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THERAPEUTIC TARGETS FOR ACUTE LUNG INJURY/ACUTE RESPIRATORY DISTRESS SYNDROME

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SUMMARY

Acute lung injury (ALI) describes the numerous symptoms and syndromes associated with hypoxemic respiratory failure, the most severe form of which is acute respiratory distress syndrome (ARDS). ALI/ARDS can be triggered by extensive lung inflammation and small blood vessel injury, such as in the setting of sepsis, trauma and pulmonary infection. Injury to the lung triggers an intense inflammatory response in pulmonary tissue, causing the alveoli to collapse or fill with fluid and hence lose their ability to mediate gas exchange with the blood. Fibrinogenesis and dysregulation of the coagulation cascade may also occur, further compromising pulmonary function. Standard treatment involves mechanical ventilation and a conservative fluid management strategy, with additional therapeutic components prescribed, such as antibiotics, immunosuppressants, vasodilators, surfactant therapy and/or diuretics. However, at present, there is no single treatment that reduces mortality in this clinical setting. Thus, the search continues for more effective treatment strategies for ALI/ARDS, with investigation focused on identifying novel targets for therapeutic intervention. This article presents the drug targets that are currently under active investigation for the treatment of ALI/ARDS.

INTRODUCTION

Acute lung injury (ALI) is a term used to describe the spectrum of symptoms and syndromes associated with hypoxemic respiratory failure, the most severe form of which is acute respiratory distress syndrome (ARDS). The distinction between ARDS and ALI, as determined at the 1994 American–European Consensus Conference on ARDS, is based on the severity of hypoxemia, measured as PaO_2/FiO_2 (partial pressure of oxygen in arterial blood/fraction of inspired oxygen) ≤ 300 and 200 mmHg, respectively, for ALI and ARDS. ARDS and direct or indirect injury to the lung trigger an intense inflammatory response in the pulmonary tissues, causing the alveoli to collapse or fill with fluid and hence lose their ability to exchange oxygen and carbon dioxide with the blood. ARDS typically develops within 12-24 h of the predisposing event, but can occur up to 3 days later. The sequence of events may occur quite rapidly, beginning in one lung and spreading quickly to the other. If inflammation persists over time, the lungs will attempt to heal the damage, leading to the formation of scar tissue. This scar tissue further compromises lung function and hinders gas exchange (1, 2).

ALI/ARDS can be triggered by extensive lung inflammation and small blood vessel injury, such as in the setting of sepsis, trauma and/or severe pulmonary infection (bacterial, viral or fungal). Other direct or indirect causes of lung injury may also lead to the development of ALI/ARDS, such as aspiration of salt water, fumes, chemicals or vomit into the lungs, an adverse reaction to blood transfusion or heart bypass surgery, asthma, emphysema, poliomyelitis, muscular dystrophy, pancreatitis, circulatory collapse, overdose of tricyclic antidepressants or shock from any cause. Heavy alcohol use and cigarette smoking, as well as certain genetic factors, may increase patient predisposition to ARDS (1, 3-6).

The pathogenesis of ARDS involves an increase in lung vascular permeability in the early or exudative stage, causing the alveolar air space and interstitium to become flooded with protein-rich edema fluid and triggering an inflammatory response. Pulmonary or systemic inflammation is both triggered by and prompts the further systemic release of proinflammatory cytokines. These cytokines activate alveolar macrophages and recruit neutrophils to the lungs, leading to release of leukotrienes, antioxidants, platelet-activating factor and proteases. All of these substances have harmful effects on the capillary endothelium and the alveolar epithelium, and hence disrupt the epithelial barrier between capillaries and air spaces. As a result, the air spaces and interstitium are flooded with edema fluid and cellular debris. In the resulting cascade of events during this exudative stage, surfactant is disrupted, air spaces collapse and there is an imbalance between ventilation and perfusion, causing hypoxemia. Fibrosis is initiated about 10 days after the initial injury and may be extensive by 3 weeks. The lungs become stiff and less compliant, and pulmonary hypertension may develop. Injury does not affect all parts of the lung equally, but rather manifests histologically as diffuse and heterogeneous alveolar damage. During this

