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CRITICAL CARE

Epigenetic susceptibility to severe respiratory viral infections and its therapeutic implications: a narrative review

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Summary

The emergence of highly pathogenic strains of influenza virus and coronavirus (CoV) has been responsible for large epidemic and pandemic outbreaks characterised by severe pulmonary illness associated with high morbidity and mortality. One major challenge for critical care is to stratify and minimise the risk of multi-organ failure during the stay in the intensive care unit (ICU). Epigenetic-sensitive mechanisms, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) methylation, histone modifications, and non-coding RNAs may lead to perturbations of the host immune-related transcriptional programmes by regulating chromatin structure and gene expression patterns. Viruses causing severe pulmonary illness can use epigenetic-regulated mechanisms during host–pathogen interaction to interfere with innate and adaptive immunity, adequacy of inflammatory response, and overall outcome of viral infections. For example, Middle East respiratory syndrome-CoV and H5N1 can affect host antigen presentation through DNA methylation and histone modifications. The same mechanisms would presumably occur in patients with coronavirus disease 2019, in which tocilizumab may epigenetically reduce microvascular damage. Targeting epigenetic pathways by immune modulators (e.g. tocilizumab) or repurposed drugs (e.g. statins) may provide novel therapeutic opportunities to control viral–host interaction during critical illness. In this review, we provide an update on epigenetic-sensitive mechanisms and repurposed drugs interfering with epigenetic pathways which may be clinically suitable for risk stratification and beneficial for treatment of patients affected by severe viral respiratory infections.

Keywords: coronavirus; COVID-19; epigenetic drugs; epigenetics; host–viral interactions; influenza virus; intensive care; severe acute respiratory syndrome

Editor's key points

- The authors reviewed the literature and ongoing clinical trials, and show that epigenetic biomarkers may stratify the risk of severe respiratory infection.
- As deoxyribonucleic acid methylation can be reversed, at least partially, some drugs (curcumin, apabetalone, and repurposed drugs such as statins and tocilizumab) may contribute to better treatment of such patients by interfering with epigenetic pathways.

Over the past two decades, we have experienced the emergence of newly recognised highly pathogenic strains of influenza virus (i.e. influenza A virus subtype H1N1 or swine

influenza, and influenza A virus subtype H5N1 or avian influenza) and coronavirus (CoV) (i.e. severe acute respiratory syndrome [SARS]-CoV, Middle East respiratory syndrome [MERS]-CoV, and SARS-CoV-2) responsible for pandemic infections associated with high morbidity and mortality.[1-5] These viruses may cause a wide spectrum of respiratory manifestations associated with massive inflammatory cell infiltration and proinflammatory cytokine/chemokine release resulting in acute lung injury (ALI); acute respiratory distress syndrome (ARDS); and, ultimately, death from multi-organ failure (MOF).[6-8] Molecular mechanisms regulating virus−host interactions can significantly affect the degree and adequacy of both immune and inflammatory responses influencing clinical outcomes.[9-11] For the critical care physicians, to identify and prevent the occurrence of this exuberant inflammatory response and to stratify the risk of MOF in the intensive care unit (ICU) are still major challenges.[12]

Epigenetics may influence host susceptibility to such viral infections.[13] Deoxyribonucleic acid (DNA) methylation, ribonucleic acid (RNA) methylation, histone tail changes, and also non-coding RNAs are heritable and acquired modifications able to alter gene expression at different levels without any changes in the primary DNA sequence. Epigenetic mechanisms, by regulating chromatin structure and gene expression patterns, modulate host immunity and inflammatory response.[14] In critical illness, such epigenetic modifications can promote the release of proinflammatory cytokines and activation of inflammatory cells, responsible for oxidative stress, endothelial dysfunction, apoptosis, and MOF.[15] Epigenetics can also regulate the interaction between host and multidrug-resistant bacteria.[16]

The interaction of viruses with host cells can cause perturbations of transcriptional programmes involving such epigenetic mechanisms leading to viral shedding and inadequate immune response.[13] Middle East respiratory syndrome-CoV and H5N1 infections can antagonise the host immune response by modulating antigen presentation through DNA methylation and histone modifications.[17-20] Similar mechanisms would occur during coronavirus disease 2019 (COVID-19).[21,22] Indeed, advanced bioinformatic tools have predicted the possibility of using microRNAs (miRNAs) to inhibit infections caused by COVID-19, SARS-CoV, and MERS-CoV by inhibiting the translation of viral proteins and viral replication.[21] As some epigenetic changes can be reversed by small agents, known as 'epidrugs', or alternatively, epigenetic pathways can be interfered by immune modulators, they might provide useful drug targets to ameliorate the clinical outcome during viral respiratory infections.[23,24] Ongoing trials will answer to this possible clinical application. The goal of the review was to provide an appropriate pathogenic scenario in which epigenetic-sensitive mechanisms and epidrugs may be clinically useful to stratify risk and treatment of patients in ICU affected by severe viral respiratory infections.

Severe respiratory viral infections: pathogenesis and clinical manifestations

Influenza viruses

Influenza viruses are enveloped, single-stranded RNA viruses classified into three major serotypes: A, B, and C. Influenza A viruses, the most extensively studied, are further classified based on the different subtypes of the two surface glycoproteins: haemagglutinin (H1−H18) and neuraminidase (N1−N11) which facilitate virus binding to host respiratory epithelial cells via a sialic acid receptor and virions released from cells, respectively.[25] Avian influenza A virus H5N1[26-28] and swine influenza A H1N1[29-34] infections have caused acute respiratory failure secondary to severe pneumonia and ARDS. The highly pathogenic avian influenza A virus H5N1, first described in 1996, caused severe pneumonia with high mortality (more than 60%), secondary to ARDS and MOF.[26-28] Risks factors for severe influenza A H1N1 infection included pregnancy,

Table 1 Clinical and immunological features of viral respiratory infections by influenza viruses.[36-44] COPD, chronic obstructive pulmonary disease; CR, conventional radiograph; CT, computed tomography; GGO, ground glass opacity; HF, heart failure; IFNγ, interferon γ; IL-1 RA, interleukin-1 receptor antagonist protein; IP-10, interferon-γ-inducible protein-10; MCP-1, monocyte chemoattractant protein-1; MIP 1-b, macrophage inflammatory protein 1-B; TLR-3, Toll-like receptor Type 3; TNF-α, tumour necrosis factor-alpha.

Clinical signs and symptoms	Complications	Lung pathological features	Immunological features	Radiographic findings
Fever; aching muscles; chills and sweats; headache; dry, persistent cough; fatigue and weakness; nasal congestion; sore throat	In young children: otitis media and respiratory complications, such as croup, bronchiolitis, myocarditis and pericarditis, severe myositis, and encephalopathy; encephalitis; transverse myelitis; and acute disseminated encephalomyelitis In all ages: dehydration and exacerbation of underlying chronic diseases (e.g. HF, myocardial infarction, cerebrovascular accident, diabetes, COPD, asthma)	Diffuse alveolar damage with alveolar haemorrhage and necrotising bronchiolitis Viral antigen localisation in Type 2 pneumocytes and alveolar lining cells	Upregulation of TLR-3, IFNα, IL-1 RA, IL-6, IL-8, MCP-1, MIP 1-b, and IP-10, and increased numbers of CD3$^+$ and CD08$^+$ T cells Initial viral immune evasion from the host immune system followed by cytokine storm	Abnormal chest CR (pulmonary infiltrate, consolidation, and pleural effusion) or CT (GGO and pleural effusion) on admission was associated with worse clinical outcomes

obesity, asthma, and chronic obstructive pulmonary disease (COPD).[29–34] Mitigation of the pathogenicity of the pandemic virus was probably caused by the presence of a cross-reactive cell response against this virus, which was boosted by seasonal vaccination.[35] In Table 1, we summarised clinical and immunological features of viral respiratory infections by influenza viruses.[36–44]

Coronaviruses

Coronaviruses, named after their crown-like structure, are enveloped, positive-sense RNA viruses, containing the largest known genome amongst RNA viruses. The CoV genome encodes for 16 non-structural proteins, which form the viral replicase transcriptase complex, and four essential structural proteins, involved in the host immune response and virion assembly: the spike (S) protein, responsible for receptor binding and viral entry into the host cell; the membrane (M) and envelope (E) proteins, responsible for virus assembly and release; and the nucleocapsid (N) protein, important for RNA synthesis and its final packaging into the viral particles.[45] The genome sequence of SARS-CoV-2 is about 79% identical to the SARS-CoV and 50% to the MERS-CoV.[46] These viruses cause severe respiratory infections (Table 2).[47–60]

Older adults with co-morbidities, especially cardiovascular diseases, diabetes, obesity, renal failure, and COPD, are at higher risk of severe disease.[51,56] ICU admission for organ support occurs in about 20–30%, 5–36%, and 50–89% of patients infected with SARS-CoV, SARS-CoV-2, and MERS-CoV, respectively.[3,51–53,56] Currently, global public health is facing the COVID-19 pandemic as the third CoV crisis in less than 20 yr (https://www.nih.gov/health-information/coronavirus).

