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Transient receptor potential (TRP) channels in pulmonary chemical injuries and as countermeasure targets

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Abstract

The lung is highly sensitive to chemical injuries caused by exposure to threat agents in industrial or transportation accidents, occupational exposures, or deliberate use as weapons of mass destruction (WMD). There are no antidotes for the majority of the chemical threat agents and toxic inhalation hazards (TIH) despite their use as WMDs for more than a century. Among several putative targets, evidence for transient receptor potential (TRP) ion channels as mediators of injury by various inhalational chemical threat agents is emerging. TRP channels are expressed in the respiratory system and are essential for homeostasis. Among TRP channels, the body of literature supporting essential roles for TRPA1, TRPV1, and TRPV4 in pulmonary chemical injuries is abundant. TRP channels mediate their function through sensory neuronal and non-neuronal pathways. TRP channels play a crucial role in complex pulmonary pathophysiologic events including, but not limited to, increased intracellular calcium levels, signal transduction, recruitment of proinflammatory cells, neurogenic inflammatory pathways, cough reflex, hampered mucus clearance, disruption of the integrity of the epithelia, pulmonary edema, and fibrosis. In this review, we summarize the role of TRP channels in chemical threat agents–induced pulmonary injuries and how these channels may serve as medical countermeasure targets for broader indications.

Keywords

TRP ion channels; toxic inhalation hazards; chlorine gas; tear gas agents; acute lung injury; medical countermeasures

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Competing interests

S.A. declares no competing interests. S.-E.J. served on the scientific advisory board of Hydra Biosciences Inc, a biopharmaceutical company that develops TRP ion channel inhibitors for the treatment of pain and inflammation.

Introduction

Several highly toxic chemicals are recognized as serious public health threats due to their potential to be released in domestic, occupational, or transportation accidents, or use as chemical warfare agents. More than accidental hazards in domestic and occupational settings, chemical warfare and terrorism have been high priority concerns because of their huge health and psychological impact and recent repeated incidents in Syria, Iraq, Egypt, and other Middle Eastern countries.^{1–6} Many inhalational chemical warfare agents, including choking agents such as chlorine gas, phosgene, chloropicrin, acrolein, ethyldichlorasine, and perfluoroisobutylene; blistering agents such as sulfur mustard, nitrogen mustard, phosgene oxime, and lewisite; riot-control agents (RCA) such as acrolein, chloropicrin, dibenz(b,f)(1,4)oxazepine (CR), chloracetophenone (CN), and *o*-chlorobenzylidenemalononitrile (CS), have been deployed dating back to historical periods.² The resurgence of chemical warfare in the last two decades has brought speculation about their use as weapons of mass destruction (WMDs) by terrorist organizations. Despite their long history of use as chemical warfare agents, there are no specific antidotes against the majority of them. Unlike diseases caused by biological warfare agents or most disease states, chemical exposures can occur quickly, with immediate casualties and higher morbidity/mortality rates. Although the use of chemicals as weapons of mass destruction captures the limelight, occupational and transportation chemical hazards also deserve special focus.^{2, 7} Reports from the Association of American Railroads (AAR) reveal that over 100,000 tank carloads of toxic inhalation hazard (TIH) chemicals are transported annually.⁸ Most of the railroads pass through densely populated cities, and accidental derailment could pose a great risk to the civilian population.⁹

Chemical weapons are defined as toxic chemicals and their precursors; munitions and devices; and any equipment specifically designed for use directly in connection with such weapons. Overall, chemical warfare agents caused more casualties and deaths in human history compared with biological and radiological warfare agents. Exposure to chemical agents manifests diverse symptoms in different organ systems. Out of the many clinical manifestations of chemical threat agent exposures, pulmonary symptoms produce the most dramatic effects. Chemical inhalational agents cause a spectrum of pulmonary symptoms depending on the concentration and duration of exposure. The consequences could be acute or chronic. There is no common mechanism of action through which most of the causative chemical threat agents work, but the clinical symptoms share several common features. Several hypotheses have been posited for the mechanism of action of these chemical agents in causing pulmonary injuries, including (but not limited to) activation of transient receptor potential (TRP) ion channels, oxidative stress, depletion of nitric oxide, the elevation of inducible nitric oxide synthase (iNOS), the elevation of tissue plasminogen activator (tPA), mitochondrial stress, the elevation of histamine levels, activation of prostanoid or β -adrenergic receptors, elevation of phosphodiesterase 4, Wnt/ β -catenin signaling, tissue factor pathway inhibitor (TFPI), coagulation, and depletion of vitamin B12.^{10–25} Out of all these putative targets, TRP ion channels have been shown to play an important role in mediating the effects of most of the chemical threat agents that cause pulmonary injuries.^{20, 22, 26–29} TRP ion channels play essential roles in the regulation of many critical respiratory

physiological mechanisms, such as the cough reflex, mucociliary clearance, epithelial barrier functions, neurogenic inflammatory pathways, alveolar–capillary barrier function, and lung perfusion.^{10, 19–23, 27–32} In this review, we summarize the role of TRP channels in chemical threat agents–induced pulmonary injuries and their potential as targets for the development of medical countermeasures based on the current literature repertoire and anecdotal reports.

Introduction to TRP channels

TRP channels are a group of relatively non-specific cationic channels located mostly on the plasma membranes of animal tissues. These channels respond to a gamut of heterogeneous stimuli, including endogenous and exogenous chemical mediators, physical stimuli such as mechanical force and temperature, free cytosolic Ca^{2+} ions, depletion of Ca^{2+} stores in the endoplasmic reticulum, and many others. Many TRP channels are voltage-sensitive and ligand-gated. These channels were originally discovered in the *trp* mutant strain of the fruit fly *Drosophila melanogaster* in 1969, and the TRP channel was subsequently cloned in 1989.³³ Later, the ubiquitous presence of these TRP channels in different tissues in mammalian species was discovered. These channels mediate a variety of functions, such as the sensation of pain, coldness, warmth or hotness, vision, taste, and pressure. Therefore, the members of the TRP ion channel family are considered polymodal. The role of TRP channels and their diverse involvement in critical pathophysiological events has been reviewed elsewhere.^{19, 34–41} There are about 33 mammalian members in the TRP channel superfamily, of which 27 are found in humans. Mammalian TRP channels are classified into 6 subfamilies based on sequence homology: canonical (TRPC), vanilloid (TRPV), melastatin (TRPM), mucolipin (TRPML), polycystin (TRPP), ankyrin (TRPA), and mucolipin (TRPML) (TRPN). Every cell in the body is likely to express at least one or more TRP ion channels. TRP channels share a common architecture having 6 membrane-spanning helices with intracellular N- and C-termini. The activation of TRP channels occurs by direct gating of channels with physical stimuli or specific ligands, or by indirect mechanisms.⁴² Many TRP ion channels mediate calcium influx into the cells. Under normal homeostasis, the tight control of cytoplasmic calcium levels is key for the regulation of many cellular processes, such as motility, secretion, action potential generation and propagation, the release of neurotransmitters, and gene expression. However, excessive cytoplasmic calcium levels are cytotoxic. Therefore, TRP channelopathies with either gain or loss of function can lead to pathophysiology.

TRPs in respiratory physiology and pathophysiology

Although TRPs exert pleiotropic functions in different organ systems, our review focuses on their role in the respiratory system. Homeostasis of intracellular Ca^{2+} is important for the normal functioning of the respiratory system and TRP channels maintain intracellular calcium homeostasis. It is widely agreed that TRP channels—in association with other molecular pathways—contribute to inflammatory and immune responses. Activation of TRP ion channels via influx of Na^+ and Ca^{2+} causes membrane depolarization, which triggers an action potential discharge, as well as induces the release of neuropeptides such as calcitonin gene-related peptide (CGRP), substance P (SP), and neurokinin (NKA).^{43, 44} The altered homeostasis of intracellular calcium levels in vascular cells leads to vasoconstriction and

altered permeability at the alveolar and vascular levels, resulting in increased bronchoconstriction and pulmonary edema. Inhalation of chemical threat agents causes chemosensory reflexes such as cough, sneezing, and pain, and may trigger bronchoconstriction, which has been associated with activation of TRP ion channels and resulting intracellular calcium levels.^{20, 32, 43, 45–50}

TRP channels are expressed throughout the respiratory system—from the nasal mucosa to the alveolar–capillary interface. Recent evidence demonstrates a fundamental role of neuronal and non-neuronal TRPs in the pathophysiology of several respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis, and cough.^{31, 51–54} TRPA1, TRPC6, TRPV1, TRPV4, and TRPM8 are the most abundantly expressed TRP subtypes in the respiratory system.^{28, 55}

Nasal irritation and secretions are the first responses experienced by the victims exposed to chemical agents. Functional studies elucidated that TRPA1, TRPV1, and TRPM8 are expressed in trigeminal C-fiber nerve endings in the nose, and activation of these ion channels by toxicants elicits the sensation of irritation. These TRP channels may be expressed in non-neuronal cells, such as epithelia, mucous glands, smooth muscles, vascular endothelium, and vasculature of the respiratory system.²⁷ Among TRP channels, TRPC1 is expressed in almost all non-neuronal cell type.⁵⁶

The outward symptom of many toxic inhalational exposures is cough. The cough reflex is triggered by the excitation of either C-fibers or A δ -fibers. Studies have implicated TRPA1 and TRPV1 in chemical irritant–induced cough.³⁴ TRP channel antagonists were shown to decrease cough frequency in several chemically induced animal models.^{32, 46–48, 57} The endogenous activators of TRP channels that elicit cough reflexes are prostaglandin E2 (PGE2) and bradykinin (Bk). Single nucleotide polymorphisms (SNPs) in TRPV1 linked to cough in humans have been described recently.⁴⁸

TRP channels are expressed in different immune and inflammatory cell types. Activation of TRP channels releases a variety of proinflammatory mediators. The role of TRP channels in chemical threat agents–induced lung injuries is primarily mediated through covalent modification of cysteine residues on the ion channel, increased intracellular calcium levels, or through lipid messengers such as diacylglycerol and phosphatidylinositol phosphate.²⁷ TRP channels work in close association with G protein–coupled receptors (GPCRs), receptor tyrosine kinases (RTKs), phospholipase C, and G_q-coupled receptors.

Exposure to toxic inhalational chemical threat agents in the airway-compromised patients and pre-existing diseased ones will cause exacerbations in symptoms.⁵⁸ Susceptibility to other diseases increased after toxic inhalational injuries.^{59, 60} TRP channels may cause additive effects for such comorbid patients due to their involvement in pulmonary diseases and chemical threat agents–induced injuries.^{61, 62} The injury will be enhanced when multifactorial agonists of TRP ion channels are present. Lipopolysaccharide (LPS), a typical cell wall component of Gram negative bacteria, activates TRPA1 and induces pain and inflammation. Concurrent administration of LPS with 4-hydroxynonenal (4-HNE), another

TRP agonist, results in enhanced inflammation.⁶² TRP channels also play an important role in sensing hypoxia or hyperoxia.

TRPs in pulmonary chemical injuries

While additional members of the TRP superfamily may be involved in mediating injuries caused by chemical threat agents, research on TRPA1, TRPV1, and TRPV4 has advanced the most, as summarized below.

