathogenesis of these conditions and the development of potential therapeutic strategies.

Chikungunya virus

Arthritogenic alphaviruses including CHIKV, Ross River virus (RRV), Barmah Forest virus, o’nyong-nyong virus, Mayaro virus, Sindbis virus and Semliki Forest virus pose significant public health threats worldwide. CHIKV, in particular, causes chronic viral arthritis outbreaks in Africa and Southeast Asia, and has emerged in South America[10]. In Makonde, *chikungunya* means ‘bending over in pain’, capturing the stooped posture resulting from severe musculoskeletal pain observed in CHIKV-infected patients[11]. Following the acute phase of symptomatic CHIKV infection, characterized by high fever, rash, headache, fatigue, muscle pain (myalgia) and joint pain (arthralgia), over 40% of CHIKV patients, predominantly women[12], develop persistent rheumatic manifestations. These chronic manifestations include inflammation of tendons, ligaments and synovial tissues, along

with bone loss[13,14], lasting for months to years after initial infection. However, patients with CHIKV experiencing chronic arthralgia are tested negative for rheumatoid arthritis (RA) biomarkers like rheumatoid factor and anti-cyclic citrullinated peptide antibodies[15]. Additionally, there is no association with predisposing RA risk alleles, such as HLA class II histocompatibility antigen, DRB1 beta chain (HLA-DRB1)[16]. Ironically, symptoms of chronic CHIKV infection fulfil diagnostic criteria for RA[14], which indicates that chronic alphavirus-induced polyarthritis mimics RA-like bone disease.

Despite CHIKV being studied for the past 70 years, the mechanisms behind its long-term rheumatic-like bone sequelae remain largely unknown. Several risk factors have been implicated in chronic CHIKV disease including comorbidities (pre-existing inflammatory joint disease, heart failure and diabetes), older age (35–45 years old or older) and high viraemia during acute CHIKV disease. In CHIKV-infected, aged rhesus macaques compared with adult macaques, a low level of plasma interferon (IFN) is associated with higher and persistent viral replication. This suggests that an aging immune system is important for CHIKV persistence and increased disease severity[17]. Although persistent CHIKV joint-related symptoms are present across all ages and in both sexes; older individuals, particularly women, with underlying comorbidities such as osteoarthritis tend to have higher risk of developing chronic CHIKV disease[18].

The impact of host response to CHIKV infection on bone pathologies.

Inflammation is associated with the onset of alphaviral polyarthritis, a condition characterized by inflammation of multiple joints. Several pro-inflammatory cytokines and chemokines including C–C motif chemokine ligand 2 (CCL2), interleukin-6 (IL-6), IL-1β, tumour necrosis factor alpha (TNF-α) and IL-17 are shown to be highly elevated in the plasma of CHIKV-infected patients, in both acute and chronic phases of disease[19]. Indeed, IL-6 is one of the predominant mediators of CHIKV-induced arthralgia[19] and this cytokine is known to stimulate RANKL (receptor activator of nuclear factor-κB ligand)-dependent osteoclastogenesis, resulting in high bone resorption activity[20]. In both CHIKV- and RRV-infected patients elevated levels of RANKL were detected in serum and synovial fluid samples. This suggests that alphavirus infection triggered osteoclastogenesis within joint tissues (Fig. 3)[21,22]. Furthermore, in vitro infection of OB with alphaviruses such as CHIKV and RRV results in elevated RANKL secretion, activation of osteoclastogenesis and bone resorption[21,22]. The direct effect of alphavirus infection on bone tissue has been demonstrated using animal models of CHIKV and RRV infection[21–23] (Box 3), where elevated RANKL expression resulted in severe periarticular bone loss in mouse tibia during peak alphavirus disease. In an RRV mouse model, a disrupted RANKL/OPG (osteoprotegerin) ratio and its associated bone loss was reversed by administering IL-6 specific antibodies, suggesting that bone loss after infection is regulated through IL-6/RANKL-dependent osteoclastogenesis[21] (Fig. 3). Infiltration of mononuclear cells, such as monocytes and macrophages, seem critical for infection-induced bone disease[24,25]. Human monocytes are susceptible to CHIKV infection[26] and non-human primate macrophages serve as a reservoir for persistent CHIKV infection[27]. Recruitment of monocytes to the site of alphavirus infection is mediated by chemotactic cytokines such as CCL2, which is also a potent osteoclastogenic factor in bone[28]. Inhibition of CCL2 with the anti-inflammatory

small molecule bindarit significantly reduced OC activation in joint tissue of CHIKV-infected mice, and thus ameliorated CHIKV-induced osteoclastogenesis[22].

CHIKV infection can trigger NLRP3 (nucleotide binding oligomerization domain (NOD)-like receptor pyrin domain containing 3) inflammasome-dependent bone loss via IL-1β activation (Fig. 3). Blocking NLRP3 activation with the small-molecule NLRP3 inhibitor MCC950[23], or IL-1 activity with an IL-1 receptor antagonist anakinra in mice, ameliorated osteoclastogenic bone loss and myositis after infection[29]. More recently, several groups showed the importance of T cell responses and IL-17-producing cells (that is, Th17 CD4 T cells, Tc17 CD8 T cells, and neutrophils) as drivers of an inflammatory response against arthritogenic alphavirus infection[30]. Overall, these studies indicate that CHIKV infection can induce high levels of IL-1β, IL-6 and CCL2, which trigger an osteoclastogenic bone microenvironment leading to bone defects and pathologies (Fig. 3). However, mechanisms behind chronic CHIKV bone pathologies remain largely unknown and warrant further investigation.

The CHIKV genome consists of ~12 kb open reading frames (ORFs) that encode for five structural (envelope glycoproteins, capsid and 6K viroporin) and four nonstructural (nsP1–4) proteins. Previously, it has been demonstrated that an R532H mutation in nsP1 reduced severe joint inflammation by lowering IL-1β production in infected mice. This suggests activation of the NLRP3–IL-1β inflammasome pathway by nsP1 (ref. 31). However, infection in mice indicated attenuation of the mutant virus compared to infection with wild-type (WT) CHIKV, suggesting that reduced NLRP3 activation could be a result of attenuated viral replication. Thus, it remains unclear how CHIKV proteins can activate the NLRP3 inflammasome leading to osteoclastogenesis.

In physiological bone resorption, high levels of lysosomal papain-like cysteine proteases, such as cathepsin K, are expressed by OCs for cleaving bone collagen into collagen fragments. By-products of this collagen-degradation process, specifically hydroxyproline, were detected at significant levels in the urine of CHIKV-infected patients[32], suggesting that bone collagen degradation occurs during alphavirus infection. The C-terminal domain of nsP2 functions as a cysteine protease with a conserved Cys–His catalytic dyad, similar to cathepsins[33]. Therefore, it is possible that nsP2 mimics host cathepsins to enhance collagen degradation and bone disease. Alternatively, nsP2 could contribute to post-translational processing of pro-cathepsins into mature, active cathepsins. However, whether CHIKV nsP2 has a role in triggering bone pathologies requires investigation and validation.

Taken together, these studies suggest that CHIKV infection activates host inflammatory signalling and disrupts the balance between bone-forming osteoblasts (OBs) and bone-resorbing OCs, resulting in bone pathologies and arthritis[22,23,29,30]. Further elucidation of the molecular mechanisms behind CHIKV and other alphaviruses altering bone metabolism and the resulting long-term persistent arthralgia will be important for the development of future therapies.

Dengue virus

DENV belongs to the Flaviviridae family and is classified into four serotypes (DENV-1 to DENV-4). With approximately 50 to 100 million symptomatic DENV cases reported annually[34], it is a major cause of illness worldwide. The outcomes of this disease range from asymptomatic to 'severe dengue', which typically occurs upon repeated infection[34]. The hallmarks of severe dengue are capillary leakage and low blood platelet count (thrombocytopenia), which can lead to organ failure and death[35]. Although approximately 5% of patients with DENV develop severe illness, 25% develop self-limiting symptomatic illness. This condition is commonly referred to as 'breakbone fever', characterized by symptoms such as high fever and excruciating inflammatory muscle, bone and joint pain[36]. DENV infects people of all ages, but infants born to seropositive mothers, and older individuals tend to incur more severe disease complications. In addition, persistent inflammation and severe joint pain is reported to be more common in women[37]. To date, the exact pathophysiology of DENV-induced arthralgia and the virus involvement in bone remodelling remains largely unknown.

Mechanisms of DENV-induced arthralgias.

Severe dengue has been associated with an exacerbated host immune response and overwhelming inflammation[35]. Key inflammatory markers (that is, IL-6, TNF, IL-1β, IL-17 and CCL2) have been shown to be upregulated in peripheral blood mononuclear cells of patients with symptomatic dengue fever[38]. Infection of a CD16+ monocyte subset with DENV, resulted in production of IL-6, TNF, IL-1β and CCL2 (ref. 39). These factors are relevant during CHIKV infection and OC-mediated bone resorption associated with inflammatory bone diseases[40]. This suggests that DENV infection could lead to a dysregulation of bone homeostasis. In vitro, DENV infection of monocytic cells (THP-1) led to upregulated osteopontin (OPN) levels[41], which is a bone remodelling factor. OPN induces OC differentiation and blocks bone mineral deposition during inflammatory processes[42]. DENV could thus promote RANKL-mediated osteoclastogenesis and osteoclastic activity, which is observed during CHIKV infection.

Inflammatory bone pain is often associated with high osteoclastogenesis due to an acidic microenvironment created by OCs during bone resorption[43]. DENV-2 infection of human OCs in vitro, resulted in an enhanced NFATc1-mediated OC activation, which also involved C-type lectin member 5A (CLEC5A). NFATc1 is speculated to be a contributor of severe bone pain in patients with dengue[44]. Furthermore, DENV infection in mice resulted in bone inflammation and increased osteoclastic-mediated trabecular bone loss, whereas CLEC5A-deficient mice were protected from osteolytic activity (Box 3). Additionally, DENV envelope protein was shown to interact with CLEC5A and activate the NLRP3 inflammasome, resulting in secretion of IL-1β, IL-18 and TNF-α (ref. 45), which are known players in osteolytic bone diseases and vascular calcification (reviewed previously[46]).

Aside from host-mediated inflammatory responses during DENV arthralgia, viral factors that cause DENV-infection-related pain have not yet been described. Chondroitin sulfate (CS) is a glycosaminoglycan that serves as a major component in the extracellular matrix of joint tissue[47]. DENV non-structural protein 1 (NS1) was shown to interact with heparan

sulfate and CS on the cell surface of epithelial cells and mesenchymal stem cell (MSCs)[48]. In patients with acute dengue, secreted NS1 can be detected in the serum[49] with high levels being associated with severe disease. Binding of NS1 to endothelial cells was shown to induce vascular permeability and leakage[50]. It is possible that secreted NS1 binds to CS that is localized in all joints. DENV NS1 was shown to induce production of inflammatory cytokines through the assembly of NS1 high-density lipoprotein complexes in monocytes and macrophages[51]. Hence, localization of NS1 at CS-rich areas in joints could promote proinflammatory cytokine production by monocytes or macrophages, favouring OC differentiation and bone resorption. Additionally, elevated levels of prostaglandins (proinflammatory mediators) were detected in synovial fluids of patients with arthritis[52], and NS1 treatment of monocytes stimulated production of prostaglandins. Therefore, NS1 could further contribute to the inflammatory process in joints. The role of DENV NS1 in dengue-related arthralgias is an interesting area for exploration and warrants experimental investigations of the hypotheses mentioned above.