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second fibroproliferative stage, connective tissue, fibrin and other structural elements in the lungs proliferate in a protective response to the original injury. Initially, fibrin deposition in alveoli exerts a beneficial effect on gas exchange, sealing off sites of leakage in the injured epithelium and endothelium and facilitating tissue repair. However, if this process becomes deregulated, it triggers a detrimental process that culminates in organ dysfunction. Pneumonia, sepsis, alveolar collapse and rupture of the lungs are the potentially lethal complications at this stage of disease. Uncontrolled host responses at this point may also have detrimental effects on the coagulation cascade. The clinical course of ALI/ARDS is variable. Whereas persistence and progression of injury lead to multiple organ failure, pulmonary fibrosis, pulmonary vascular obliteration with pulmonary hypertension, or death in some patients, in others the pulmonary edema and lung inflammation resolve, leading to repair and recovery from ALI (1, 3, 6-12).

Because of the heterogeneous nature of patients meeting the diagnostic criteria for ALI/ARDS, no single treatment has proven effective in decreasing the mortality rate in this clinical setting. Standard management of the patient presenting with ALI or ARDS includes treatment both of ALI/ARDS and of the predisposing or underlying condition or injury. Mechanical ventilation with a conservative fluid management strategy and supportive care are the mainstays of treatment for ARDS in the intensive care unit. Additional components of therapy may include any of the following: antibiotics to prevent and treat secondary bacterial infections, immunosuppressants (e.g., corticosteroids), vasodilators, surfactant therapy, targeting of the coagulation cascade, β-adrenoceptor agonists to aid in removal of pulmonary edema fluid from the alveolar space, elastase inhibitors and diuretics to reduce fluid in the lungs (1, 6, 7, 13, 14).

The search for effective treatment strategies for ALI/ARDS continues, with research focusing on the identification of novel targets for drug development. Those targets currently under active investigation are discussed below (see Figure 1). Table I provides a selection of products under active development for each target and Table II includes selected patents.