Transient receptor potential channel ankyrin repeat 1 (TRPA1)

TRPA1 consists of six transmembrane subunits (S1–S6) that assemble as a tetramer with a cation-permeable central pore, with a pore-forming helix between the 5th and 6th transmembrane domains. Figure 1 displays the structure, localization of TRPA1 ion channels in the respiratory system, and agonists of TRPA1. The N-terminus contains 16 ankyrin repeats in human TRPA1, whereas other species have a varying number. The chemical and thermal sensitivity of TRPA1 ion channels in different species is determined by the N-terminal sequence and specific N-terminal cysteine residues. Advances in cryo-electron microscopy (cryo-EM) have revealed the high-resolution structure of the TRPA1 ion channel, providing mechanistic insights into activation and resulting conformational changes.^{63, 64} The evidence for the expression of TRPA1 ion channels on neuronal cells is abundant compared with non-neuronal cells.^{19, 65} In mammals, TRPA1 is expressed in a subset of C-fibers. TRPA1 also co-localizes with TRPV1-expressing nociceptive neurons with cell bodies in trigeminal (TG), jugular-nodose (vagal, JNG), and dorsal root (DRG) ganglia. Although the functional role of TRPA1 has been described primarily in neuronal context, some studies detected the expression of this ion channel on human lung fibroblast cell lines, alveolar type II pneumocytes in the A549 (human lung carcinoma) cell line, and small cell lung cancer cell lines.^{65–67} The neuronal and non-neuronal expression of TRPA1 ion channels was demonstrated in porcine and human lung sections using immunohistochemistry.⁶⁵

TRPA1 channels are activated in response to exogenous electrophile chemical irritants, endogenous proinflammatory mediators, physical stimuli, pathogen-associated molecular patterns (PAMPs), and danger-associated molecular patterns (DAMPs).^{34, 68} Many studies have demonstrated TRPA1 functioning as a direct chemosensor.^{69–71} The role of TRPA1 in inflammation and chemosensation has been reviewed in detail elsewhere.^{43, 69, 72}

Endogenous agonists of TRPA1 such as 4-hydroxy-2-nonenal (4-HNE), 5,6-epoxyeicosatrienoic acid (5–6-EET), NO, H₂O₂, 15-deoxy- δ (12,14)-prostaglandin J2 (15d-PGJ2), and H₂S can accumulate and activate TRPA1 directly without any exogenous agonists. Exogenous agonists of TRPA1 ion channels such as allyl isothiocyanate (AITC, mustard oil), cinnamaldehyde, acrolein, and thiosulfinates (allicin) activate by direct covalent modification of specific cysteine and lysine residues located within the long N-terminal ankyrin repeat domain.^{20, 68, 73–75} For TRPA1 and other TRP channels, modulation by G protein-coupled receptors (GPCRs) through second-messenger signaling is another regulatory mechanism. For instance, bradykinin (Bk) activates TRPA1 indirectly through downstream signaling of GPCRs in a phospholipase C-dependent manner.⁴³ Upon activation

of TRPA1 ion channels, depolarization causes generation and propagation of an action potential to signal pain to the central nervous system. The depolarization of nerve terminals expressing TRPA1 also activates the release of neuropeptides such as SP, NKA, and CGRP. Among commercially available TRPA1 agonists, JT010 (2-chloro-*N*-(4-(4-methoxyphenyl)thiazol-2-yl)-*N*-(3-methoxypropyl)-acetamide) is the most potent (EC_{50} of 0.65 nM).⁷⁶ TRPA1 knockout mouse and rat models have been described.^{54, 68, 77, 78} As expected, some of the studies with the knockout mouse models described compensatory physiological mechanisms^{79, 80}. However, thorough compensatory mechanistic studies are lacking.

Within the respiratory system, TRPA1 channels are ubiquitously present on C-fibers that innervate beginning from the oral cavity, oropharynx, airways, and alveoli. Expression of TRPA1 on TG, JNG, DRG, and lung afferent fibers has been documented in different species.^{29, 81} Stimulation of TRPA1 ion channels in the upper and lower respiratory system by TIHs results in sneezing, coughing, mucus hypersecretion, shallow breathing, and bronchoconstriction to limit further exposure to imminent chemical threat agents and induce protective expulsion of irritants. These immediate responses to TIHs are protective reflexes and serve as warning signs to move away from the source of the insult. However, prolonged or repeated exposure to TIHs can lead to acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) and result in chronic conditions such as allergic rhinitis, chronic cough, chronic obstructive pulmonary disease (COPD), asthma, fibrosis, and reactive airway dysfunction syndrome (RADS), wherein endogenous TRPA1 agonists such as 4HNE and H_2O_2 remain elevated. TRPA1 can remain active chronically in the continuous presence of eliciting mediators. TRPA1 agonists evoked cough in animal models and human volunteers.^{32, 46} Using *in vitro* and *in vivo* studies, the efficacy of the potent and selective TRPA1 antagonist GRC 17536 has been validated in a citric acid-induced cough model.⁸² Increasing evidence suggests that TRPA1 plays an important role in hypersensitivity to noxious chemical stimuli.⁸³ The role of TRPA1 in asthma and several other injury models has been described.^{20, 54, 72, 75, 84–87}

Most of the chemical threat agents-induced pulmonary injuries cause hypoxia. Studies demonstrated that TRPA1 and TRPC6 can be activated in hypoxic states.^{81, 88} In hypoxia or hypoxemia, decreased oxygen concentrations activate TRPA1 through relief from prolyl hydroxylation. Insertion of unmodified TRPA1 proteins to the plasma membrane and internalization of hydroxylated TRPA1 proteins initiates relief from prolyl hydroxylation.^{81, 88} Under hyperoxia conditions, oxidation of Cys633, Cys856, or both leads to activation of TRPA1.^{81, 88}

Interspecies differences in agonism and antagonism of TRPA1 ion channels have been reported. Therefore, careful considerations should be taken when selecting the species for pharmacologic and toxicologic testing and when interpreting the outcomes.^{89–93} Species differences in TRPA1 agonism and antagonism significantly impeded the drug discovery pipeline for this ion channel. Also, the percentage of expression of TRPA1 is different between humans and rodents. Sex- and age-specific differences have been noted in different animal models, possibly mediated by TRPA1 ion channels.^{75, 94, 95}

Several pharmaceutical companies have active research portfolios to develop and optimize small molecule inhibitors of TRPA1. Table 1 shows representative TRPA1 antagonists. Hydra Biosciences (MA, USA) was the first company to discover and disclose xanthine derivatives as TRPA1 antagonists, with HC-030031 one of the representative compounds. HC-030031 has been shown to be effective in multiple animal models.^{54, 79, 96, 97} Orion Pharma studied another xanthine derivative, Chembridge-5861528, which is more efficacious than HC-030031 in pain models.⁹⁸ Abbott discovered the oxime derivative A-967079, which has also been shown to be an effective TRPA1 antagonist in several animal models.⁷⁹ Hydra Biosciences/Cubist Pharmaceuticals and Orion Pharma conducted Phase I clinical trials of their CB-625 and ODM-108 compounds; however, both of these antagonists failed to move forward further because of low solubility and discouraging pharmacokinetic issues, respectively. In 2012, Glenmark conducted Phase IIa clinical trials of GRC17536 in diabetic peripheral neuropathy patients. GRC17536, an orally bioavailable and highly potent TRPA1 inhibitor (>1000-fold more potent than other TRPA1 antagonists), was well tolerated with no adverse events, although long-term efficacy was not established.⁹⁹ Recently, several pharmaceutical companies, including Amgen, Abbott, AstraZeneca, Janssen, Roche, Genentech, Merck, Orion, Pfizer, and others, have disclosed their TRPA1 antagonist research findings, which have been summarized elsewhere.^{100–102} Genentech recently published their novel and potent (4R,5S)-4-fluoro-5-methylproline sulfonamide series of TRPA1 inhibitors.¹⁰³ GDC-0334 advanced to Phase I clinical trials (Genentech; [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03381144) Identifier: [NCT03381144](https://clinicaltrials.gov/ct2/show/study/NCT03381144)). More recently, the efficacy of a novel TRPA1 inhibitor, BI01305834, was investigated in a guinea pig model of allergic asthma.⁸⁷ BI01305834 protected against airway hyperresponsiveness (AHR) and early and late asthmatic response in an acute ovalbumin-induced asthma model. Therapeutic effects were also tested *ex vivo* in histamine-induced airway narrowing in precision-cut lung slices and allergen-induced bronchoconstriction in tracheal strips.⁸⁷ Despite the lack of TRPA1 antagonists that have completed clinical trials and been approved, no safety concerns regarding any of the potential drug candidates have yet been reported. Given the diverse indications in several diseases, TRPA1 antagonists have the potential to counteract chemical threat agents–induced pulmonary injuries and may emerge as broad-spectrum antidotes.

20, 79, 92, 104

Transient receptor potential channel vanilloid 1 (TRPV1)

TRPV1, a member of the vanilloid family of TRP ion channels, was originally identified as a neuronally expressed ion channel.^{34, 105–107} Figure 2 displays the structure of the TRPV1 ion channel, its localization in the respiratory system, and its agonists. The high-resolution cryo-EM technique revealed the near-atomic resolution (3.4 Å) structure of TRPV1 ion channel in both resting (closed) and ligand-bound (open) states.¹⁰⁸ TRPV1 has 6 transmembrane domains (S1–S6) and long cytosolic N- and C-terminal modules. Other key structural modules in TRPV1 are the ankyrin (ANK) repeat domain, linker domain, pre-S1 helix, S4–S5 linker, pore helix, pore loop, and TRP domain. Critical residues involved in the binding of various ligands, calmodulin, phosphatidylinositol 4,5-bisphosphate (PIP₂), adenosine triphosphate (ATP), and phosphorylation are also shown in Figure 2A.¹⁰⁹ TRPV1 is expressed in C-fibers of the vagus nerve, which innervates airways. Although expression levels of TRPV1 are reportedly low in respiratory systems, TRPV1-positive nerve fibers

innervate nose, larynx, trachea, lung parenchyma, alveoli, smooth muscle, and blood vessels.¹¹⁰ Non-neuronal expression of TRPV1 has also been observed in airway epithelial cells.^{111, 112} TRPV1 ion channels are activated by various stimuli, such as capsaicin, heat (>43 °C), acidic pH (<5.3), and endogenous mediators, and they sense noxious heat and pain.¹⁰⁹ Capsaicin and resiniferatoxin are classic noxious exogenous TRPV1 irritants. Other exogenous agonists are camphor, piperine, voltage, and sweeteners. Endogenous mediators such as acid and anandamide (an endocannabinoid eicosanoid derivative), 12-hydroperoxy-eicosatetraenoic acid (12-HPETE), and N-arachidonoyl-dopamine (NADA) also act as TRPV1 agonists. TRPV1 activation is mediated through calcium influx and depolarization. The subsequent release of tachykinins, nerve growth factors (NGFs), prostaglandins, SP, and CGRP initiate the activation of several kinases such as protein kinase A (PKA), protein kinase C (PKC), and MAP kinases (ERK and p38). Phosphorylation mechanisms play an important role in the modulation of TRPV1 ion channel.¹⁰⁹ Interestingly, the role of TRPV1 ion channels has been described in longevity.^{113, 114}

Activation of TRPV1 ion channels causes respiratory tract irritation, which results in sneezing, cough, mucus hypersecretion, and pain. Activation of the TRPV1 ion channels can also lead to desensitization of the airway response to other irritants.^{29, 34} TRPV1 agonists such as capsaicin and citric acid induced cough. Studies showed increased sensitization or upregulation of cough reflex in humans is associated with increased expression of TRPV1 in airway nerves in comparison with healthy control subjects.^{115, 116} In a rat model of allergic asthma, expression of TRPV1 increased in tracheal Aδ-fibers.¹¹⁷

Several potential TRPV1 antagonists have been developed and at least a dozen of them were tested in human clinical trials, primarily for pain management.^{118–120} Table 2 shows representative TRPV1 antagonists. A detailed summary of TRPV1 antagonists has been described elsewhere.¹¹⁹ In a mouse model of ovalbumin-induced asthma, treatment with capsazepine (TRPV1 antagonist) or TRPV1 small interfering RNA (siRNA) reduced AHR.¹²¹ TRPV1 knockout mice protected against LPS-induced airway inflammation and bronchial hyperreactivity.¹²² While some of the TRPV1 antagonists are still undergoing clinical trials, many of the preclinical and clinical drug candidates did not reach advanced phases of testing due to drug-induced altered thermosensation.^{119, 120} AMG-517 caused significant and long-lasting hyperthermia in phase I clinical trials before even evaluating the analgesic efficacy, resulting in termination of the study.¹²³ SB-705498 is a potent, selective, orally bioavailable, competitive TRPV1 antagonist with minimal disturbance in temperature perception or control.¹²⁴ SB-705498 was tested in a clinical trial in patients with refractory chronic cough, with a modest therapeutic benefit at the tested dosing regimen.¹²⁵ XEN-D0501, a TRPV1 antagonist, has been tested in two different Phase 2 clinical trials against chronic idiopathic cough ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02233699) Identifier: [NCT02233699](https://clinicaltrials.gov/ct2/show/study/NCT02233699)) and chronic obstructive pulmonary disease ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02233686) Identifier: [NCT02233686](https://clinicaltrials.gov/ct2/show/study/NCT02233686)).¹²⁰ As strong evidence from preclinical and clinical studies suggest the role of TRPV1 ion channel in pulmonary diseases, it remains an interesting target.