Coronaviruses are capable of infecting several other organs, as demonstrated by the presence of SARS-CoV in circulating immune cells, neurones, intestinal mucosa, and epithelium of renal distal tubules.[61] Coronaviruses–host interaction can influence susceptibility to CoV infection and progression to severe disease.[3,11] After entering the body via the respiratory system, a critical step for cell entry and infection is the binding of the envelope S glycoprotein to the epithelial cells through specific receptors. The S protein of the SARS-CoV and SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) molecule present on cells through the receptor-binding domain,[62,63] whilst MERS-CoV binds to the host cell protein dipeptidyl peptidase 4.[64] After binding to the host, the S protein needs to be activated by a cellular protease, the transmembrane protease serine 2, which cleaves S in two subunits liberating the fusion peptide that mediates the fusion of the viral envelope with the cellular membrane. Differences in the structural and dynamic state of the receptor-binding domain[65] and S protein priming[66] between SARS-CoV and SARS-CoV-2 cause higher ACE2-binding affinity of the SARS-CoV-2 and favour its evasion of the immune surveillance,[64] suggesting a potential explanation of the higher SARS-CoV-2 infectivity.[63] Angiotensin-converting enzyme 2 can play a protective role in lung injury,[67] and its downregulation by SARS-CoV can contribute to progression to severe lung injury.[68] On the contrary, MERS-CoV regulates dipeptidyl peptidase 4 receptor in the lungs of smokers and COPD, and this could explain their susceptibility to severe disease.[69]

To survive in the host cells, the CoVs adopt multiple strategies to evade detection by the host immune system, allowing active virus replication. The virus can downregulate genes involved in the antigen presentation, such as retinoic-acid-inducible gene and melanoma differentiation-associated protein 5, and interfere with intracellular signalling pathways through structural (proteins M and N) and non-structural proteins, so delaying interferon (IFN) expression.[3,17,70,71]

Ultimately, a delayed but excessive reaction of the immune system with an uncontrolled expression of cytokines and chemokines (the so-called cytokine storm) associated with virus-induced cytopathic effects will result in lung epithelial and endothelial cell apoptosis and activation of the coagulation cascade, leading to vascular leakage; alveolar oedema; microvascular thrombosis; and, later, cell proliferation with pulmonary fibrosis.[3,11,47] Cytokine storm in such patients is associated with more severe lung injury, ICU admission, and worse outcome.[3,51,71] To date, no definitive treatment for

Table 2 Clinical and immunological features of viral respiratory infections by highly pathogenic coronaviruses.[47–60] ARDS, acute respiratory distress syndrome; BOOP, bronchiolitis obliterans organising pneumonia; CT, computed tomography; HF, heart failure; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus.

| Clinical signs and symptoms	Transmissibility and mortality rate	Lung pathological features	Immunological features	Radiographic findings
Early stage: moderate disease with flu-like symptoms Advanced stage: ARDS, septic shock, acute kidney injury, HF, coagulation dysfunction, and death (mainly in older patients)	SARS-CoV-2 shows a higher transmissibility rate with respect to SARS-CoV and MERS-CoV MERS-CoV shows a mortality rate higher than SARS-CoV and SARS-CoV-2	Early stage: diffuse alveolar damage (necrosis of alveolar epithelial cells, hyaline membrane formation, infiltration with inflammatory cells, and presence of giant multinucleated cells) Proliferative phase: hyperplasia of Type II pneumocytes (with mild-to-moderate fibrosis and a BOOP pattern)	Initiation phase: antiviral innate immunity in the lung Resolution phase: viral clearance and the process Restoration phase: repair processes associated with viral respiratory tract infections	Chest CT shows bilateral ground glass opacities and subsegmental areas of consolidation
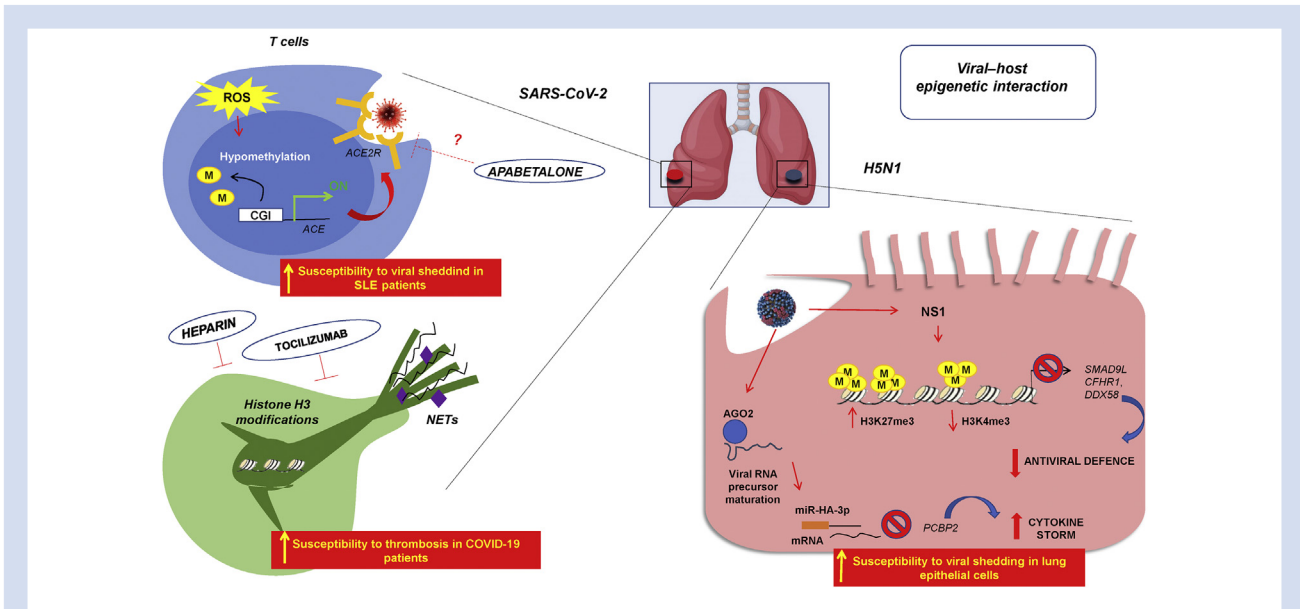
Fig 1. Viral—host epigenetic interactions. We illustrated three putative cell-specific epigenetic-sensitive mechanisms by which SARS-CoV-2 and H5N1 may impact on individual sensitiveness to severe pulmonary critical illness. Mainly, T cells and neutrophils can undergo deoxyribonucleic acid hypomethylation and histone modifications, respectively, in COVID-19 patients. Otherwise, *in vitro* lung epithelial cells upon H5N1 infection can undergo modifications of micro-ribonucleic acid (RNA) patterns and histone tail marks, leading to down-regulation of antiviral defence (see the text for more details). ACE, angiotensin-converting enzyme; AGO2, argonaute 2; CGI, CpG island; COVID-19, coronavirus disease 2019; mRNA, messenger RNA; NET, neutrophil extracellular trap; NS1, non-structural protein 1; PCBP2, poly(RC)-binding protein 2; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SLE, systemic lupus erythematosus.

SARS-CoV, MERS-CoV, and SARS-CoV-2 is available, although several strategies have been studied.[72]

Epigenetic mechanisms and respiratory viral infections

Viruses causing severe pulmonary illness can use three epigenetic-regulated ways during host—pathogen interaction: (i) they can affect host DNA methylation signatures and miR-NAs regulating a cassette of genes underlying innate and adaptive antiviral responses; (ii) they can encode for viral proteins that directly interact with the host modified histones; and (iii) they can manipulate the host miRNA processing nuclear machinery to encode viral non-canonical miRNA-like RNA fragments (v-miRNAs) regulating the viral life cycle and immune response.[73] Here, we focus on epigenetic-sensitive mechanisms by which H5N1 and SARS-CoV-2 may affect susceptibility to pulmonary illness by interfering with both innate and adaptive immune responses in humans[74,75] (Fig. 1 and Table 3).

