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RESEARCH ARTICLE

Targeting the ATP-Axis in Lungs as a New Therapeutic Modality for COPD

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ABSTRACT

Adenosine 5'-triphosphate (ATP) is found in every cell of the body where it plays a critical role in cellular metabolism and energetics. ATP is released from cells under physiologic and pathophysiologic conditions; extracellular ATP acts as an autocrine and paracrine agent. Its effects on targeted cells are mediated by subtypes of purinergic receptors (P2R). In the lungs, relatively large amounts of ATP are released under inflammatory conditions. Extracellular ATP triggers a central vagal reflex by activating purinergic receptor P2XR localized on pulmonary vagal sensory nerve terminals. This results in cough, bronchoconstriction and the release of pro-inflammatory neuropeptides via axon reflex. COPD patients manifest higher sensetivity to aerosolized ATP than healthy subjects, and the levels of ATP in COPD patients' lungs are 3x that found in healthy subjects. This review succinctly details (i) the sources amd mechanisms of ATP's release into the extracellular space, (ii) the ways extracellular ATP is eliminated, (iii) the deleterious effects of ATP in the lungs in general and in COPD in particular, and (iv) the rationale for the blockade of these actions of ATP in the lungs as a novel therapeutic approach in the management of COPD patients.

Introduction

In recent years, it has become overwhelmingly clear that extracellular adenosine 5′-triphosphate (ATP) is a ubiquitous component of damage-associated molecular patterns (an alarmin)[1], which plays a major role in systemic and localized inflammatory responses. Here we focus on the mechanistic role of ATP in inflammatory processes in the lungs in general and COPD in particular.

ATP is found in every cell of the human body at a concentration range of 5-10 mM, except for platelets, in which its concentration is far higher[2]. Intracellular ATP plays a critical role in cellular metabolism[3]. It is the final source of energy for all body functions at the cellular, tissue, organ, and organism levels. ATP is stored in intracellular vesicles of multiple cell types[4]. In particular, it is stored as a co-transmitter in neurotransmitter vesicles[4]. ATP is released from cells under physiologic and pathophysiologic conditions; extracellular ATP is rapidly degraded by ecto-enzymes to adenosine, which is eliminated from the extracellular space by ecto-adenosine deaminase and active transport into cells (see below). Extracellular ATP may act as an autocrine and paracrine agent[5]. The actions of extracellular ATP are mediated by P2 purinergic receptors (P2R)[6]. These receptors are divided into two families: P2YR, which are seven trans-cell membrane domain G-protein coupled receptors (GPCR; metabotropic)[7], and P2XR, which are cationic channels (ionotropic)[8]. Eight P2YR and seven P2XR have now been cloned. Several P2XR heterotrimers have also been identified including P2X2/3R, which manifests combined characteristics of P2X2R and P2X3R[9,10]. P2R are highly expressed in the lungs[11,12], and purinergic signaling plays important roles in alveolar homeostasis, but is also involved in the development and progression of severe pathological conditions like acute lung injury, fibrosis and cancer[13-15].

The continued exponential growth of research activities dealing with the role of purine nucleosides and nucleotides in pulmonary physiology and pathophysiology is manifested in the large number of papers that have been published since the publication of our recent reviews[14,15]. Accordingly, it is inevitable that the coverage of the relevant research in this review is succinct, and article citation is limited.

ATP release: ATP is released from cells under physiologic and pathophysiologic conditions. Multiple mechanisms mediate the release of ATP including exocytosis, large membrane pores and specific trans-cell membrane ionic channels[16-19]. There are several sources for extracellular ATP[20]: Large amounts of ATP are found in platelets and ATP is released during platelet activation[21-23]. ATP is also stored in red blood cells (RBC), from which it is released under conditions of imbalance between O_2 supply and O_2 demand[24-26]. In addition, several biologic substances as well as increased blood flow can induce the release of ATP from vascular endothelial cells[27-31] and smooth muscle cells[32,33]. Pannexin channels and connexin hemichannels play a critical role in this release[34]. ATP is also released from nerves as a co-transmitter [35] and from exercising skeletal muscles[36]. In the heart, ATP is released into the extracellular fluid under various conditions. Specifically, ATP's release is evoked by sympathetic nerve stimulation and by catecholamines[37-40]. In addition, ATP is released in the heart during acute myocardial ischemia[41], and from cardiac myocytes in response to hypoxia [42,43]. During inflammation, ATP is

released from inflammatory cells[44,45]. For example, ATP is released from mast cells following FcεRI-cross linking[46]. Elevated extracellular concentrations of ATP have been found in the lungs of COPD patients[47,48]. Increased plasma concentrations of ATP have also been reported in COPD patients and are correlated with disease severity. Hlapčić I, Hulina-Tomašković A, Somborac-Bačura A, Rajković MG, Dugac AV, Popović-Grle S, Rumora L. Extracellular adenosine triphosphate is associated with airflow limitation severity and symptoms burden in patients with chronic obstructive pulmonary disease[49]. Pulmonary ATP concentrations are also increased in a mouse model of smoke-induced acute lung inflammation and emphysema[50,51] and in human smokers[52].

