Targeting Abl Kinases to Regulate Vascular Leak During Sepsis and Acute Respiratory Distress Syndrome

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Abstract—The vascular endothelium separates circulating fluid and inflammatory cells from the surrounding tissues. Vascular leak occurs in response to wide-spread inflammatory processes, such as sepsis and acute respiratory distress syndrome, because of the formation of gaps between endothelial cells. Although these disorders are leading causes of mortality in the intensive care unit, no medical therapies exist to restore endothelial cell barrier function. Recent evidence highlights a key role for the Abl family of nonreceptor tyrosine kinases in regulating vascular barrier integrity. These kinases have well-described roles in cancer progression and neuronal morphogenesis, but their functions in the vasculature have remained enigmatic until recently. The Abl family kinases, c-Abl (Ab1) and Abl related gene (Arg, Abl2), phosphorylate several cytoskeletal effectors that mediates vascular permeability, including nonmuscle myosin light chain kinase, cortactin, vinculin, and β-catenin. They also regulate cell–cell and cell–matrix junction dynamics, and the formation of actin-based cellular protrusions in multiple cell types. In addition, both c-Abl and Arg are activated by hyperoxia and contribute to oxidant-induced endothelial cell injury. These numerous roles of Abl kinases in endothelial cells and the current clinical usage of imatinib and other Abl kinase inhibitors have spurred recent interest in repurposing these drugs for the treatment of vascular barrier dysfunction. This review will describe the structure and function of Abl kinases with an emphasis on their roles in mediating vascular barrier integrity. We will also provide a critical evaluation of the potential for exploiting Abl kinase inhibition as a novel therapy for inflammatory vascular leak syndromes. (Arterioscler Thromb Vasc Biol. 2015;35:1071-1079. DOI: 10.1161/ATVBAHA.115.305085.)

Key Words: acute lung injury ■ c-abl gene ■ cytoskeleton ■ endothelium ■ imatinib ■ respiratory distress syndrome, adult ■ sepsis

Inflammation-induced vascular leak underlies the pathophysiology of multiple disorders that affect critically ill patients, including sepsis and acute respiratory distress syndrome (ARDS). These conditions are major causes of morbidity and mortality in the intensive care unit affecting 750000 and 200000 patients/y in the United States, respectively. The lack of medical therapies capable of attenuating vascular leak necessitates additional research into the mechanisms underlying endothelial cell (EC) barrier dysfunction and clinical trials of novel therapeutic strategies.

Inflammatory Vascular Leak: Overview
Multiple inflammatory mediator-initiated cellular signaling pathways alter the actin–myosin contractile apparatus of ECs to regulate vascular permeability. Contractile pathways, which cause disruption of cell–cell junctions, cell rounding, and ultimately vascular leak, are counterbalanced by tethering forces that cause lamellipodia formation, restore cell–cell junctions, and resolve paracellular gaps. Inflammatory disorders, including sepsis and ARDS, create an imbalance in these pathways in favor of vascular leak. Current work aims to characterize the signaling pathways that determine vascular permeability and identify therapeutic strategies to enhance barrier function. The Abl family kinases, c-Abl (Ab1) and Abl related gene (Arg, Abl2), have recently emerged as key mediators of vascular permeability because of their well-characterized roles in the dynamic regulation of the actin cytoskeleton and cell–cell and cell–matrix junctions. Several groups have recently reported that the Food and Drug Administration (FDA)–approved Abl kinase inhibitor imatinib attenuates vascular leak induced by thrombin, histamine, vascular endothelial growth factor, lipopolysaccharide, and oxidative stress.

Although the mechanisms are not fully characterized, it is clear that Abl kinase inhibition has potent and pleiotropic effects on vascular barrier function. This is not surprising given that Abl kinases phosphorylate several cytoskeletal effectors that have established roles in vascular permeability and contribute to nuclear factor (NF)-κB and reactive oxygen species (ROS) signaling pathways. In addition to sepsis and ARDS, inflammatory vascular injury contributes to the
pathogenesis of atherosclerosis, ischemia-reperfusion injury, and pulmonary hypertension, thus broadening the potential clinical relevance of this work.20 This review will (1) describe the structure and functions of the Abl family kinases, (2) discuss the mechanisms by which they mediate EC barrier function, (3) evaluate the therapeutic potential of Abl kinase inhibition in inflammatory vascular leak syndromes.