Transient receptor potential channel vanilloid 4 (TRPV4)

TRPV4 ion channels are calcium (Ca^{2+})-permeable, nonselective ion channels that play an important role in the homeostasis of different organ systems, including lungs, heart, brain, liver, placenta, and salivary glands. Similar to other TRP family members, TRPV4 has intracellular N- and C-termini and 6 transmembrane spanning α -helices (S1-S6). The putative cation-permeable pore is located between S5 and S6. The full length of the TRPV4 ion channel is 871 amino acids. Cryo-EM and X-ray structures of TRPV4 have provided mechanistic insights.^{126, 127} Figure 3 shows the structure of the TRPV4 ion channel, its localization in the pulmonary system, and representative agonists. The N terminus has 6 ankyrin repeat domains and a proline-rich domain (PRD). The PRD plays an important role in mediating the mechanosensitive properties of the channel. The C terminus has calmodulin-binding domains, which are important for Ca^{2+} -dependent activation of the TRPV4 ion channel, and a PDZ (PSD95/SAP90-Discs-large-Zonula-occludentes-1) domain, which may contribute to interactions between TRPV4 and PDZ-domain proteins.^{128, 129} The mammalian homologs of TRPV4 also show similarities in length and a high degree of sequence identity. Sonkusare *et al.* elucidated the concept of cooperative channel gating to characterize the transcellular mechanism responsible for endothelium-dependent regulation of vascular smooth muscle tone.¹³⁰

Within the lungs, TRPV4 ion channels are expressed in different tissues. Several studies have demonstrated the localization of TRPV4 on pseudostratified ciliated epithelia of major airways, the alveolar septal barrier, pulmonary endothelium, airway smooth muscle, alveolar type I and type II cells, alveolar–capillary endothelium, alveolar macrophages, and neutrophils.^{131–136} Thorne and colleagues found TRPV4 expression in pulmonary vessels in human lungs, with increased expression in congestive heart failure.¹³² TRPV4 ion channels open in response to mechanical stimuli, hypo-osmolarity, exogenous small molecule ligands, temperature ($>27^\circ\text{C}$), phosphorylation by Src, protein kinase A (PKA), protein kinase C (PKC), arachidonic acid metabolites, and endocannabinoids (anandamide and 2-arachidonoylglycerol).

Synthetic TRPV4 agonists have been studied extensively and used in several functional studies. Among the potent agonists of TRPV4 ion channels, 4 α -phorbol 12,13-didecanoate (4 α -PDD, EC_{50} : 200–400 nM), 4 α -phorbol 12,13-dihexanoate (4 α -PDH, $\text{EC}_{50} \approx 70$ nM), and GSK1016790A ($\text{EC}_{50} \approx 1$ –10 nM) are widely used.^{137, 138, 139} Phorbol esters directly bind at a binding pocket formed by residues between S3 and S4.¹³⁷ GSK1016790A is considered the most potent and specific agonist of TRPV4 and is widely used in studying TRPV4 pharmacology.^{138, 141} The arachidonic acid metabolite 5,6-epoxyeicosatrienoic acid (5,6-EET) and botanical extracts such as bis-andrographolide are also agonists. The availability of TRPV4 knockout mice enhanced the studies on TRPV4 biology. Currently, there are 3 knockout mouse models developed independently and some differences have been reported among the models.^{129, 142, 143, 138}

A recent review summarized the significant advances in TRPV4 drug discovery, emphasizing small molecule agonists and antagonists of TRPV4 ion channels.¹³⁹ Table 3 summarizes representative TRPV4 antagonists that have been tested in preclinical and clinical studies. Initially, non-specific inhibitors such as ruthenium red, gadolinium, and

lanthanum were tested. Hydra Biosciences disclosed their potent and reversible inhibitor HC-067047. GlaxoSmithKline (GSK) reported several tool compounds, including GSK2193874, GSK2220691, and GSK2337429A, that have been tested successfully in preclinical pulmonary injury models.^{21, 30, 132, 139, 144} More recently, GSK disclosed their clinical drug candidate GSK2798745, which has been successfully tested in clinical trials.^{139, 145–147} A first-time-in-human study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of this TRPV4 inhibitor has been completed in healthy and heart failure subjects.^{145, 148} No significant safety issues were observed in healthy volunteers (dosed up to 14 days) or in heart failure patients (dosed up to 7 days), which will allow GSK2798745 to be evaluated in clinical studies in heart failure and other indications. The clinical pharmacokinetics of GSK2798745 revealed a systemic half-life of approximately 13 hours. Therefore, patients can be prescribed a once-daily dosing regimen without any meal restrictions.¹⁴⁵

Blockade of TRPV4 ion channels for treating pulmonary congestion in heart failure subjects has been studied in a pilot trial. The lung diffusing capacity for carbon monoxide (DLCO) was assessed as the primary endpoint after treatment with GSK2798745 for 7 days. GSK2798745 has been safe and well-tolerated, and an anticipated trend toward improvement in the gas transfer has been reported.¹⁴⁹ A Phase I proof-of-mechanism clinical trial to study the effects of GSK2798745 on alveolar–septal barrier disruption in a segmental lipopolysaccharide (LPS) challenge model in healthy volunteer subjects has been terminated due to low probability of achieving a positive outcome on the primary endpoint.¹⁵⁰ While GSK2798745 has several potential therapeutic benefits, a clinical trial to assess the effectiveness and side effects of GSK2798745 in participants with chronic cough shows no efficacy against chronic cough.¹⁵¹

Originally, TRPV4 was considered to have primarily an osmoregulatory role.¹⁵² However, later studies have revealed that TRPV4 plays a role in various physiologic functions and pathophysiologic conditions.^{21, 30, 129, 134, 136, 140, 144, 153} The diverse roles of TRPV4 are reviewed in detail elsewhere.^{128, 129, 140} These channels have been shown to play an important role in limiting the movement of intravascular fluid into the interstitial and alveolar air spaces of the lung.^{132, 154} *In vitro* and preclinical animal studies have shown diverse indications of TRPV4 antagonism for several pathological conditions, including pulmonary diseases (ARDS/ALI, mechanical ventilator-induced lung injury, asthma, pulmonary fibrosis, cough, and COPD), congestive heart failure, colitis, Crohn's disease, interstitial cystitis, overactive bladder, glaucoma, diabetic retinopathy, cerebral edema, pain, and itch.^{30, 129, 133, 135, 136, 139, 144, 155–158}

As shown in Figure 4, activation of TRPV4 ion channels in the respiratory system leads to disruption of the alveolar–septal barrier, resulting in alveolar flooding and hypoxemia, which causes acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Stimuli that cause endothelial cell contraction and detachment activate TRPV4 and, in turn, increase vasoconstriction and pulmonary venous pressure.^{133, 134} Disruption of alveolar–capillary barrier function and increased vasoconstriction results in protein leakage into the alveolar parenchyma, leading to pulmonary edema.^{133, 134, 159} Hamanaka *et al.* found that TRPV4 plays an important role in mechanical ventilator–induced acute lung injury (ALI).

^{49, 50} Rapid calcium entry through TRPV4 channels is a major determinant of the increased acute vascular permeability in the lungs following high peak inflation pressure ventilation. TRPV4 channels also play a key role in mucociliary clearance.¹³¹ Upon activation of TRPV4 channels, the increased intracellular calcium levels activate the ciliary activity of the bronchial system.²⁷ Emerging evidence suggests that TRPV4 plays a role in the activation and differentiation of innate immune cells such as neutrophils, monocytes, and macrophages.^{49, 133, 144} TRPV4 ion channels serve as a cellular mechanosensor in response to such forces as shear, stretch, osmotic swelling and shrinking, stiffening, and surface expansion.^{49, 50, 133, 152, 160, 161}

Dalsgaard *et al.* showed that the activation of the TRPV4 ion channel is associated with edema formation and circulatory collapse in rodent sepsis models.¹⁶² The TRPV4 inhibitors GSK2193874 and HC067047 protected mice from the lethality of intraperitoneal administration of LPS and the cecal ligation and puncture (CLP) procedure.¹⁶² A recent study showed that TRPV4 protects the lung from injury caused by intratracheal *Pseudomonas aeruginosa* in mice.¹⁵⁵ TRPV4 enhances macrophage bacterial clearance and downregulates secretion of proinflammatory cytokines through a novel mechanism of molecular switching of LPS signaling from predominant activation of MAPK/JNK signaling to activation of MAPK/p38 signaling.¹⁵⁵ Herbal extracts of *Nigella sativa*, *Anthemis hyalina*, and *Citrus sinensis* were tested to study the replication of coronavirus and expression of the TRP gene family.¹⁶³ Viral loads and TRPV4 gene expression were decreased with *Anthemis hyalina* extract treatment, suggesting a possible role for TRPV4 ion channels.¹⁶³ These studies lay the foundation for studying the role of TRPV4 antagonism in ALI/ARDS resulting from bacterial and viral diseases. In the face of the global COVID-19 pandemic, TRPV4 inhibitors for the treatment of ARDS symptoms in COVID-19 patients may offer some hope.¹⁶⁴

Pulmonary chemical threat agents

Since World War I, chlorine gas, hydrochloric acid, nitrogen/sulfur mustard, tear gas agents, ammonia, chloropicrin, phosgene, and hydrogen sulfide have been the most commonly used chemical warfare agents. Unfortunately, most of these dangerous chemicals can be synthesized with simple ingredients and easily available protocols and, thus, may become weapons of mass destruction (WMDs).

Chemical threat agents cause a spectrum of lung injuries because of variation in their water solubility, which dictates their localization in the pulmonary system.²⁷ However, they share some clinical symptoms upon inhalational exposure. Acute symptoms are nasal irritation, copious nasal secretions, cough, chest tightness, gasping for air, bronchoconstriction, vasoconstriction, pulmonary hemorrhage, and pulmonary edema. Chronic symptoms can result in chronic obstructive pulmonary disease, asthma, pulmonary fibrosis, susceptibility to other bacterial and viral infections because of damage in airway epithelia and decreased airway clearance. Some of the chronic symptoms of sulfur mustard-induced lung injuries continue to develop for several decades after the initial exposure.