Degradation of extracellular ATP:

Extracellular ATP is rapidly and sequentially degraded by ectonucleotidases, including ectonucleoside triphosphate dephosphorylase-1 (CD39) and ecto-5'-nucleotidase (CD73) to ADP, adenosine monophosphate (AMP), and adenosine; the latter, exerts its own effects on targeted cells by activating P1 purinergic cell-surface receptors (A1R, A2aR, A2bR, and A3R) [53-56]. CD73 is widely expressed in a variety of tissues, including the colon, kidney, brain, liver, heart, lung, spleen, and bone marrow[57]. CD39 is expressed by multiple cell-types including epithelial, endothelial and immune cells. It is highly expressed in different human tumor types[58]. Adenosine is rapidly eliminated from the extracellular space by ecto-adenosine deaminase and active transport into cells[59]. Extracellular adenosine acts as an anti-inflammatory agent, the actions of which are mediated by A2aR, A2bR and A3R[60]. However, adenosine can indirectly cause bronchoconstriction by activating airways mast cells[61], and cause CD73-dependent excessive neutrophil infiltration through the upregulated expression of the A2aR receptor in a murine model of asthma[62]. Therefore, the levels of CD39 and CD73 and their enzymatic activities play a critical role in controlling the duration and magnitude of autocrine and paracrine effects of ATP and adenosine. Multiple studies have shown that the level of these enzymes is altered during pathophysiologic conditions. For example, increased expression of CD39 and CD73 by pulmonary epithelial and endothelial cells was observed during high inspiratory pressure-induced lung injury[63]. Also, upregulation of CD39/CD73 expression has been observed in patients with small cell lung cancer as well as in patients with a broad spectrum of solid cancers[64]. It has most recently recognized that CD73 manifest a bimodal activity in the lungs, specifically, certain pathophysiologic conditions are associated with harmful effects of CD73, while others are associated with beneficial effects[65].

ATP axis in the lungs: In 1996, Pelleg and Hurt reported for the first time that extracellular (intravenous) ATP stimulates canine vagal sensory nerve terminals in the lungs by activating P2XR localized on slowly conducting C-fibers[66] as well as fast conducting Aδ fibers (see Figure 1 in [15]). The former are bimodal receptors, i.e., they respond to either mechanical (stretch) or chemical (ATP, capsaicin) stimuli[66]. Also in 1996, a study in human subjects demonstrated that aerosolized ATP is a potent bronchoconstrictor in healthy subjects and more so, in asthmatic patients[67]. A subsequent study in 1998 established

the cause-and-effect relationship between these two fundamental observations; in that study, bronchoconstriction in the canine lungs was caused by extracellular ATP, thereby indicating that ATP's stimulation of vagal sensory nerve terminals in the lungs triggers a central pulmonary-pulmonary vagal reflex[68]. Since C- and Aδ fibers mediate cough[69,70], the fact that ATP stimulates both fiber types strongly suggest that extracellular ATP is an important tussigenic agent[71-77]. The stimulation by ATP of these nerve terminals could also lead to localized release of pro-inflammatory neuropeptides via the axon reflex[78-80].

A year later, the spectrum of ATP's effects in the lungs was significantly broaden by the observation that extracellular ATP markedly enhanced the IgE-dependent histamine release in human lung mast-cells[81]. Based on these early studies, a seminal review put forward the hypothesis that extracellular ATP plays a major mechanistic role in pulmonary disorders, what was termed as "*ATP Axis in Obstructive Airway Diseases*" (Figure 1)[82]. Since then, voluminous data obtained in numerous studies have validated this original claim that extracellular ATP plays a major mechanistic role in multiple pulmonary disorders[14,15,83].

In addition to P2X3R and P2X2/3R that mediate the action of ATP on vagal sensory nerve terminals in the lungs[84-86], P2X4R has been more recently implicated in the deleterious effects of ATP in the lungs. Specifically, the expression of P2X4R that are predominantly expressed in secretory cells of the airways, is upregulated in those cells under inflammatory conditions, and activation of P2X4R enhances mucin secretion and potentially contributes to mucus hypersecretion and mucus plaque formation[87]. In addition, P2X4R has also been implicated in ATP-induced contraction of tracheal and bronchial smooth muscle cells[88,89] as well as airway remodeling by acting on the phenotype switching of bronchial smooth muscle cells[90]. Last but not least, P2X4R mediates the ATP-induced augmentation of immune response-induced mast cell degranulation[91].

