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**Perspective**

Background Severe manifestations of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), such as acute respiratory distress syndrome (ARDS), are characterized by hyperinflammation, exuberant cytokine release, profound and progressive hypoxia, deranged coagulation, and multiorgan failure (1). COVID-19 also presents not infrequently with a devastating thrombotic diathesis, manifesting as arterial thrombosis, pulmonary embolism, deep vein thrombosis, and thrombotic microangiopathy. Not surprisingly, dysregulation of tonic vascular homeostatic functions of endothelial and hematopoietic lineage cells has been implicated in the pathophysiology of COVID-19. Reminiscent of neutrophil and macrophage hyperactivation syndromes, the COVID-19–associated systemic inflammatory response syndrome is likely driven by the activation and/or death of infected cells and collateral damage triggered by explosive production of cytokines and chemokines. Recruited leukocytes spew a fibrin/platelet-entrapping meshwork of DNA and associated histones, which in a vicious cycle further recruits leukocytes and triggers intravascular coagulation through endothelial, platelet, and leukocyte dysfunction (2). These concepts are supported by autopsy studies that have detected clusters of activated and degenerating myeloid cells in both intravascular and extravascular spaces (3) as well as an endotheliitis that is characterized by viral inclusion bodies and SARS-CoV-2 nucleoprotein in the walls of blood vessels (4, 5).Immunothrombosis in COVID-19 Autopsy specimens from patients with overwhelming SARS-CoV-2 infection have demonstrated histopathologic findings of pulmonary microvascular thrombosis (3). When combined with the […]

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# New (re)purpose for an old drug: purinergic modulation may extinguish the COVID-19 thromboinflammatory firestorm

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## Background

Severe manifestations of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), such as acute respiratory distress syndrome (ARDS), are characterized by hyperinflammation, exuberant cytokine release, profound and progressive hypoxia, deranged coagulation, and multiorgan failure (1). COVID-19 also presents not infrequently with a devastating thrombotic diathesis, manifesting as arterial thrombosis, pulmonary embolism, deep vein thrombosis, and thrombotic microangiopathy. Not surprisingly, dysregulation of tonic vascular homeostatic functions of endothelial and hematopoietic lineage cells has been implicated in the pathophysiology of COVID-19. Reminiscent of neutrophil and macrophage hyperactivation syndromes, the COVID-19–associated systemic inflammatory response syndrome is likely driven by the activation and/or death of infected cells and collateral damage triggered by explosive production of cytokines and chemokines. Recruited leukocytes spew a fibrin/platelet-entrapping meshwork of DNA and associated histones, which in a vicious cycle further recruits leukocytes and triggers intravascular coagulation through endothelial, platelet, and leukocyte dysfunction (2). These concepts are supported by autopsy studies that have detected clusters of activated and degenerating myeloid cells in both intravascular and extravascular spaces (3) as well as an endotheliitis that is characterized by viral inclusion bodies and SARS-CoV-2 nucleoprotein in the walls of blood vessels (4, 5).