Severe acute respiratory syndrome coronavirus 2.

The betacoronavirus SARS-CoV-2 is the causative agent of COVID-19. Although acute COVID-19 can be asymptomatic, symptomatic patients often present non-specific and broad spectrum symptoms such as fever, cough, myalgia, fatigue, acute respiratory distress syndrome or pneumonia[53]. Some SARS-CoV-2-infected individuals have reported persistent chronic manifestations affecting normal function of the lungs, brain, heart, kidneys, liver and/or musculoskeletal bone joints (reviewed previously[54]). Musculoskeletal sequelae (such as joint pain) is a common symptom, persisting up to two years post-infection[55]. Additionally, patients with SARS-CoV-2 and no prior joint disorder reported morning stiffness and polyarthralgia of the knees, hands or ankles[4,5]. However, many symptoms are self-reported and well curated studies of articular manifestations, including details of standardized musculoskeletal examination on a systematic basis, are lacking. Diagnostic imaging from isolated cases have described ankle joint bone erosion resembling rheumatoid nodules in hospitalized patients with COVID-19[56]. Indeed, bone loss has been identified as a prognostic symptom of severe clinical outcomes and future mortality in hospitalized patients with COVID-19[57].

Sex-related (abundance of viral entry receptors, hormones) and age-related (comorbidities, immune responses) factors may impact pathogenesis of COVID-19. Older individuals and men have an increased risk of developing severe COVID-19, whereas women are at increased risk of long COVID and musculoskeletal pain[58]. Severity of COVID-19 in pregnant women is correlated with an increased level of serum bone morphogenetic proteins (BMP), such as BMP4, in infants from severe cases[59]. Additionally, SARS-CoV-2 infection has been linked to reactive arthritis in children[6]. Adult patients with pre-existing rheumatic diseases also reported increased arthritic ‘flares’ upon SARS-CoV-2 infection, who typically have a higher risk of severe inflammation, long-term complications, hospitalization and mortality.

Mechanisms of SARS-CoV-2-associated arthralgias.

Insights into the cause of COVID-19-associated joint pain are limited. Angiotensin converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) have been identified as entry receptor and factor for SARS-CoV-2[60]. Apart from lung tissue, both ACE2 and TMPRSS2 are expressed in human cortical and trabecular bone, chondrocytes and OB-enriched synovium tissue[61]. This suggests that bone may be a potential site for viral replication. However, clinical and experimental findings have clearly demonstrated the absence of detectable SARS-CoV-2 in bone tissues of patients with COVID-19[62] or in COVID-19 animal models[63] (Box 3). Hence, this implies that alternative mechanisms may be involved in driving SARS-CoV-2-associated arthralgias in patients with acute and long COVID-19.

The cytokine storm is a characteristic of COVID-19, driving hyper-inflammation and further aggravating pre-existing respiratory syndromes[64]. Coincidentally, SARS-CoV-2 induced pro-inflammatory cytokines are also known osteoclastogenic immune mediators, including IL-6, IL-17, C-X-C motif chemokine ligand 10 (CXCL10), TNF-α and vascular endothelial growth factor A (VEGF-A)[65]. These cytokines could be contributing to an OC-skewed bone resorption process. Further, SARS-CoV-2 infection has been shown to induce robust inflammasome activation, particularly the activation of NLRP3 inflammasome, which is associated with severity of COVID-19 disease[66].

Although it is likely that SARS-CoV-2-mediated inflammation may contribute to bone loss[67], the exact viral mechanism underlying SARS-CoV-2-induced bone loss remains ill-defined. To date, several SARS-CoV-2 proteins have been identified to be directly involved in triggering host inflammatory responses, (1) structural proteins—envelope (E)[68], spike (S)[69], nucleocapsid (N)[70], and (2) accessory proteins—ORF3a (ref. 71), ORF7a and ORF8 (ref. 72). Specifically, E and S proteins have been shown to induce pro-inflammatory cytokines through toll-like receptor (TLR) 2 signalling pathway[68,69]. SARS-CoV-2 N and ORF3a proteins induced NLRP3 activation by direct interaction with NLRP3, promoting the assembly of the NLRP3 inflammasome[70]. Another study showed ORF3a serving as a virus ion channel protein, viroporin, facilitating the efflux of potassium ions and mitochondrial reactive oxygen species production, which triggers NLRP3 inflammasome activation[71]. Finally, SARS-CoV-2 ORF7a and ORF8 have been shown to bind to IL-17 receptors (IL-17RA and IL17-RC) for induction of IL-17 signalling[72]. Nevertheless, the role of these viral proteins in SARS-CoV-2-associated arthralgias has not been investigated.

Virus-mediated calcifications

Congenital viral infections can have profound and long-lasting effects on a developing fetus, often leading to a spectrum of health complications. Interestingly, they can induce pathogenic calcification, characterized by the abnormal accumulation of calcium phosphate in soft tissues, often linked to cellular injury or death following viral infection[73] (Box 1). Calcification observed in the brain shares similarities with physiological processes in bone and cartilage formation[74]. These are driven by osteogenic signalling pathways activated by morphogens from the transforming growth factor-β (TGF-β) family, such as BMPs. Although over 20 BMPs have been identified to date[75], BMP2, BMP4, BMP6, BMP7 and

BMP9 stand out for their strong osteogenic properties. In this section, we delve into recent advancements on host osteogenic activation and the occurrence of pathogenic calcification in response to congenital viral infections. Our discussion encompasses clinical observations and sheds light on the known molecular mechanisms underpinning soft tissue calcifications triggered by these viral infections.

Zika virus

ZIKV is a neurotropic flavivirus mainly spread by an infectious *Aedes* mosquito bite[76]. Contemporary ZIKV strains, compared with ancestral strains, have acquired mutations that enhance neurovirulence, leading to anomalies within the human fetal brain[77]. Placental ZIKV transmission is associated with infantile complications such as microcephaly (abnormally small head), intracranial calcification (calcium deposits in brain) and arthrogryposis (stiffness in multiple joints), which limits movement. These complications are collectively known as congenital ZIKV syndrome (CZS). Although prenatal ZIKV infection in any trimester may result in CZS, the risk is greatest during the first trimester[76]. Although children born with CZS have a high likelihood of mortality, surviving children may exhibit several structural (craniofacial disproportion), functional (hearing loss, dystonia) and/or neurologic impairments (seizures, epilepsy) up to three years following birth[78]. In 2016, among 400,000 reported ZIKV cases in Brazil, 42% of babies born to ZIKV-positive mothers had congenital craniofacial deformities[76].

ZIKV-associated calcification of brain regions (such as corticol–subcorticol white matter junctions, basal ganglia and thalamus) is one of the most common clinical findings of ZIKV-infected infants[79]. Typically, brain calcification is associated with aging. But abnormal calcium deposition in the brains of children infected with ZIKV before birth, remains poorly studied. While calcium deposition induced by ZIKV infection is often partially or completely resolved in infants by one year of age[80], however clearance does not mitigate motor skill disorders (epilepsy andbbc feeding problems). This suggests that calcification causes permanent brain tissue damage and neurological impairment in the developing child[81]. Exemplifying this, ZIKV-exposed children in a prospective cohort study in Rio de Janeiro, Brazil displayed severely delayed neurodevelopment[82].

Mechanisms of ZIKV-induced fetal brain calcification.

Acute ZIKV infection disrupts neurogenesis by killing neural-lineage cells, such as neural progenitor[83] and glial cells[84]. Pericytes (specialized cells located around blood vessels) were found to play a role during early stages of ZIKV brain infection[85] and are thought to be involved in the overall process of ZIKV-induced fetal brain calcification[74] (Fig. 4). ZIKV-infected human pericytes express high levels of BMP2/4, resulting in osteogenic activation and calcium deposition[74]. BMP2 is also affiliated with osteoblastic differentiation of MSCs in vascular calcification[86], suggesting that perivascular cells may function as osteogenic precursors in ZIKV brain calcification. Furthermore, ZIKV-infected human pericytes were able to activate master osteogenic regulators (for example, *RUNX2* and *OSX/SP7*), differentiate into osteogenic cells, and activate mineralization genes (for example *TNAP*, *DMP1* and *E11*/*PDPN*) that initiated calcium deposition (Fig. 4)[74]. However, it

remains unclear how ZIKV infection triggers calcification within the fetal brain on a molecular level.

Structural and non-structural viral proteins may influence host cellular responses and subsequent pathogenesis[87]. For example, unbiased virus protein screens performed in human brain pericytes showed that ZIKV NS3 serine protease could effectively process the mature form of BMP2/4, resulting in SMAD1/5/9 phosphorylation and in vitro calcium deposition[74] (Fig. 4). Interestingly, both African and Asian/American ZIKV strains activate early osteogenic signalling, but cellular calcification is limited to Asian/American ZIKV strains and not seen in ancestral African ZIKV strains. This is likely due to the rapid cell death induced by African ZIKV strains during replication[88], and this cytopathic effect masks the onset of cell-mediated calcium deposition[74]. Taken together, these findings highlight the importance of perivascular cells in promoting osteogenic differentiation into osteoblastic-like cells and their association in the process of ZIKV-related brain calcification.

Coxsackievirus B

Coxsackie B viruses are a collection of serotypes under the *Enterovirus* genus known for their ability to cause a broad spectrum of diseases. Outbreaks have been recorded globally and recent retrospective studies attempt to better understand CVB epidemiology[89]. Depending on the virus subtype and the affected tissue, CVB can cause vomiting, diarrhoea, severe pain in chest or abdomen (pleurodynia), as well as inflammation of meninges, brain and pancreas[90]. CVB is considered the predominant cause of cardiomyopathy (up to 25–40% of myocarditis cases) and myocardial calcifications in neonates and young children[91]. Irreversible myocardial damage caused after neonatal CVB infection is associated with severe disease and high mortality in young children[92], however there are currently no specific treatment options available or commercial vaccines for CVB.

Most of our understanding of CVB myocardial calcification and its pathogenesis is based on in vivo animal studies[90] (Box 3). Cardiac fibroblasts and myocytes are highly susceptible to CVB infection[93] resulting in myocardial inflammation, necrotic cell death, fibrosis and dystrophic calcification[94]. Previously, inflammatory infiltrates were shown to be absent from focal calcified lesions during the early viraemic phase. This suggests that cardiac myocyte injury was due to direct myocyte infection and virus-mediated cell death. In the sub-acute phase of infection, extensive CVB replication in heart tissue[94] triggers migration of activated innate immune cells such as macrophages, neutrophils and natural killer cells, to the infection site. This results in the release of cytokines (for example, TNF, IFN-γ, IL-1β, IL-2)[95] and a secondary wave of adaptive immune responses aimed at clearing the virus[96]. However, persistent immune responses were reported to exacerbate myocardial damage[97]. Chronic CVB-induced myocarditis results when viral replication has halted, but the viral genome persists in heart tissue. This persistence potentially triggers severe myocardial calcification and dilated cardiomyopathy[98].

While there is some understanding of the association between CVB infection and myocardial calcification, the specific pathways and factors involved in this process are complex and not fully elucidated. The exact mechanisms by which persisting virus genomes trigger calcification in the heart are not entirely clear and remain a subject of ongoing

research. Exploring various factors, including the role of persistent inflammation, immune responses and potential interactions between viral components and host cellular pathways, in driving the calcification process is warranted, as targeted therapies and preventive measures for associated heart conditions are still sought after.