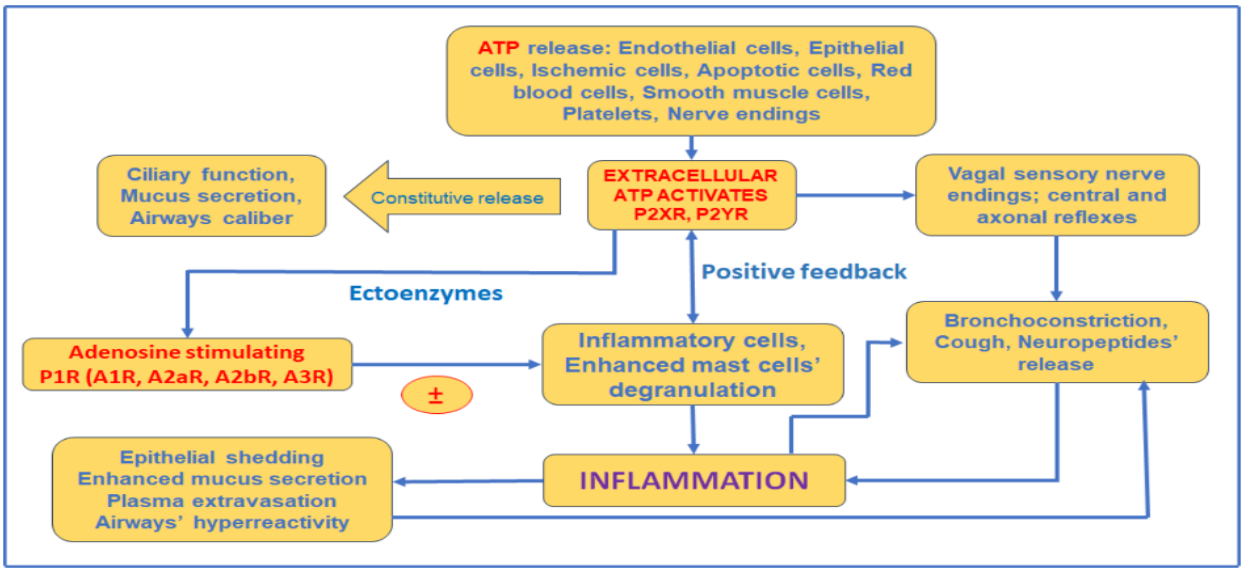

Figure 1: A schematic outline of the "ATP Axis in the Lungs." ATP released from multiple cell types activates P2

purinergic receptors (P2R). The latter triggers a pulmonary central vagal reflex leading to bronchoconstriction and cough, as well as an axonal reflex leading to the localized release of pro-inflammatory neuropeptides. In addition, ATP stimulates inflammatory cells and enhances IgE-dependent degranulation and histamine release from lung mast cells, and acting as an autocrine agent, causing the release of ATP in a positive feedback loop. Extracellular ATP is degraded by ectoenzymes to adenosine; adenosine acts either as an anti- or pro-inflammatory agent, the action of which are mediated by P1R (i.e., A2aR, A2bR and A3R).

ATP and COPD: COPD is associated with chronic inflammation of the airways and lung parenchyma as well as systemic inflammation[92]. This inflammatory process increases further during acute exacerbations episodes in COPD patients[93]. Chronic and acute inflammations[94] are complex pathological processes manifested by a variety of molecular events, including the activation of immune cells and the release of pro-inflammatory cytokines[95]. Extracellular ATP acts as a major pro-inflammatory agent[95]; it is chemotactic to and activator of inflammatory cells such as neutrophils, macrophages, dendritic cells, and memory T cells[96]. As noted above, the involvement of ATP in pulmonary inflammation is well documented[97-99]. The facts that (i) ATP is a pro-inflammatory agent, (ii) its levels are increased in the lungs of COPD patients and (iii) several P2R mediating the effects of ATP in the lungs have been identified, constitute a strong rationale for the targeting of these P2R and their signal transduction pathways as a new therapeutic modality in the management of patients with COPD and chronic cough. COPD remains a critical unmet clinical need and therefore, the potential addition of ATP-P2R based therapy in this arena would be attractive. Indeed, several P2X3R antagonists are currently being developed as oral medications for the treatment of chronic cough. Several P2X3R antagonists are currently being developed as oral medication for the treatment of chronic cough. These include the phenoxy-diaminopyrimidines including gefapixant (AF-219)[100,101], the imidazo-pyridines like camlipixant (BLU-5937)[72], which are in phase III clinical trials and eliapixant (BAY-1817080)[102], and sivopixant (S-600918)[103] which are in Phase II clinical trials (Figure 2).