Most of the chemical agents that cause pulmonary injuries are deployed as gas bombs, hand-thrown grenades, or rocket-launched gas tanks. These chemical agents cause maximum injury in their gaseous state. Most are heavier than air and remain close to the ground for longer periods. Children are more susceptible to these inhalational toxic agents than adults due to their short height and their relatively higher lung surface area and higher minute volumes normalized to their body weight.

Chlorine gas

Chlorine (Cl_2) is a yellowish-green gas with a strong odor of bleach and is one of the most highly produced and used chemicals around the world (among the top 10 most-produced chemicals in the United States). Chlorine is used for water treatment, whitening paper, and industrial manufacture of numerous chemicals. In the hospital setting, especially in developing countries, bleach plays an important role in sanitation, and is particularly effective in killing viral contamination, including COVID-19.¹⁶⁵

Cl_2 has been used as a chemical weapon since World War I (WWI) as it is easy to produce by electrolysis of aqueous sodium chloride (table salt). Since then, there have been several reports on the use of Cl_2 in Iran, Iraq, and Syria. UN-supported OPCW fact-finding missions have proven that Syria used Cl_2 , allegedly on the civilian population.^{3, 4}

Accidental exposure to Cl_2 in domestic and occupation settings are another big concern. It is estimated that Cl_2 accounts for 84% of the total TIHs transported every year.^{9, 166} The train derailment incident in Graniteville, SC in 2005 is considered one of the largest transportation accidents in the United States.^{7, 167, 168} A relatively small number of people were exposed in this event; however, an intentional terrorist attack could result in a huge death toll. Based on data from hazardous release of Cl_2 in the Graniteville, SC train derailment, detailed epidemiologic modeling of Cl_2 plumes has been performed.¹⁶⁹ Predictions of fatalities due to Cl_2 release under high and less extreme conditions have also been modeled.^{170, 171} In a worst-case scenario, up to 100,000 deaths may be expected if a chlorine gas tank car on the rail line that passes near the Capitol Mall in Washington, DC is attacked during a major public event.¹⁷²

Cl_2 causes a spectrum of clinical symptoms depending on the concentration of Cl_2 and the duration of exposure.^{10, 173–175} White *et al.* summarized the clinical manifestations of exposure of the pulmonary system to Cl_2 .¹⁷⁴ Mortality rates vary depending on the level of exposure, but typically range from 1–2%.¹⁷⁶ Death within a few hours of exposure is considered to be primarily due to pulmonary edema and hemorrhage. The National Institute for Occupational Safety and Health suggests that a Cl_2 concentration of 10 ppm is dangerous to health or life. Although symptoms of moderate exposure to Cl_2 do resolve, several epidemiologic studies in victims of the Cl_2 leak in the Graniteville, SC train derailment show long-term respiratory problems.^{7, 173, 177}

The treatment of Cl_2 intoxication is largely supportive. Specifically, humidified oxygen is appropriate for all victims along with the use of inhaled bronchodilators. No mechanism-based treatments for Cl_2 injury are yet available. Although some benefit has been suggested with agents such as nebulized sodium bicarbonate and corticosteroids, the evidence is

anecdotal.^{7, 11, 178} Given the potential of Cl₂ to cause mass casualties, the development of a medical countermeasure to treat lung injury caused by Cl₂ remains one of the highest priorities of the Biomedical Advanced Research and Development Authority (BARDA) Chemical Medical Countermeasures Program. Our recent review on toxic effects of Cl₂ and potential treatments describe in detail some of the progress in preclinical research.¹⁰

TRPA1 and TRPV4 ion channels have been well studied in *in vitro* and animal models as potential targets for the treatment of Cl₂-induced lung injuries.^{10, 19, 21, 29, 30} Chlorine reacts with mucus on the epithelial lining to form hydrochloric acid and hypochlorous acid. Studies have shown that the hypochlorite ion (OCl⁻) is an important oxidative product of Cl₂ that plays an important role in mediating the effects of Cl₂.

Bessac *et al.* used an *in vitro* pharmacological functional assay and *in vivo* mouse studies to uncover the role of the TRPA1 ion channel in mediating the effects of Cl₂.¹⁹ When NaOCl-containing physiologic buffer was added to cultured dorsal root ganglia, a robust increase in intracellular calcium was noted. Cultured neurons from trigeminal ganglia and nodose ganglia also showed robust responses to NaOCl. However, a calcium influx was absent in neurons cultured from *Trpa1*^{-/-} mice. The *in vitro* findings were validated in *in vivo* mouse studies. Wild-type and *Trpa1* knockout mice were exposed to a NaOCl aerosol and evaluated using barometric plethysmography. *Trpa1* knockout mice displayed profound deficiencies in hypochlorite- and hydrogen peroxide-induced respiratory depression. Oxidant-induced pain behavior was also decreased in *Trpa1* knockout mice, compared with wild-type mice.¹⁹

Exposure to Cl₂ results in the recruitment of inflammatory neutrophils and the production of reactive oxygen and nitrogen species.^{21, 174} In mouse models of acute lung injury caused by Cl₂, desquamation of epithelia, infiltration of leucocytes, hemorrhage, edema, atelectasis, emphysema, and necrosis were observed.²¹ Furthermore, Cl₂ exposure resulted in increased expression of proinflammatory markers and endogenous mediators of inflammation as well as reactive airway dysfunction syndrome, protein leakage, edema, and alveolar damage leading to hypoxia.²¹ Type II pneumocyte hyperplasia may be seen in ALI-induced by Cl₂ or other TIHs.¹⁷⁹ Cl₂ exposure also causes overexpression of inducible nitric oxide synthase (iNOS) in the vasculature, which in turn, mediates inflammation and the generation of oxidative stress.¹⁸⁰

TRPV4 is abundant in vascular endothelium. In response to increased intracellular calcium levels, the downstream effects result in plasma extravasation, vascular leakage, and edema.¹³¹ This cascade of events is further supported by preclinical *in vitro* and *in vivo* pharmacologic studies using selective TRPV4 inhibitors and genetic knockout studies.^{30, 49, 132, 133, 161, 181} Balakrishna and colleagues used two structurally different novel tool TRPV4 inhibitors (GSK2220691 and GSK2337429A) in a mouse model of nonlethal Cl₂-induced acute lung injury.²¹ Mimicking the real chlorine exposure situation, treatment was administered after exposure to the Cl₂. These TRPV4 antagonists reduced critical hallmarks of chemical lung injury such as airway hyperreactivity and increase in lung elastance, protein leakage, and neutrophil and macrophage infiltration of the lungs, as well as the associated production of oxidative mediators and inflammatory chemokines and cytokines.²¹ Studies in *Trpv4* knockout mice recapitulated these findings. Overall, administration of

either inhibitor beginning immediately after Cl₂ exposure reduced pulmonary edema, improved pulmonary function and oxygenation, reduced airway hyperreactivity, and reduced bronchoalveolar lavage fluid (BALF), neutrophils, macrophages, and cytokines, suggesting reduced inflammation.^{21, 159}

As noted earlier, exposure to Cl₂ resulted in the recruitment of inflammatory neutrophils and macrophages and the production of reactive oxygen and nitrogen species.^{21, 182, 183} TRPV4 ion channels have been identified as novel regulators of neutrophil activation and downstream effects that contribute to the pathophysiology of ALI.^{21, 133, 144, 182, 184} The recruited inflammatory neutrophils also contribute to the generation of myeloperoxidase (MPO), which catalyzes the formation of reactive oxygen intermediates, including HOCl, which may further augment chlorine injury.^{21, 183} MPO has been shown to be a local mediator of tissue damage and has been implicated in various inflammatory diseases.^{183, 185}

N-acyl amides, fatty acid-derived products related to anandamide and similar endocannabinoids, were analyzed in BALF samples and lung tissues collected from mice exposed to either Cl₂ or air.^{21, 186} *N*-acyl amides are known to activate TRP ion channels, including TRPV4 ion channels.^{186, 187} Exposure to Cl₂ increased the concentration of fatty acyl amides.²¹ These findings suggest that the endogenous agonists of TRP ion channels continue to show their effects long after the Cl₂ exposure or other toxic inhalational insult has ended.

Long-term studies have shown the development of fibrosis and airway remodeling following chlorine exposure.^{188–191} TRPV4 is also believed to activate innate immune cells and establish a proinflammatory loop in fibrotic diseases, with increased deposition of extracellular matrix (ECM) and substrate stiffness, via an overall mechanosensation path.^{133, 192} Figure 5 summarizes the role of TRPs in chemical threat agents–induced respiratory pathophysiology.

The research pipeline for TRPV4 inhibitors is active. GSK2798745, a potent and selective TRPV4 antagonist, has completed early clinical trials^{145, 147–151}. The TRPV4 inhibitors GSK2798745 and GSK3527497 are now under advanced testing for efficacy in treating for chlorine gas–induced pulmonary injuries with the support of BARDA, based on the mechanism of action.^{193, 194}

Tear gas agents

The deployment of tear gas agents to control riots has increased in the past few years in several countries.¹⁹⁵ The most commonly used tear gas agents are *o*-chlorobenzylidene malononitrile (CS), oleoresin capsicum (OC, pepper spray), 1-chloroacetophenone (CN), and dibenz [*b,f*]-1,4-oxazepine (CR). These riot control agents are deployed as either sprays or hand grenades. The common and immediate respiratory symptoms of exposure to tear gas agents include, but are not limited to, irritation of the nasal cavity, cough, rhinorrhea, breathlessness, and chest tightness. Other symptoms include a sore throat, wheezing, laryngospasm, bronchoconstriction, and rarely, respiratory arrest. Although the respiratory symptoms are generally perceived transient in nature, laryngospasm, pulmonary edema, and reactive airway dysfunction have been reported in long-term follow-up studies.^{196, 197}

Exposure to CS tear gas agents during mask confidence training in new military recruits is associated with significant increased risk of acute respiratory illness such as influenza, bronchitis, pneumonia, and cough.^{60, 198} A recent retrospective study of 93 cases shows the long-term effects of tear gas agents.¹⁹⁶ Cough, phlegm, and rhinorrhea are commonly seen for more than 3 months in victims exposed to tear gas, compared with control subjects. Severe or repeated exposure to tear gas agents in persons with pre-existing chronic respiratory illness can lead to death.¹⁹⁹

CS, CN, and CR tear gas agents are electrophilic agents. TRPA1 is an electrophilic irritant receptor.^{19, 20, 200} *In vitro* and *in vivo* studies have shown that TRPA1 mediates the noxious effects of tear gas agents.^{85, 200} *Trpa1*^{-/-} mice displayed no or only minimal acute pain behavior when exposed to CN or CS, confirming the essential role of TRPA1 in their sensory detection.²⁰⁰ Treatment with HC-030031, a first-generation TRPA1 inhibitor, or genetic ablation of the *Trpa1* gene abolished nocifensive behavior in mouse models of CS and CN tear gas agents-induced facial and paw pain.²⁰⁰ Exposure to CN tear gas agent increased levels of neuropeptides such as calcitonin gene-related peptide (CGRP), substance P (SP), and neurokinin A (NK-A) in bronchoalveolar lavage fluid (BALF) of wild-type mice, whereas reduced levels were detected in *Trpa1*^{-/-} mice.⁵⁴ The role of human TRPA1 ion channel was further clarified *in vitro* and by retrospective analysis of human volunteers exposed to benzylidenemalononitriles (BMN), including CS tear gas agent.²⁰¹ Volunteers were exposed to BMN in chambers and assessed for their tolerance and physiologic responses, that is, lachrymatory or sternutatory effects or irritation of the eyes or respiratory tract. Prior physiological data from trials highly correlated with the EC₅₀ of the hTRPA1 ion channel agonists studied.²⁰¹

The active noxious agent in pepper spray (oleoresin capsicum) is capsaicin, a purified extract of chili peppers. TRPV1 is the molecular target of capsaicin. TRPV1 is expressed in nociceptor receptors in mucous membranes of the upper and lower respiratory tract. When a person inhales tear gas agents, cough is one of the outward respiratory symptoms.^{104, 202} Cough is elicited when tear gas agents come in contact with the vagal sensory nerve endings in the mucosal surface of the larynx.^{34, 46} TRPV1 and TRPA1 are highly expressed in vagal sensory nerve endings in the larynx. Rhinorrhea and profuse airway secretions are the results of sensory-autonomic reflexes. TRPA1 plays an important role in allergen-induced asthma.⁵⁴ One of the long-term severe consequences of tear gas exposure is asthma.²⁰³ Tear gas sprays generate particulate matter. TRPA1 has been shown to modulate asthmatic responses in children living in areas with high particulate matter.²⁰⁴ A review of an epidemiological and mechanistic reassessment of tear gas agents has been published recently, and the effects of tear gas agents on skin, eyes, and pulmonary systems are discussed in detail.¹⁰⁴

No mechanism-based treatment options are yet available for tear gas agents-induced respiratory illness. Symptomatic treatment is the current line of therapy. In some of the preclinical studies in different animal models, TRP channel inhibitors have shown promise results in mitigating the symptoms of tear gas exposure. More detailed preclinical studies are needed, however, to bridge this knowledge gap.