Mechanisms of CVB myocardial calcification.

Although CVB pathogenesis has been well characterized, current research focuses on elucidating both direct and indirect mechanisms of CVB-induced myocardial calcification.

The CVB3 protease 2A was previously shown to cleave dystrophin, a major constituent of muscle structural integrity. Furthermore, dystrophin cleavage by CVB3 protease 2A was shown to exacerbate virus release[99] and myocyte death[100]. Significantly less cardiac calcification was observed in CVB3-infected mice carrying cleavage-resistant dystrophin[100]. Similar to ZIKV-induced brain calcification[74], CVB3 infection in the heart results in an upregulation of osteogenic signalling (for example, *BMP2*, *RUNX2*, *OSX/SP7*) and osteoblast-like differentiation, leading to abnormal calcification[101]. High serum concentrations of inorganic phosphate, were detected in CVB3-infected mice. Thus, calcification of heart tissue during CVB infection could be caused by phosphate dysregulation[94].

Although some studies have suggested a connection between CVB infection and myocardial calcification, the exact nature of this relationship is not fully understood, and opposing effects complicate the interpretation. Unexpectedly, tibias of CVB3-infected mice demonstrated severe bone loss mediated through increased RANKL-dependent osteoclastogenesis, similar to bone loss during alphavirus infecition[21,22]. Interestingly, blocking the RANKL–RANK interaction in CVB-infected mice ameliorated bone loss and inhibited heart calcification, suggesting that RANKL could contribute to CVB-induced cardiac calcification[94]. These opposing effects during CVB infection and calcification could be explained by immune responses triggered after CVB infection causing acute or chronic inflammation. Also, RANKL–RANK signalling or calcium-regulating parathyroid hormones, could be responses that actively counteract the calcification processes[102].

Although CVB3 protease 2A has been linked to myocardial calcification, open questions on the mechanisms of CVB-associated myocardial calcifications remain. (1) How does CVB infection specifically regulate RANKL–RANK signalling during calcification? Are there viral factors involved and if so, how? (2) What are the host factors leading to calcification related heart conditions after CVB infection? Are there genetic factors determining susceptibility? (3) What is the timeline of RANKL–RANK involvement in CVB-related calcification? Does it occur primarily during the acute phase of infection or persist into chronic stages? (4) Could RANKL–RANK signalling be targeted for therapies against CVB-related calcification and associated heart conditions?

Therapeutic strategies for virus-related bone diseases

Although joint inflammation, bone loss and soft-tissue calcification have been connected to viral infection, disease-specific treatment options are lacking. Repurposing of approved drugs that treat osteoporosis (low bone mineral density and mass) could be a feasible

therapeutic approach. Specifically, treatment using antiresorptive bisphosphonates (such as, etidronate, clodronate or alendronate), hormones (like oestrogen or parathyroid hormone with calcitonin) or monoclonal antibodies targeting RANK (denosumab) or sclerostin (romosozumab) have proven effective in ameliorating osteoporosis. Additionally, arthralgias after viral infection may be alleviated with anti-inflammatory drugs (for example, ibuprofen, diclofenac, dipyrone) and anti-rheumatics (for example, methotrexate, hydroxychloroquine)[103]. Site-specific calcium chelation therapy with ethylenediaminetetraacetic acid (EDTA), and targeting RUNX2 activity by small-molecule inhibitors could be used for preventing virus-induced calcification[104]. These treatments function largely by immunosuppressive means or general symptomatic relief. However, there is a need to develop treatment options that target virus factors that drive bone diseases. Hence, we explore potential therapeutic targets and drugs against bone loss and calcifications (Table 1) after viral infection, in the following section.

As mentioned previously, CHIKV nsP2 protease can trigger arthritis making it a potential target for therapy[105]. The protease inhibitor E64d was shown to bind between the interface the SAM MTase and protease domains of nsP2, and inhibit alphavirus replication in vitro[106]. Similarly, riboflavin (vitamin B2) was shown to inhibit CHIKV protease activity and replication in vitro[107].

DENV E protein is needed for virus attachment and fusion, thus it is a promising drug target for treating dengue disease[108]. Synthetic peptides such as DET4 and MLH40 target the E protein domain III and inhibit virus entry, as well as replication in vitro. The small molecule BP34610 was identified as an inhibitor of DENV-1–4 E protein, displaying synergistic antivirus activity with ribavirin in vitro[108].

Secreted NS1 during flavivirus infection can trigger proinflammatory signals in human primary macrophages, making NS1 another attractive target[51]. A pan-flavivirus NS1-specific antibody termed 1G5.3, blocks NS1 resulting in reduced viraemia while improving survival in DENV murine models[109].

ZIKV NS3 is a major drug target for calcification after infection, because it enhances osteogenic factors BMPs and OPG[74]. In vitro, niclosamide and nitazoxanide were shown to block NS2B–NS3 interaction, which is needed for efficient virus replication[110]. In addition, the NS3 helicase domain could be targeted with small molecules to block viral replication[111]. Furthermore, ZIKV NS3-based vaccines could be promising, as blocking vertical transmission from mother to child could prevent fetal brain calcification[112].

The capsid protein VP1 and non-structural protein NS2A have been shown to contribute to heart calcification after CVB infection[113]. The small molecule pirodavir was shown to inhibit replication CVB1, CVB3 and CVB4 by binding to VP1 in vitro[114]. In vitro and in vivo studies showed that polyamines are essential for virus replication of picornaviruses, including CVB. As such, the polyamine analogue DENSpm can block CVB3 replication by impairing genome packaging and NS2A protease activity[115], and thus could prevent myocardial calcification. However, many of these candidates need to be evaluated in pre-clinical settings using established animal models described in Box 3.

Conclusion and future perspectives

Various viral infections have the propensity to induce osteogenic pathways of bone resorption and bone formation, resulting in chronic debilitating bone diseases. Despite the broad range of viruses describe here and the potential socio-economic burden of virus-related bone disease, research focusing on the underlying molecular mechanisms is limited. While arthritis-like symptoms caused by alphaviruses may not be fatal, their impact on quality of life is considerable, often leading to symptoms that persist for years after infection. Furthermore, prenatal CVB infections can lead to heart calcification with subsequent life-threatening cardiac failure during early childhood. In addition, prenatal infections with ZIKV result in fetal brain calcifications following neurodevelopmental delays later in childhood.

Here, we have discussed clinically relevant viruses, including CHIKV, DENV, SARS-CoV-2, ZIKV and CVB, as well as their bone-related diseases and underlying mechanisms. We have presented potential drugs for repurposing that could relieve symptoms or target host immune responses, and proposed drugs that could target the causative virus. It is important to acknowledge that our understanding of SARS-CoV-2 and its potential impact on bone loss remains limited, making it challenging to speculate about potential therapies. Nevertheless, we can leverage knowledge gained from studying the effects of other viruses on bone health as we move forward in our research and exploration of this area.

Going further, congenital pathogens known as TORCH pathogens (*Toxoplasma gondii*; ‘other’ including syphilis, varicella-zoster virus, parvovirus B19, and human immunodeficiency virus; rubella; cytomegalovirus; herpes simplex virus) frequently cause calcifications in the brain. But the mechanisms remain largely unclear. TORCH pathogens could produce factors increasing BMPs, or brain calcification could be triggered by host-mediated pathways such as inflammatory responses.

Animal models are crucial for identifying cell targets, disease mechanisms involved in virus-induced bone pathology, as well as pre-clinical evaluation of treatments (Box 3). Mice has the advantage of being a comparatively established model, with available tools and numerous genetically modified strains that enable investigation of specific target genes[116]. Collaborative Cross mice[117] could provide an avenue for mimicking divergent host genetics and clinical disease. Although mice would be suited to study acute viral infection, non-human primates would be more fitting for investigating chronic viral infections and disease manifestations, due to a closer physiological resemblance to humans. Combining animal models with technologies such as those described in Box 2, is needed to address questions in this field. Our current understanding is that virus-induced bone pathologies stem from the host’s inflammatory signalling, which disrupts the balance between bone-forming OBs and bone-resorbing OCs. The timing and genetic factors related to virus-induced bone loss are poorly defined for most viruses. Ex vivo bone cultures, organoid or in vivo models that replicate human genetic diversity would be valuable for unravelling host–pathogen interactions in virus-induced bone disease.

In summary, to advance the field of osteovirology and to improve patient outcomes, we propose prioritizing the following research: (1) deciphering virus–host protein interactions,

and immune responses in the bone microenvironment to ascertain molecular mechanisms behind virus-associated bone pathologies; (2) understanding the long-term effects of viral infections on bone health, structure and metabolism; (3) determine physiologically relevant animal models of infection (Box 3) to aid understanding of other potential soft tissue sites also affected by osteogenic dysregulation (for example, liver, placenta or lung); (4) utilizing recent advancements in multi-cellular culture model systems and multi-omics technologies to gain molecular insight into virus-mediated bone pathologies (Box 2); and (5) evaluation of pre-existing co-morbidities (for example, autoimmune diseases) in relation to viral bone pathology severity. Overall, the identification of virus determinants and comprehending the underlying mechanisms ultimately responsible for bone diseases will facilitate the development of virus-specific therapeutic interventions.

Acknowledgements

W.C. was supported by grants R00DE028573 and R01DE033391 from the National Institute of Dental and Craniofacial Research.

References

1. Takayanagi H. et al. T-Cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma. Nature 408, 600–605 (2000). [PubMed: 11117749]
2. Walsh MC et al. Osteoimmunology: interplay between the immune system and bone metabolism. Annu Rev. Immunol. 24, 33–63 (2006). [PubMed: 16551243]
3. Ohlsson C. & Sjogren K. Effects of the gut microbiota on bone mass. Trends Endocrinol. Metab. 26, 69–74 (2015). [PubMed: 25497348]
4. Baimukhamedov C, Barskova T. & Matucci-Cerinic M. Arthritis after SARS-CoV-2 infection. Lancet Rheumatol. 3, e324–e325 (2021).
5. Honge BL, Hermansen MF & Storgaard M. Reactive arthritis after COVID-19. BMJ Case Rep. 14, e241375 (2021).
6. Sinaei R. et al. Post SARS-CoV-2 infection reactive arthritis: a brief report of two pediatric cases. Pediatr. Rheumatol. Online J. 19, 89 (2021). [PubMed: 34118941]
7. Burt FJ et al. Chikungunya virus: an update on the biology and pathogenesis of this emerging pathogen. Lancet Infect. Dis. 17, e107–e117 (2017). [PubMed: 28159534]
8. Filgueiras IS et al. The clinical spectrum and immunopathological mechanisms underlying ZIKV-induced neurological manifestations. PLoS Negl. Trop. Dis. 15, e0009575 (2021). [PubMed: 34351896]
9. Al Senaidi K, Lacson A, Rebeyka IM & Mackie AS Echocardiographic detection of early myocardial calcification in acute neonatal myocarditis due to Coxsackie virus type B. Pediatr. Cardiol. 30, 862–863 (2009). [PubMed: 19412563]
10. Harris E. WHO: concerning spread of dengue, chikungunya in Latin America. JAMA 329, 1341–1341 (2023).
11. Burt FJ, Rolph MS, Rulli NE, Mahalingam S. & Heise MT Chikungunya: a re-emerging virus. Lancet 379, 662–671 (2012). [PubMed: 22100854]
12. Rodriguez-Morales AJ et al. Post-chikungunya chronic inflammatory rheumatism: results from a retrospective follow-up study of 283 adult and child cases in La Virginia, Risaralda, Colombia. F1000Res. 5, 360 (2016). [PubMed: 27081477]
13. Manimunda SP et al. Clinical progression of chikungunya fever during acute and chronic arthritic stages and the changes in joint morphology as revealed by imaging. Trans. R. Soc. Trop. Med Hyg. 104, 392–399 (2010). [PubMed: 20171708]
14. Mogami R. et al. Ultrasonography of hands and wrists in the diagnosis of complications of chikungunya fever. J. Ultrasound Med. 37, 511–520 (2018). [PubMed: 28786505]