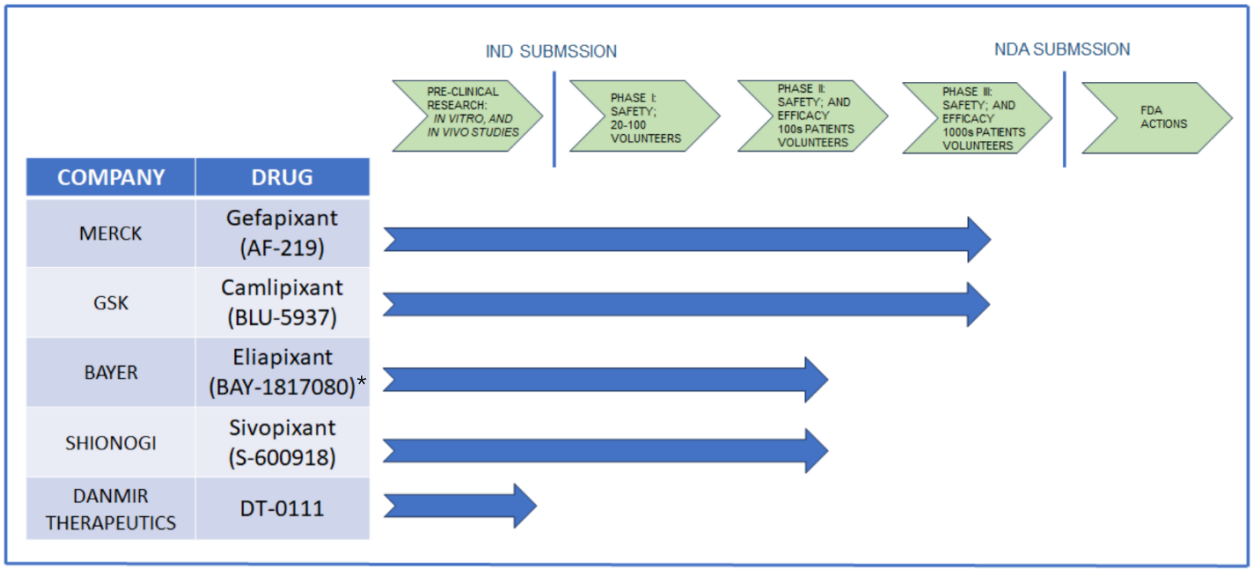

Figure 2: Current developmental stages of drug-candidates aimed at treating patients with chronic cough. Of the five drug candidates only DT-0111 is being developed as an aerosol. *On February 4, 2023, Bayer

announced that it will discontinue the clinical development of BAY-1817080. (https://www.bayer.com/media/en-us/bayer-will-discontinue-phase-ii-development-candidate-eliapixant/)

Inhalation is the preferred administration route for COPD therapy because it facilitates reaching localized relatively high drug concentrations within the lungs, leading to increased efficacy and decreased systemic adverse events versus other administration routes (e.g., oral or intravenous)[104]. Indeed, inhalations of long-acting muscarinic antagonists and long-acting adrenergic agonists (LAMA and LABA, respectively) with and without corticosteroids has been the hallmark of therapy for COPD, and pending approval, phosphodiesterase 3/4 inhibitor[105]. The first inhaled drug-candidate targeting ATP-axis in the lung as a novel therapy for COPD is DT-0111 (DT), which is a novel small-water soluble molecule that acts as a selective P2X3R antagonist[74,106]. In preclinical proof-of-concept studies, aerosolized DT effectively (i.e., an optimal dose of 0.14 mg/kg) suppressed aerosolized ATP-induced bronchoconstriction and cough in free moving conscious animals[74]. While these observations are yet to be replicated in human subjects, they indicate that DT might be the first inhaled drug to combine bronchodilatory, anti-tussive and anti-inflammatory actions.

Conclusions:

Over more than two decades since it was originally proposed in 2002 that extracellular ATP plays an important mechanistic role in pulmonary disorders[82], numerous studies have generated voluminous data supporting this hypothesis. The identification of specific signal transduction pathways activated by extracellular ATP's binding to pulmonary cell-surface P2R, has facilitated the novel drug discovery process based on the pharmacologic manipulation of these pathways. We suggest here that future development in this arena could go beyond anti-tussive therapies to include novel therapeutic approach in the treatment of COPD.

Conflict of Interest Statement:

AP is the CEO and CSO of Danmir Therapeutics, LLC (Danmir), the lead drug-candidate of which is being developed as a novel treatment of COPD. PJB is a medical consultant of Danmir. ESS declares no conflict.

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References:

1. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* 2007; 81(1): 1-5.

2. Meyers KM, Holmsen H, Seachord CL. Comparative study of platelet dense granule constituents. *The American journal of physiology* 1982; 243(3): R454-61.

3. Nirody JA, Budin I, Rangamani P. ATP synthase: Evolution, energetics, and membrane interactions. *J Gen Physiol* 2020; 152(11).

4. Bonora M, Patergnani S, Rimessi A, et al. ATP synthesis and storage. *Purinergic Signal* 2012; 8(3): 343-57.

5. Corriden R, Insel PA. Basal release of ATP: an autocrine-paracrine mechanism for cell regulation. *Sci Signal* 2010; 3(104): re1.

6. Burnstock G. Purine and pyrimidine receptors. *Cell Mol Life Sci* 2007; 64(12): 1471-83.

7. Jacobson KA, Delicado EG, Gachet C, et al. Update of P2Y receptor pharmacology: IUPHAR Review 27. *Br J Pharmacol* 2020; 177(11): 2413-33.