H5N1 avian influenza A

Histone modifications

By combining multi-omics data, H5N1 antagonised the early host antiviral response by altering histone methylation at Type I IFN-sensitive genes.[18] In detail, NS1 viral protein was

associated with parallel increased H3K27me3 (repressive mark) and decreased H3K4me3 (active mark) levels favouring a heterochromatin state surrounding the *SMAD9L*, *CFHR1*, and *DDX58* genes in human airway cells.[18]

Viral non-canonical miRNA-like RNA fragments

Comparing with DNA virus, a few reports provided proofs of v-miRNAs originating from RNA virus. Varble and colleagues[79] incorporated a hairpin miRNA-124 into the influenza virus genome showing that the engineered virus was able to produce a functional miR-124 without any harmful consequence on the viral life cycle. This suggested that RNA viruses may also exploit the host nuclear RNA machinery to synthesise v-miRNAs, which might contribute to the typical influenza-virus-induced cytokine storm. Successively, Umbach and colleagues[80] demonstrated that the 5′ end of all the eight segments of the influenza virus can codify for small viral leader RNAs, which are involved in the genomic RNA encapsidation to provide a new progeny of virions, suggesting a relevant role in the viral life cycle. Li and colleagues[76] have demonstrated that H5N1 virus encodes miR-HA-3p, a miRNA-like small RNA exacerbating the production of antiviral cytokines by downregulating the poly(RC)-binding protein 2 gene, a master regulator of the retinoic acid-inducible gene-I (RIG)/mitochondrial *antiviral signalling* protein (MAVS) antiviral signalling, in human macrophages. This proved a novel virulence factor underlying H5N1-

induced cytokine storm and high mortality[76]; however, v-miRNA biogenesis and function remain to be clarified.[81] Overall, the virus skill to produce functional miRNAs can be also exploited to construct delivery systems of miRNAs based on RNA viruses as molecular vectors.[82]

Severe acute respiratory syndrome coronavirus 2

DNA methylation

The high transmissibility and asymptomatic infection rates of SARS-CoV-2 may be caused by a more efficient virus replication and reduced IFN production in lung tissues.[83] As both SARS-CoV-2 and MERS can reprogramme the host epigenome, we hypothesise a possible role for epigenetic drivers underlying susceptibility to COVID-19.

Sawalha and colleagues[77] have proposed oxidative-stress-induced epigenetic pathways linked to ACE2 deregulation to increase susceptibility and severity of COVID-19 in patients affected by systemic lupus erythematosus (SLE). Indeed, the ACE2 gene CpG hypomethylation status characterising SLE patients could be exacerbated upon SARS-CoV-2 infection leading to further ACE2 protein overexpression in T cells, thus promoting viral infections and dissemination.[77] Disease-related epigenetic perturbations might be hotspots favouring viral infection and provide risk biomarkers useful to stratify sensitiveness to infection and disease severity in patients more prone to disseminate SARS-CoV-2 infection. Consistent with this, a bioinformatic analysis focusing on *ACE2* gene has supported the hypothesis for which DNA methylation signatures are dependent on host cell type and gender and age, which are risk factors associated with increased susceptibility to COVID-19 and poor prognosis.[84]

NETosis

Patients affected by COVID-19 are at higher risk of thromboembolic events and disseminated intravascular coagulation.[85] As evidence of neutrophil lung infiltration, Barnes and colleagues[78] have emphasised the key role of NETosis in contributing to organ damage and mortality of patients affected by COVID-19. Physiologically, NETosis is a form of innate immunity, in which the neutrophil cell death is guided from histone H3 modifications and release of neutrophil extracellular traps (NETs), complexes of DNA fibres, histones, and proteins aimed to provide a scaffold for platelet adhesion and aggregation to entrap pathogens and avoid their diffusion.[86] Moreover, NETs can induce macrophages to secrete IL1B to further sustain the signalling loop between macrophages and neutrophils, leading to progressive inflammatory damage. Previously, NETosis dysregulation was linked to thrombotic events,[86] ARDS, pulmonary inflammation, and extensive lung damage.[87] Interestingly, there is evidence for which heparin can dismantle NETs and prevent histone-induced platelet aggregation. This might represent the molecular basis for which heparin treatment reduces mortality in subjects affected by severe COVID-19, which develop sepsis-induced coagulopathy.[88] Thus, deoxyribonuclease I-mediated degradation of NETs could provide a therapeutic avenue to suppress excess injury in patients severely affected by COVID-19.[89]

Table 3 Major epigenetic mechanisms during viral respiratory infections. ACE2, angiotensin-converting enzyme 2; CFHR1, complement factor H-related 1; DDX58, DExD/H-box helicase 58; NET, neutrophil extracellular trap; PCBP2, poly(RC) binding protein 2; SAMD9L, sterile alpha motif domain-containing protein 9-like; v-miRNA, viral micro-ribonucleic acid.

Sample source	Epigenetic modification	Region	Gene target	Effect	References
H5N1 avian influenza A Human airway cells	Increased levels of H3K27me3 and decreased levels of H3K4me3	Regulatory regions	SMAD9L, CFHR1, and DDX58	Impaired host antiviral response	Chiu and Openshaw[75]
Human macrophages	miR-HA-3p (v-miRNA)	—	PCBP2	Downregulation of PCBP2 antiviral signalling	Li and colleagues[76]
Sars-CoV-2 T cells of lupus patients	DNA hypomethylation	CpG sites	ACE2	Increased expression of ACE2 protein leading to higher susceptibility to infection	Sawalha and colleagues[77]
Lung biopsy	NETosis	Nucleosomes	—	Increased risk for thromboembolic events in patients affected by COVID-19	Barnes and colleagues[78]

Table 4 Epidrugs and repurposed drugs in viral respiratory infections. ACE2, angiotensin-converting enzyme 2; A549, human Type II pneumocyte cell line; BETi, bromodomain and extra-terminal domain (BET) inhibitor; HDACi, histone deacetylase inhibitor; IL-6, interleukin-6; miRNA, micro-ribonucleic acid; MYD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa-light-chain enhancer of activated B cell; NHBE, normal human bronchial epithelial cell; RA, rheumatoid arthritis; RANTES, C—C chemokine ligand 5; TCZ, tocilizumab; TLR, Toll-like receptor; TNF-α, tumour necrosis factor-alpha; T2D, Type 2 diabetes.

Chemical compound	Epigenetic-oriented mechanism of action	Viral infection	Proinflammatory/cytokine storm target	Epigenetic-related effects	Sample source	References
De novo epidrug						
Curcumin	HDACi	H1N1	NF-κB	Downregulation of inflammation	Human macrophages	Xu and Liu[93]
Apabetalone	BET2/4i	SARS-CoV-2	ACE2	Possible reduction of viral infection and replication	Human cells	Gordon and colleagues[94]
Epidrug repurposing						
Statins	HDACi	H1N1	RANTES	Block of key factors in virus infectivity	NHBE, A549	Lee and colleagues[95]
		—	TLR-MYD88-NFKB axis	Possible use in MERS-CoV infection	—	Yuan[96]
Metformin	HDACi	H1N1	TNF-α	Reduction of proinflammatory late/exhausted memory B-cell subset and increased antibody response to the influenza vaccine	B cells from diabetics (T2D) *vs* healthy subjects	Diaz and colleagues[97]
Immunomodulator repurposing						
TCZ	Not properly defined epidrug	SARS-CoV-2	IL-6	Reduction of inflammation by impacting on NETosis and upregulating circulating miRNA-23, miRNA-146, and miRNA-223	Neutrophils and plasma collected from AR patients	Ruiz-Limón and colleagues[98] and Müller and colleagues[99]

Table 5 Novel and repurposed drugs modulating epigenetic pathways in clinical trials to treat viral pulmonary infections.

Epidrug	Conditions	Study type	Study title	Aim	Status/phase	ID
Influenza viruses						
Repurposed drugs						
Statins	Influenza	Interventional; 116 participants; randomised	Statin therapy in acute influenza	To test whether the anti-inflammatory effects of statins will decrease the severity of illness in patients who are infected with influenza	Completed/Phase 2	NCT02056340
	Patients hospitalised with community-acquired pneumonia	Adults; older patients	Statins in the prevention of myocardial damage in pneumonia	To test the efficacy in preventing cardiovascular complications	Ongoing	EudraCT number: 2013-002799-42
	Patients affected by sepsis	Adults; older patients	Randomised, double-blind, placebo-controlled trial of 40 mg day^{-1} of atorvastatin on reduction in severity of sepsis in ward patients	To test the efficacy in preventing sepsis-related complications	Ongoing	EudraCT number: 2005-004636-52
Coronaviruses						
Drugs interfering with epigenetic pathways						
Ruxolitinib with simvastatin	Coronavirus infection	Interventional; 94 participants; randomised	Study of Ruxolitinib Plus Simvastatin in the Prevention and Treatment of Respiratory Failure of COVID-19 (Ruxo-Sim-20)	To test the combined use of ruxolitinib with simvastatin looking for a synergistic effect in the inhibition of viral entry and in the anti-inflammatory effect	Phases 1 and 2/recruiting	NCT04348695
Epigenetic-oriented repurposed drugs						
Artemisinin, curcumin, frankincense, and vitamin C (ArtemiC)	COVID-19 coronavirus infection; SARS-CoV-2 coronavirus infection	Interventional; 50 participants; randomised	A Phase II controlled clinical study designed to evaluate the effect of ArtemiC in patients diagnosed with COVID-19	To test the efficacy in treatment for the COVID-19 as a food supplement	Phase 2/recruiting	NCT04382040
Vitamin C	Vitamin C, pneumonia, viral, pneumonia, ventilator associated	Interventional; 140 participants; randomised	Vitamin C infusion for the treatment of severe 2019-nCoV infected pneumonia	To test vitamin C infusion in improving the prognosis of patients	Phase 2/recruiting	NCT04264533
Tocilizumab	COVID-19 pneumonia	Interventional; 398 participants; randomised	Efficacy of early administration of tocilizumab in COVID-19 patients	To test whether early administration of tocilizumab compared with late administration of tocilizumab can reduce the number of patients with COVID-19 who require mechanical ventilation	Phase 2/recruiting	NCT04346355