15. Amaral JK, Taylor PC, Teixeira MM, Morrison TET & Schoen RT The clinical features, pathogenesis and methotrexate therapy of chronic chikungunya arthritis. Viruses 11, 289 (2019). [PubMed: 30909365]
16. Chaaithanya IK et al. HLA class II allele polymorphism in an outbreak of chikungunya fever in Middle Andaman, India. Immunology 140, 202–210 (2013). [PubMed: 23710940]
17. Messaoudi I. et al. Chikungunya virus infection results in higher and persistent viral replication in aged rhesus macaques due to defects in anti-viral immunity. PLoS Negl. Trop. Dis. 7, e2343 (2013). [PubMed: 23936572]
18. Bertolotti A. et al. Prevalence of chronic chikungunya and associated risks factors in the French West Indies (La Martinique): a prospective cohort study. PLoS Negl. Trop. Dis. 14, e0007327 (2020). [PubMed: 32163420]
19. Chow A. et al. Persistent arthralgia induced by chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. J. Infect. Dis. 203, 149–157 (2011). [PubMed: 21288813]
20. De Benedetti F. et al. Impaired skeletal development in interleukin-6-transgenic mice: a model for the impact of chronic inflammation on the growing skeletal system. Arthritis Rheum. 54, 3551–3563 (2006). [PubMed: 17075861]
21. Chen W. et al. Arthritogenic alphaviral infection perturbs osteoblast function and triggers pathologic bone loss. Proc. Natl Acad. Sci. USA 111, 6040–6045 (2014). [PubMed: 24733914]
22. Chen W. et al. Bindarit, an inhibitor of monocyte chemotactic protein synthesis, protects against bone loss induced by chikungunya virus infection. J. Virol. 89, 581–593 (2015). [PubMed: 25339772]
23. Chen W. et al. Specific inhibition of NLRP3 in chikungunya disease reveals a role for inflammasomes in alphavirus-induced inflammation. Nat. Microbiol. 2, 1435–1445 (2017). [PubMed: 28848230]
24. Gardner J. et al. Chikungunya virus arthritis in adult wild-type mice. J. Virol. 84, 8021–8032 (2010). [PubMed: 20519386]
25. Foo SS et al. Role of pentraxin 3 in shaping arthritogenic alphaviral disease: from enhanced viral replication to immunomodulation. PLoS Pathog. 11, e1004649 (2015). [PubMed: 25695775]
26. Her Z. et al. Active infection of human blood monocytes by chikungunya virus triggers an innate immune response. J. Immunol. 184, 5903–5913 (2010). [PubMed: 20404274]
27. Labadie K. et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J. Clin. Invest. 120, 894–906 (2010). [PubMed: 20179353]
28. Khan UA, Hashimi SM, Bakr MM, Forwood MR & Morrison NA CCL2 and CCR2 are essential for the formation of osteoclasts and foreign body giant cells. J. Cell. Biochem. 117, 382–389 (2016). [PubMed: 26205994]
29. Wolf S. et al. Inhibition of interleukin-1β signaling by anakinra demonstrates a critical role of bone loss in experimental arthritogenic alphavirus infections. Arthritis Rheumatol. 71, 1185–1190 (2019). [PubMed: 30747500]
30. Liu X. et al. Interleukin-17 contributes to chikungunya virus-induced disease. mBio 13, e0028922 (2022).
31. Chan Y-H et al. Mutating chikungunya virus non-structural protein produces potent live-attenuated vaccine candidate. EMBO Mol. Med. 11, e10092 (2019).
32. Lokireddy S, Vemula S. & Vadde R. Connective tissue metabolism in chikungunya patients. Virol. J. 5, 31 (2008). [PubMed: 18302795]
33. Saha A. et al. Development of nsP2 protease based cell free high throughput screening assay for evaluation of inhibitors against emerging chikungunya virus. Sci. Rep. 8, 10831 (2018). [PubMed: 30018455]
34. Huits R. et al. Clinical characteristics and outcomes among travelers with severe dengue: a geosentinel analysis. Ann. Intern. Med. 176, 940–948 (2023). [PubMed: 37335991]
35. Her Z. et al. Severity of plasma leakage is associated with high levels of interferon γ-inducible protein 10, hepatocyte growth factor, matrix metalloproteinase 2 (MMP-2), and MMP-9 during dengue virus infection. J. Infect. Dis. 215, 42–51 (2017). [PubMed: 28077582]

36. Dengue: clinical presentation. Centers for Disease Control and Prevention https://www.cdc.gov/dengue/healthcare-providers/clinical-presentation.html#:~:text=Severe%20dengue%20is%20defined%20by,impaired%20consciousness%2C%20or%20heart%20impairment (2023).
37. Zambrano LI et al. Assessment of post-dengue rheumatic symptoms using the WOMAC and DAS-28 questionnaires in a Honduran population after a four-month follow-up. Trop. Med. Infect. Dis. 7, 394 (2022). [PubMed: 36548649]
38. Friberg H. et al. Protective versus pathologic pre-exposure cytokine profiles in dengue virus infection. PLoS Negl. Trop. Dis. 12, e0006975 (2018). [PubMed: 30557313]
39. Wong KL et al. Susceptibility and response of human blood monocyte subsets to primary dengue virus infection. PLoS One 7, e36435 (2012). [PubMed: 22574162]
40. McInnes IB & Schett G. The pathogenesis of rheumatoid arthritis. N. Engl. J. Med. 365, 2205–2219 (2011). [PubMed: 22150039]
41. Pascapurnama DN et al. Induction of osteopontin by dengue virus-3 infection in THP-1 cells: inhibition of the synthesis by brefelamide and its derivative. Front. Microbiol. 8, 521 (2017). [PubMed: 28405192]
42. Lund SA, Giachelli CM & Scatena M. The role of osteopontin in inflammatory processes. J. Cell Commun. Signal. 3, 311–322 (2009). [PubMed: 19798593]
43. Zhu S. et al. Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain. J. Clin. Invest. 129, 1076–1093 (2019). [PubMed: 30530994]
44. Huang YL et al. CLEC5A is critical for dengue virus-induced osteoclast activation and bone homeostasis. J. Mol. Med. 94, 1025–1037 (2016). [PubMed: 27033255]
45. Wu MF et al. CLEC5A is critical for dengue virus-induced inflammasome activation in human macrophages. Blood 121, 95–106 (2013). [PubMed: 23152543]
46. Tseng H-W, Samuel SG, Schroder K, Lévesque J-P & Alexander KA Inflammasomes and the IL-1 family in bone homeostasis and disease. Curr. Osteoporos. Rep. 20, 170–185 (2022). [PubMed: 35567665]
47. Wang JY & Roehrl MH Glycosaminoglycans are a potential cause of rheumatoid arthritis. Proc. Natl Acad. Sci. USA 99, 14362–14367 (2002). [PubMed: 12391302]
48. Avirutnan P. et al. Secreted NS1 of dengue virus attaches to the surface of cells via interactions with heparan sulfate and chondroitin sulfate E. PLoS Pathog. 3, e183 (2007). [PubMed: 18052531]
49. Ambrose JH, Sekaran SD & Azizan A. Dengue virus NS1 protein as a diagnostic marker: commercially available ELISA and comparison to qRT-PCR and serological diagnostic assays currently used by the state of Florida. J. Trop. Med. 2017, 8072491 (2017). [PubMed: 28740517]
50. Beatty PR et al. Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. Sci. Transl. Med. 7, 304ra141 (2015).
51. Benfrid S. et al. Dengue virus NS1 protein conveys pro-inflammatory signals by docking onto high-density lipoproteins. EMBO Rep. 23, e53600 (2022). [PubMed: 35607830]
52. Fattahi MJ & Mirshafiey A. Prostaglandins and rheumatoid arthritis. Arthritis 2012, 239310 (2012). [PubMed: 23193470]
53. Huang C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506 (2020). [PubMed: 31986264]
54. Ramos-Casals M, Brito-Zerón P. & Mariette X. Systemic and organ-specific immune-related manifestations of COVID-19. Nat. Rev. Rheumatol. 17, 315–332 (2021). [PubMed: 33903743]
55. Bowe B, Xie Y. & Al-Aly Z. Postacute sequelae of COVID-19 at 2 years. Nat. Med. 29, 2347–2357 (2023). [PubMed: 37605079]
56. Ramani SL et al. Musculoskeletal involvement of COVID-19: review of imaging. Skeletal Radiol. 50, 1763–1773 (2021). [PubMed: 33598718]
57. Kottlors J. et al. Early extrapulmonary prognostic features in chest computed tomography in COVID-19 pneumonia: bone mineral density is a relevant predictor for the clinical outcome - a multicenter feasibility study. Bone 144, 115790 (2021). [PubMed: 33301962]
58. Khoja O. et al. Clinical characteristics and mechanisms of musculoskeletal pain in long COVID. J. Pain. Res. 15, 1729–1748 (2022). [PubMed: 35747600]