Abl Family Kinase Structure and Function
The cytoplasmic tyrosine kinase c-Abl was originally identified as a virally transduced oncogene that causes murine lymphosarcoma.21 Subsequently, the human ortholog of this protein was identified as a part of the breakpoint cluster region–Abl fusion protein that causes chronic myelogenous leukemia.22 Arg was identified later based on its sequence homology with c-Abl, and together these kinases make up the Abl subfamily of cytoplasmic tyrosine kinases.23

Structure and Regulation of Abl Kinases
In mammals, both c-Abl and Arg have a myristoylated isoform and a nonmyristolated isoform.7 In both isoforms, the N-terminal regions of c-Abl and Arg are composed of a highly homologous Src homology 3-Src Homology 2-Tyrosine kinase domain cassette and an upstream Cap region.14 This region regulates Abl kinase activity via autoinhibitory interactions that disrupt specific tyrosine phosphorylation events.14 In contrast, the C-terminal regions of these kinases, termed the last exon region, are distinct and are composed of multiple cytoskeletal binding elements (Figure 1). Although c-Abl and Arg both contain a calponin homology F-actin–binding domain, c-Abl has a unique proline-rich region that binds to G-actin, whereas Arg has a unique F-actin–binding domain that resembles the I/LWEQ domain of talins.24,25 The last exon region of c-Abl also contains a DNA-binding domain and nuclear localization and nuclear export sequences, which allow it to move back and forth between the nucleus and the cytoplasm under various conditions.26 Because of the fact that the substrates of these kinases are similar, their functional differences are likely mediated by differential subcellular targeting and diversity in their cytoskeletal binding elements.

Overview of Abl Family Kinase Functions
Not surprisingly given their structural properties, the Abl kinases have well-described roles in regulating cytoskeletal structure, including binding and bundling F-actin filaments, phosphorylating cytoskeletal effector proteins, and modulating the activity of nonmuscle myosin light chain kinase (MLCK) and Rho family GTPases.14 Together these functions allow Abl kinases to promote actin-based cellular protrusion formation and alter cell–cell and cell–matrix junction dynamics, which underlie their crucial roles in regulating cell shape and migration.27 Fibroblasts deficient in these kinases display decreased membrane ruffling and lamellipodia formation.30 However, Abl−/−/Arg−/− mouse embryonic fibroblasts migrate faster than control cells.6 This seemingly paradoxical effect has been attributed Abl kinase inhibition of Rho-mediated cellular contractility.7,29 Abl kinases also modulate mechanotransduction pathways to alter the stability of cell–cell and cell–matrix junctions.30 The broad implications of these processes are highlighted by the roles of Abl kinases in diverse processes, including immune synapse formation, dendrite branching, endocytosis, and epithelial morphogenesis.7,14 Although cytoskeletal rearrangements are critically involved in EC barrier function, the role of Abl kinases in regulating vascular permeability has been recognized only recently.

Given its multitude of cellular functions, it comes as no surprise that c-Abl knockout mice (c-Abl−/−) die shortly after birth with diverse phenotypes, including splenic and thymic atrophy, osteoporosis, and cardiomegaly.31–34 In contrast, Arg−/− mice survive to adulthood with behavioral abnormalities as their main phenotype.35 Combined knockout of c-Abl
and Arg (c-Abl<sup>−/−</sup>;Arg<sup>−/−</sup>) is embryonic lethal because of impaired neurulation, pericardial edema, and hemorrhage. Endothelial-specific c-Abl knockout mice on an Arg<sup>−/−</sup> background (c-Abl<sup>IECKO</sup>;Arg<sup>−/−</sup>) die during late embryonic development because of focal loss of vasculature and tissue apoptosis and necrosis. However, c-Abl<sup>IECKO</sup> mice on an Arg<sup>−/+</sup> background (c-Abl<sup>IECKO</sup>;Arg<sup>+/−</sup>) are viable to adulthood and display a phenotype with multiple cardiovascular defects including dilation of the left atrium and loss of EC in the left ventricle. Although these studies demonstrate that Abl kinases are critical to vascular function, additional work is necessary to differentiate the roles of these kinases and determine the extent to which they can compensate for each other.