Continued

Table 5 Continued

Epidrug	Conditions	Study type	Study title	Aim	Status/phase	ID
	COVID-19 pneumonia	Interventional; 400 participants; randomised	A RCT—Safety & Efficacy of Tocilizumab—Tx of Severe COVID-19: ARCHITECTS (ARCHITECTS)	To evaluate the clinical efficacy and safety of tocilizumab relative to placebo amongst approximately 300 hospitalised adult patients who have severe COVID-19	Phase 3/recruiting	NCT04412772
	COVID-19 pneumonia	Interventional; 206 participants; randomised	An Open Randomized Therapeutic Trial Using ANAKINRA, TOCILIZUMAB Alone or in association with RUXOLITINIB in Severe Stage 2b and 3 of COVID19-associated Disease (INFLAMMACOV)	To test the efficacy in treatment for the COVID-19	Phase 3/not yet recruiting	NCT04424056
Tocilizumab	COVID-19 pneumonia	Interventional; 60 participants; randomised	Experimental use of tocilizumab (RoActemra®) in severe SARS-CoV-2-related pneumonia	To evaluate the safety and efficacy of tocilizumab (RoActemra) in hospitalised adults diagnosed with COVID-19	Ongoing	EudraCT number: 2020-001770-30
	COVID-19 pneumonia	Interventional; 500 participants	A multicentre, open-label clinical trial to evaluate the effectiveness and safety of i.v. tocilizumab for treating patients with COVID-19 pneumonia: the BREATH-19 study	To evaluate the effectiveness of i.v. tocilizumab in treating patients with COVID-19 pneumonia	Ongoing	EudraCT number: 2020-001995-13
	COVID-19 pneumonia with hypoxia	Interventional; 354 participants; randomised	Pre-emptive tocilizumab in hypoxic COVID-19 patients, a prospective randomised trial	To assess in a randomised comparison the effect of pre-emptive tocilizumab in patients with hypoxia attributable to COVID-19 on 30-day mortality (from randomisation)	Ongoing	EudraCT number: 2020-001375-32
	COVID-19 pneumonia	Interventional; 276 participants; randomised	Pilot, randomised, multicentre, open-label clinical trial of combined use of hydroxychloroquine, azithromycin, and tocilizumab for the treatment of SARS-CoV-2 infection (COVID-19)	To evaluate in-hospital mortality or mechanical ventilation in the ICU, or need for a rescue dose of tocilizumab in patients with confirmed infection by COVID-19 in treatment with hydroxychloroquine and azithromycin combined or non-tocilizumab	Ongoing	EudraCT number: 2020-001442-19

Continued

Table 5 Continued

Epidrug	Conditions	Study type	Study title	Aim	Status/phase	ID
	COVID-19 pneumonia	Interventional; 78 participants; randomised	Single-centre, randomised, open-label clinical trial on the efficacy of tocilizumab in modifying the inflammatory parameters of patients with COVID-19	To assess the impact of administering two different tocilizumab regimens *vs* the standard of care on IL-12 levels in patients with non-severe COVID-19 pneumonia	Ongoing	EudraCT number: 2020-002032-69
	COVID-19 pneumonia	Interventional; 330 participants	Multicentre study on the efficacy and tolerability of tocilizumab in the treatment of patients with COVID-19 pneumonia	To describe: (i) whether IL-6 and CRP levels are predictive of treatment efficacy (ii) trend of the Pa_{O_2}/F_{IO_2} ratio (iii) trend of lymphocyte count (iv) change of the 'Sequential organ failure assessment' (v) remission of respiratory symptoms to describe the toxicity of tocilizumab	Ongoing	EudraCT number: 2020-001110-38
Tocilizumab	COVID-19 severe pneumonia	Interventional; 450 participants; randomised	A randomised, double-blind, placebo-controlled, multicentre study to evaluate the safety and efficacy of tocilizumab in patients with severe COVID-19 pneumonia	To evaluate the efficacy, safety, pharmacokinetics, and pharmacodynamics of TCZ compared with placebo in combination with SOC for the treatment of severe COVID-19 pneumonia	Ongoing	EudraCT number: 2020-001154-22
	COVID-19 pneumonia	Interventional; 24 participants; randomised	A randomised, controlled, open-label, Phase II trial to evaluate the efficacy and safety of tocilizumab combined with pembrolizumab (MK-3475) in patients with coronavirus disease 2019 (COVID-19) pneumonia who are unresponsive to standard care	To assess the efficacy—as determined by the proportion of patients with normalisation of $Sp_{O_2} \geq 96\%$—of continued standard care together with tocilizumab plus pembrolizumab (MK-3475) in patients with COVID-19 pneumonia who are non-responsive to front-line therapy within 48 h from treatment initiation	Ongoing	EudraCT number: 2020-001160-28
	COVID-19 severe pneumonia	Interventional; 200 participants; randomised	Effectiveness of interleukin-6 receptor inhibitors in the management of patients	To investigate the effect of different types of IL-6 inhibition *vs* no adjuvant treatment compared	Ongoing	EudraCT number: 2020-001275-32

Table 5 Continued

Epidrug	Conditions	Study type	Study title	Aim	Status/phase	ID
			with severe SARS-CoV-2 pneumonia: an open-label, multicentre, sequential RCT	with standard of care in patients with severe SARS-CoV-2 pneumonia		
Bevacizumab	COVID-19 severe pneumonia	Interventional; 20 participants	A Pilot Study of Bevacizumab in the Treatment of Severe or Critical Patients with COVID-19 Pneumonia (BEST-CP)	To evaluate bevacizumab for the treatment of ALI/ARDS and reduction of mortality in severe and critical COVID-19 patients through suppression of pulmonary oedema	Recruiting	NCT04348695
	COVID-19 severe pneumonia	Interventional; 140 participants; randomised	Bevacizumab in severe or critically severe patients with COVID-19 pneumonia RCT	To evaluate the efficacy in treatment for COVID-19	Recruiting	NCT04305106

Epitranscriptome

The epitranscriptome of SARS-CoV-2 was analaysed as a possible strategy to dissect the hidden layer of viral regulation. By using nanopore direct RNA sequencing, almost 41 RNA modification sites, mostly located in the AAGAA motif on viral transcripts with shorter poly(A) tails, have been suggested.[90] As poly(A) tails play a relevant role in RNA turnover and stability, the proposed modifications may represent one of the molecular mechanisms by which SARS-CoV-2 evades the host immune response and indices the cytokine storm.[90] Besides, the authors did not identify what type of RNA modifications occur at these sites and the mechanisms underlying COVID-19 pathogenesis.[90]

Epitherapy and immune modulators

Nowadays, the emergence of drug-resistant pathogens continuously increases, thus the discovery of novel drugs or the repositioning of already-approved drugs is needed.[16] Epitherapy may provide further therapeutic opportunities to control viral—host interaction during critical illness.[91] In particular, the current emergence of COVID-19 is guiding researchers towards the possible repurposing of Food and Drug Administration (FDA)-approved epidrugs,[92] including metformin and statins, which may be effective against the novel SARS-CoV-2 infection. Here, we give an update on clinical evidence about the usefulness of novel and FDA-approved drugs interfering with epigenetic pathways, which were applied to ICU patients affected by highly pathogenic strains of influenza virus and CoV, with a particular interest about the novel SARS-CoV-2 (Table 4).

In recent years, a few epidrugs have been introduced into clinic use (e.g. vorinostat and belinostat mainly to treat haematological malignancies), and a wide range of epigenetic-based drugs are undergoing trials, which will clarify whether pharmacological epigenetic modulation is of clinical interest (Table 5). The road from discovery to clinical approval requires long timelines; thus, the repurposing of old drugs interfering with epigenetic pathways is another goal. In this case, epigenetic effect is inevitably going to be 'off-target' in comparison with the drug action used in the first place. Experimental evidence demonstrated that the effects of epidrugs were achieved at lower doses with prolonged exposure, whereas higher drug concentrations were detrimental. However, when ongoing trials will be completed, we will establish clinically if the dose used for epigenetic modification reversal would be the same as, less than, or more than that used for the original purpose.