Acrolein

Acrolein (2-propenal) is a highly reactive unsaturated aldehyde and a byproduct of burning organic matter, gasoline, diesel, and plastic. Within households, acrolein is formed when frying or heating oils/fats at high temperatures.²⁰ Traditional cigarette smoke may contain >50 ppm acrolein. While acrolein finds many uses in industrial applications, it is unfortunately a toxic inhalation hazard. The Environmental Protection Agency (EPA) has classified acrolein as high priority water and air pollutant/toxicant. Toxic effects of acrolein have been described following inhalation, ingestion, and cutaneous exposures. The primary target of acrolein toxicity is the pulmonary system. Exposure to a low concentration of acrolein leads to airway hyperreactivity, hampered mucociliary clearance, and disruption of the epithelial and endothelial barrier.²⁰⁵ Prolonged inhalation of acrolein causes ALI and edema, and can be lethal depending on the concentration and duration of exposure. The role of acrolein has also been described in COPD and cancer. The role of TRPA1 in the cardiopulmonary effects of acrolein has been studied in detail in mouse models.^{72, 75, 206}

Acrolein is a sensory irritant that excites peripheral sensory nerves and induces pain and respiratory irritation.^{20, 205} Acrolein was used as a tear gas agent in World War I because of its irritant and lachrymator properties. Activation of trigeminal nerve endings in the cornea causes stinging ocular pain and lachrymatory reflex. Acrolein also stimulates nerve endings in upper airways and causes tingling, stinging, painful sensations, sneezing, and mucus hypersecretion. Inhalation of acrolein triggers cough reflex by stimulating vagal laryngeal sensory nerve endings.

Acrolein activated both rodent and human TRPA1 ion channels expressed in heterologous cells.⁶⁸ Cultured sensory neurons from *Trpa1*^{-/-} mice did not show calcium influx, suggesting a role for the TRPA1 ion channel in neuronal sensitivity to acrolein.⁶⁸ Subsequently, using wild-type and *Trpa1*^{-/-} mice, the role of TRPA1 ion channel in mediating the effects of acrolein was studied.^{19, 34, 207, 208} The EC₅₀ values of acrolein for human TRPA1 are reported in the range of 1–5 μ M.^{68, 208} Andre *et al.* showed that aqueous extracts of cigarette smoke containing crotonaldehyde and acrolein caused rapid intracellular Ca²⁺ in cultured guinea pig jugular ganglia neurons and also promoted contraction of bronchi isolated from guinea pig.²⁰⁸ However, HC-030031, a selective TRPA1 inhibitor, abolished these responses. When acrolein activates TRPA1 ion channels in sensory nerve endings in the upper and lower respiratory tract, proinflammatory neuropeptides such as SP, CGRP, and NK-A are released.²⁰⁸ Birrell *et al.* showed that acrolein activated cloned human TRPA1 ion channels in HEK293 cells and vagal sensory nerves in murine, guinea pig, and human tissues.⁴⁶ Furthermore, the role of TRPA1 was confirmed using HC-030031, a selective TRPA1 inhibitor, and *Trpa1* knockout mice⁴⁶. In human subjects and guinea pigs, cinnamaldehyde (3-phenyl-acrolein), a TRPA1 agonist, evoked the cough reflex⁴⁶.

Conklin *et al.* observed upper airway epithelial sloughing, bradypnea and oral gasping, hypothermia, and cardiac depression and mortality when mice were exposed to acrolein (100–275 ppm, 10–30min).⁷⁵ Male wild-type (WT) mice (C57BL/6; 5–52 weeks old) were significantly more sensitive to high-level acrolein than age-matched, female WT mice. Treatment of WT male mice either with a TRPA1 antagonist (HC030031; 200 mg/kg) alone or with combined TRPA1 (100mg/kg) and TRPV1 (capsazepine, 10mg/kg) antagonists at 30

min post-acrolein exposure significantly reduced mortality.⁷⁵ The sex difference in acrolein-induced mortality is intriguing and additional studies are warranted for further investigation. While sex-specific regulation of TRPA1 expression may be possible for the noted differences, sexually dimorphic mechanisms such as the differential production of antioxidants, protective enzymes, proinflammatory cytokines, and other mediators may contribute as well.²⁰

Methyl isocyanate

Methyl isocyanate (MIC), also known as mustard cyanate, isocyanomethane, isocyanatomethane, and methylcarbylamine, is produced by reacting methylamine with phosgene. MIC is an intermediate product in the manufacture of pesticides such as fumigants. It is also used to produce several polymers such as polyurethane foams and plastics. The 1984 methyl isocyanate leak in Bhopal, Madhya Pradesh, India is considered one of the worst industrial gas leaks in the world. The Bhopal tragedy caused 558,128 injuries, including 3900 severe permanent injuries and 38,478 temporary partial injuries. Within 2 weeks of the gas leak, 8000 people died due to inhalational injury. Another 8000 died from gas-related diseases. Because methyl isocyanate has a higher density than air, it displaces air on the ground. Humans can smell its pungent odor from 2 to 5 ppm. However, adverse health effects have been reported at or below the human odor threshold. Therefore, odor detection is not a reliable indicator of exposure. The Occupational Safety and Health Administration (OSHA) time-weighted average (TWA) permissible exposure limit (PEL) of 0.02 ppm is almost 100 to 250 times lower than the odor threshold for MIC.

The pungent MIC vapors are highly irritating and corrosive to the mucosa of the respiratory tract. The inhalational effects of MIC are very rapid as vapors are readily absorbed through the respiratory mucosa. Exposure to MIC leads to respiratory symptoms such as cough, dyspnea, and chest pain, and can even lead to coma and death. Bronchoconstriction and pulmonary edema may occur immediately following exposure to MIC. Pulmonary edema may lead to the destruction of alveoli and pneumonia, which can result in severe respiratory complications and death. Follow-up studies on the Bhopal MIC leak revealed long-term respiratory effects, including asthma²⁰⁹.

TRPA1 ion channels present in trigeminal and associated peripheral afferent nerves have shown particular sensitivity to isothiocyanates.^{74, 210} Activation of heterologously expressed TRPA1 ion channels in HEK cells with industrial isocyanates has been shown previously.²⁰⁰ The interaction is a reversible covalent bonding between these ion channels and methyl isocyanate. The electrophilic carbon atom in methyl isocyanate makes it reactive with nucleophilic materials in tissue, including cysteine residues of the TRPA1 channels.⁷⁴ In mouse models of facial and paw pain, pharmacological blockade or genetic ablation of the *Trpa1* gene significantly abolished pain behaviors.²⁰⁰ These TRPA1 channels also play an important role in warning organisms against any potentially harmful chemicals/materials. Taylor-Clark *et al.* showed the role of TRPA1 in toluene diisocyanate (TDI)-evoked respiratory irritation.²¹¹ TDI activated TRPA1 ion channels *in vitro* using HEK293 cells stably transfected with human TRPA1 channels and in dissociated trigeminal nerves. The findings were validated with mouse *Trpa1*^{-/-} studies. TDI caused pronounced decreased

breathing rate, suggesting respiratory sensory irritation, whereas this reflex was abolished in *Trpa1*-deficient mice.²¹¹

Phosgene

Phosgene (carbonyl chloride, COCl_2 ; military designation = CG) is a highly toxic substance that exists as a colorless gas at room temperature, or may appear as a white to pale yellow cloud. Phosgene gas is nonflammable. At low concentrations, the odor of phosgene gas is pleasant, resembling freshly cut hay or green corn. At higher concentrations, the odor may not be pleasant. However, one should not rely on odor to detect phosgene, as some people may not notice.

Phosgene is one of the highly toxic inhalational agents that can cause sudden death due to the failure of the respiratory system. The first toxic effects of phosgene gas were reported as early as 1899 by a group of surgeons and anesthesiologists when chloroform converted to phosgene in the presence of a flame from an inhaler used during that period.²¹² Phosgene gas is widely used as a chemical intermediate in the manufacture of pharmaceuticals, foam rubber, dyes, and other products. Due to its ease of synthesis and lethal effects, CG became an agent of interest for chemical warfare.

Following a latency period of about 6–8 hours after inhalation, phosgene gas manifests its toxic pulmonary effects, causing bronchoconstriction, bronchitis, and severe pulmonary edema, leading to high mortality. Chronic effects include inflammation, airway and pulmonary remodeling, and asphyxia. Survivors of CG exposure may develop chronic obstructive pulmonary disease (COPD) over time. Despite the use of phosgene gas as a chemical weapon since World War I, there is no effective antidote. Therefore, phosgene gas remains a serious threat, as it could be released in an industrial accident or diverted or synthesized by terrorist groups.

Since no mechanism-based treatment options are available, symptomatic treatment to relieve edema and provide mechanical ventilation is considered standard of care. Only a few experimental therapeutics are in preclinical testing.²¹³ In a recent study conducted by Andres *et al.*, a potential role for TRP ion channels in CG-induced pulmonary toxicity was demonstrated using SKF96365, a non-specific TRP antagonist, and ruthenium red (RR), a non-specific inhibitor of TRPV1 and TRPV4, in *in vitro* and *in vivo* rodent models.²¹⁴ Although the TRP channel inhibitors used in this proof-of-concept study were non-specific, and more definitive work is thus warranted to clarify the role of TRP channels, the study does suggest an important role for TRPV4 in lower lung edema associated with phosgene gas-induced toxicity, as edema decreased in response to RR treatment. Another study demonstrated a potential role for the TRPA1 ion channel in CG-induced lung injury and protection with HC-030031, a TRPA1 inhibitor.²¹⁵

Hydrochloric acid

Acid inhalation from transportation and occupational accidents is another big concern.²¹⁵ In addition to dangers from accidents or intentional terrorist attacks with hydrochloric acid, acid aspiration can occur in patients who are under anesthesia or mechanical ventilation for a long time. Indeed, acid aspiration pneumonia accounts for 10% of respiratory diseases. Cl_2

and phosgene react with water in the mucus of the respiratory tract to form hydrochloric acid.