**Abl Kinase Signaling in Inflammatory Vascular Leak**

Because of their multitude of substrates, the Abl kinases likely mediate vascular barrier function via several mechanisms. Table 1 describes functions of major Abl kinase substrates that have established roles in EC permeability. The following section will discuss the role of Abl kinases in (1) responding to barrier altering agonists and regulating, (2) actin cytoskeletal structure, (3) cell–cell and cell–matrix junctions, (4) NFkB signaling, and (5) ROS signaling.

**Role of Abl Kinases in Responding to Barrier Altering Agonists**

Multiple groups have reported attenuation of inflammatory vascular leak with the Abl kinase inhibitor imatinib. The first report identified a protective effect of Arg inhibition on vascular leak induced by thrombin, histamine, and vascular endothelial growth factor. Each of these stimuli increase Arg activity and induce a downstream decrease in Rac activity and focal adhesion (FA) number. However, c-Abl inhibition also attenuates vascular endothelial growth factor–induced EC permeability, suggesting that these kinases may have some overlapping functions in regulating EC barrier integrity. Although the mechanisms are not completely understood, they include increased Rac1/Rap1 activity and decreased Ca<sup>2+</sup> mobilization, nonmuscle MLCK activation, and stress fiber formation. In addition, vascular barrier disruption induced by both lipopolysaccharide and oxidative stress are attenuated by imatinib treatment. These potent barrier protective effects, in response to several barrier disruptive agonists, strongly support the hypothesis that Abl kinases are central mediators of vascular integrity. This work is supported by in vivo studies that demonstrate a protective effect of imatinib in vascular leak induced by injection of vascular endothelial growth factor (intradermal), thrombin receptor activating peptide (intravenuous), lipopolysaccharide (intratracheal), and the cecal ligation and puncture sepsis model. In addition, imatinib restores blood–brain barrier integrity and decreases intracerebral hemorrhage in murine models. Although other kinase targets of imatinib may play a role in these effects, a clear contribution of c-Abl is evidenced by decreased vascular leak in c-Abl<sup>IECKO</sup>;Arg<sup>−/−</sup> mice compared with Arg<sup>−/−</sup> control mice.

In contrast with these barrier protective effects, Abl kinase inhibition worsens endothelial barrier disruption in EC challenged with 18% cyclic stretch and worsens vascular leak in a murine model of ventilator-induced lung injury induced by high tidal volume mechanical ventilation. These observations indicate differential pathophysiology of ventilator-induced lung injury and ARDS because of other causes and suggest that Abl kinase inhibition in patients with ARDS undergoing mechanical ventilation should be approached with caution. Abl kinases also contribute to the barrier protective response to the endogenous bioactive sphingolipid sphingosine 1-phosphate (S1P). S1P causes c-Abl activation and formation of a c-Abl/nonmuscle MLCK/cortactin complex, which facilitates peripheral actin polymerization. Similar to S1P, the response to the barrier protective agent activated protein C is dependent on the S1P receptor 1, Rac1 activation, and cortical MLCK phosphorylation. The critical role of S1P receptor 1 in the barrier protective effects induced by activated protein C suggests that c-Abl may also participate in this response. In addition, the barrier protective response to FTY720, a pharmaceutical S1P analog with a distinct mechanism of action, involves c-Abl.

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**Table 1. Abl Family Kinase Targets Involved in Vascular Barrier Function**