8. Illes P, Muller CE, Jacobson KA, et al. Update of P2X receptor properties and their pharmacology: IUPHAR Review 30. *Br J Pharmacol* 2021; 178(3): 489-514.

9. Burnstock G. Purine and purinergic receptors. *Brain Neurosci Adv* 2018; 2: 2398212818817494.

10. Jacobson KA, Muller CE. Medicinal chemistry of adenosine, P2Y and P2X receptors. *Neuropharmacology* 2016; 104: 31-49.

11. Burnstock G, Brouns I, Adriaensen D, Timmermans JP. Purinergic signaling in the airways. *Pharmacol Rev* 2012; 64(4): 834-68.

12. Lazarowski ER, Boucher RC. Purinergic receptors in airway epithelia. *Curr Opin Pharmacol* 2009; 9(3): 262-7.

13. Wirsching E, Fauler M, Fois G, Frick M. P2 Purinergic Signaling in the Distal Lung in Health and Disease. *Int J Mol Sci* 2020; 21(14).

14. Pelleg A. Extracellular adenosine 5'-triphosphate in pulmonary disorders. *Biochem Pharmacol* 2021; 187: 114319.

15. Pelleg A, Schulman ES, Barnes PJ. Extracellular Adenosine 5'-Triphosphate in Obstructive Airway Diseases. *Chest* 2016; 150(4): 908-15.

16. Taruno A. ATP Release Channels. *Int J Mol Sci* 2018; 19(3).

17. Penuela S, Gehi R, Laird DW. The biochemistry and function of pannexin channels. *Biochim Biophys Acta* 2013; 1828(1): 15-22.

18. Chekeni FB, Elliott MR, Sandilos JK, et al. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. *Nature* 2010; 467(7317): 863-7.

19. Adamson SE, Leitinger N. The role of pannexin1 in the induction and resolution of inflammation. *FEBS Lett* 2014; 588(8): 1416-22.

20. Bodin P, Burnstock G. Purinergic signalling: ATP release. *Neurochem Res* 2001; 26(8-9): 959-69.

21. Mills DCB, Robb I.A., Roberts, G.C.K. The release of nucleotides, 5-hydroxytryptamine and enzymes from human blood platelets during aggregation. *J Physiol (Lond)* 1968; 195(3): 715-29.

22. Day HJ, Holmsen, H. Concepts of the blood platelet release reaction. *Semin Hematol* 1971; 4(1): 3-27.

23. Holmsen H. Platelet metabolism and activation. *Semin Hematol* 1985; 22(3): 219-40.

24. Dean BM, Perrett D. Studies on adenine and adenosine metabolism by intact human erythrocytes using high performance liquid chromatography. *Biochim Biophys Acta* 1976; 437(1): 1-5.

25. Bergfeld GR, Forrester T. Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. *Cardiovasc Res* 1992; 26(1): 40-7.

26. Ellsworth ML, Forrester T, Ellis CG, Dietrich HH. The erythrocyte as a regulator of vascular tone. *The American journal of physiology* 1995; 269(6 Pt 2): H2155-61.

27. Pearson JD, Gordon JL. Vascular endothelial and smooth muscle cells in culture selectively release adenine nucleotides. *Nature* 1979; 281(5730): 384-6.

28. Bodin P, Bailey D, Burnstock G. Increased flow-induced ATP release from isolated vascular endothelial cells but not smooth muscle cells. *Br J Pharmacol* 1991; 103(1): 1203-5.

29. Ralevic V, Milner P, Kirkpatrick KA, Burnstock G. Flow-induced release of adenosine 5'-triphosphate from endothelial cells of the rat mesenteric arterial bed. *Experientia* 1992; 48(1): 31-4.

30. Yang S, Cheek DJ, Westfall DP, Buxton IL. Purinergic axis in cardiac blood vessels. Agonist-mediated release of ATP from cardiac endothelial cells. *Circ Res* 1994; 74(3): 401-7.

31. Bodin P, Burnstock G. ATP-stimulated release of ATP by human endothelial cells. *J Cardiovasc Pharmacol* 1996; 27(6): 872-5.

32. Katsuragi T, Tamesue S, Sato C, Sato Y, Furukawa T. ATP release by angiotensin II from segments and cultured smooth muscle cells of guinea-pig taenia coli. *Naunyn Schmiedebergs Arch Pharmacol* 1996; 354(6): 796-9.

33. Katsuragi T, Soejima O, Tokunaga T, Furukawa T. Evidence for postjunctional release of ATP evoked by stimulation of muscarinic receptors in ileal longitudinal muscles of guinea pig. *J Pharmacol Exp Ther* 1992; 260(3): 1309-13.

34. Begandt D, Good ME, Keller AS, et al. Pannexin channel and connexin hemichannel expression in vascular function and inflammation. *BMC Cell Biol* 2017; 18(Suppl 1): 2.