Several acid-sensitive TRP channels have been identified, including TRPV1, TRPV4, TRPC4, TRPC5, and TRPP2 (PKD2L1). The acid-sensing properties of TRPV1 have been studied in detail.²¹⁶ Different subtypes of TRP channels respond differently to a drop in pH. Most of these molecular acid sensors are differentially expressed in the sensory neurons in pulmonary airways. The literature demonstrates a role for TRP acid-sensing ion channels in acid-induced inflammation. Several studies have reported acidic conditions in different forms of inflammation. Decreased pH has also been reported in the vicinity of metastatic tumors, which has led to a growing interest in the role of TRPs in tumor biology and diagnosis and the use of TRP antagonists as potential chemotherapeutic agents. Increased upregulation and hyperactivity of acid-sensing TRP ion channels under nonphysiologic acidic conditions leads to various forms of chronic pain. Taken together then, these findings support a growing interest in TRP acid-sensing ion channels as countermeasure targets for treating acid-induced lung injuries.

The role of TRPV4 ion channels and their antagonists in acid-induced lung injuries has been well studied. Compared with wild-type mice, TRPV4 ion channel knockout mice did not show acute lung injury after the intratracheal administration of HCl. In a mouse model of hydrochloric acid-induced ALI, the use of the TRPV4 inhibitors GSK2220691 and GSK2337429A recapitulated the findings in knockout mice.²¹ Post-exposure treatment with TRPV4 antagonists reduced critical hallmarks of chemical lung injury such as airway hyperreactivity and increased lung elastance, protein leakage, and neutrophil and macrophage infiltration of the lungs and the associated production of oxidative mediators and inflammatory chemokines and cytokines.²¹ Exposure to hydrochloric acid increases the concentrations of fatty acyl amides, which are known to activate TRP ion channels, including TRPV4 ion channels.^{21, 186, 187}

Yin *et al.* validated the role of TRPV4 ion channels in mediating hydrochloric acid-induced ALI, confirming the previous findings of Balakrishna and colleagues.^{21, 144} The TRPV4 inhibitor GSK2193874 was injected prophylactically before the intratracheal administration of HCl in wild-type or *Trpv4*^{-/-} mice. HC-067047, another commercially available TRPV4 inhibitor, was also tested, but was administered after HCl exposure to mimic a clinical situation. Intratracheal administration of HCl in wild-type mice resulted in characteristic features of murine ALI including hypoxemia, progressive arterial deoxygenation, reduced lung compliance, increased pulmonary edema, protein leakage, increased MPO levels in BALF, and elevated proinflammatory cytokines. Prophylactic pharmacologic TRPV4 inhibition with GSK2193874 or genetic deletion of the *Trpv4* gene attenuated HCl-induced ALI, whereas, post-exposure treatment with HC-067047 (45 min after HCl administration) showed no therapeutic benefits.¹⁴⁴

Chloropicrin

Chloropicrin (Cl₃CNO₂, nitrochloroform, trichloronitromethane) has broad biocidal fungicidal properties, and is commonly used as a pre-emergent soil fumigant. Chloropicrin is highly reactive and a dangerous explosive hazard. Chloropicrin was used as a chemical

warfare agent during World War I due to its toxic effects in all routes of exposure and its ease of synthesis and has also been used as a tear gas agent. Chloropicrin is formed when Cl_2 or hypochlorous acid reacts with picric acid (2,4,6-trinitrophenol). Exposure to chloropicrin for 30 min at 119 ppm is considered lethal. Chloropicrin is a powerful lachrymator, and inhalational exposure leads to nasal and pharyngeal irritation, coughing, shortness of breath, and pulmonary edema.²¹⁷

In vitro studies have shown that chloropicrin is a TRP ion channel agonist due to its electrophilic nature.²⁰⁰ However, systemic preclinical studies are needed to characterize the role of TRP channels in chloropicrin-induced pulmonary injuries, and to test the effects of TRP antagonists in mitigating those injuries.

Hydrogen sulfide

Hydrogen sulfide (H_2S) is a colorless volcanic gas with a peculiar foul odor of rotten eggs. It is very poisonous, corrosive, flammable, and explosive. In one oil drilling accident, 9 people died because of hydrogen sulfide inhalation. Hydrogen sulfide was also used during World War I. Hydrogen sulfide readily complexes with iron molecules in mitochondrial cytochrome enzymes, and prevents cellular oxygen uptake. Exposure to lower levels leads to sore throat, cough, nausea, shortness of breath, and pulmonary edema. Hydrogen sulfide is present in the human body at very low concentrations, and is a putative signal transmitter for normal homeostasis. Thus, low levels are usually tolerated.

In a murine sepsis model and a guinea pig lung inflammation model, H_2S -induced inflammation was shown to be mediated by TRPV1.^{218, 219} Capsazepine, a TRPV1 inhibitor, protected against hydrogen sulfide-induced systemic inflammation.²¹⁹ *In vitro* and *in vivo* functional studies demonstrated that hydrogen sulfide activates TRPA1.^{220, 221} Further, acidic pH under pathophysiologic conditions potentiates the capacity of hydrogen sulfide to activate TRPA1.^{220, 221}

Sulfur mustard

Sulfur mustard (mustard gas, SM) has been used as a chemical WMD since World War I and was recently used in the Iran–Iraq war (1980–1988). SM has caused more than 80% of all documented chemical warfare casualties. The short-term and long-term effects of sulfur mustard-induced lung injuries are reviewed in Poursaleh *et al.* (2012).²²² There are no specific and universally accepted antidotes for sulfur mustard.

Due to the safety restrictions on handling bifunctional sulfur mustard gas, most preclinical studies have investigated the effects of monofunctional sulfur mustard (2-chlor-ethyl-ethyl sulfide, CEES). In one study, human A549 lung epithelial cells, which express TRPA1 endogenously, reacted to CEES exposure with a transient calcium influx. Furthermore, cytotoxicity was mitigated by the TRPA1 antagonist AP18 in *in vitro* studies.²²³ Achanta *et al.* demonstrated the therapeutic efficacy of the TRPA1 inhibitors HC-030031 and A-967079 in a CEES mouse ear vesicant model.⁸⁴ The TRPA1 inhibitors were administered after CEES exposure to mimic clinical conditions. The role of TRPA1 in mediating the effects of sulfur mustard analogs has also been demonstrated independently by other investigators.^{28, 223, 224}

Ammonia

Anhydrous ammonia was also used during World War I. Ammonia (NH₃) is commonly used as a fertilizer and refrigerant and in several industrial chemical reactions. Accidental exposure during stockpiling, occupational and household handling, and transportation are the current major concerns about ammonia toxicity. NH₃ readily reacts with mucous secretions of the respiratory tract to form ammonium hydroxide. Ammonia gas has a pungent odor and irritating properties. Inhalation of ammonia causes severe respiratory toxicity, and symptoms depend on the concentration and duration of exposure. Respiratory symptoms are rhinorrhea, bronchitis, coughing, and edema. Acutely, ammonia causes pneumonitis, and chronically, it causes bronchitis. There is no specific antidote for ammonia-induced ALI. Treatment is symptomatic and supportive care, which includes mechanical ventilation with humidified oxygen, nebulized bronchodilators, and managing acid–base imbalance.

In vitro studies in dorsal root ganglia showed that both TRPA1 and TRPV1 sense ammonia.²²⁵ In the same 2009 study, Dhaka *et al.* hypothesized that TRPA1 detects a basic pH environment via cysteine modification whereas TRPV1 detects a basic environment via histidine residue modification.²²⁵ There are no *in vivo* studies that demonstrate the role of TRP ion channels in mediating ammonia-induced pulmonary injuries. However, based on parsimonious *in vitro* studies and the role of TRP channels in mediating diverse noxious stimuli, TRP ion channels likely play an important role in ammonia pulmonary toxicity. *In vivo* studies are needed to study their role and to develop countermeasures.

Conclusions

The role of TRPs in various chemical-induced pulmonary injury models is unfolding rapidly. There is abundant literature evidence to support the role of TRPs in several diseases and TRP ion channels as potential targets for treatment. There is great potential to unlock the therapeutic efficacy of TRPs in different chemical-induced pulmonary injuries. The growing threat of using chemical agents to create mass casualties demands the immediate development of medical countermeasures. Because of the absence of specific antidotes for most of the chemical threat agents that cause severe pulmonary injuries, TRP ion channel antagonists are putatively accepted as good candidates for preclinical studies of countermeasures in different injury models. The existing preclinical evidence for the therapeutic efficacy of TRPA1 and TRPV4 antagonists in different chemical injury models is promising, and paves the way for the advanced development of these candidates. We believe that TRP ion channels will become blockbuster targets for several indications, including chemical medical countermeasures.

Future directions

- It is impractical to develop scientific countermeasures/antidotes against every dangerous chemical threat agent. The generation of chemical threat agents should be curbed, and the transportation and handling of these agents should be monitored by a global alliance. When global efforts to mitigate TIH as chemical

weapons fail and an effective antidote for each TIH is unrealistic, broad-spectrum antidotes such as TRP ion channel antagonists at least offer hope.

- Most of the chemical threat agents have been deployed since World War I, but there are no specific antidotes. Testing approved therapeutic agents for relabeling and broadening the list of indications should be the priority to hasten the process of discovering specific antidotes for each chemical agent. To expedite the process of approval of therapeutic agents for chemical and biologic countermeasures, the U.S. FDA has established an alternative approval process that is unlike the approval process for traditional therapeutic agents against other indications (FDA's animal rule).²²⁶
- High throughput screening and genomics/transcriptomics analysis should be considered for identifying specific targets for chemical injuries. Quantitative structure-activity relationship models (QSAR) and *in silico* predictive models should be used to identify lead candidates.
- Although some of the TRP antagonists are showing great therapeutic efficacy in preclinical and clinical studies, we may have to wait a few more years to see TRP antagonists on the market. However, with the support of the BARDA, the NIH CounterACT program, and FDA's animal rule (21 CFR 314.600 through 314.650 [drugs]), this gap may be filled soon.
- Combination therapies may be more beneficial. For example, TRPA1 and TRPV1 antagonists in combination completely diminished cough reflex compared with single treatments.²²⁷ However, the literature on this subject is very limited.

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References:

1. Ala'Aldeen AA 1991. "Death Clouds: Saddam Hussein's Chemical War Against the Kurds 5/1/1991". In.
2. Johnston WR 2017. Summary of historical attacks using chemical or biological weapons. Accessed March 16th, 2020. <http://www.johnstonsarchive.net/terrorism/chembioattacks.html>.
3. Weapons, O.f.t.P.o.C. 2014. OPCW Fact Finding Mission: "Compelling Confirmation" That Chlorine Gas Used as Weapon in Syria. Accessed 04/08/2020, 2020. <https://www.opcw.org/news/article/opcw-fact-finding-mission-compelling-confirmation-that-chlorine-gas-used-as-weapon-in-syria/>.
4. Weapons, O.f.t.P.o.C. 2020. OPCW Releases First Report by Investigation and Identification Team. Accessed 04/08/2020, 2020. <https://www.opcw.org/media-centre/news/2020/04/opcw-releases-first-report-investigation-and-identification-team>.
5. SCHNEIDER T & LÜTKEFEND T Nowhere to Hide: The Logic of Chemical Weapons Use in Syria. G.P.P. Institute.
6. Jones R, Wills B & Kang C 2010. Chlorine gas: an evolving hazardous material threat and unconventional weapon. *West J Emerg Med* 11: 151–156. [PubMed: 20823965]