| Protein | Function in Vascular Barrier Function |
|---------|--------------------------------------|
| c-Abl   | Mediates vascular leak downstream of edemagenic agents via effects on AJs and nmMLCK activation<sup>11</sup> |
|         | Attenuates vascular leak downstream of S1P and FTY720 via effects on cortactin and nmMLCK phosphorylation<sup>40</sup> |
| Arg     | Mediates vascular leak downstream of edemagenic agents via effects on FAs and Rac activity<sup>18</sup> |
| β-Catenin | Binds to VE-Cadherin, which structurally and functionally couples AJs to the actin cytoskeleton<sup>15</sup> |
| Cav1    | Major component of caveolae, which are responsible for transcellular transport of fluid and albumin<sup>90</sup> |
| Crk and CrkL | Adaptor proteins involved in AJ remodeling<sup>44</sup> |
|         | Crk phosphorylation is involved in Rac activation and unphosphorylated Crk promotes AJ disassembly<sup>45</sup> |
| Cortactin | Attenuates vascular leak by promoting actin polymerization at lamellipodia<sup>46</sup> |
| nmMLCK  | Central mediator of permeability via effects on actin-myosin contraction and ultimately cell shape. Involved in formation of both stress fibers and lamellipodia depending on subcellular localization<sup>3</sup> |
| Myosin IIB | Component of actin-myosin contractile apparatus<sup>3</sup> |
| p190RhoGAP | Associates with p120RhoGEF as a part of a complex that inhibits Rho activity, decreases actomyosin contractility, and focal adhesion remodeling<sup>4,29</sup> |
| Paxillin | Component of FAs that binds to the integrin cytoplasmic tail, serving as a link to the actin cytoskeleton<sup>15</sup> |
| WAVE2/3 and N-WASP | Promote actin polymerization at lamellipodia<sup>2</sup> |

Multiple targets of c-Abl and Arg have established roles in the regulation of vascular permeability, which are briefly described. A complete listing of known Abl kinase substrates is available in a recent comprehensive review. AJ indicates adherens junctions; FA, focal adhesions; nmMLCK, nonmuscle myosin light chain kinase; S1P, sphingosine 1-phosphate; and WAVE, Wasp-Family Verprolin-Homologous Protein.
activation. Furthermore, Abl kinases are necessary for Tie2 expression and EC survival–mediated angiopoietin-1, a potent barrier enhancing agonist. Together this body of work provides strong evidence that Abl kinases play a central role in EC barrier regulation. The published effects of imatinib on permeability are summarized in verbal and schematic form (Table 2; Figure 2). Although the roles of Abl kinases in other barrier protective pathways remain poorly characterized, Abl kinase inhibition is likely to affect a wide-variety of signaling pathways because of their ability to regulate Rac1/Rap1 activity. This highly complex picture necessitates additional work to differentiate the roles of c-Abl and Arg and defines the pathways by which they alter actin cytoskeletal structure, cell–cell and cell–matrix junctions, and inflammation.

### Abl Kinase Regulation of the Actin Cytoskeleton

Barrier disrupting agents cause nonmuscle MLCK and Rho family kinase–mediated cytoskeletal rearrangements, including loss of the stabilizing cortical band of actin microfilaments and the formation of cytoplasmic actin stress fibers.

### Table 2. Preclinical Data on the Effects of Imatinib on EC Barrier Dysfunction

| In vitro models | Agonist | Effect on EC Barrier |
|-----------------|---------|----------------------|
| Rat aortic EC   | VEGF    | Protective<sup>10</sup> |
| HUVEC           | Thrombin, histamine | Protective<sup>10</sup> |
| HLMVEC          | Thrombin | Protective<sup>10</sup> |
| Immortalized EC | VEGF    | Protective<sup>11</sup> |
| HUVEC           | SCF     | Protective<sup>19</sup> |
| Mouse lung microvascular EC | H<sub>2</sub>O<sub>2</sub> | Protective<sup>15</sup> |
| HPAEC           | LPS     | Protective<sup>12</sup> |
| HPAEC           | 18% CS  | Disruptive<sup>12</sup> |
| Murine models   | Bleomycin-induced ALI | Anti-inflammatory<sup>11</sup> |
| CLP (sepsis)    | Fecal peritonitis | Protective<sup>10</sup> |
| Isolated perfused lung | TRAP | Protective<sup>15</sup> |
| Isolated perfused lung | Ischemia/ reperfusion | *Attenuates cytotoxicity*<sup>13</sup> |
| IT LPS (neutropenic mice) | LPS | Protective<sup>9</sup> |
| IT LPS          | LPS     | Protective<sup>12</sup> |
| Miles assay     | VEGF    | Protective<sup>10</sup> |
| Miles assay     | VEGF    | Protective<sup>19</sup> |
| VILI            | MV (30 mL/kg tidal volume) | Disruptive<sup>12</sup> |

Several independent groups have demonstrated that imatinib enhances endothelial barrier function and protects against vascular leak in vitro and in vivo. However, some data demonstrate increased leak after imatinib in other injury models. ALI indicates acute lung injury; CLP, cecal ligation and puncture; CS, cyclic stretch; EC, endothelial cell; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; HLMVEC, human lung microvascular endothelial cells; HPAEC, human pulmonary artery EC; HUVEC, human umbilical vein EC; IT, intratracheal; LPS, lipopolysaccharide; MV, mechanical ventilation; SCF, stem cell factor; TRAP, thrombin receptor activating peptide; VEGF, vascular endothelial growth factor; and VILI, ventilator-induced lung injury.