35. Lundberg JM. Pharmacology of cotransmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. *Pharmacol Rev* 1996; 48(1): 113-78.

36. Forrester T. An estimate of adenosine triphosphate release into the venous effluent from exercising human forearm muscle. *The Journal of physiology* 1972; 224(3): 611-28.

37. Fredholm BB, Hedqvist P, Lindstrom K, Wennmalm M. Release of nucleosides and nucleotides from the rabbit heart by sympathetic nerve stimulation. *Acta Physiol Scand* 1982; 116(3): 285-95.

38. Darius H, Stahl GL, Lefer AM. Pharmacologic modulation of ATP release from isolated rat hearts in response to vasoconstrictor stimuli using a continuous flow technique. *J Pharmacol Exp Ther* 1987; 240(2): 542-7.

39. Katsuragi T, Tokunaga T, Ohba M, Sato C, Furukawa T. Implication of ATP released from atrial, but not papillary, muscle segments of guinea pig by isoproterenol and forskolin. *Life Sci* 1993; 53(11): 961-7.

40. Tokunaga T, Katsuragi T, Sato C, Furukawa T. ATP release evoked by isoprenaline from adrenergic nerves of guinea pig atrium. *Neurosci Lett* 1995; 186(2-3): 95-8.

41. Paddle BM, Burnstock G. Release of ATP from perfused heart during coronary vasodilatation. *Blood Vessels* 1974; 11(3): 110-9.

42. Forrester T, Williams CA. Release of adenosine triphosphate from isolated adult heart cells in response to hypoxia. *The Journal of physiology* 1977; 268(2): 371-90.

43. Williams CA, Forrester T. Possible source of adenosine triphosphate released from rat myocytes in response to hypoxia and acidosis. *Cardiovasc Res* 1983; 17(5): 301-12.

44. Dosch M, Gerber J, Jebbawi F, Beldi G. Mechanisms of ATP Release by Inflammatory Cells. *Int J Mol Sci* 2018; 19(4).

45. Baxter M, Eltom S, Dekkak B, et al. Role of transient receptor potential and pannexin channels in cigarette smoke-triggered ATP release in the lung. *Thorax* 2014; 69(12): 1080-9.

46. Tsai Shihn H, Kinoshita M, Kusu T, et al. The Ectoenzyme E-NPP3 Negatively Regulates ATP-Dependent Chronic Allergic Responses by Basophils and Mast Cells. *Immunity* 2015; 42(2): 279-93.

47. Bucchioni E CZ, Polkey M, Collins JV, Cramer D, Allegra L, Barnes PJ, Kharitonov SA. Adenosine 5'-triphosphate (ATP) is increased in exhaled breath condensate in COPD. *Am J Respir Crit Care Med* 2002; 165: A599.

48. Basoglu OK, Barnes PJ, Kharitonov SA, Pelleg A. Effects of Aerosolized Adenosine 5'-Triphosphate in Smokers and Patients With COPD. *Chest* 2015; 148(2): 430-5.

49. Hlapčić I, Hulina-Tomašković A, Somborac-Bačura A, Rajković MG, Dugac AV, Popović-Grle S, Rumora L. Extracellular adenosine triphosphate is associated with airflow limitation severity and symptoms burden in patients with chronic obstructive pulmonary disease. Scientific Reports 2019; 9:15349. https://doi.org/10.1038/s41598-019-51855

50. Mortaz E, Braber S, Nazary M, Givi ME, Nijkamp FP, Folkerts G. ATP in the pathogenesis of lung emphysema. *Eur J Pharmacol* 2009; 619(1-3): 92-6.

51. Cicko S, Lucattelli M, Muller T, et al. Purinergic Receptor Inhibition Prevents the Development of Smoke-Induced Lung Injury and Emphysema. *J Immunol* 2010; 185(1): 688-97.

52. Lommatzsch M, Cicko S, Müller T, et al. Extracellular Adenosine Triphosphate and Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2010; 181(9): 928-34.

53. Colgan SP, Eltzschig HK, Eckle T, Thompson LF. Physiological roles for ecto-5'-nucleotidase (CD73). *Purinergic Signal* 2006; 2(2): 351-60.

54. Knapp K, Zebisch M, Pippel J, El-Tayeb A, Müller CE, Sträter N. Crystal structure of the human ecto-5'-nucleotidase (CD73): insights into the regulation of purinergic signaling. *Structure* 2012; 20(12): 2161-73.

55. Schetinger MR, Morsch VM, Bonan CD, Wyse AT. NTPDase and 5'-nucleotidase activities in physiological and disease conditions: new perspectives for human health. *Biofactors* 2007; 31(2): 77-98.

56. Zhao H, Bo C, Kang Y, Li H. What Else Can CD39 Tell Us? *Frontiers in Immunology* 2017; 8.

57. Antonioli L, Pacher P, Vizi ES, Hasko G. CD39 and CD73 in immunity and inflammation. *Trends Mol Med* 2013; 19(6): 355-67.