59. Foo S-S et al. The systemic inflammatory landscape of COVID-19 in pregnancy: extensive serum proteomic profiling of mother-infant dyads with in utero SARS-CoV-2. Cell Rep. Med. 2, 100453 (2021). [PubMed: 34723226]
60. Jackson CB, Farzan M, Chen B. & Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat. Rev. Mol. Cell Biol. 23, 3–20 (2022). [PubMed: 34611326]
61. Disser NP et al. Musculoskeletal consequences of COVID-19. J. Bone Joint Surg. Am. 102, 1197–1204 (2020). [PubMed: 32675661]
62. Mi B. et al. SARS-CoV-2-induced overexpression of miR-4485 suppresses osteogenic differentiation and impairs fracture healing. Int J. Biol. Sci. 17, 1277–1288 (2021).
63. Qiao W. et al. SARS-CoV-2 infection induces inflammatory bone loss in golden Syrian hamsters. Nat. Commun. 13, 2539 (2022). [PubMed: 35534483]
64. Fajgenbaum DC & June CH Cytokine storm. N. Engl. J. Med. 383, 2255–2273 (2020). [PubMed: 33264547]
65. Chen G. et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J. Clin. Invest. 130, 2620–2629 (2020). [PubMed: 32217835]
66. Rodrigues TS et al. Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients. J. Exp. Med. 218, e20201707 (2021). [PubMed: 33231615]
67. Chevrier S. et al. A distinct innate immune signature marks progression from mild to severe COVID-19. Cell Rep. Med. 2, 100166 (2021). [PubMed: 33521697]
68. Zheng M. et al. TLR2 senses the SARS-CoV-2 envelope protein to produce inflammatory cytokines. Nat. Immunol. 22, 829–838 (2021). [PubMed: 33963333]
69. Khan S. et al. SARS-CoV-2 spike protein induces inflammation via TLR2-dependent activation of the NF-kappaB pathway. eLife 10, e68563 (2021). [PubMed: 34866574]
70. Pan P. et al. SARS-CoV-2 N protein promotes NLRP3 inflammasome activation to induce hyperinflammation. Nat. Commun. 12, 4664 (2021). [PubMed: 34341353]
71. Chen IY, Moriyama M, Chang MF & Ichinohe T. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. Front. Microbiol. 10, 50 (2019). [PubMed: 30761102]
72. Wu X. et al. Viral mimicry of interleukin-17A by SARS-CoV-2 ORF8. mBio 13, e0040222 (2022). [PubMed: 35343786]
73. Carson DA An infectious origin of extraskeletal calcification. Proc. Natl Acad. Sci. USA 95, 7846–7847 (1998). [PubMed: 9653101]
74. Chen W. et al. Zika virus NS3 protease induces bone morphogenetic protein-dependent brain calcification in human fetuses. Nat. Microbiol. 6, 455–466 (2021). [PubMed: 33510473]
75. Katagiri T. & Watabe T. Bone morphogenetic proteins. Cold Spring Harb. Perspect. Biol. 8, e021899 (2016).
76. Brasil P. et al. Zika virus infection in pregnant women in Rio de Janeiro. N. Engl. J. Med. 375, 2321–2334 (2016). [PubMed: 26943629]
77. Yuan L. et al. A single mutation in the prM protein of Zika virus contributes to fetal microcephaly. Science 358, 933–936 (2017). [PubMed: 28971967]
78. Paixao ES et al. Mortality from congenital Zika syndrome — nationwide cohort study in Brazil. N. Engl. J. Med. 386, 757–767 (2022). [PubMed: 35196428]
79. Pool KL et al. Association between neonatal neuroimaging and clinical outcomes in zika-exposed infants from Rio de Janeiro, Brazil. JAMA Netw. Open 2, e198124 (2019). [PubMed: 31365112]
80. Petribu NCL et al. Follow-up brain imaging of 37 children with congenital Zika syndrome: case series study. Br. Med. J. 359, j4188 (2017). [PubMed: 29030384]
81. Proudfoot D. Calcium signaling and tissue calcification. Cold Spring Harb. Perspect. Biol. 11, a035303 (2019). [PubMed: 31138543]
82. Nielsen-Saines K. et al. Delayed childhood neurodevelopment and neurosensory alterations in the second year of life in a prospective cohort of ZIKV-exposed children. Nat. Med. 25, 1213–1217 (2019). [PubMed: 31285631]

83. Ferraris P. et al. Zika virus differentially infects human neural progenitor cells according to their state of differentiation and dysregulates neurogenesis through the Notch pathway. Emerg. Microbes Infect. 8, 1003–1016 (2019). [PubMed: 31282298]
84. Chen J. et al. AXL promotes Zika virus infection in astrocytes by antagonizing type I interferon signalling. Nat. Microbiol. 3, 302–309 (2018). [PubMed: 29379210]
85. Kim J. et al. Zika virus infects pericytes in the choroid plexus and enters the central nervous system through the blood-cerebrospinal fluid barrier. PLoS Pathog. 16, e1008204 (2020). [PubMed: 32357162]
86. Speer MY et al. Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. Circ. Res. 104, 733–741 (2009). [PubMed: 19197075]
87. Ding Q. et al. Species-specific disruption of STING-dependent antiviral cellular defenses by the Zika virus NS2B3 protease. Proc. Natl Acad. Sci. USA 115, E6310–E6318 (2018). [PubMed: 29915078]
88. Crooks CM et al. African-lineage Zika virus replication dynamics and maternal-fetal interface infection in pregnant rhesus macaques. J. Virol. 95, e0222020 (2021). [PubMed: 34076485]
89. Centers for Disease Control and Prevention (CDC). Nonpolio enterovirus and human parechovirus surveillance—United States, 2006–2008. MMWR Morb. Mortal. Wkly Rep. 59, 1577–1580 (2010). [PubMed: 21150865]
90. Yin Z. et al. Development of a neonatal mouse model for Coxsackievirus B1 antiviral evaluation. Virol. Sin. 36, 1575–1584 (2021). [PubMed: 34581960]
91. Gaaloul I. et al. Coxsackievirus B detection in cases of myocarditis, myopericarditis, pericarditis and dilated cardiomyopathy in hospitalized patients. Mol. Med. Rep. 10, 2811–2818 (2014). [PubMed: 25241846]
92. Stallion A, Rafferty JF, Warner BW, Ziegler MM & Ryckman FC Myocardial calcification: a predictor of poor outcome for myocarditis treated with extracorporeal life support. J. Pediatr. Surg. 29, 492–494 (1994). [PubMed: 8014800]
93. Bode MF et al. Cell type-specific roles of PAR1 in Coxsackievirus B3 infection. Sci. Rep. 11, 14264 (2021). [PubMed: 34253819]
94. Lee K. et al. Targeting of the osteoclastogenic RANKL–RANK axis prevents osteoporotic bone loss and soft tissue calcification in Coxsackievirus B3–infected mice. J. Immunol. 190, 1623–1630 (2013). [PubMed: 23303667]
95. Wan F. et al. Vγ1+γδT, early cardiac infiltrated innate population dominantly producing IL-4, protect mice against CVB3 myocarditis by modulating IFNγ+ T response. Mol. Immunol. 81, 16–25 (2017). [PubMed: 27886550]
96. Opavsky MA et al. Susceptibility to myocarditis is dependent on the response of αβ T lymphocytes to Coxsackieviral infection. Circ. Res. 85, 551–558 (1999). [PubMed: 10488058]
97. Dennert R, Crijns HJ & Heymans S. Acute viral myocarditis. Eur. Heart J. 29, 2073–2082 (2008). [PubMed: 18617482]
98. Chapman N. & Kim K-S Persistent coxsackievirus infection: enterovirus persistence in chronic myocarditis and dilated cardiomyopathy. Curr. Top. Microbiol Immunol. 323, 275–292 (2008). [PubMed: 18357775]
99. Xiong D. et al. Dystrophin deficiency markedly increases enterovirus-induced cardiomyopathy: a genetic predisposition to viral heart disease. Nat. Med. 8, 872–877 (2002). [PubMed: 12118246]
100. Lim BK et al. Inhibition of Coxsackievirus-associated dystrophin cleavage prevents cardiomyopathy. J. Clin. Invest. 123, 5146–5151 (2013). [PubMed: 24200690]
101. Kim HS et al. α-Lipoic acid attenuates coxsackievirus B3-induced ectopic calcification in heart, pancreas, and lung. Biochem. Biophys. Res. Commun. 432, 378–383 (2013). [PubMed: 23357417]
102. Hak AE, Pols HAP, Hemert AMV, Hofman A. & Witteman JCM Progression of aortic calcification is associated with metacarpal bone loss during menopause. Arterioscler. Thromb. Vasc. Biol. 20, 1926–1931 (2000). [PubMed: 10938013]
103. Reid IR & Billington EO Drug therapy for osteoporosis in older adults. Lancet 399, 1080–1092 (2022). [PubMed: 35279261]

104. Kim WJ, Shin HL, Kim BS, Kim HJ & Ryoo HM RUNX2-modifying enzymes: therapeutic targets for bone diseases. Exp. Mol. Med 52, 1178–1184 (2020). [PubMed: 32788656]
105. Prescott L. Alphavirus nsP2 protease structure and cleavage prediction: possible relevance to the pathogenesis of viral arthritis. Preprint at bioRxiv 10.1101/2022.01.22.477317 (2022).
106. Tan YB, Law MCY & Luo D. Targeting the alphavirus virus replication process for antiviral development. Antivir. Res 210, 105494 (2022). [PubMed: 36574906]
107. Eberle RJ et al. Riboflavin, a potent neuroprotective vitamin: focus on flavivirus and alphavirus proteases. Microorganisms 10, 1331 (2022). [PubMed: 35889050]
108. Norshidah H, Vignesh R. & Lai NS Updates on dengue vaccine and antiviral: where are we heading? Molecules 26, 6768 (2021). [PubMed: 34833860]
109. Modhiran N. et al. A broadly protective antibody that targets the flavivirus NS1 protein. Science 371, 190–194 (2021). [PubMed: 33414219]
110. Nunes D. Ad. F. et al. NS2B-NS3 protease inhibitors as promising compounds in the development of antivirals against Zika virus: a systematic review. J. Med. Virol. 94, 442–453 (2022). [PubMed: 34636434]
111. Zhu S. et al. Discovery and computational analyses of novel small molecule zika virus inhibitors. Molecules 24, 1465 (2019). [PubMed: 31013906]
112. Elong Ngono A. et al. CD8+ T cells mediate protection against Zika virus induced by an NS3-based vaccine. Sci. Adv. 6, eabb2154 (2020).
113. Wang Y. et al. The capsid protein VP1 of Coxsackievirus B induces cell cycle arrest by up-regulating heat shock protein 70. Front. Microbiol. 10, 1633 (2019). [PubMed: 31379784]
114. Alhazmi A. et al. Combating coxsackievirus B infections. Rev. Med Virol. 10.1002/rmv.2406 (2022).
115. Hulsebosch BM & Mounce BC Polyamine analog diethylnorspermidine restricts coxsackievirus B3 and is overcome by 2A protease mutation in vitro. Viruses 13, 310 (2021). [PubMed: 33669273]
116. Baxter VK & Heise MT Genetic control of alphavirus pathogenesis. Mamm. Genome 29, 408–424 (2018). [PubMed: 30151711]
117. Noll KE et al. Complex genetic architecture underlies regulation of influenza-A-virus-specific antibody responses in the collaborative cross. Cell Rep. 31, 107587 (2020). [PubMed: 32348764]
118. Salhotra A, Shah HN, Levi B. & Longaker MT Mechanisms of bone development and repair. Nat. Rev. Mol. Cell Biol. 21, 696–711 (2020). [PubMed: 32901139]
119. Teitelbaum SL Bone resorption by osteoclasts. Science 289, 1504–1508 (2000). [PubMed: 10968780]
120. Ikebuchi Y. et al. Coupling of bone resorption and formation by RANKL reverse signalling. Nature 561, 195–200 (2018). [PubMed: 30185903]
121. Chen Q. et al. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death Differ. 23, 1128–1139 (2016). [PubMed: 26868907]
122. Liu Q. et al. Recent advances of osterix transcription factor in osteoblast differentiation and bone formation. Front. Cell Dev. Biol. 8, 601224 (2020). [PubMed: 33384998]
123. Uenaka M. et al. Osteoblast-derived vesicles induce a switch from bone-formation to bone-resorption in vivo. Nat. Commun. 13, 1066 (2022). [PubMed: 35210428]
124. Vidal NO, Brandstrom H, Jonsson KB & Ohlsson C. Osteoprotegerin mRNA is expressed in primary human osteoblast-like cells: down-regulation by glucocorticoids. J. Endocrinol. 159, 191–195 (1998). [PubMed: 9795357]
125. Veis DJ & O'Brien CA Osteoclasts, master sculptors of bone. Annu Rev. Pathol. 18, 257–281 (2023). [PubMed: 36207010]
126. Liang B, Burley G, Lin S. & Shi Y-C Osteoporosis pathogenesis and treatment: existing and emerging avenues. Cell. Mol. Biol. Lett. 27, 72 (2022). [PubMed: 36058940]
127. Doherty TM et al. Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads. Proc. Natl Acad. Sci. USA 100, 11201–11206 (2003). [PubMed: 14500910]
128. Zarb Y, Franzoso FD & Keller A. Pericytes in primary familial brain calcification. Adv. Exp. Med Biol. 1147, 247–264 (2019). [PubMed: 31147881]