Abl kinases alter the activity of both nonmuscle MLCK and Rho kinases, indicating that they have the potential to regulate EC cytoskeletal dynamics and barrier integrity via multiple mechanisms. Although the direct effects of Abl kinases on the location of actin-myosin contraction have not been investigated in EC, work in other cell types suggests that these kinases play distinct roles in regulating cytoskeletal structure by this mechanism. In mouse embryonic fibroblasts, c-Abl<sup>−/−</sup> causes central contractile apparatus localization, whereas Arg<sup>−/−</sup> causes peripheral contractile apparatus localization.

In addition to direct effects on the actin-myosin contractile apparatus, Abl kinases phosphorylate cytoskeletal effectors that promote actin-based cellular protrusion formation, including cortactin, Wiskott–Aldrich syndrome protein, Wiskott–Aldrich syndrome protein-family verprolin-homologous proteins, and Dok1. Both overlapping and distinct roles of these kinase-mediated phosphorylation events have been identified in the responses to growth factors, including platelet-derived growth factor (PDGF) and epidermal growth factor. In fibroblasts, PDGF induces cortactin phosphorylation, downstream actin polymerization, and cortical wave formation as a part of a signaling pathway that is dependent on c-Abl or Arg. In addition, the formation of lamellipodia and filopodia in fibroblasts, and the actin comet tail of Shigella, are dependent on c-Abl–mediated phosphorylation of Wasp-Family Verprolin-Homologous Protein 3, Dok1, and N-Wiskott–Aldrich syndrome protein, respectively. These findings need to be confirmed in ECs to determine whether barrier altering agonists lead to Abl kinase–mediated phosphorylation of these proteins and elucidate the subsequent downstream effects.

### Abl Kinase Regulation of Cell–Cell and Cell–Matrix Junction Dynamics

Barrier disruptive agents also disrupt the cell–cell and cell–matrix junctions that maintain barrier integrity. In human umbilical vein EC, Abl kinase inhibition with imatinib restores the integrity of adherens junctions (AJ) after thrombin stimulation. The mechanisms underlying these effects have not been described; however, the AJ protein β-catenin is a known target of Abl kinases, and its phosphorylation contributes to AJ internalization. Although these data suggest that Abl kinases disrupt AJ in EC, work in c-Abl<sup>−/−</sup>/Arg<sup>−/−</sup> mouse embryonic fibroblasts demonstrates that Abl kinases are also necessary for Rac activation and AJ formation, suggesting that Abl kinases function both upstream and downstream of Rac to promote AJ integrity.

In addition to cell–cell junctions, cell–matrix junctions (focal adhesions [FA]) are critical to EC barrier integrity. In human umbilical vein EC, imatinib blocks Arg-mediated barrier disruption by promoting FA junction stability. The exact mechanism underlying this effect remains unclear, but in fibroblasts, Arg-mediated activation of p190RhoGAP at the periphery decreases Rho activity downstream of integrin-mediated cellular adhesion, which ultimately decreases FA dynamics. However, Abl kinases also phosphorylate the FA
proteins Crk/CrkL, and paxillin, which suggests other possible mechanisms for Abl-mediated changes in FA dynamics. For example, in response to cellular adhesion, c-Abl is recruited to FA, where it phosphorylates paxillin and promotes FA formation. Studies in mouse neurons have demonstrated that integrin signaling through both c-Abl and Arg promotes cortical dendrite branching in response to cellular adhesive cues. Abl kinases are, therefore, critical to both cell–cell and cell–matrix junctional dynamics, providing additional mechanisms by which Abl kinase inhibitors alter vascular integrity.