58. Timperi E, Barnaba V. CD39 Regulation and Functions in T Cells. *Int J Mol Sci* 2021; 22(15).

59. Jacobson KA, Pradhan B, Wen Z, Pramanik A. New paradigms in purinergic receptor ligand discovery. *Neuropharmacology* 2023; 230: 109503.

60. Pasquini S, Contri C, Borea PA, Vincenzi F, Varani K. Adenosine and Inflammation: Here, There and Everywhere. *Int J Mol Sci* 2021; 22(14).

61. Polosa R. Adenosine-receptor subtypes: their relevance to adenosine-mediated responses in asthma and chronic obstructive pulmonary disease. *Eur Respir J* 2002; 20(2): 488-96.

62. Liu TT, Wang YL, Zhang Z, et al. Abnormal adenosine metabolism of neutrophils inhibits airway inflammation and remodeling in asthma model induced by Aspergillus fumigatus. *BMC Pulm Med* 2023; 23(1): 258.

63. Eckle T, Fullbier L, Wehrmann M, et al. Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. *Journal of immunology* 2007; 178(12): 8127-37.

64. Li J, Wang L, Chen X, et al. CD39/CD73 upregulation on myeloid-derived suppressor cells via TGF-beta-mTOR-HIF-1 signaling in patients with non-small cell lung cancer. *Oncoimmunology* 2017; 6(6): e1320011.

65. Hu XM, Shi NR, Zhang JZ, et al. CD73: Friend or Foe in Lung Injury. *Int J Mol Sci* 2023; 24(6).

66. Pelleg A, Hurt CM. Mechanism of action of ATP on canine pulmonary vagal C fibre nerve terminals. *The Journal of physiology* 1996; 490 (Pt 1): 265-75.

67. Pellegrino R, Wilson O, Jenouri G, Rodarte JR. Lung mechanics during induced bronchoconstriction. *J Appl Physiol* 1996; 81(2): 964-75.

68. Katchanov G, Xu J, Schulman ES, Pelleg A. ATP causes neurogenic bronchoconstriction in the dog. *Drug Dev Res* 1998; 45(3-4): 342-9.

69. Canning BJ, Chang AB, Bolser DC, Smith JA, Mazzone SB, McGarvey L. Anatomy and Neurophysiology of Cough. *Chest* 2014; 146(6): 1633-48.

70. Widdicombe JG. Neurophysiology of the cough reflex. *Eur Respir J* 1995; 8(7): 1193-202.

71. Kamei J, Takahashi Y, Yoshikawa Y, Saitoh A. Involvement of P2X receptor subtypes in ATP-induced enhancement of the cough reflex sensitivity. *Eur J Pharmacol* 2005; 528(1-3): 158-61.

72. Abdulqawi R, Dockry R, Holt K, et al. P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study. *The Lancet* 2015; 385(9974): 1198-205.

73. Garceau D, Chauret N. BLU-5937: A selective P2X3 antagonist with potent anti-tussive effect and no taste alteration. *Pulm Pharmacol Ther* 2019; 56: 56-62.

74. Pelleg A, Xu F, Zhuang J, Undem B, Burnstock G. DT-0111: a novel drug-

candidate for the treatment of COPD and chronic cough. *Ther Adv Respir Dis* 2019; 13: 1753466619877960.

75. Burgoyne DS. Managed care considerations for the treatment of chronic cough. *Am J Manag Care* 2022; 28(9 Suppl): S166-S74.

76. Sykes DL, Zhang M, Morice AH. Treatment of chronic cough: P2X3 receptor antagonists and beyond. *Pharmacol Ther* 2022; 237: 108166.

77. Basoglu OK, Pelleg A, Essilfie-Quaye S, Brindicci C, Barnes PJ, Kharitonov SA. Effects of aerosolized adenosine 5'-triphosphate vs adenosine 5'-monophosphate on dyspnea and airway caliber in healthy nonsmokers and patients with asthma. *Chest* 2005; 128(4): 1905-9.

78. Barnes PJ. Asthma as an axon reflex. *The Lancet* 1986; 327(8475): 242-5.

79. Barnes PJ. Neurogenic Inflammation in Airways. *Int Arch Allergy Immunol* 1991; 94(1-4): 303-9.

80. Barnes PJ, Baraniuk JN, Belvisi MG. Neuropeptides in the Respiratory Tract: Part I. *Am Rev Respir Dis* 1991; 144(5): 1187-98.

81. Schulman ES, Glaum MC, Post T, et al. ATP Modulates Anti-IgE–Induced Release of Histamine from Human Lung Mast Cells. *Am J Respir Cell Mol Biol* 1999; 20(3): 530-7.

82. Pelleg A, Schulman ES. Adenosine 5'-Triphosphate Axis in Obstructive Airway Diseases. *Am J Therap* 2002; 9(5): 454-64.