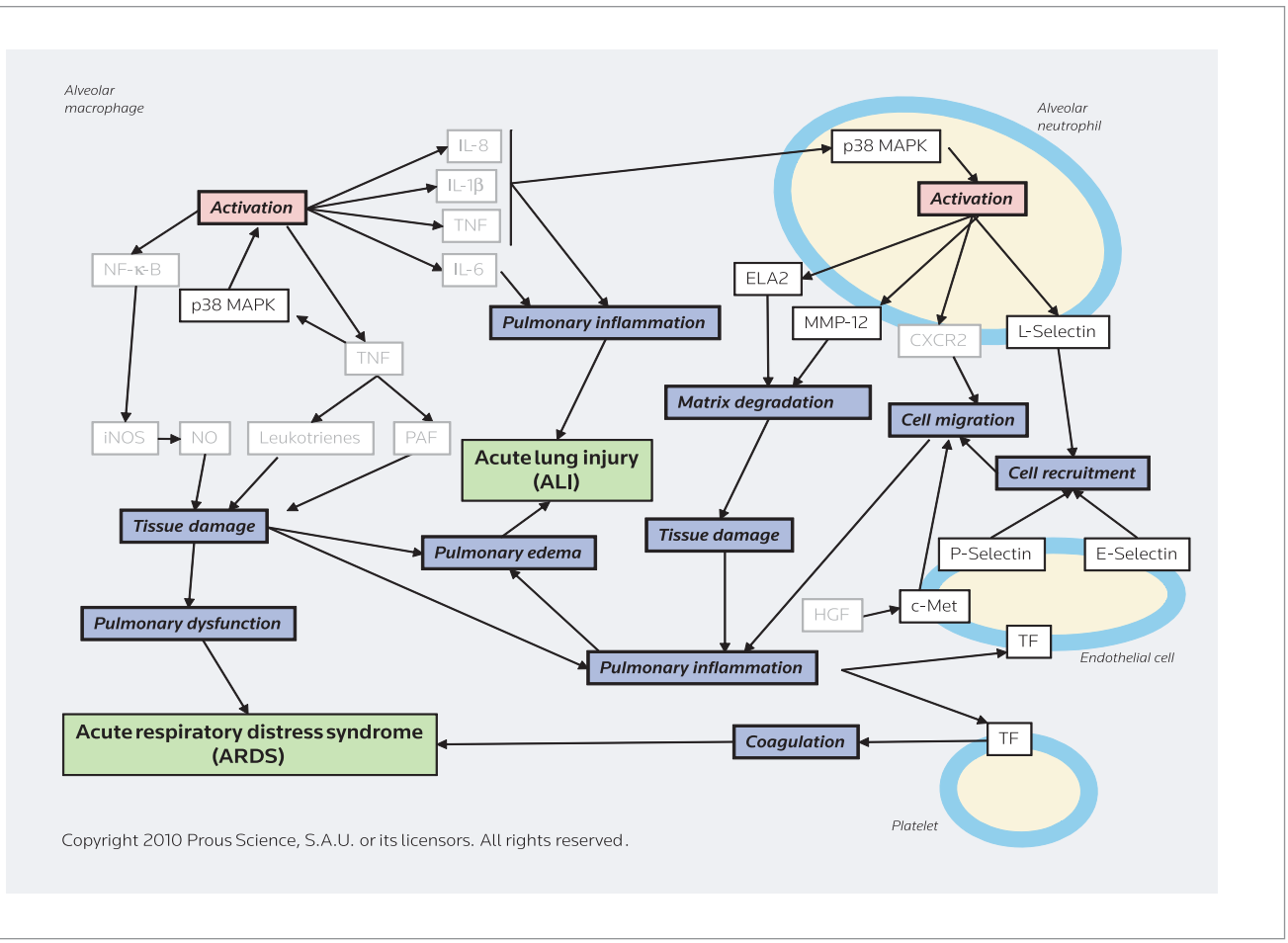

Figure 1. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) targetscape. A diagram showing an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of ALI/ARDS and their biological actions. Gray or lighter symbols are targets that are not validated (i.e., targets not associated with a product that is currently under active development for ALI/ARDS). c-Met, proto-oncogene c-Met (hepatocyte growth factor receptor); CXCR2, chemokine receptor CXCR2; HGF, hepatocyte growth factor; HLE, neutrophil elastase; IL, interleukin; iNOS, inducible nitric oxide synthase; MMP-12, matrix metalloproteinase-12; NF-κ-B, nuclear factor NF-κ-B; NO, nitric oxide; p38 MAPK, p38 mitogen-activated protein kinases; PAF, platelet-activating factor; TNF, tumor necrosis factor; TF, tissue factor.

Table I. *Selected targets and products launched or being actively investigated for acute lung injury/acute respiratory distress syndrome (from Thomson Reuters Integrity®).*

Target	Product	Source	Phase
E-selectin	Bimosiamose	Revotar	I
Hepatocyte growth factor receptor	BB3	Angion Biomedica	II
L-selectin	Bimosiamose	Revotar	I
Matrix metalloelastase	V-85546	Vernalis	I
Neutrophil elastase	Sivelestat sodium hydrate	Ono Pharmaceutical	L-2002
p38 MAP kinases (nonspecified subtype)	Dilmapimod	GlaxoSmithKline	II
P-selectin	Bimosiamose	Revotar	I
Tissue factor	ALT-836	Altor Biosciences	II

Table II. *Selected patents for targets being validated for acute lung injury/acute respiratory distress syndrome (from Thomson Reuters Integrity®).*

Target	Patent	Source	Phase
Chemokine CXCR1/CXCR2 receptor antagonists	WO 2007124423	GlaxoSmithKline	Biological testing
	WO 2007131352	University of Saskatchewan	Biological testing
	WO 2007150016	GlaxoSmithKline	Biological testing
L-selectin	WO 2000034255	NV Organon	Biological testing
Neutrophil elastase	WO 2000032216	Cortech/Ono Pharmaceutical	Biological testing
	WO 2000052032	Dainippon Sumitomo Pharma	Preclinical
	WO 2001044245	sanofi-aventis	Discontinued/biological testing
	WO 2005021509	AstraZeneca	Biological testing
	WO 2005021512	AstraZeneca	Biological testing
	WO 2005026123	AstraZeneca	Biological testing
	WO 2005026124	AstraZeneca	Biological testing
	WO 2006098683	AstraZeneca	Biological testing
	WO 2007129060	Argenta Discovery	Biological testing
	WO 2007129962	AstraZeneca	Biological testing
	WO 2008030158	AstraZeneca	Biological testing
	WO 2008104752	AstraZeneca/AstraZeneca	Biological testing
	WO 2009013444	Argenta Discovery	Biological testing
	WO 2009058076	AstraZeneca	Biological testing
	WO 2009060158	Argenta Discovery	Biological testing
	WO 2009060206	Argenta Discovery	Biological testing
	WO 2009080199	Bayer Schering Pharma	Biological testing
	WO 2009135599	Bayer Schering Pharma	Biological testing
	US 6228853	GlaxoSmithKline	Preclinical
	US 6294648	Aerovance	Biological testing
	US 6358928	Enzyme System Products	Biological testing
P-selectin	WO 2000034255	NV Organon	Biological testing