Role of Abl Kinases in NFκB-Mediated Inflammation
Several barrier disruptive agents activate EC to increase transcriptional activation of NFκB, release of inflammatory cytokines, and upregulation of multiple cellular adhesion molecules that are involved in neutrophil recruitment and extravasation. Imatinib attenuates lipopolysaccharide-induced vascular leak in human pulmonary artery EC, and these effects are mimicked by silencing of c-Abl, but not of Arg. Our unpublished data indicate that imatinib prevents lipopolysaccharide-induced NFκB phosphorylation and nuclear translocation in human pulmonary artery EC, suggesting that lipopolysaccharide increases vascular cellular adhesion molecule expression and cytokine production via a c-Abl/NFκB-dependent signaling pathway. In a murine model of lipopolysaccharide-induced acute lung injury during recovery from neutropenia, imatinib decreased production of proinflammatory cytokines (tumor necrosis factor-α, IL-8, and IL-1β) and attenuated lung injury. Suppression of NFκB activation and inflammatory cytokine production after imatinib treatment has also been reported in peripheral blood mononuclear cells, cells of the monocyte–macrophage lineage, and leukocytes isolated from chronic myelogenous leukemia patients. These data are supported by a murine model of tumor necrosis factor-α-dependent acute hepatic inflammation, in which imatinib suppressed production of tumor necrosis factor-α, IL-6, and IL-8 induced by both lipopolysaccharide and Con-A. Although these data provide strong evidence for the anti-inflammatory effects of imatinib, the effects of imatinib on NFκB activation seem to increase or decrease depending on cell type and length of imatinib exposure. In pancreatic β cells, imatinib causes an initial increase in NFκB activation, followed by a decrease in activation and cytokine production. This work provides evidence for a key role of Abl kinase inhibition in NFκB activation. The distinct Abl kinases (c-Abl versus Arg) and the downstream targets that mediate this effect have yet to be determined.

Abl Kinase Regulation of ROS Signaling Pathways
Prolonged mechanical ventilation with high fraction of inspired oxygen during ARDS treatment can lead to hyperoxic lung
injury. This involves increased production of ROS, including superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), which overwhelms antioxidant defenses, leading to cell death and EC barrier dysfunction. Abl kinases are activated in response to oxidative stress in multiple cell types. In EC, H$_2$O$_2$ leads to c-Abl activation and nuclear translocation, while increasing protein kinase G activity with the cGMP analog 8pCPT-cGMP blocks this effect. Inhibition of c-Abl, indirectly by increasing protein kinase G activity or directly with imatinib, attenuates the oxidant-induced decline in expression of the antioxidant proteins catalase and glutathione peroxidase, which ultimately decreases EC injury and barrier dysfunction. Although the role of Arg in oxidant-induced EC injury has not been investigated, work in MCF-7 breast cancer cells and mouse embryonic fibroblast suggests that both Abl kinases are critical to oxidant-induced injury. In these cell types, H$_2$O$_2$ leads to heterodimerization of c-Abl and Arg, inhibition of antioxidant enzymes, and ultimately oxidant-induced apoptosis. The growing amount of evidence for a role of Abl kinases in ROS signaling pathways paves the way for future research, in which the role of Arg deserves specific attention.

### Therapeutic Potential of Abl Kinase Inhibition in Inflammatory Vascular Leak Syndromes

The mechanistic studies described above, which were initiated in part because of the serendipitous observation that imatinib quickly reversed pulmonary edema in some patients, suggest that inhibition of Abl kinases may have therapeutic potential for decreasing vascular permeability. The broad clinical experience with current FDA-approved Abl kinase inhibitors may facilitate repurposing of these drugs for inflammatory vascular leak syndromes. In contrast to some other anticancer agents that specifically target oncogenic proteins, the Abl kinase inhibitors have potent effects on nontransformed cells because they block cellular c-Abl/Arg activity. This section will provide a brief review of the FDA-approved Abl kinase inhibitors and describe their known clinical effects on vascular permeability.