83. Mortaz E, Folkerts G, Nijkamp FP, Henricks PA. ATP and the pathogenesis of COPD. *Eur J Pharmacol* 2010; 638(1-3): 1-4.

84. Pelleg A, Undem BJ. A-317491 Inhibits the activation of guinea-pig pulmonary vagal sensory nerve terminals by α,β methylene_ATP. *Clin Immunol* 2005; 115(Suppl 1): S59-60.

85. Weigand LA, Ford AP, Undem BJ. A role for ATP in bronchoconstriction-induced activation of guinea pig vagal intrapulmonary C-fibres. *The Journal of physiology* 2012; 590(16): 4109-20.

86. Ford AP, Undem BJ. The therapeutic promise of ATP antagonism at P2X3 receptors in respiratory and urological disorders. *Front Cell Neurosci* 2013; 7: 267.

87. Winkelmann VE, Thompson KE, Neuland K, et al. Inflammation-induced upregulation of P2X(4) expression augments mucin secretion in airway epithelia. *Am J Physiol Lung Cell Mol Physiol* 2019; 316(1): L58-L70.

88. Obara K, Inaba R, Kawakita M, Murata A, Yoshioka K, Tanaka Y. Effects of NP-1815-PX, a P2X4 Receptor Antagonist, on Contractions in Guinea Pig Tracheal and Bronchial Smooth Muscles. *Biol Pharm Bull* 2022; 45(8): 1158-65.

89. Nagaoka M, Nara M, Tamada T, et al. Regulation of adenosine 5'-triphosphate (ATP)-gated P2X(4) receptors on tracheal smooth muscle cells. *Respir Physiol Neurobiol* 2009; 166(1): 61-7.

90. Wang L, Feng X, Hu B, Xia Q, Ni X, Song Y. P2X4R promotes airway remodeling by acting on the phenotype switching of bronchial smooth muscle cells in rats. *Purinergic Signal* 2018; 14(4): 433-42.

91. Yoshida K, Ito MA, Sato N, et al. Extracellular ATP Augments Antigen-Induced Murine Mast Cell Degranulation and Allergic Responses via P2X4 Receptor Activation. *Journal of immunology* 2020; 204(12): 3077-85.

92. Barnes PJ. Cellular and molecular mechanisms of chronic obstructive pulmonary disease. *Clin Chest Med* 2014; 35(1): 71-86.

93. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2016; 138(1): 16-27.

94. Kuprash DV, Nedospasov SA. Molecular and Cellular Mechanisms of Inflammation. *Biochemistry (Mosc)* 2016; 81(11): 1237-9.

95. Liu JP, Liu SC, Hu SQ, et al. ATP ion channel P2X purinergic receptors in inflammation response. *Biomed Pharmacother* 2023; 158: 114205.

96. Linden J, Koch-Nolte F, Dahl G. Purine Release, Metabolism, and Signaling in the Inflammatory Response. *Annu Rev Immunol* 2019; 37: 325-47.

97. Adriaensen D. Purinergic signalling in the lung: important in asthma and COPD? *Curr Opin Pharmacol* 2004; 4(3): 207-14.

98. Idzko M, Hammad H, van Nimwegen M, et al. Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nature Med* 2007; 13(8): 913-9.

99. Vieira RP, Müller T, Grimm M, et al. Purinergic Receptor Type 6 Contributes to Airway Inflammation and Remodeling in Experimental Allergic Airway Inflammation. *Am J Respir Crit Care Med* 2011; 184(2): 215-23.

100.Birring SS, Dicpinigaitis PV, Smith JA, et al. Efficacy and Safety of Gefapixant for Refractory or Unexplained Chronic Cough over 52 Weeks. *Am J Respir Crit Care Med* 2023; 207(11): 1539-42.

101. McGarvey L, Sher M, Shvarts YG, et al. The Efficacy and Safety of Gefapixant in a Phase 3b Trial of Patients with Recent-Onset Chronic Cough. *Lung* 2023; 201(2): 111-8.

102. Morice A, Smith JA, McGarvey L, et al. Eliapixant (BAY 1817080), a P2X3 receptor antagonist, in refractory chronic cough: a randomised, placebo-controlled, crossover phase 2a study. *Eur Respir J* 2021; 58(5).

103. McGarvey L, Smith JA, Morice A, et al. A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Phase 2b Trial of P2X3 Receptor Antagonist Sivopixant for Refractory or Unexplained Chronic Cough. *Lung* 2023; 201(1): 25-35.

104. Barjaktarevic IZ, Milstone AP. Nebulized Therapies in COPD: Past, Present, and the Future. *Int J Chron Obstruct Pulmon Dis* 2020; 15: 1665-77.

105. Labaki WW, Rosenberg SR. Chronic Obstructive Pulmonary Disease. *Ann Intern Med* 2020; 173(3): ITC17-ITC32.

106. Pelleg A, Sirtori E, Rolland JF, Mahadevan A. DT-0111: a novel P2X3 receptor antagonist. *Purinergic Signal* 2023.