De novo epidrugs

Curcumin

Curcumin, belonging to the histone deacetylase inhibitor (HDACi) group, is a natural polyphenol extracted from turmeric with a wide range of molecular targets and drug activities, including anti-inflammatory properties. Interestingly, after H1N1 infection, curcumin treatment downregulated the secretion of proinflammatory cytokines and expression of the nuclear factor kappa-light-chain enhancer of activated B cell (NF-κB) gene in human macrophages without affecting cell viability.[93] This evidence suggests that curcumin may confer protection against influenza A virus-induced ALI by counteracting the cytokine signalling without damaging the immune

system.[93] Interestingly, curcumin and demetoxycurcumin have been indicated as possible inhibitors of COVID-19 virus main protease, which plays a crucial function in controlling viral replication and transcription of SARS-CoV-2, suggesting a putative useful drug–target interaction to be validated in clinical trials.[100]

Apabetalone

An international consortium of scientists has identified 50 proteins as putative drug targets against COVID-19. Amongst these targets, bromodomain (BRD) 2/4 would be relevant during interaction with the (E) envelope proteins of SARS-CoV-2 and viral reproduction.[94] By mimicking the histone structure, the (E) envelope proteins might potentially disrupt BRD-histone complexes. BRD proteins are epigenetic players that bind acetylated groups on histone proteins to aid in the recruitment of transcriptional machinery at promoter genes. Apabetalone can directly inhibit BET2/4 protein–SARS-CoV-2 interaction and may downregulate the expression of ACE2 receptors, which are exploited by the surface S glycoprotein to enter into human cells.[101] Currently, apabetalone is not approved by FDA, but has already shown clinical safety as demonstrated during the Phase 3 trial (BETonMACE) focusing on secondary prevention of cardiovascular dysfunction in diabetics.[102] Overall, this evidence suggests that apabetalone may potentially reduce viral infection and replication. In this way, Resverlogix Corporation (https://bit.ly/2CBaEKB) invites collaborators for further research on apabetalone as a putative therapeutic strategy for COVID-19.

Epidrug repurposing

Statins

Influenza viruses

Statins are hydroxymethylglutaryl-coenzyme A reductase inhibitors normalising lipid levels with pleiotropic epigenetic-oriented effects by acting as HDACi. As statins also show anti-inflammatory effects,[103] they were thought to block the cytokine storm triggered by influenza viruses.[95] Indeed, experimental evidence reported that statins can affect various molecular pathways underlying the virus life cycle, representing promising alternatives for influenza treatments by limiting apoptosis.[95,104] However, retrospective clinical trials and epidemiological studies provided mixed results regarding the relationship between statin use and reduction of influenza virus morbidity.[104] Evidence from four large clinical trials demonstrated that patients treated with statins may have a reduced immune response to the influenza vaccine with respect to controls (untreated patients).[103,105–107] However, it should be noted that these studies enrolled patients with underlying acute respiratory or cardiovascular diseases; thus, the reduced response to influenza vaccine and the apparent major risk of influenza-like diseases in patients treated with statins might arise from pre-existent chronic diseases, independently from statin therapy. A completed clinical trial (NCT01427309) that enrolled 31 989 participants demonstrated that the administration of high-dose influenza vaccine to patients treated with statins may result in higher levels of antibodies and protection against the influenza viruses *vs* standard-dose vaccine.[108] However, as statins are widely prescribed cardioprotective drugs, major knowledge about the molecular mechanisms by which they can control the cytokine overexpression and modulate the intense inflammatory response is needed to design novel combination therapies to improve customised treatments.

Coronaviruses

Clinical evidence about the use of statins in the treatment of viral pneumonia is limited and provided mixed results. Indeed, two trials reported that the anti-inflammatory effects of statins may reduce cardiovascular risk and mortality in ICU patients affected by pneumonia.[109,110] Otherwise, the results of one randomised clinical trial did not support statin use in those patients with ventilator-associated pneumonia.[111] Currently, there is no clinical or experimental evidence supporting the assertion that statins can improve clinical management of COVID-19. As the rates of acute events and mortality associated with COVID-19 infection are extremely high in patients with cardiovascular diseases (10.8%) and diabetics (7.3%), which generally use statins as primary or secondary prevention, these patients should continue the treatment when SARS-CoV-2 infection is suspected or diagnosed (https://www.acc.org/latest-in-cardiology/features/~/media/Non-Clinical/Files-PDFs-Excel-MS-Word-etc/2020/02/S20028-ACC-Clinical-Bulletin-Coronavirus.pdf). Statins are cleared by the liver metabolism and can increase the level of transaminases in cardio-hepatic patients[112]; thus, strict evaluation and monitoring of statin therapy should be provided for COVID-19 patients, which commonly show an elevation of the aminotransferases (aspartate transaminase and alanine aminotransferase), with occasional alkaline phosphatase and total bilirubin elevations underlying a high risk for hepatotoxicity.[113,114] Whether a *de novo* use of statin therapy may play a key role in preventing COVID-19 complications remains to be elucidated. Remarkably, experimental studies supported the hypothesis for which an early and high dose of statins might be a useful strategy for the treatment of MERS-CoV infections by directly affecting the TLR–MYD88–NF-κB axis, which plays a pivotal role during CoV infections.[96,115,116] Statins are the most common FDA-approved drugs classified as TLR–MYD88 antagonists; moreover, under normal conditions, statins did not strongly alter MYD88 levels, whereas they maintain basal MYD88 levels during stress and hypoxia.[96] This supports that statins might be protective for patients affected by COVID-19. Thus, the putative regulation of MYD88 pathway *via* statins may be an attractive field of research to explore how to protect innate immune response against novel viral respiratory infections, including SARS-CoV-2.

Metformin

Influenza viruses

Metformin, belonging to HDACi class, is the first-line anti-hyperglycaemic drug for type 2 diabetes (T2D) patients, which can indirectly reduce chronic inflammation by normalising glucose levels or directly impact on inflammatory pathways. Recently, Saenwongsa and colleagues[117] have demonstrated

that after the trivalent inactivated influenza vaccine (TIV), both the IgG antibody response and IFN-α expression were impaired in T2D patients treated with metformin *via* repression of rapamycin (mTOR)-mediated pathway and impaired IgG avidity index, leading to increased sensitiveness to H1N1 and H3N2 infection. This suggests that the TIV may not be suitable for T2D patients treated with metformin by emphasising the necessity of developing a more customised strategy for influenza prevention in high-risk groups. Metformin may recover the influenza vaccine responses in T2D patients (treated and non-treated with metformin) by improving the B-cell function *via* parallel downregulation of inflammation and upregulation of AMPK phosphorylation (active form), a metabolic enzyme involved also in antibody responses.[97] Understanding of the metformin effects on the immune system may guide the repurposing of this drug focused on therapeutic intervention on metabolism in inflammatory diseases.

Immunomodulators and antivirals: could they impact on epigenetic pathways underlying cardiovascular dysfunction in COVID-19?

The repurposing of both immunomodulators and antivirals, including tocilizumab (TCZ), remdesevir, favipiravir, hydroxychloroquine, and chloroquine, could be a fast way to get effective treatments whilst a preventive vaccine will be available (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). In the current COVID-19 pandemic, TCZ is one of the most promising repurposed drugs under clinical investigation for the treatment of severe hospitalised pneumonia patients. Tocilizumab is a humanised monoclonal antibody that can counteract the cytokine storm by blocking the interleukin-6 (IL-6) receptor signalling associated with a high risk of cardiovascular mortality.[118] Immunomodulators can counteract the overactive inflammatory response, which seems to be the driver of increased disease severity. Many old anti-inflammatory drugs are in clinical trials, such as sarilumab (NCT04315298; Phases 2 and 3) and TCZ (NCT04320615; Phase 3). Besides, the efficacy of antivirals inhibiting viral replication, such as favipiravir (NCT04358549; Phase 2) and remdesevir (NCT04292730; Phase 3), is being evaluated in clinical trials compared with standard of care. The impact of COVID-19 on cardiovascular health is an urgent question for physicians.[119] As epigenetics plays an increasing role in cardiovascular diseases and inflammation,[102,120] and also during SARS-CoV-2 infection,[77] we emphasised the need to clarify if these drugs could potentially impact the associated cardiac dysfunction modulating the severity of the disease.