## Immunothrombosis in COVID-19

Autopsy specimens from patients with overwhelming SARS-CoV-2 infection have demonstrated histopathologic findings of pulmonary microvascular thrombosis (3). When combined with the clinical observations of ventilation-perfusion mismatching and resultant profound hypoxemia, renal failure, pulmonary emboli, ischemic stroke, mesenteric ischemia, and digital necrosis, a common theme emerges — microvascular and macrovascular accretion of thrombus contributes to patient demise. Biomarker studies have revealed a marked imbalance of coagulation and fibrinolysis, which has emerged as a compelling marker of disease severity in COVID-19. Elevated levels of D-dimer, a fibrin(ogen) degradation product, are a hallmark of patients hospitalized with moderate and severe COVID-19, where they predict not only severity, but also mortality (6). Furthermore, greater than 25% of patients with severe COVID-19 will suffer a thrombotic complication characterized by accrual of intravascular fibrin, leukocytes, platelets, and erythrocytes (2). Thrombosis in critical illnesses such as ARDS occurs at the convergence of inflammation and coagulation. Endothelial cells release Weibel-Palade bodies containing vWF, along with a preformed membrane-based storage pool of cell adhesion proteins. Monocytes undergo pyroptosis, liberating the prototypical inflammatory cytokine IL-1β, in addition to shedding microvesicles bearing procoagulant tissue factor (5, 7). Platelets activate neutrophils and self-aggregate in response to ADP and thrombin. Neutrophils release cytokines and extracellular chromatin traps (NETs) decorated with oxidant enzymes and microbicidal proteins. If not contained or degraded, NETs may function as damage-associated molecular patterns to amplify inflammation and thrombosis (8). Indeed, sera from patients with COVID-19 potently trigger healthy neutrophils to undergo NETosis, demonstrating a potential mechanism by which soluble factors in blood may remotely trigger immunothrombosis. Not surprisingly, molecular signatures of neutrophil hyperactivity correlate with clinical thrombosis and the requirement for mechanical ventilation in COVID-19 (9–11). Taken together, these findings provide a framework to understand and target
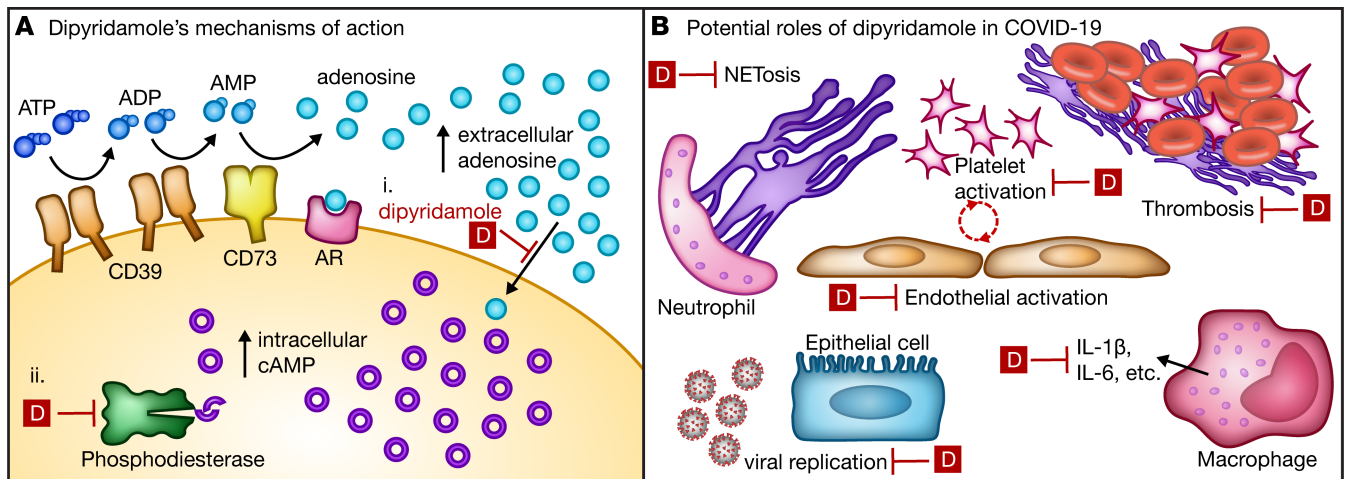
**Figure 1. Dipyridamole's mechanisms of action and potential role in the treatment of COVID-19.** (**A**) Dipyridamole potentiates homeostatic adenosine receptor signaling by preventing reuptake of ectonucleoside (i) and by stabilizing the intracellular cAMP pools via inhibition of cytoplasmic phosphodiesterase (PDE) activity (ii). (**B**) Left unchecked, purinergic signaling exacerbates both inflammation and thrombosis; therefore, dipyridamole has the potential to inhibit multiple processes that have been recently linked to COVID-19 severity. Illustrated by Rachel Davidowitz.

the thromboinflammation that drives severe disease and mortality in COVID-19. Based on the lynchpin role that purinergic nucleotides (ATP, ADP) play in platelet and leukocyte activation and the contribution of the nucleoside adenosine to endothelial and leukocyte quiescence, we hypothesize that harnessing purinergic signaling could amplify vascular homeostasis and potentially limit some of the devastating clinical sequelae driven at the nexus of inflammation and coagulation.