#### FDA-Approved Abl Family Kinase Inhibitors

Imatinib (STI-571; Gleevec), the prototype Abl kinase inhibitor, was developed to inhibit the breakpoint cluster region–Abl fusion protein that causes chronic myelogenous leukemia. Resistance and intolerance to imatinib led to the development of second-generation breakpoint cluster region–Abl inhibitors, nilotinib (AMN107; Tasigna) and dasatinib (BMS-354825; Sprycel), and third-generation breakpoint cluster region–Abl inhibitors, ponatinib (AP24534; Iclusig) and bosutinib (SKI606; Bosulif; Table 3). A common feature of these Abl kinase inhibitors is their mechanism of action involving competitive inhibition by binding to the ATP-binding pocket of the kinase. High-sequence homology in the ATP-binding pocket of c-Abl, Arg, and closely related kinases (including Src family kinases) has led to difficulty in development of specific kinase inhibitors. However, off-target kinase inhibition has allowed for imatinib to be repurposed for other conditions, including gastrointestinal stromal tumor (c-Kit dependent) and dermatofibrosarcoma protubersans (PDGF receptor dependent). Recent work has suggested that they may also be beneficial in the treatment of the proliferative lung diseases pulmonary hypertension and idiopathic pulmonary fibrosis, as well as systemic inflammatory disorders including rheumatoid arthritis.

Another consequence of the lack of specificity is interference with the physiological function of target kinases, which results in unwanted side effects (Table 3). Of note, all Abl kinase inhibitors cause varying degrees of subcutaneous edema, as well as pulmonary, peritoneal, and pericardial effusions. Because these side effects depend on treatment dose and duration, this paradox may be explained by the chronic kinase inhibition that is present in patients undergoing treatment for chronic myelogenous leukemia. Specifically, long-term inhibition of PDGF receptor on pericytes may impair vascular integrity. Imatinib has also been occasionally associated with congestive heart failure, but larger patient series failed to show a relationship between imatinib treatment and cardiac failure. In addition, side effects of these drugs may occur because of the wide-spread expression of the Abl family kinases. Although both c-Abl and Arg are broadly expressed throughout the tissues of the body, c-Abl expression is high in smooth muscle cells and in the face of its role in migration and cytokinesis.

Neurons express a large amount of Arg, which promotes the development of the neural tube and dendritic spines. Both c-Abl and Arg are highly expressed in

| Drug | Imatinib | Dasatinib | Nilotinib | Ponatinib | Bosutinib |
|------|----------|-----------|-----------|-----------|-----------|
| Off-target effects | c-KIT, PDGFR, DDR1, NQ02 | c-KIT, PDGFR, DDR1/2, SRC, YES, FYN, LYN, HCK, LCK, FGR, BLK, FRK, CSK, BTK, TEC, BMX, TXK, ACK, BRAF, EGFR, EPHA, MAPK, RAF, SLK, ZAK | c-KIT, PDGFR, DDR1, NQ02 | c-KIT, PDGFR, FLT3, FGFR, RET, VEGFR, SRC, More specific to Abl kinases | ALK, CSK, FGR, LYN, PKA, CK1, CK2, SRC, RET, SYK |
| $T_{1/2}$, h | 18 | 3–5 | 15–17 | 24 | 22.5 |
| F, % | 98 | <34 | 30 | 65 | Unknown |
| Vd, L/kg | 2–6 | 36 | 2 | 17.5 | 87 |
| Metabolism | CYP3A4, CYP3A5, CYP2C8 | CYP3A4 | CYP3A4, CYP2C8 | CYP3A4, CYP3A5, CYP2C8, CYP2D6 | CYP3A4 |
| Edema, % | 53 | 50 | 11 | 13–22 | 14 |

A comparison of the pharmacokinetic and pharmacodynamic properties, and the edema-related side effects, of the Abl kinase inhibitors that are currently in clinical usage. BCR indicates breakpoint cluster region; PDGFR, platelet-derived growth factor receptor; VEGFR; vascular endothelial growth factor receptor; F, bioavailability; and Vd, volume of distribution.
Clinical Effects of Abl Family Kinase Inhibitors on Vascular Permeability

Although there are no clinical studies about the effects of Abl kinase inhibitors on inflammatory vascular leak, evidence from a series of case reports indicates that Abl kinase inhibition may decrease permeability in certain patients. The first report indicating the potential of imatinib to reduce EC permeability described a pulmonary hypertension patient with peripheral veno-occlusive disease and edema symptoms who experienced improvement of dyspnea and reduction of pulmonary edema (evidenced by computed tomography) within 24 hours after initiation of imatinib therapy. A second report describes a patient with bleomycin-induced pneumonitis with clinical and radiographic signs of pulmonary edema and fibrosis. Imatinib treatment was followed by a quick improvement in respiratory status and resolution of radiographic findings. More recently, a patient diagnosed with idiopathic vascular leak experienced almost complete resolution of her symptoms, including dyspnea and cough, and normalization of parameters of vascular leak including serum albumin levels, body weight, and pulmonary leak index after initiation of imatinib treatment.