Clinical evidence from rheumatoid arthritis patients demonstrated that TCZ therapy can prevent cardiovascular dysfunction via two main epigenetic-sensitive mechanisms: (i) reduction of NETosis; and (ii) upregulation of miRNA-23, miRNA-146, and miRNA-223 serum levels.[98] Overall, this suggests that TCZ can improve the pro-atherosclerotic status by regulating dyslipidaemias, endothelial dysfunction, inflammation, and oxidative stress.[98] Moreover, TCZ-treated rheumatoid arthritis patients demonstrated a differential expression of 85 lncRNAs in CD14$^+$ monocytes regulated by IL-6 or tumour necrosis factor-alpha.[99]

Preliminary trials have suggested the usefulness of chloroquine or hydroxychloroquine repurposing in the treatment of COVID-19, which is correlated with the ability of these antimalarial agents in interfering with the cellular-mediated viral endocytosis.[121] However, a randomised trial demonstrated that hydroxychloroquine failed in preventing symptomatic infection when taken within 4 days after exposure.[122] At epigenetic level, hydroxychloroquine can exert an inhibitory activity against the polycomb repressive complex 2 (PRC2), responsible of switching chromatin towards a compacted state (inactive gene expression) in blood malignancies.[123] Interestingly, influenza A viruses, MERS-CoV, and SARS-CoV are able to activate the PRC2, which, in turn, increases the levels of H3K27me3 (repressive mark) at the promoters of targeted IFN-stimulated genes to counteract the host antiviral immune response.[124] This let us suggest that also SARS-CoV-2 might use the same mechanisms to inactive IFN-related pathways in infected cells; besides, since polycomb inhibitors have shown a general antiviral activity,[125] it might be useful to investigate whether hydroxychloroquine can directly impact on PRC2 activity in COVID-19.

As reported, the clinical effect of i.v. remdesivir seems to be relatively modest; however, a randomised Phase 3 clinical trial (NCT04292899) did not find a significant difference in efficacy between 5- and 10-day courses of this drug.[126] Until now, there is no direct evidence that antivirals, such as remdesevir and favipiravir, would impact epigenetic-sensitive ways during their mechanism of action.

Concluding remarks

Targeted epigenetic-sensitive molecular networks are temporally manipulated during virus−host interactions, providing further risk factors for viral shedding and inadequate immune response also in patients affected by COVID-19, in which ACE2 promoter hypomethylation may be one of the relevant drivers. During the current COVID-19 pandemic, we need to understand why a part of the population becomes critically infected when exposed to low viral load, whilst other subjects are less responsive when exposed to high viral loads. As the influenza virus may also promote acute coronary syndromes (which can be reduced by vaccine),[127] the issue of co-infection (i.e. SARS-CoV-2 and influenza viruses) needs to be further explored both in terms of epigenetic-sensitive tandem events and in critical care. For better risk stratification, it would be needed to clarify the SARS-CoV-2 basic mechanisms of action and how these impact on the individual genetic/epigenetic background and pre-existent cardio-metabolic diseases highly correlated with mortality rate, especially in the elderly.[128,129]

Clinical treatment of mild forms of COVID-19 should not be phobic from fever, which can promote an effective immune response and virus clearance from the body.[130] Ongoing controlled clinical trials would clarify the repositioning of TCZ, whereas curcumin, apabetalone, metformin, and statins could be an effective treatment to protect innate immune response against severe viral respiratory infections. In the era of network medicine, predictive analysis tools are playing a relevant role in addressing the COVID-19 pandemic by providing maps of human proteins interacting with SARS-CoV-2 proteins[101] and a list of candidate repurpose drugs and potential drug combinations targeting SARS-CoV-2.[131] The epigenetic susceptibility to pulmonary viral infections requires further investigation by using the network-oriented analysis to clarify the molecular routes underlying perturbation of the human interactome,[132] in particular in COVID-19 and its impact on cardiovascular health.

Authors' contributions

Design/implementation of the review: EC, GB, CN
Critical feedback: NF-M, MG
Writing of paper: all authors

Declarations of interest

The authors declare that they have no conflicts of interest.

References

1. Jhung MA, Swerdlow D, Olsen SJ, et al. Epidemiology of 2009 pandemic influenza A (H1N1) in the United States. *Clin Infect Dis* 2011; **52**: 13—26
2. Qun L, Lei Z, Minghao Z, Zhiping C, Furong L, Huanyu W. Epidemiology of human infections with avian influenza A(H7N9) virus in China. *N Engl J Med* 2014; **370**: 520—32
3. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 2016; **14**: 523—34
4. Perlman S. Another decade, another coronavirus. *N Engl J Med* 2020; **382**: 760—2
5. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727—33
6. Nicholls JM, Poon LL, Lee KC, et al. Lung pathology of fatal severe acute respiratory syndrome. *Lancet* 2003; **361**: 1773—8
7. Bradley BT, Bryan A. Emerging respiratory infections: the infectious disease pathology of SARS, MERS, pandemic influenza, and *Legionella*. *Semin Diagn Pathol* 2019; **36**: 152—9
8. Ding Y, He L, Zhang Q, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J Pathol* 2004; **203**: 622—30
9. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* 2012; **76**: 16—32
10. Tavares LP, Teixeira MM, Garcia CC. The inflammatory response triggered by Influenza virus: a two edged sword. *Inflamm Res* 2017; **66**: 283—302
11. Li G, Fan Y, Lai Y, et al. Coronavirus infections and immune responses. *J Med Virol* 2020; **92**: 424—32
12. Zaim S, Chong JH, Sankaranarayanan V, Harky A. COVID-19 and multi-organ response. *Curr Probl Cardiol* 2020; **45**: 100618
13. Schäfer A, Baric RS. Epigenetic landscape during coronavirus infection. *Pathogens* 2017; **6**: 8
14. Busslinger M, Tarakhovsky A. Epigenetic control of immunity. *Cold Spring Harb Perspect Biol* 2014; **6**: a019307
15. Crimi E, Cirri S, Benincasa G, Napoli C. Epigenetics mechanisms in multiorgan dysfunction syndrome. *Anesth Analg* 2019; **129**: 1422—32
16. Crimi E, Benincasa G, Cirri S, Mutesi R, Faenza M, Napoli C. Clinical epigenetics and multidrug-resistant bacterial infections: host remodelling in critical illness. *Epigenetics* 2020; **1**—**13**
17. Comar CE, Goldstein SA, Li Y, Yount B, Baric RS, Weiss SR. Antagonism of dsRNA-induced innate immune pathways by NS4a and NS4b accessory proteins during MERS coronavirus infection. *mBio* 2019; **10**. e00319—19
18. Menachery VD, Eisfeld AJ, Schäfer A, et al. Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. *mBio* 2014; **5**. e01174—14
19. Marazzi I, Ho JS, Kim J, et al. Suppression of the antiviral response by an influenza histone mimic. *Nature* 2012; **483**: 428—33
20. Qin S, Liu Y, Tempel W, et al. Structural basis for histone mimicry and hijacking of host proteins by influenza virus protein NS1. *Nat Commun* 2014; **5**: 3952
21. Ivashchenko A, Rakhmetullina A, Akimniyazova A, Aisina D, Pyrkova A. The miRNA complexes against coronaviruses COVID-19, SARS-CoV, and MERS-CoV. *Res Sq* 2020. https://doi.org/10.21203/rs.3.rs-19592/v1. Advance Access published on March 31
22. Zhi L, Jianwei W, Yuyu X, et al. Implications of the virus-encoded miRNA and host miRNA in the pathogenicity of SARS-CoV-2. *arXiv* 2020. Advance Access published on April 10, https://arxiv.org/abs/2004.04874
23. Vachharajani V, Liu T, McCall CE. Epigenetic coordination of acute systemic inflammation: potential therapeutic targets. *Expert Rev Clin Immunol* 2014; **10**: 1141—50
24. Schiano C, Benincasa G, Franzese M, et al. Epigenetic-sensitive pathways in personalized therapy of major cardiovascular diseases. *Pharmacol Ther* 2020; **210**: 107514
25. Pascua PN, Choi YK. Zoonotic infections with avian influenza A viruses and vaccine preparedness: a game of mix and match. *Clin Exp Vaccine Res* 2014; **3**: 140—8
26. Claas EC, Osterhaus AD, van Beek R, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet* 1998; **351**: 472—7
27. Beigel JH, Farrar J, Han AM, et al. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 2005; **353**: 1374—85
28. Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus, Abdel-Ghafar AN, Chotpitayasunondh T, *et al.* Update on avian influenza A (H5N1) virus infection in humans. *N Engl J Med* 2008; **358**: 261—73
29. Domínguez-Cherit G, Lapinsky SE, Macias AE, et al. Critically ill patients with 2009 influenza A(H1N1) in Mexico. *JAMA* 2009; **302**: 1880—7
30. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009; **360**: 2605—15. https://doi.org/10.1056/NEJMoa0903810

31. Jain S, Kamimoto L, Bramley AM, et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April–June 2009. N Engl J Med 2009; 361: 1935–44