129. Lees-Shepard JB et al. Activin-dependent signaling in fibro/adipogenic progenitors causes fibrodysplasia ossificans progressiva. Nat. Commun. 9, 471 (2018). [PubMed: 29396429]
130. Wancket LM Animal models for evaluation of bone implants and devices: comparative bone structure and common model uses. Vet. Pathol. 52, 842–850 (2015). [PubMed: 26163303]
131. Chen RE & Diamond MS Dengue mouse models for evaluating pathogenesis and countermeasures. Curr. Opin. Virol. 43, 50–58 (2020). [PubMed: 32950933]
132. Clark KB, Onlamoon N, Hsiao H-M, Perng GC & Villinger F. Can non-human primates serve as models for investigating dengue disease pathogenesis? Front. Microbiol. 4, 305 (2013). [PubMed: 24130557]
133. Awosanya OD et al. Osteoclast-mediated bone loss observed in a COVID-19 mouse model. Bone 154, 116227 (2022). [PubMed: 34607050]
134. Chu H, Chan JF-W & Yuen K-Y Animal models in SARS-CoV-2 research. Nat. Methods 19, 392–394 (2022). [PubMed: 35396468]
135. Shou S. et al. Animal models for COVID-19: hamsters, mouse, ferret, mink, tree shrew, and non-human primates. Front. Microbiol. 12, 626553 (2021). [PubMed: 34531831]
136. Gorman MJ et al. An immunocompetent mouse model of zika virus infection. Cell Host Microbe 23, 672–685.e676 (2018). [PubMed: 29746837]
137. Nem de Oliveira Souza I. et al. Acute and chronic neurological consequences of early-life Zika virus infection in mice. Sci. Transl. Med. 10, eaar2749 (2018).
138. Haese NN et al. Nonhuman primate models of Zika virus infection and disease during pregnancy. Viruses 13, 2088 (2021). [PubMed: 34696518]
139. Martinot AJ et al. Fetal neuropathology in Zika virus-infected pregnant female rhesus monkeys. Cell 173, 1111–1122.e1110 (2018). [PubMed: 29606355]
140. Błyszczuk P. Myocarditis in humans and in experimental animal models. Front. Cardiovasc. Med. 6, 64 (2019). [PubMed: 31157241]
141. Fairweather D. & Rose NR Coxsackievirus-induced myocarditis in mice: a model of autoimmune disease for studying immunotoxicity. Methods 41, 118–122 (2007). [PubMed: 17161308]
142. Stone V. et al. A hexavalent Coxsackievirus B vaccine is highly immunogenic and has a strong protective capacity in mice and nonhuman primates. Sci. Adv. 6, eaaz2433 (2020).
143. Skottke J, Gelinsky M. & Bernhardt A. In vitro co-culture model of primary human osteoblasts and osteocytes in collagen gels. Int. J. Mol. Sci. 20, 1998 (2019). [PubMed: 31018582]
144. Drakhlis L. et al. Human heart-forming organoids recapitulate early heart and foregut development. Nat. Biotechnol. 39, 737–746 (2021). [PubMed: 33558697]
145. Su X. et al. Human brain organoids as an in vitro model system of viral infectious diseases. Front. Immunol. 12, 792316 (2022). [PubMed: 35087520]
146. Park Y. et al. Trabecular bone organoid model for studying the regulation of localized bone remodeling. Sci. Adv. 7, eabd6495 (2021).
147. Wang T. et al. Single-cell RNA sequencing in orthopedic research. Bone Res. 11, 10 (2023). [PubMed: 36828839]
148. Debnath S. & Greenblatt MB Specimen preparation for single-cell sequencing analysis of skeletal cells. Methods Mol. Biol. 2221, 89–100 (2021). [PubMed: 32979200]
149. Tilburg J. et al. Spatial transcriptomics of murine bone marrow megakaryocytes at single-cell resolution. Res Pract. Thromb. Haemost. 7, 100158 (2023). [PubMed: 37255850]
150. Ratnasiri K, Wilk AJ, Lee MJ, Khatri P. & Blish CA Single-cell RNA-seq methods to interrogate virus-host interactions. Semin. Immunopathol. 45, 71–89 (2022). [PubMed: 36414692]

BOX 1 Bone homeostasis and pathogenesis

Bone remodelling is a continuous metabolic process that mediates the breakdown and synthesis of bone matrix throughout life. This process is facilitated by three cell types, which reside in the bone microenvironment, namely the bone-forming osteoblasts (OBs), bone-resorbing osteoclasts (OCs) and bone-matrix-residing osteocytes (Ocys), as reviewed elsewhere[118] (Fig. 1).

Briefly, OCs are multinucleated giant cells derived from the fusion of myeloid precursor cells (for example, myeloid/monocyte), known as osteoclastogenesis (Fig. 1). They mediate bone resorption, a physiological process that involves the breakdown of bone tissues through the secretion of acids and proteases[119]. This process is predominantly mediated by OCs and/or immune cells (for example, T cells and B cells) in bone tissues[120], with the local presence of pro-osteoclastogenic signalling molecules: (1) macrophage colony stimulating factor (M-CSF/CSF1) and (2) RANKL (ref. 119). Conversely, OBs are differentiated from MSC-derived osteoprogenitor cells in a process called osteoblastogenesis[121] (Fig. 1). They regulate bone-matrix formation through Wnt/β-catenin, BMP-SMAD, Hedgehog and Notch signalling cascades[122]. Subsequently, OBs cease to synthesize bone matrix and terminally differentiate into Ocys, a mature form of OBs that coordinate bone remodelling through the lacuna-canalicular system in bone[123]. In addition, OBs express the RANKL-binding ‘decoy’ receptor, OPG, which competes with RANK for RANKL and thus inhibits osteoclastogenesis[124]. Hence, the RANKL–RANK–OPG axis plays a central role in regulating bone homeostasis (Fig. 1).

Altered functions of bone-resorbing OCs and bone-forming OBs can result in pathogenic bone loss or calcification (Fig. 2a,b). During homeostasis, specific bone sites are resorbed by OCs for bone matrix maintenance, but imbalance of osteoclastic coupling factors (for example, RANKL) results in excessive OC formation and pathological bone-loss disorders, such as osteoporosis and periodontal disease (reviewed previously[125]). OCs play a crucial role in pathologic bone loss by producing high levels of cathepsin K and collagenase, enzymes that breaks down the bone matrix (Fig. 2a). Several underlying molecular mechanisms have been proposed, including: (1) disruption in bone remodelling with accelerated bone resorption or attenuated bone formation; (2) direct hormonal actions on OC differentiation; (3) inflammatory factors such as TNF-α, IL-1β, IL-6, IL-17 and CCL2 (reviewed previously[126]; Fig. 2a). Furthermore, calcification (both micro and macro) is a highly regulated physiological process in bone formation, but a pathologic process in soft tissues (for example, brain, placenta, joints and blood vessels; Fig. 2b). Several mechanisms known to be involved in the onset of pathologic calcification[127] include: (1) abnormal differentiation of osteogenic-potential cells, such as smooth muscle cells, pericytes and fibro/adipogenic progenitors, into OB-like cells[128,129] (Fig. 2b); (2) local increase in osteogenic morphogens; (3) dysregulated calcium and phosphate ion levels that are elevated above physiological levels; (4) inflammation; (5) cell damage, apoptosis and necrosis; and (6) imbalance or loss of calcification inhibitors (reviewed previously[81]; Fig. 2b).

BOX 2 Technological advances for osteovirology

Two-dimensional co-cultures of multiple cell types in vitro have the ability to mimic a tissue microenvironment, thus being a useful experimental tool for osteovirology. Co-cultures in trans-well systems can be used to investigate the exchange of secreted factors relevant for bone formation. A 3D co-culture model involving primary human Ocys collagen-embedded on the apical side with OBs on the basal side of a porous membrane closely mimics bone morphology and demonstrates the expression of osteosclerostin and RANKL (ref. 143).

The use of ex vivo models, such as primary bone explants, preserve multicellular 3D environments allowing the analysis of osteogenesis. Alternatively, 3D models based on co-culturing human pluripotent stem cells with primary cells in a physiological dense matrix can generate cultures with cellular morphology, architecture and transcriptomic profile similar to those found in brain, heart and bone[144–146]. Recently, a bone organoid has been developed by demineralization and micro-slicing of primary bone samples, then co-culturing the samples with murine OBs and bone marrow mononuclear cells, creating a microenvironment for studying the bone remodelling cycle[146].

Single-cell RNA sequencing (scRNAseq) is a valuable tool for characterizing cellular heterogeneity, identifying new cell subtypes and subclusters, understanding potential molecular mechanisms, and unravelling cell−cell interactions[147]. In response to the challenges posed by the hardness of bone tissue, standardized protocols have been devised to enhance sample preparation, with a primary focus on the generation of a viable cell suspension from skeletal cells. For example, the processing of mouse femur fragments entails a series of steps, including collagenase P digestion, DNase treatment, and subsequent filtration[148]. Recognizing that scRNAseq methodologies lack the spatial context of cells within their tissue environment, spatial transcriptomics has emerged as a potent complementary technique. To facilitate spatial transcriptomics analysis of bone tissues, specimens can be prepared by fixing and decalcifying them using EDTA before micro-sectioning[149]. Additionally, it is feasible to quantify virus transcripts through the application of virus-inclusive scRNAseq (viscRNA-Seq), thereby facilitating the integration of host and virus assessments[150]. Most scRNAseq methods are carried out by 3′ poly-A mRNA capture, hence, viruses which generate polyadenylated transcripts, such as alphaviruses and SARS-CoV-2, are able to be analysed by these methods. However, non-polyadenylated viruses, such as flaviviruses, can be analysed by a capture strategy based on oligo(dT) with virus-specific primers[150]. Spatial transcriptomics can be achieved either by laser capture microdissection, or by annealing fixed tissue directly to barcoded primers attached to microscope slides, with a resolution of 10–30 μm. In the context of basic bone biology, these technologies have successfully aided in identification of novel OC recycling mechanisms, suggesting their promise in osteovirology[146,150].

BOX 3 Experimental animal models for virus-induced bone pathologies

CHIKV arthritis models

The primary animal models used to study the pathogenesis of CHIKV are mice and nonhuman primates (NHPs). Immunocompetent WT adolescent C57BL/6 (3–4 weeks old[22]) or adult C57BL/6 (>6 weeks old[24]) mice have been used to recapitulate CHIKV arthritis, myositis and bone pathology. Being the natural host of CHIKV sylvatic cycle, cynomolgus macaques exhibit typical acute CHIKV disease, followed by macrophage activation and virus persistence in synovial and musculoskeletal tissues for up to 44 days after infection[27]. CHIKV-infected, aged rhesus macaques are associated with increased and persistent virus replication along with lower levels of plasma IFN when compared to adult macaques[17]. Hence, NHPs provide a more comprehensive animal model for studying CHIKV musculoskeletal disease due to their closer physiological resemblance to humans in immune system function, joint structure and bone remodelling[27,130].