TARGETS

E-selectin

E-selectin is a cellular adhesion molecule (CAM) and CD antigen (CD62E) expressed only on endothelial cells and activated by cytokines. It plays a crucial role in inflammation, mediating leukocyte recruitment to the sites of injury or, more specifically, neutrophil, monocyte and memory T-cell adhesion to cytokine-activated endothelial cells. E-selectin recognizes sialylated carbohydrate groups present on the surface proteins of certain leukocytes, including Lewis X or Lewis A family carbohydrates on proteins expressed by monocytes, granulocytes and T lymphocytes. Neutrophilic inflammation is a pathogenic feature of many airways diseases and inhibition of E-selectin may be an effective therapy for the treatment of chronic obstructive pulmonary disease (COPD) and ALI/ARDS (15-18).

Hepatocyte growth factor receptor

Hepatocyte growth factor (HGF) receptor (EC 2.7.10.1) is a transmembrane receptor tyrosine kinase encoded by the *MET* proto-oncogene. The receptor undergoes activation via HGF (or scatter fac-

tor) binding and induces signaling through proto-oncogene tyrosine-protein kinase Src, growth factor receptor-bound protein 2 (GRB2)/son of sevenless homolog (SOS), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and/or GRB2-associated-binding protein 1 pathways. HGF is required for normal mammalian development and has been shown to be particularly important in cell migration, morphogenic differentiation, proliferation and organization of three-dimensional tubular structures (e.g., renal tubular cells, gland formation, etc.). HGF was first identified as a mitogen for hepatocyte regeneration. However, it is also a multifunctional mesenchymal factor for epithelial regeneration, including the regeneration of alveolar type II epithelial cells. Disruption of vascular endothelial cell barrier integrity is involved in inflammation, tumor angiogenesis, atherosclerosis and ALI/ARDS. Thus, activators of the HGF receptor could be effective in the treatment of these disorders (19-22).

L-selectin

L-selectin is a CAM (also known as CD antigen CD62L) and member of the selectin family of proteins. It is constitutively expressed on leukocytes/neutrophils but is shed upon cellular activation as a result of cleavage mediated by disintegrin and metalloproteinase domain-containing protein 17 (ADAM 17), a membrane-bound metalloproteinase. It has been identified as a peripheral lymph node-homing receptor and several receptors (e.g., GlyCAM-1, CD34, MAdCAM-1) for this CAM have been identified on lymph node high endothelial venules. However, the ligand for L-selectin on inflamed venular endothelium has not been identified. L-selectin recognizes sialylated carbohydrate groups (e.g., members of the Lewis X or Lewis A family) present on surface proteins of certain monocytes, granulocytes and T lymphocytes. Because neutrophilic inflammation is a pathogenic feature of many airways diseases, inhibition of L-selectin may be a therapeutic option for the treatment of conditions such as COPD and ALI/ARDS (17, 23-25).

Macrophage metalloelastase

Macrophage metalloelastase (EC 3.4.24.65; also known as macrophage elastase or matrix metalloproteinase-12 [MMP-12]) belongs to a family of zinc-dependent enzymes (matrixins) that catalyze the hydrolysis of peptide chains, and therefore have the ability to degrade a variety of proteins (i.e., elastin, collagen, proteoglycans, laminin, fibronectin) of the extracellular matrix. These enzymes are functionally categorized into three groups according to their substrate target: collagenases, stromelysins and gelatinases. They are responsible for the degradation of fibrillar collagen, proteoglycans and glycoproteins and denatured and basement membrane collagens, respectively. MMPs, and MMP-12 in particular, are produced by neutrophils, alveolar macrophages and airways epithelial cells. MMP-12 hydrolyzes soluble and insoluble elastin and specifically cleaves between alanine-14 and leucine-15 or tyrosine-16 and leucine-17 of the insulin B chain. Under normal conditions, MMP levels are tightly regulated at both the transcriptional and post-transcriptional levels. However, they are upregulated in pathological states (e.g., inflammation). MMP-12 has been implicated in several clinical conditions, such as arthritis, tumor growth and metastasis, periodontal disease, hepatitis C, multiple sclerosis, COPD and ALI/ARDS. In ALI/ARDS, inhibition of MMP-12 may be an effective therapeutic strategy as it reduces the influx of neutrophils into the alveolar space (26-29).