32. Kumar A, Zarychanski R, Pinto R, et al. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. JAMA 2009; 302: 1872–9

33. ANZIC Influenza Investigators, Webb SA, Pettilä V, Seppelt I, et al. Critical care services and 2009 H1N1 influenza in Australia and New Zealand. N Engl J Med 2009; 361: 1925–34. https://doi.org/10.1056/NEJMoa0908481

34. Dominguez-Cherit G, De la Torre A, Rishu A, et al. Influenza A (H1N1pdm09)-related critical illness and mortality in Mexico and Canada, 2014. Crit Care Med 2016; 44: 1861–70

35. Iorio AM, Bistoni O, Galdiero M, et al. Influenza viruses and cross-reactivity in healthy adults: humoral and cellular immunity induced by seasonal 2007/2008 influenza vaccination against vaccine antigens and 2009 A(H1N1) pandemic influenza virus. Vaccine 2012; 30: 1617–23

36. To KF, Chan PK, Chan KF, et al. Pathology of fatal human infection associated with avian influenza A H5N1 virus. J Med Virol 2001; 63: 242–6

37. Peiris JS, Yu WC, Leung CW, et al. Re-emergence of fatal human influenza A subtype H5N1 disease. Lancet 2004; 363: 617–9

38. Mauad T, Hajjar LA, Callegari GD, et al. Lung pathology in fatal novel human influenza A (H1N1) infection. Am J Respir Crit Care Med 2010; 181: 72–9

39. Shieh WJ, Blau DM, Denison AM, et al. 2009 Pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. Am J Pathol 2010; 177: 166–75

40. Gao R, Bhatnagar J, Blau DM, et al. Cytokine and chemokine profiles in lung tissues from fatal cases of 2009 pandemic influenza A (H1N1): role of the host immune response in pathogenesis. Am J Pathol 2013; 183: 1258–68

41. Zheng J, Perlman S. Immune responses in influenza A virus and human coronavirus infections: an ongoing battle between the virus and host. Curr Opin Virol 2018; 28: 43–52

42. Liu Q, Zhou Y-H, Yang Z-Q. The cytokine storm of severe influenza and development of immunomodulatory therapy. Cell Mol Immunol 2016; 13: 3–10

43. Schoen K, Horvat N, Guerreiro NFC, de Castro I, de Giassi KS. Spectrum of clinical and radiographic findings in patients with diagnosis of H1N1 and correlation with clinical severity. BMC Infect Dis 2019; 19: 964

44. de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med 2006; 12: 1203–7

45. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019; 17: 181–92

46. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020; 395: 565–74

47. Liu J, Zheng X, Tong Q, et al. Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV. J Med Virol 2020; 92: 491–4

48. Li JY, You Z, Wang Q, et al. The epidemic of 2019-novel-coronavirus (2019-nCoV) pneumonia and insights for emerging infectious diseases in the future. Microbe. Infect 2020; 22: 80–5

49. Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology 2018; 23: 130–7

50. Hui DS, Azhar EI, Kim YJ, Memish ZA, Oh MD, Zumla A. Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. Lancet Infect Dis 2018; 18: e217–27

51. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395: 497–506

52. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395: 507–13

53. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020; 382: 1708–20

54. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. JAMA 2020; 323: 2052–9

55. Mehra MR, Desai SS, Kuy S, Henry TD, Patel AN. Cardiovascular disease, drug therapy, and mortality in Covid-19. N Engl J Med 2020; 382: e102

56. Grasselli G, Zangrillo A, Zanella A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region. Italy JAMA 2020; 323: 1574–81

57. Chen T, Wu D, Chen H, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ 2020; 368: m1091

58. Shi H, Han X, Jiang N, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis 2020; 20: 425–34

59. Tian S, Xiong Y, Liu H, et al. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. Mod Pathol 2020; 33: 1007–14

60. Ng DL, Al Hosani F, Keating MK, et al. Clinicopathologic, immunohistochemical, and ultrastructural findings of a fatal case of Middle East respiratory syndrome coronavirus infection in the United Arab Emirates. Am J Pathol 2016; 186: 652–8. April 2014

61. Gu J, Gong E, Zhang B, et al. Multiple organ infection and the pathogenesis of SARS. J Exp Med 2005; 202: 415–24

62. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 2020; 46: 586–90

63. Shang J, Wan Y, Luo C, et al. Cell entry mechanisms of SARS-CoV-2. Proc Natl Acad Sci U S A 2020; 117: 11727–34

64. Meyerholz DK, Lambertz AM, McCray Jr PB. Dipeptidyl peptidase 4 distribution in the human respiratory tract: implications for the Middle East respiratory syndrome. Am J Pathol 2016; 186: 78–86

65. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 367: 1260–3

66. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antivir Res 2020; 176: 104742

67. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005; **436**: 112−6

68. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005; **11**: 875−9

69. Seys LJM, Widagdo W, Verhamme FM, et al. DPP4, the Middle East respiratory syndrome coronavirus receptor, is upregulated in lungs of smokers and chronic obstructive pulmonary disease patients. *Clin Infect Dis* 2018; **66**: 45−53

70. Josset L, Menachery VD, Gralinski LE, et al. Cell host response to infection with novel human coronavirus EMC predicts potential antivirals and important differences with SARS coronavirus. *mBio* 2013; **4**. e00165−13

71. Faure E, Poissy J, Goffard A, et al. Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside? *PLoS One* 2014; **9**, e88716

72. Wu R, Wang L, Kuo HD, et al. An update on current therapeutic drugs treating COVID-19. *Curr Pharmacol Rep* 2020: 1−15

73. Gómez-Díaz E, Jordà M, Peinado MA, Rivero A. Epigenetics of host−pathogen interactions: the road ahead and the road behind. *PLoS Pathog* 2012; **8**, e1003007

74. Iwasaki A, Foxman E, Molony R. Early local immune defences in the respiratory tract. *Nat Rev Immunol* 2017; **17**: 7−20

75. Chiu C, Openshaw P. Antiviral B cell and T cell immunity in the lungs. *Nat Immunol* 2015; **16**: 18−26

76. Li X, Fu Z, Liang H, et al. H5N1 influenza virus-specific miRNA-like small RNA increases cytokine production and mouse mortality via targeting poly(rC)-binding protein 2. *Cell Res* 2018; **28**: 157−71

77. Sawalha AH, Zhao M, Coit P, Lu Q. Epigenetic dysregulation of ACE2 and interferon-regulated genes might suggest increased COVID-19 susceptibility and severity in lupus patients. *Clin Immunol* 2020; **215**: 108410

78. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J Exp Med* 2020; **217**, e20200652

79. Varble A, Chua MA, Perez JT, Manicassamy B, García-Sastre A, tenOever BR. Engineered RNA viral synthesis of microRNAs. *Proc Natl Acad Sci U S A* 2010; **107**: 11519−24

80. Umbach JL, Yen HL, Poon LL, Cullen BR. Influenza A virus expresses high levels of an unusual class of small viral leader RNAs in infected cells. *mBio* 2010; **1**. e00204−10

81. Aguado LC, tenOever B. RNA virus building blocks−miRNAs not included. *PLoS Pathog* 2018; **14**, e1006963

82. tenOever BR. RNA viruses and the host microRNA machinery. *Nat Rev Microbiol* 2013; **11**: 169−80

83. Chu H, Chan JF, Wang Y, et al. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. *Clin Infect Dis* 2020. https://doi.org/10.1093/cid/ciaa410. Advance Access published on April 9

84. Corley MJ, Ndhlovu LC. DNA methylation analysis of the COVID-19 host cell receptor, angiotensin I converting enzyme 2 gene (ACE2) in the respiratory system reveal age and gender differences. *Preprints* 2020. https://doi.org/10.20944/preprints202003.0295.v1. Advance Access published on March 19

85. Cattaneo M, Bertinato EM, Birocchi S, et al. Pulmonary embolism or pulmonary thrombosis in COVID-19? Is the recommendation to use high-dose heparin for thromboprophylaxis justified? *Thromb Haemost* 2020. https://doi.org/10.1055/s-0040-1712097. Advance Access published on April 29

86. Benincasa G, Costa D, Infante T, Lucchese R, Donatelli F, Napoli C. Interplay between genetics and epigenetics in modulating the risk of venous thromboembolism: a new challenge for personalized therapy. *Thromb Res* 2019; **177**: 145−53

87. Bendib I, de Chaisemartin L, Granger V, et al. Neutrophil extracellular traps are elevated in patients with pneumonia-related acute respiratory distress syndrome. *Anesthesiology* 2019; **130**: 581−91

88. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost* 2020; **18**: 1094−9

89. Golonka RM, Saha P, Yeoh BS, et al. Harnessing innate immunity to eliminate SARS-CoV-2 and ameliorate COVID-19 disease. *Physiol Genomics* 2020; **52**: 217−21