7. Mackie E, Svendsen E, Grant S, et al. 2014. Management of chlorine gas-related injuries from the Graniteville, South Carolina, train derailment. *Disaster Med Public Health Prep* 8: 411–416. [PubMed: 25225966]
8. Railroads A.o.A. 2015 “STATEMENT OF EDWARD R. HAMBERGER PRESIDENT & CHIEF EXECUTIVE OFFICER, ASSOCIATION OF AMERICAN RAILROADS, BEFORE THE U.S. HOUSE OF REPRESENTATIVES COMMITTEE ON TRANSPORTATION AND INFRASTRUCTURE SUBCOMMITTEE ON RAILROADS, PIPELINES AND HAZARDOUS MATERIALS HEARING TO EXAMINE THE EFFECTS OF THE U.S. ENERGY RENAISSANCE ON THE TRANSPORTATION SYSTEM “. In.
9. Branscomb L, Fagan M, Auerswald PE, et al. 2010. Rail Transportation of Toxic Inhalation Hazards: Policy Responses to the Safety and Security Externalities. Available at SSRN:
10. Achanta S & Jordt SE 2019. Toxic Effects of Chlorine Gas and Potential Treatments: A Literature Review. *Toxicol Mech Methods*. 1–34.
11. McGovern T, Day BJ, White CW, et al. 2011. AEOL10150: a novel therapeutic for rescue treatment after toxic gas lung injury. *Free Radic Biol Med* 50: 602–608. [PubMed: 21156205]
12. Wigenstam E, Koch B, Bucht A, et al. 2015. N-acetyl cysteine improves the effects of corticosteroids in a mouse model of chlorine-induced acute lung injury. *Toxicology*. 328: 40–47. [PubMed: 25497111]
13. Carlisle M, Lam A, Svendsen ER, et al. 2016. Chlorine-induced cardiopulmonary injury. *Ann N Y Acad Sci* 1374: 159–167. [PubMed: 27303906]
14. Jurkuvenaite A, Benavides GA, Komarova S, et al. 2015. Upregulation of autophagy decreases chlorine-induced mitochondrial injury and lung inflammation. *Free Radic Biol Med* 85: 83–94. [PubMed: 25881550]
15. Chang W, Chen J, Schlueter CF, et al. 2012. Inhibition of chlorine-induced lung injury by the type 4 phosphodiesterase inhibitor rolipram. *Toxicol Appl Pharmacol* 263: 251–258. [PubMed: 22763362]
16. Hoyle GW 2010. Mitigation of chlorine lung injury by increasing cyclic AMP levels. *Proc Am Thorac Soc* 7: 284–289. [PubMed: 20601633]
17. Zhang J, Shao Y, He D, et al. 2016. Evidence that bone marrow-derived mesenchymal stem cells reduce epithelial permeability following phosgene-induced acute lung injury via activation of wnt3a protein-induced canonical wnt/beta-catenin signaling. *Inhalation toxicology*. 28: 572–579. [PubMed: 27644345]
18. Ahmad S, Ahmad A, Hendry-Hofer TB, et al. 2015. Sarcoendoplasmic reticulum Ca(2+) ATPase. A critical target in chlorine inhalation-induced cardiotoxicity. *Am J Respir Cell Mol Biol* 52: 492–502. [PubMed: 25188881]
19. Bessac BF, Sivula M, von Hehn CA, et al. 2008. TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J Clin Invest* 118: 1899–1910. [PubMed: 18398506]
20. Achanta S & Jordt SE 2017. TRPA1: Acrolein meets its target. *Toxicol Appl Pharmacol* 324: 45–50. [PubMed: 28284857]
21. Balakrishna S, Song W, Achanta S, et al. 2014. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 307: L158–172. [PubMed: 24838754]
22. Summerhill EM, Hoyle GW, Jordt SE, et al. 2017. An Official American Thoracic Society Workshop Report: Chemical Inhalational Disasters. Biology of Lung Injury, Development of Novel Therapeutics, and Medical Preparedness. *Ann Am Thorac Soc* 14: 1060–1072. [PubMed: 28418689]
23. Andres D, Keyser B, Benton B, et al. 2016. Transient receptor potential (TRP) channels as a therapeutic target for intervention of respiratory effects and lethality from phosgene. *Toxicol Lett* 244: 21–27. [PubMed: 26562769]
24. Filipczak PT, Senft AP, Seagrave J, et al. 2015. NOS-2 Inhibition in Phosgene-Induced Acute Lung Injury. *Toxicol Sci* 146: 89–100. [PubMed: 25870319]
25. Zarogiannis SG, Wagener BM, Basappa S, et al. 2014. Postexposure aerosolized heparin reduces lung injury in chlorine-exposed mice. *Am J Physiol Lung Cell Mol Physiol* 307: L347–354. [PubMed: 25038191]

26. Dietrich A, Steinritz D & Gudermann T 2017. Transient receptor potential (TRP) channels as molecular targets in lung toxicology and associated diseases. *Cell Calcium*
27. Buch T, Schafer E, Steinritz D, et al. 2013. Chemosensory TRP channels in the respiratory tract: role in toxic lung injury and potential as “sweet spots” for targeted therapies. *Rev Physiol Biochem Pharmacol* 165: 31–65. [PubMed: 23532495]
28. Steinritz D, Stenger B, Dietrich A, et al. 2018. TRPs in Tox: Involvement of Transient Receptor Potential-Channels in Chemical-Induced Organ Toxicity-A Structured Review. *Cells*. 7.
29. Bessac BF & Jordt SE 2010. Sensory detection and responses to toxic gases: mechanisms, health effects, and countermeasures. *Proc Am Thorac Soc* 7: 269–277. [PubMed: 20601631]
30. Achanta S, Liu B, Caceres AI, et al. 2017. “TRPV4 Inhibitor Improves Pulmonary Function and Oxygen Saturation in a Pig Translational Model of Chemically-Induced Acute Lung Injury “. In Society of Toxicology Annual Meeting. *The Toxicologist: Supplement to Toxicological Sciences*.
31. Grace MS, Baxter M, Dubuis E, et al. 2014. Transient receptor potential (TRP) channels in the airway: role in airway disease. *Br J Pharmacol* 171: 2593–2607. [PubMed: 24286227]
32. Bonvini SJ, Birrell MA, Smith JA, et al. 2015. Targeting TRP channels for chronic cough: from bench to bedside. *Naunyn Schmiedeberg Arch Pharmacol* 388: 401–420. [PubMed: 25572384]
33. Montell C & Rubin GM 1989. Molecular characterization of the *Drosophila* trp locus: a putative integral membrane protein required for phototransduction. *Neuron* 2: 1313–1323. [PubMed: 2516726]
34. Bessac BF & Jordt SE 2008. Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. *Physiology (Bethesda)* 23: 360–370. [PubMed: 19074743]
35. Julius D 2013. TRP channels and pain. *Annual review of cell and developmental biology*. 29: 355–384.
36. Holzer P 2011. TRP channels in the digestive system. *Curr Pharm Biotechnol* 12: 24–34. [PubMed: 20932260]
37. Holzer P & Izzo AA 2014. The pharmacology of TRP channels. *Br J Pharmacol* 171: 2469–2473. [PubMed: 24773265]
38. Pedersen SF, Owsianik G & Nilius B 2005. TRP channels: an overview. *Cell Calcium*. 38: 233–252. [PubMed: 16098585]
39. Premkumar LS & Abooj M 2013. TRP channels and analgesia. *Life Sci* 92: 415–424. [PubMed: 22910182]
40. Sexton JE, Vernon J & Wood JN 2014. TRPs and Pain. *Handb Exp Pharmacol* 223: 873–897. [PubMed: 24961972]
41. Sun S & Dong X 2015. Trp channels and itch. *Semin Immunopathol*
42. Christensen AP & Corey DP 2007. TRP channels in mechanosensation: direct or indirect activation? *Nat Rev Neurosci* 8: 510–521. [PubMed: 17585304]
43. Bautista DM, Pellegrino M & Tsunozaki M 2013. TRPA1: A gatekeeper for inflammation. *Annu Rev Physiol* 75: 181–200. [PubMed: 23020579]
44. Caterina MJ 2015. “Chapter 1 - An Introduction to Transient Receptor Potential Ion Channels and Their Roles in Disease”. In *TRP Channels as Therapeutic Targets From Basic Science to Clinical Use*. Szallasi A, Ed.: 1–12. Academic Press.
45. Inoue T & Bryant BP 2009. Multiple Cation Channels Mediate Increases in Intracellular Calcium Induced by the Volatile Irritant, Trans-2-Pentenal in Rat Trigeminal Neurons. *Cell Mol Neurobiol*
46. Birrell MA, Belvisi MG, Grace M, et al. 2009. TRPA1 agonists evoke coughing in guinea pig and human volunteers. *Am J Respir Crit Care Med* 180: 1042–1047. [PubMed: 19729665]
47. Leung SY, Niimi A, Williams AS, et al. 2007. Inhibition of citric acid- and capsaicin-induced cough by novel TRPV-1 antagonist, V112220, in guinea-pig. *Cough* 3: 10. [PubMed: 18154688]
48. Smit LA, Kogevinas M, Anto JM, et al. 2012. Transient receptor potential genes, smoking, occupational exposures and cough in adults. *Respir Res* 13: 26. [PubMed: 22443337]
49. Hamanaka K, Jian MY, Townsley MI, et al. 2010. TRPV4 channels augment macrophage activation and ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 299: L353–362. [PubMed: 20562229]

50. Hamanaka K, Jian MY, Weber DS, et al. 2007. TRPV4 initiates the acute calcium-dependent permeability increase during ventilator-induced lung injury in isolated mouse lungs. *Am J Physiol Lung Cell Mol Physiol* 293: L923–932. [PubMed: 17660328]
51. De Logu F, Patacchini R, Fontana G, et al. 2016. TRP functions in the broncho-pulmonary system. *Semin Immunopathol* 38: 321–329. [PubMed: 27083925]
52. Banner KH, Igney F & Poll C 2011. TRP channels: emerging targets for respiratory disease. *Pharmacol Ther* 130: 371–384. [PubMed: 21420429]
53. Abbott-Banner K, Poll C & Verkuyl JM 2013. Targeting TRP channels in airway disorders. *Curr Top Med Chem* 13: 310–321. [PubMed: 23506455]
54. Caceres AI, Brackmann M, Elia MD, et al. 2009. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc Natl Acad Sci U S A* 106: 9099–9104. [PubMed: 19458046]
55. Kaneko Y & Szallasi A 2014. Transient receptor potential (TRP) channels: a clinical perspective. *Br J Pharmacol* 171: 2474–2507. [PubMed: 24102319]
56. Parenti A, De Logu F, Geppetti P, et al. 2016. What is the evidence for the role of TRP channels in inflammatory and immune cells? *Br J Pharmacol* 173: 953–969. [PubMed: 26603538]
57. Jia Y, McLeod RL, Wang X, et al. 2002. Anandamide induces cough in conscious guinea-pigs through VR1 receptors. *Br J Pharmacol* 137: 831–836. [PubMed: 12411414]
58. Song W, Yu Z, Doran SF, et al. 2015. Respiratory syncytial virus infection increases chlorine-induced airway hyperresponsiveness. *Am J Physiol Lung Cell Mol Physiol* 309: L205–210. [PubMed: 26071553]
59. Gessner MA, Doran SF, Yu Z, et al. 2013. Chlorine gas exposure increases susceptibility to invasive lung fungal infection. *Am J Physiol Lung Cell Mol Physiol* 304: L765–773. [PubMed: 23564508]
60. Hout JJ, White DW, Artino AR, et al. 2014. O-chlorobenzylidene malononitrile (CS riot control agent) associated acute respiratory illnesses in a U.S. Army Basic Combat Training cohort. *Military medicine*. 179: 793–798. [PubMed: 25003867]
61. Zholos AV 2015. TRP Channels in Respiratory Pathophysiology: the Role of Oxidative, Chemical Irritant and Temperature Stimuli. *Curr Neuropharmacol* 13: 279–291. [PubMed: 26411771]
62. Meseguer V, Alpizar YA, Luis E, et al. 2014. TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. *Nat Commun* 5: 3125. [PubMed: 24445575]
63. Paulsen CE, Armache JP, Gao Y, et al. 2015. Structure of the TRPA1 ion channel suggests regulatory mechanisms. *Nature*. 525: 552.
64. Suo Y, Wang Z, Zubcevic L, et al. 2020. Structural Insights into Electrophile Irritant Sensing by the Human TRPA1 Channel. *Neuron* 105: 882–894 e885. [PubMed: 31866091]
65. Buch TR, Schafer EA, Demmel MT, et al. 2013. Functional expression of the transient receptor potential channel TRPA1, a sensor for toxic lung inhalants, in pulmonary epithelial cells. *Chem Biol Interact* 206: 462–471. [PubMed: 23994502]
66. Ramsey IS, Delling M & Clapham DE 2006. An introduction to TRP channels. *Annu Rev Physiol* 68: 619–647. [PubMed: 16460286]
67. Mukhopadhyay I, Gomes P, Aranake S, et al. 2011. Expression of functional TRPA1 receptor on human lung fibroblast and epithelial cells. *J Recept Signal Transduct Res* 31: 350–358. [PubMed: 21848366]
68. Bautista DM, Jordt SE, Nikai T, et al. 2006. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell*. 124: 1269–1282. [PubMed: 16564016]
69. Guimaraes MZP & Jordt SE 2007. “TRPA1 : A Sensory Channel of Many Talents”. In *TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades*. Liedtke WB & Heller S, Eds. Boca Raton (FL).
70. Macpherson LJ, Dubin AE, Evans MJ, et al. 2007. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature*. 445: 541–545. [PubMed: 17237762]
71. Zurborg S, Yurgionas B, Jira JA, et al. 2007. Direct activation of the ion channel TRPA1 by Ca²⁺. *Nat Neurosci* 10: 277–279. [PubMed: 17259981]