DENV disease models

Several mouse models have been established to study DENV pathogenesis, including immunocompetent WT, immunocompromised AG129 mice (IFN-α/β-receptor and IFN-γ-receptor double knockout), humanized and transgenic mice[131]. Immunocompetent WT mice generally exhibit resistance to infection and develop mild disease or neuropathogenesis. By contrast, immunocompromised AG129 mice are highly susceptible to DENV infection and are commonly used to evaluate antivirus therapies, vaccine effectiveness and pathogenesis. Additionally, STAT1-deficient mice have been utilized to demonstrate osteolytic activity and inflammation in bone tissue upon DENV infection[44]. Humanized DENV mouse models are ideal for understanding human immune responses towards infection but do not recapitulate severe DENV manifestations[131]. NHPs such as rhesus macaques, cynomolgus macaques and marmosets are susceptible to DENV infection, develop viraemia and display an antibody response together with clinical signs recapitulating DENV disease manifestations in humans[132].

SARS-CoV-2 bone-loss models

To date, several COVID-19 animal models have been utilized to investigate SARS-CoV-2-associated skeletal complications. Within two weeks of infection, 19-week-old K18 human ACE2 (hACE2) transgenic mice infected with SARS-CoV-2 exhibits bone resorption and bone loss[133]. Other COVID-19 animal models include naturally infected, non-transgenic ferrets, hamsters and NHPs that exhibit mild-to-moderate clinical disease[134]. SARS-CoV-2 infection of golden Syrian hamsters displayed extrapulmonary manifestations and inflammatory bone loss even up to 60 days post-infection[63]. The NHP macaque model has been utilized to evaluate the severity of disease and age-related pathogenesis due to the virus's pervasiveness in several tissue types, serving as an ideal model for studying long-term complications of post-acute COVID-19 (reviewed previously[135]).

ZIKV brain calcification models

Immunocompetent human *STAT2* knock-in (h*STAT2* KI) pregnant dam on C57BL/6 background is susceptible to ZIKV and exhibits prenatal infection with vertical transmission across the placenta, infecting the fetal brain[136]. Pups born to ZIKV-infected pregnant h*STAT2* KI mice display abnormal intracranial calcification, recapitulating human fetal brain calcifications[74]. Dystrophic subcortical brain calcifications and behavioural deficits were observed at 12 days post-infection in Swiss mice infected with ZIKV at postnatal day three[137]. NHP ZIKV prenatal infection models including rhesus macaques, pigtail macaques and squirrel monkeys develop brain calcification in surviving infants[138]. In particular, rhesus macaques infected with ZIKV early in pregnancy exhibit brain calcifications and neuropathology at birth[139].

CVB disease models

Several CVB-susceptible infection models were established to mimic human infection specifically focusing on virus myocardial injury, including adolescent (3-week-old) and adult (6–7-week-old) BALB/c, A/J or C57BL/6 mice[140]. Following acute myocarditis, CVB3-infected mice developed multi-organ virus tropism (for example, lung and pancreas) along with cardiac fibrosis and ectopic calcification[94]. Acute myocarditis and high mortality are observed in BALB/c, A/J or C57BL/6 mice infected with in-vitro-passaged CVB. However, use of heart-passaged CVB in BALB/c and A/J mice resulted in chronic myocarditis and fibrosis[141]. Although NHPs have not been widely used CVB animal models, rhesus macaques have proven valuable for studying CVB-induced myocarditis and vaccines[142].

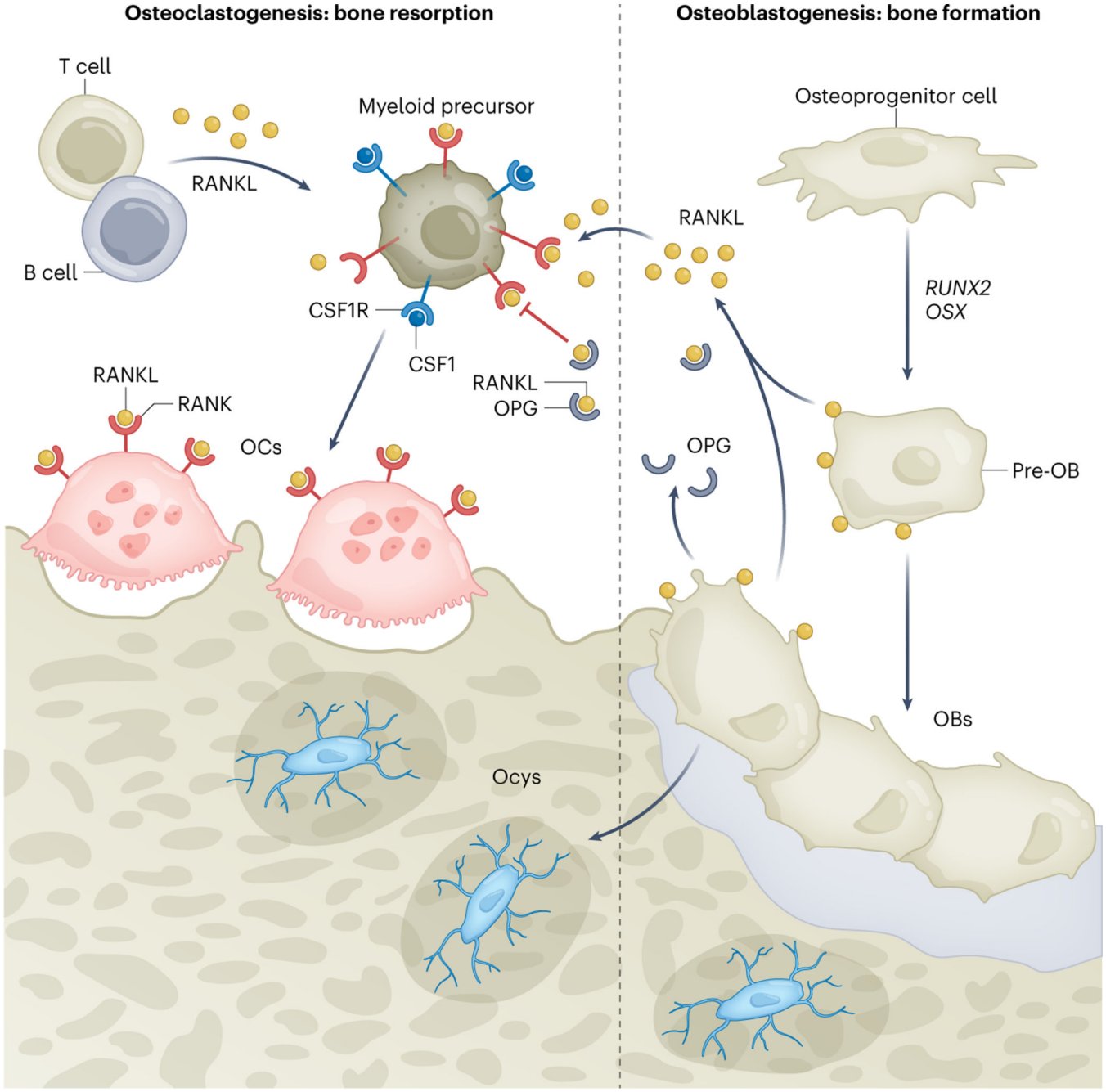

Fig. 1 |. The cross-talk of bone remodelling.

The bone remodelling process involves bone resorption (osteoclastogenesis) and bone formation (osteoblastogenesis). Osteoclastogenesis (left) starts with RANKL production by immune cells, such as T and B lymphocytes, and OBs. The binding of RANKL to the RANK expressed on monocytic CSF1R^{+} OC precursor cells drives differentiation into bone-resorptive OCs. Bone resorption can be negatively regulated though the production of OPG by OBs, which compete with RANK for RANKL, blocking osteoclastogenesis. Osteoblastogenesis (right) starts with the differentiation of osteoprogenitor cells into pre-OBs, regulated by transcription factors *RUNX2* and *OSX*. Upon maturation to OBs, the cells

promote calcification and can subsequently differentiate into Ocys which help maintain the equilibrium between OBs and OCs.

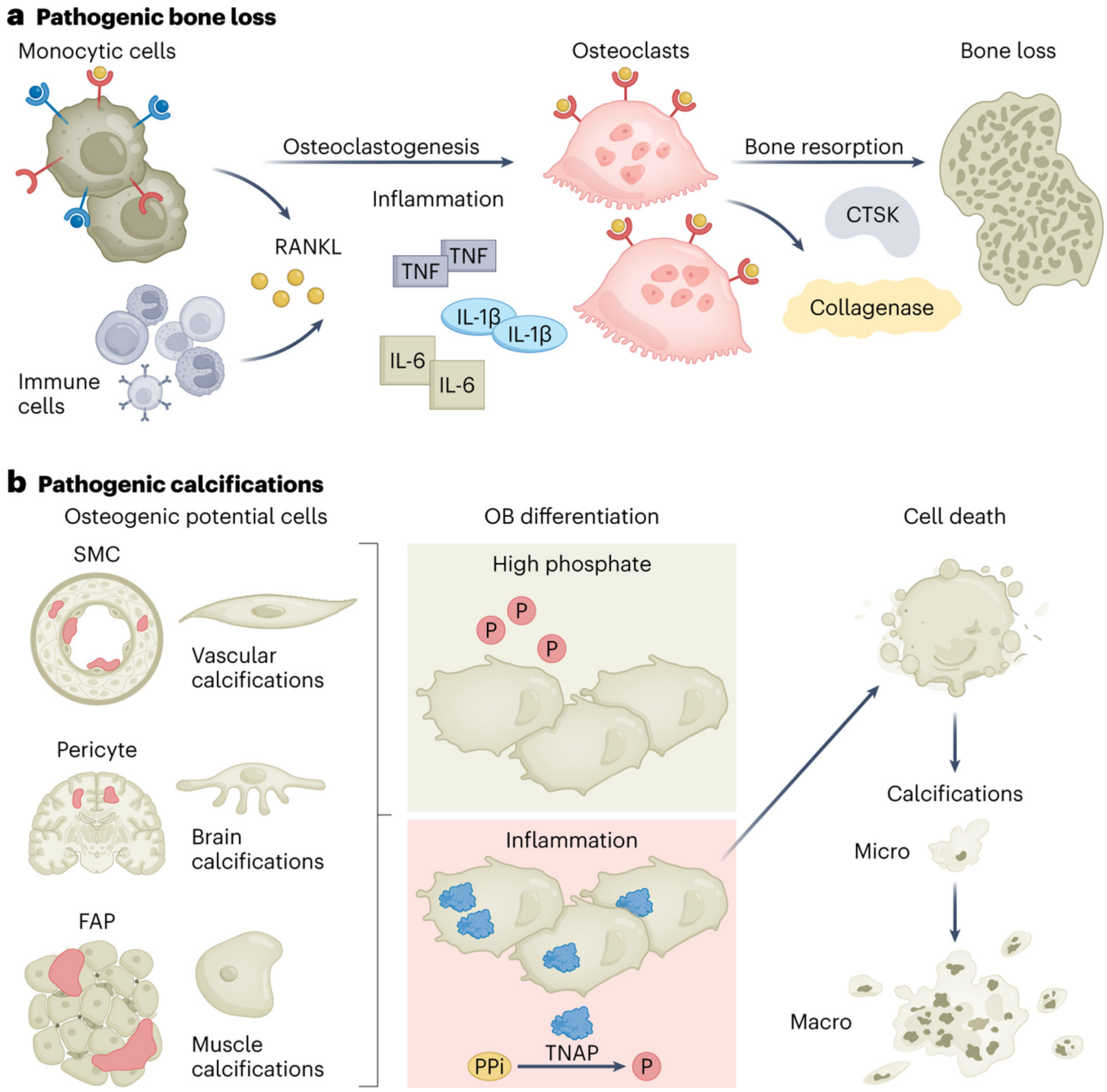

Fig. 2 |. Pathogenic bone loss and calcifications.

a, Pathogenic bone loss is typically driven by inflammation. Immune cells produce RANKL and inflammatory mediators (for example, TNF, IL-1B and IL-6) leading to the differentiation of monocytic cells into OCs producing cathepsin K (CTSK) and collagenase that breaks down the bone matrix giving rise to bone loss. **b**, Pathogenic calcification is abnormal osteogenesis driven by non-OB cells with osteogenic potential such as smooth muscle cells (SMCs), pericytes and fibro/adipogenic progenitors (FAPs). These can cause vascular, brain and muscle calcifications, respectively. Non-OB osteogenic cells differentiate into OB-like cells under conditions such as high phosphate (P) or inflammatory-driven

overt tissue-nonspecific alkaline phosphatase (TNAP) production (convert pyrophosphate; PPi to P). Subsequently, these cells undergo apoptosis, leading to soft-tissue calcifications beginning with small calcium deposits (microcalcifications) that can grow into large calcification sites (macrocalcifications).