90. Dongwan K, Joo-Yeon L, Jeong-Sun Y, Jun Won K. The architecture of SARS-CoV-2 transcriptome. *Cell* 2020; **181**: 914−21. e10

91. Nehme Z, Pasquereau S, Herbein G. Control of viral infections by epigenetic-targeted therapy. *Clin Epigenetics* 2019; **11**: 55

92. Ivanov M, Barragan I, Ingelman-Sundberg M. Epigenetic mechanisms of importance for drug treatment. *Trends Pharmacol Sci* 2014; **35**: 384−96

93. Xu Y, Liu L. Curcumin alleviates macrophage activation and lung inflammation induced by influenza virus infection through inhibiting the NF-κB signaling pathway. *Influenza Other Respir Virus.* 2017; **11**: 457−63

94. Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* 2020; **583**: 459−68

95. Lee CS, Yi EH, Lee JK, et al. Simvastatin suppresses RANTES-mediated neutrophilia in polyinosinic−polycytidylic acid-induced pneumonia. *Eur Respir J* 2013; **41**: 1147−56

96. Yuan S. Statins may decrease the fatality rate of Middle East respiratory syndrome infection. *mBio* 2015; **6**. e01120-15

97. Diaz A, Romero M, Vazquez T, Lechner S, Blomberg BB, Frasca D. Metformin improves in vivo and in vitro B cell function in individuals with obesity and type-2 diabetes. *Vaccine* 2017; **35**: 2694−700

98. Ruiz-Limón P, Ortega R, Arias de la Rosa I, et al. Tocilizumab improves the proatherothrombotic profile of rheumatoid arthritis patients modulating endothelial dysfunction, NETosis, and inflammation. *Transl Res* 2017; **183**: 87−103

99. Müller N, Döring F, Klapper M, et al. Interleukin-6 and tumour necrosis factor-α differentially regulate lincRNA transcripts in cells of the innate immune system in vivo in human subjects with rheumatoid arthritis. *Cytokine* 2014; **68**: 65−8

100. Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S, Soetjipto S. Potential inhibitor of COVID-19 main protease (M^{pro}) from several medicinal plant compounds by molecular docking study. *Preprints* 2020. https://doi.org/10.20944/preprints202003.0226.v1. Advance Access published on March 13

101. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 2020; **367**: 1444−8

102. Napoli C, Benincasa G, Schiano C, Salvatore M. Differential epigenetic factors in the prediction of cardiovascular risk in diabetic patients. *Eur Heart J Cardiovasc Pharmacother* 2020; **6**: 239—47

103. Black S, Nicolay U, Del Giudice G, Rappuoli R. Influence of statins on influenza vaccine response in elderly individuals. *J Infect Dis* 2016; **213**: 1224—8

104. Mehrbod P, Omar AR, Hair-Bejo M, Haghani A, Ideris A. Mechanisms of action and efficacy of statins against influenza. *Biomed Res Int* 2014; **2014**: 872370

105. Omer SB, Phadke VK, Bednarczyk RA, Chamberlain AT, Brosseau JL, Orenstein WA. Impact of statins on influenza vaccine effectiveness against medically attended acute respiratory illness. *J Infect Dis* 2015; **213**: 1216—23

106. McLean HQ, Chow BD, VanWormer JJ, King JP, Belongia EA. Effect of statin use on influenza vaccine effectiveness. *J Infect Dis* 2016; **214**: 1150—8

107. Izurieta HS, Chillarige Y, Kelman JA, et al. Statin use and risks of influenza-related outcomes among older adults receiving standard-dose or high-dose influenza vaccines through Medicare during 2010—2015. *Clin Infect Dis* 2018; **67**: 378—87

108. DiazGranados CA, Dunning AJ, Kimmel M, et al. Efficacy of high-dose versus standard-dose influenza vaccine in older adults. *N Engl J Med* 2014; **371**: 635—45

109. Makris D, Manoulakas E, Komnos A, et al. Effect of pravastatin on the frequency of ventilator-associated pneumonia and on intensive care unit mortality: open-label, randomized study. *Crit Care Med* 2011; **39**: 2440—6

110. Douglas I, Evans S, Smeeth L. Effect of statin treatment on short term mortality after pneumonia episode: cohort study. *BMJ* 2011; **342**: d1642

111. Papazian L, Roch A, Charles PE, et al. Effect of statin therapy on mortality in patients with ventilator-associated pneumonia: a randomized clinical trial. *JAMA* 2013; **310**: 1692—700

112. Benincasa G, Cuomo O, Vasco M, et al. Epigenetic-sensitive challenges of cardio-hepatic interactions: clinical and therapeutic implications in heart failure patients. *EJGD* 2020. in press

113. Fan Z, Chen L, Li J, et al. Clinical features of COVID-19-related liver damage. *Clin Gastroenterol Hepatol* 2020; **18**: 1561—6

114. Xu L, Liu J, Lu M, Yang D, Zheng X. Liver injury during highly pathogenic human coronavirus infections. *Liver Int* 2020; **40**: 998—1004

115. Gallelli L, Falcone D, Scaramuzzino M, et al. Effects of simvastatin on cell viability and proinflammatory pathways in lung adenocarcinoma cells exposed to hydrogen peroxide. *BMC Pharmacol Toxicol* 2014; **15**: 67

116. Yuan X, Deng Y, Guo X, Shang J, Zhu D, Liu H. Atorvastatin attenuates myocardial remodeling induced by chronic intermittent hypoxia in rats: partly involvement of TLR-4/MYD88 pathway. *Biochem Biophys Res Commun* 2014; **446**: 292—7

117. Saenwongsa W, Nithichanon A, Chittaganpitch M, et al. Metformin-induced suppression of IFN-α via mTORC1 signalling following seasonal vaccination is associated with impaired antibody responses in type 2 diabetes. *Sci Rep* 2020; **10**: 3229

118. Lindmark E, Diderholm E, Wallentin L, Siegbahn A. Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy. *JAMA* 2001; **286**: 2107—13

119. Clerkin KJ, Fried JA, Raikhelkar J, et al. Coronavirus disease 2019 (COVID-19) and cardiovascular disease. *Circulation* 2020; **141**: 1648—55

120. Napoli C, Benincasa G, Donatelli F, Ambrosio G. Precision medicine in distinct heart failure phenotypes: focus on clinical epigenetics. *Am Heart J* 2020; **224**: 113—28

121. Devaux CA, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? *Int J Antimicrob Agents* 2020; **55**: 105938

122. Boulware DR, Pullen MF, Bangdiwala AS, et al. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. *N Engl J Med* 2020; **383**: 517—25. https://doi.org/10.1056/NEJMoa2016638. Advance Access published on June 3

123. Catalano R, Rocca R, Juli G, et al. A drug repurposing screening reveals a novel epigenetic activity of hydroxychloroquine. *Eur J Med Chem* 2019; **183**: 111715

124. Ayaz S, Crea F. Targeting SARS-CoV-2 using polycomb inhibitors as antiviral agents. *Epigenomics* 2020; **12**: 811—2

125. Arbuckle JH, Gardina PJ, Gordon DN, et al. Inhibitors of the histone methyltransferases EZH2/1 induce a potent antiviral state and suppress infection by diverse viral pathogens. *mBio* 2017 Aug 15; **8**. https://doi.org/10.1128/mBio.01141-17. e01141-17. Advance Access published on August 15

126. Goldman JD, Lye DCB, Hui DS, et al. Remdesivir for 5 or 10 days in patients with severe Covid-19. *N Engl J Med* 2020. https://doi.org/10.1056/NEJMoa2015301. Advance Access published on May 27

127. Phrommintikul A, Kuanprasert S, Wongcharoen W, Kanjanavanit R, Chaiwarith R, Sukonthasarn A. Influenza vaccination reduces cardiovascular events in patients with acute coronary syndrome. *Eur Heart J* 2011; **32**: 1730—5

128. Napoli C, Tritto I, Benincasa G, Mansueto G, Ambrosio G. Cardiovascular involvement during COVID-19 and clinical implications in elderly patients. A Review. *Ann Med Surg (Lond)* 2020; **57**: 236—43

129. Mansueto G, Niola M, Napoli C. Can COVID 2019 disease induce a specific cardiovascular damage or it exacerbates pre-existing cardiovascular diseases? *Pathol Res Pract* 2020; **216**: 153086. https://doi.org/10.1016/j.prp.2020.153086

130. Galdiero M, Napoli C. COVID-19: do not be phobic from fever. *J Infect Public Health* 2020; **13**: 938

131. Zhou Y, Hou Y, Shen J, Huang Y, Martin W, Cheng F. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Discov* 2020; **6**: 14

132. Silverman EK, Schmidt HHH, Anastasiadou E, et al. Molecular networks in network medicine: development and applications. *Wiley Interdiscip Rev Syst Biol Med* 2020, e1489

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