## Targeting purinergic signaling to prevent thromboinflammation

Purinergic signaling represents a critical checkpoint in the self-amplifying thromboinflammatory loop of endothelial dysfunction and hyperactivation of neutrophils, monocytes, and platelets. Activated and injured cells release their stores of ATP and ADP, creating a purinergic cloud in the extracellular space, where these nucleotides function in an autocrine and paracrine fashion as "danger" signals for neighboring and downstream tissues. Activation of extracellular ATP receptors (such as P2X7) on monocytes and neutrophils engages the canonical NLRP3 inflammasome, resulting in exuberant IL-1β production (12, 13). Moreover, blockade of this pathway prevents accretion of venous and arterial thrombi in mice (14–16). Similarly, thienopyridine antagonists of the ADP P2Y12 receptor are used clinically to suppress platelet activation in patients with cardiovascular disease (17).

CD39 and CD73, the dominant vascular ectonucleotidases, sequentially phosphohydrolyze these nucleotides to generate adenosine, which sustains homeostasis and a quiescent, antiinflammatory microenvironment. Furthermore, efficient activation of the extracellular adenosine receptor 2A ($A_{2A}R$) by the FDA-approved drug dipyridamole tempers both neutrophil ROS formation and NET release in a cAMP- and PKA-dependent manner, while preventing intraluminal vascular thrombi in mice (18, 19). Dipyridamole also protects the endothelium from ROS generation and acquiring a tissue factor–rich procoagulant phenotype induced by TNF-α (20–22). Dipyridamole potentiates homeostatic adenosine receptor signaling through at least 2 mechanisms: (a) inhibition of ectonucleoside reuptake and (b) stabilization of intracellular cAMP via inhibition of cytoplasmic phosphodiesterase activity (Figure 1). Dipyridamole is an old and relatively safe drug, with FDA-approved indications for use in combination with aspirin to prevent recurrent strokes and in combination with vitamin K antagonists for thromboprophylaxis following mechanical heart valve replacement (23). Dipyridamole was once one of the fifty most prescribed drugs in the US (24), with a favorable safety profile and a bleeding risk similar to that of aspirin.

In addition to its antithrombotic properties, human and animal studies have demonstrated that dipyridamole both induces synthesis of antiviral type I IFNs and protects against harmful inflammatory responses in the context of endotoxemia (25, 26). Intriguingly, dipyridamole has recently been shown to inhibit SARS-CoV-2 replication in vitro, likely via direct interaction with the SARS-CoV-2 replication complex at concentrations below those achieved in plasma with FDA-approved dosing. In a small study in

China, dipyridamole significantly suppressed D-dimer elevation and improved platelet counts in patients with COVID-19; however, larger studies are needed to evaluate clinical outcomes (27).

## COVID-19 therapy on (re)purpose: purinergic modulation in clinical trials

There remains a paucity of drugs approved to treat the thromboinflammatory milieu of COVID-19. Current trials to address this problem either block specific inflammatory cytokines or broadly paralyze the coagulation system. For example, protocols using inhibitors of IL-6 signaling are ongoing in patients with severe disease, while a large observational study conducted in New York has suggested that therapeutic anticoagulation with heparin may reduce mortality in patients with severe COVID-19 (albeit with little effect on patients with mild or moderate disease) (28). To date, marked reductions in mortality or progression of COVID-19 to the severest complications such as ARDS have not been observed. The lack of noted effect may suggest that these treatments are incomplete, as each drug targets only one facet of a complex local and systemic thromboinflammatory milieu (29).

These observations provide a rationale for pursuing purinergic modulators, such as dipyridamole, as a means to amplify the body's natural homeostatic pathways and, in so doing, target the myriad cellular and molecular pathways that appear to be relevant to COVID-19 pathogenesis. In particular, prospective randomized clinical trials would be necessary to determine whether dipyridamole has a therapeutic benefit in the treatment of patients with COVID-19. Along with others, our group is developing such protocols to launch multiple clinical trials with dipyridamole at different stages of COVID-19 (NCT04391179), which will be made available on http://clinicaltrials.gov. If successful in clinical trials, purinergic modulation will be an accessible and scalable approach to break the relentless and lethal cycle of thromboinflammation in COVID-19.

## Author contributions

YK, JSK, YZ, and DJP participated in the writing of the manuscript and gave approval before submission.

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