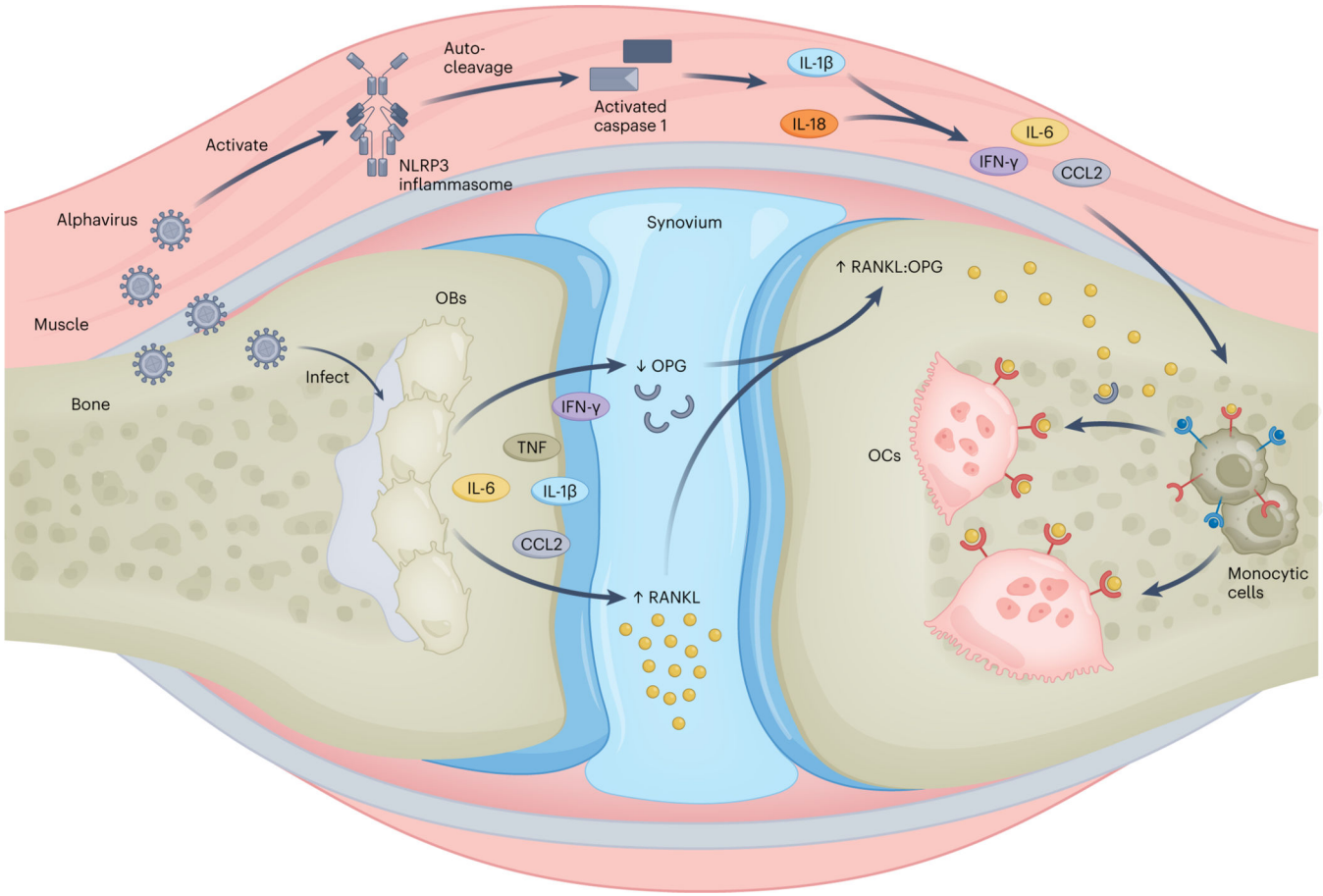

Fig. 3 |. Inflammatory bone loss induced by arthritogenic alphaviruses.

The activation of the NLRP3 inflammasome significantly contributes to inflammation induced by alphaviruses, ultimately disrupting bone remodelling in the context of virus-induced arthritis. The activation of the NLRP3 inflammasome promotes the expression of IL-1β, IL-18, IL-6, MCP-1/CCL2 and IFN-γ, further intensify the inflammatory response. The resulting inflammation creates a pro-inflammatory signalling loop that significantly contributes to the pathophysiology of bone resorption. OBs infected with alphaviruses produce elevated levels of RANKL, while showing decreased OPG expression. This disruption in the RANKL/OPG ratio within the synovial fluid plays a pivotal role in the maturation and activation of OCs, which are central to virus-induced bone resorption through RANKL-mediated osteoclastogenesis. Furthermore, alphavirus-infected OBs produce elevated levels of IL-1β, IL-6, TNF, IFN-γ, and MCP-1/CCL2, which promote the recruitment of OC precursors to the infection site. This cascade of events leads to heightened OC activity and subsequent bone loss.

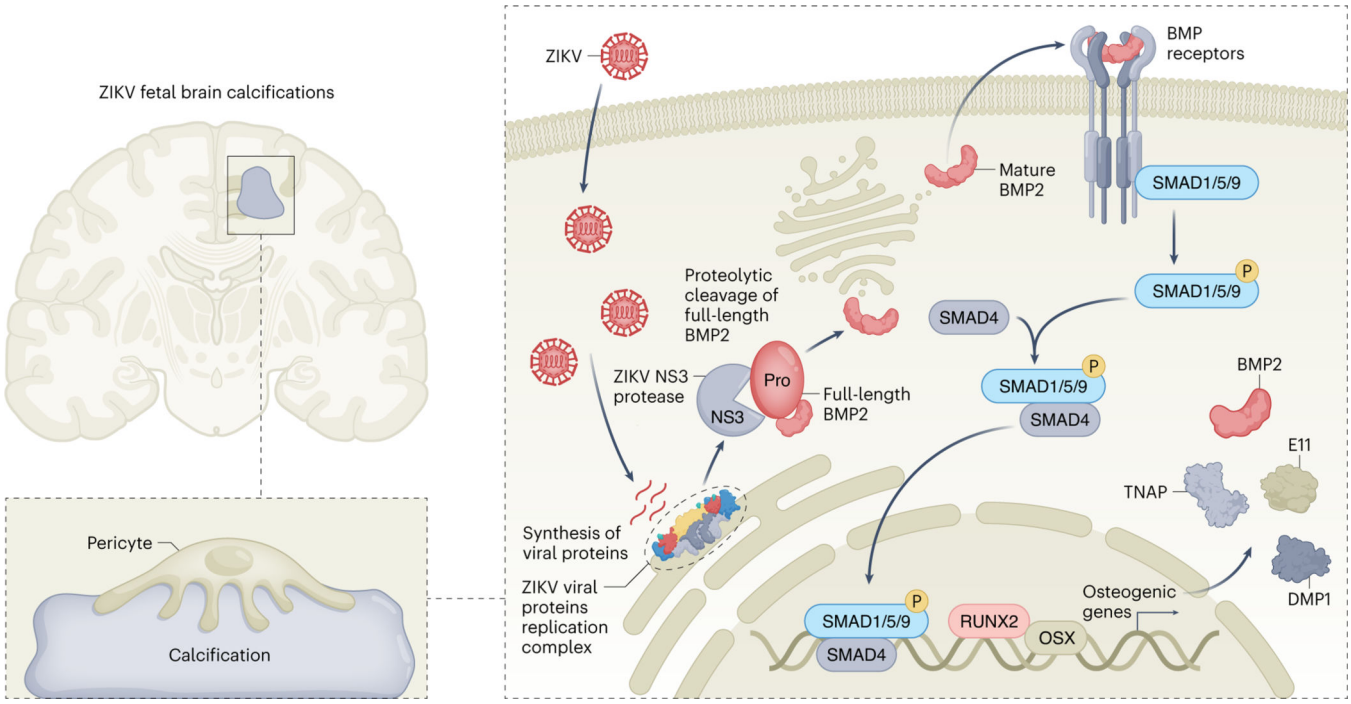

Fig. 4 |. Mechanisms of ectopic calcification induced by ZIKV.

Intra-uterine exposure to ZIKV culminates with fetal brain calcification, mainly localized at the cortical–subcortical white matter junctions, basal ganglia and thalamus. This process is triggered by ZIKV infection of pericytes, which initiates a process similar to physiological osteogenesis. Upon virus replication, the ZIKV NS3 protease domain cleaves the pro-BMP2/4, generating mature forms of BMP2/4. In turn, BMP2/4 heterodimers transduce signals by binding to type I and II serine/threonine kinase receptors which induce the phosphorylation of SMAD1/5/9 and interaction with SMAD4, forming an osteogenic transcription factor. Its translocation to the nucleus activates the expression of master osteogenic regulators (*RUNX2* and *OSX/SP7*), culminating with the upregulation of osteogenic genes (*TNAP, E11/PDPN, DMP1* and *BMP2*) and brain calcification.

Table 1 |

Therapeutic strategies for virus bone loss and calcification

Target (virus/protein)	Therapeutic	Category	Mechanisms	Reference
Bone loss				
Alphavirus/NSP2	E64d	Peptide-like protease inhibitor	Binding of alphavirus NSP2 SAM MTase and protease domains	106
	Riboflavin	B vitamin (B2)	Inhibitory effect on alphavirus NSP2 protease domain	107
DENV/E	DET4, MLH40	Synthetic peptides	Inhibitory effect on envelope domain III	108
	BP34610	Small molecule	Inhibitor of DENV-1–4 E	108
DENV/NS1	1G5.3	Monoclonal antibody	Neutralization of DENV and ZIKV NS1	109
Calcification				
ZIKV/NS3	Niclosamide nitazoxanide	Protease inhibitor	Inhibitory effect on NS3 2B53 pockets (NS2B–NS3 binding site)	110
	Bromocriptine	Protease inhibitor	Inhibitory effect on active residues of the NS2B–NS3 proteolytic cavity	110
	Small molecules NSC10580, ZINC01706300 and NSC45741, ZINC263598830	Enzyme inhibitor	Inhibitory effect on NS3 helicase domain	111
CVB/VP1	Pirodavir	Small molecule	Inhibitory effect on picornavirus capsid	114
CVB/NS2	DENSpm	Polyamine analogue	Mutagenesis of NS2	115