The rapid responses in these cases suggest a direct effect on the endothelium, supporting the experimental studies described above, but the exact mechanisms remain incompletely understood. In addition, inhibition of other imatinib-sensitive kinases may have contributed to the observed effects. Among these kinases is PDGF receptor, a target of all of the FDA-approved inhibitors, that is expressed in the pulmonary vasculature and contributes to imatinib’s barrier protective effects in tissue-type plasminogen activator–induced blood–brain barrier permeability. In addition, stem cell factor increases vascular permeability via increased vascular endothelial-cadherin internalization, as well as increased endothelial nitric oxide (NO) synthase phosphorylation and NO production, which can be attenuated by inhibition of the intracellular kinase domain of the stem cell factor receptor (c-Kit) with imatinib.

We anticipate that the array of FDA-approved Abl kinase inhibitors with differing specificities will aid in determining the contribution of each of the targets of these inhibitors to vascular leak pathophysiology to maximize potential treatment efficacy.

Conclusions

The recent studies discussed in this review point toward a central role of Abl kinases in endothelial barrier regulation and inflammatory signaling. Abl kinases are activated downstream of several barrier altering agonists and regulate EC cytoskeletal structure and junctional dynamics, NFnX signaling, and oxidant-induced EC injury. The protective effects of the Abl kinase inhibitor imatinib observed in most studies not only establishes the relevance of Abl kinases in vascular biology but also provides a relevant link to clinical conditions associated with vascular leak. Although the majority of this work indicates barrier protective effects of Abl kinase inhibition, Abl kinases play a barrier protective role in the EC response to S1P, FTY720, and ventilator-induced lung injury. A likely explanation for these paradoxical observations might be the differential role of c-Abl versus Arg, and their downstream targets. This body of work suggests that c-Abl and Arg have distinct functions in the response to barrier altering agonists involved in the pathophysiology of inflammatory vascular leak syndromes.

Development of specific c-Abl and Arg inhibitors will help to elucidate the biological roles of c-Abl and Arg and contribute to tailored therapy. Because little is still known from pericytes and smooth muscle cells, the role of Abl kinases in vascular biology may extend well beyond endothelial barrier regulation and inflammation. In support of the experimental work, a series of recent case reports describe protective effects of imatinib in the treatment of inflammatory vascular leak. Because inflammatory vascular leak is prevalent in the intensive care unit population, and data on use of Abl kinase inhibitors in this population are scarce, testing their safety and pharmacokinetics in this population are critical first steps in clinical development of this strategy. To support these initial steps, the European Medicines Agency granted Orphan Drug Designation for application of imatinib in ARDS (http://www.orphan-drugs.org/2014/10/ema-orphan-drug-designations-october-2014). Although additional work is necessary to differentiate the roles of these 2 kinases and determine the clinical scenarios and patient populations in which Abl kinase inhibition would be most beneficial, therapeutic manipulation of the Abl kinase family holds great promise as a novel and highly effective intervention for inflammatory vascular leak syndromes.

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Inflammatory vascular leak syndromes, such as sepsis and acute respiratory distress syndrome, are leading causes of mortality in critically ill patients; however, no pharmacological treatments exist to attenuate vascular leak. Recent work demonstrates that the ABL family kinases are involved in the cellular signaling pathways initiated by both barrier protective and barrier disruptive stimuli. Preclinical studies using both cell culture and animal models indicate that imatinib, an Food and Drug Administration–approved Abl kinase inhibitor, attenuates vascular leak induced by diverse stimuli including thrombin, histamine, vascular endothelial growth factor, lipopolysaccharide, and oxidative stress. A series of recent case reports support these findings, further arguing for clinical trials to determine the efficacy of this novel treatment strategy.