Neutrophil elastase

Neutrophil elastase (EC 3.4.21.37; also known as human leukocyte elastase, HLE) is an elastin-hydrolyzing serine protease that is a major component of the lung's elastolytic activity and a stimulant of mucus secretion. It also induces the release of interleukin-8 (IL-8) from epithelial cells and may therefore prolong inflammation (MMP-12 hydrolyzes soluble and insoluble elastin). Neutrophils, neutrophil elastase, macrophages, macrophage-derived metalloproteases, lymphocytes, TNF-α and oxidants have all been shown to play a role in the pathogenesis of emphysema, cystic fibrosis, COPD and ALI/ARDS. Thus, inhibition of elastase is a potential therapeutic strategy for the treatment of these and other respiratory disorders (30-33).

p38 Mitogen-activated protein kinases

The class of the p38 mitogen-activated protein kinases (MAPKs) is composed of four isoforms: MAP kinase p38 α (MAPK 14), MAP kinase p38 β (MAPK 11), MAP kinase p38 γ (MAPK 12) and MAP kinase p38 δ (MAPK 13). p38 MAPKs are activated by a variety of cellular stress factors, including osmotic shock, inflammatory cytokines, lipopolysaccharides, ultraviolet light and growth factors. Activation occurs via MAP kinase kinase and MAPK/ERK kinase-mediated phosphorylation at tyrosine residues 180 and 182. Activated p38 MAPKs have been shown to phosphorylate and activate MAPK-activated protein kinase 2 (MAPKAPK-2) and cyclic AMP-dependent transcription factor ATF-2, the Mac transcription factor and myocyte-specific enhancer factor 2A. p38 MAPKs are implicated in mucin secretion and inhibitors of this kinase could be potentially effective as a treatment for the hypersecretion of mucus in the airways seen in COPD and other respiratory disorders. Inhibitors may also reduce lung fibroblast proliferation, which contributes to the pathology of COPD and ALI/ARDS (34-37).

P-selectin

P-selectin is a CAM (also known as CD antigen CD62P) that is involved in acute inflammation and hemostasis. It is expressed by platelets and the endothelium and mediates adhesion, an essential step in the initial recruitment of leukocytes to the site of injury during inflammation; the majority of P-selectin-binding lymphocytes are memory cells. P-selectin is stored in intracellular granules and expression can be rapidly upregulated by several mediators, such as histamine, thrombin and leukotriene C4. P-selectin binds P-selectin glycoprotein ligand 1 (PSGL-1), expressed on most leukocytes, and also recognizes sialylated carbohydrate groups related to the Lewis X or Lewis A family expressed on monocytes, granulocytes and T lymphocytes. Neutrophilic inflammation is a pathogenic feature of COPD, ALI/ARDS and other respiratory disorders, and inhibition of P-selectin may be an effective therapy for these conditions (17, 38, 39).

Tissue factor

Tissue factor (TF; also known as coagulation factor III and CD antigen CD142) initiates the coagulation protease cascade, where it

mediates the formation of thrombin from the zymogen prothrombin. It is a high-affinity receptor for coagulation factor VIIa and is involved in cellular immune responses. TF initiates blood coagulation by forming a complex with circulating coagulation factor VII or VIIa. It is expressed on cells that are not exposed to blood flow (i.e., subendothelial cells), such as fibroblasts and vascular smooth muscle cells, and has three distinct domains: extracellular, transmembrane and cytoplasmic. Under inflammatory conditions and in response to IL-1 and TNF, it is induced on monocytes, platelets and vascular endothelial cells. Studies have shown that plasma and pulmonary edema TF levels were elevated in patients with ALI/ARDS compared to control patients with hydrostatic pulmonary edema. These higher levels were associated with increased mortality and fewer ventilation-free days and the presence of disseminated intravascular coagulation and sepsis, suggesting that systemic activation of coagulation may be involved in the severity and progression of ALI/ARDS. Thus, inhibition of TF may be an effective therapeutic strategy for the treatment of ALI/ARDS (40-42).

DISCLOSURES

The authors state no conflicts of interest.

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