72. Conklin DJ 2016. Acute cardiopulmonary toxicity of inhaled aldehydes: role of TRPA1. *Ann N Y Acad Sci*
73. Bautista DM, Movahed P, Hinman A, et al. 2005. Pungent products from garlic activate the sensory ion channel TRPA1. *Proc Natl Acad Sci U S A* 102: 12248–12252. [PubMed: 16103371]
74. Jordt SE, Bautista DM, Chuang HH, et al. 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature*. 427: 260–265. [PubMed: 14712238]
75. Conklin DJ, Haberzettl P, Jagatheesan G, et al. 2016. Role of TRPA1 in acute cardiopulmonary toxicity of inhaled acrolein. *Toxicol Appl Pharmacol*
76. Takaya J, Mio K, Shiraishi T, et al. 2015. A Potent and Site-Selective Agonist of TRPA1. *J Am Chem Soc* 137: 15859–15864. [PubMed: 26630251]
77. Reese RM, Dourado M, Anderson K, et al. 2020. Behavioral characterization of a CRISPR-generated TRPA1 knockout rat in models of pain, itch, and asthma. *Sci Rep* 10: 979. [PubMed: 31969645]
78. Kwan KY, Allchorne AJ, Vollrath MA, et al. 2006. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* 50: 277–289. [PubMed: 16630838]
79. Achanta S, Chintagari NR, Brackmann M, et al. 2018. TRPA1 and CGRP antagonists counteract vesicant-induced skin injury and inflammation. *Toxicol Lett*
80. Petrus M, Peier AM, Bandell M, et al. 2007. A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. *Molecular pain*. 3: 40. [PubMed: 18086313]
81. Mori Y, Takahashi N, Kurokawa T, et al. 2017. TRP channels in oxygen physiology: distinctive functional properties and roles of TRPA1 in O₂ sensing. *Proc Jpn Acad Ser B Phys Biol Sci* 93: 464–482.
82. Mukhopadhyay I, Kulkarni A, Aranake S, et al. 2014. Transient receptor potential ankyrin 1 receptor activation in vitro and in vivo by pro-tussive agents: GRC 17536 as a promising anti-tussive therapeutic. *PloS one*. 9: e97005. [PubMed: 24819048]
83. Jha A, Sharma P, Anaparti V, et al. 2015. A role for transient receptor potential ankyrin 1 cation channel (TRPA1) in airway hyper-responsiveness? *Can J Physiol Pharmacol* 93: 171–176. [PubMed: 25654580]
84. Achanta S, Chintagari NR, Brackmann M, et al. 2018. TRPA1 and CGRP antagonists counteract vesicant-induced skin injury and inflammation. *Toxicol Lett* 293: 140–148. [PubMed: 29535050]
85. Brone B, Peeters PJ, Marrannes R, et al. 2008. Tear gasses CN, CR, and CS are potent activators of the human TRPA1 receptor. *Toxicol Appl Pharmacol* 231: 150–156. [PubMed: 18501939]
86. Martinez JM & Eling TE 2019. Activation of TRPA1 by volatile organic chemicals leading to sensory irritation. *ALTEX* 36: 572–582. [PubMed: 31026039]
87. van den Berg M, Nijboer-Brinksma S, Bos S, et al. 2020. The novel TRPA1 antagonist BI01305834 inhibits ovalbumin-induced bronchoconstriction in guinea pigs. *Br J Pharmacol*
88. Takahashi N, Kuwaki T, Kiyonaka S, et al. 2011. TRPA1 underlies a sensing mechanism for O₂. *Nat Chem Biol* 7: 701–711. [PubMed: 21873995]
89. Lindsay CD & Timperley CM 2020. TRPA1 and issues relating to animal model selection for extrapolating toxicity data to humans. *Hum Exp Toxicol* 39: 14–36. [PubMed: 31578097]
90. Chen J, Zhang XF, Kort ME, et al. 2008. Molecular determinants of species-specific activation or blockade of TRPA1 channels. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 28: 5063–5071. [PubMed: 18463259]
91. Chen J, Kang D, Xu J, et al. 2013. Species differences and molecular determinant of TRPA1 cold sensitivity. *Nat Commun* 4: 2501. [PubMed: 24071625]
92. Tan J, Hunsberger G, Neipp C, et al. 2015. “TRPA1 Antagonists as Potential Therapeutics for Respiratory Diseases”. In *TRP Channels As Therapeutic Targets : From Basic Science to Clinical Use*. Szallasi A, Ed.: 167–173. Elsevier Science & Technology.
93. Koivisto A, Jalava N, Bratty R, et al. 2018. TRPA1 Antagonists for Pain Relief. *Pharmaceuticals (Basel)* 11.

94. Garrison SR & Stucky CL 2014. Contribution of transient receptor potential ankyrin 1 to chronic pain in aged mice with complete Freund's adjuvant-induced arthritis. *Arthritis Rheumatol* 66: 2380–2390. [PubMed: 24891324]
95. Xiao R, Zhang B, Dong Y, et al. 2013. A genetic program promotes *C. elegans* longevity at cold temperatures via a thermosensitive TRP channel. *Cell*. 152: 806–817. [PubMed: 23415228]
96. Liu B, Tai Y, Caceres AI, et al. 2016. Oxidized Phospholipid OxPAPC Activates TRPA1 and Contributes to Chronic Inflammatory Pain in Mice. *PloS one*. 11: e0165200. [PubMed: 27812120]
97. McNamara CR, Mandel-Brehm J, Bautista DM, et al. 2007. TRPA1 mediates formalin-induced pain. *Proc Natl Acad Sci U S A* 104: 13525–13530. [PubMed: 17686976]
98. Wei H, Hamalainen MM, Saarnilehto M, et al. 2009. Attenuation of mechanical hypersensitivity by an antagonist of the TRPA1 ion channel in diabetic animals. *Anesthesiology*. 111: 147–154. [PubMed: 19512877]
99. India GPL 2014. "A Clinical Trial to Study the Effects GRC 17536 in Patients With Painful Diabetic Peripheral Neuropathy (Painful Extremities Due to Peripheral Nerve Damage in Diabetic Patients).". In. [ClinicalTrials.gov](https://clinicaltrials.gov).
100. Skerratt S 2017. Recent Progress in the Discovery and Development of TRPA1 Modulators. *Prog Med Chem* 56: 81–115. [PubMed: 28314413]
101. Chen H & Terrett JA 2020. Transient receptor potential ankyrin 1 (TRPA1) antagonists: a patent review (2015–2019). *Expert Opin Ther Pat*
102. Pryde DC, Marron BE, West CW, et al. 2017. Discovery of a Series of Indazole TRPA1 Antagonists. *ACS Med Chem Lett* 8: 666–671. [PubMed: 28626530]
103. Chen H, Volgraf M, Do S, et al. 2018. Discovery of a Potent (4 R,5 S)-4-Fluoro-5-methylproline Sulfonamide Transient Receptor Potential Ankyrin 1 Antagonist and Its Methylene Phosphate Prodrug Guided by Molecular Modeling. *J Med Chem* 61: 3641–3659. [PubMed: 29590749]
104. Rothenberg C, Achanta S, Svendsen ER, et al. 2016. Tear gas: an epidemiological and mechanistic reassessment. *Ann N Y Acad Sci*
105. Kollarik M & Undem BJ 2004. Activation of bronchopulmonary vagal afferent nerves with bradykinin, acid and vanilloid receptor agonists in wild-type and TRPV1^{-/-} mice. *J Physiol* 555: 115–123. [PubMed: 14634201]
106. Goswami SC, Mishra SK, Maric D, et al. 2014. Molecular signatures of mouse TRPV1-lineage neurons revealed by RNA-Seq transcriptome analysis. *J Pain* 15: 1338–1359. [PubMed: 25281809]
107. Caterina MJ, Schumacher MA, Tominaga M, et al. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 389: 816–824. [PubMed: 9349813]
108. Cao E, Liao M, Cheng Y, et al. 2013. TRPV1 structures in distinct conformations reveal activation mechanisms. *Nature*. 504: 113–118. [PubMed: 24305161]
109. Cui M, Gosu V, Basith S, et al. 2016. Polymodal Transient Receptor Potential Vanilloid Type 1 Nociceptor: Structure, Modulators, and Therapeutic Applications. *Adv Protein Chem Struct Biol* 104: 81–125. [PubMed: 27038373]
110. Watanabe N, Horie S, Michael GJ, et al. 2006. Immunohistochemical co-localization of transient receptor potential vanilloid (TRPV)1 and sensory neuropeptides in the guinea-pig respiratory system. *Neuroscience*. 141: 1533–1543. [PubMed: 16765524]
111. Agopyan N, Head J, Yu S, et al. 2004. TRPV1 receptors mediate particulate matter-induced apoptosis. *Am J Physiol Lung Cell Mol Physiol* 286: L563–572. [PubMed: 14633515]
112. Costa SK, Kumagai Y, Brain SD, et al. 2010. Involvement of sensory nerves and TRPV1 receptors in the rat airway inflammatory response to two environment pollutants: diesel exhaust particles (DEP) and 1,2-naphthoquinone (1,2-NQ). *Arch Toxicol* 84: 109–117. [PubMed: 19399481]
113. Riera CE, Huising MO, Follett P, et al. 2014. TRPV1 pain receptors regulate longevity and metabolism by neuropeptide signaling. *Cell*. 157: 1023–1036. [PubMed: 24855942]
114. Steculorum SM & Bruning JC 2014. Die another day: a painless path to longevity. *Cell*. 157: 1004–1006. [PubMed: 24855937]

115. Groneberg DA, Niimi A, Dinh QT, et al. 2004. Increased expression of transient receptor potential vanilloid-1 in airway nerves of chronic cough. *Am J Respir Crit Care Med* 170: 1276–1280. [PubMed: 15447941]
116. Mitchell JE, Campbell AP, New NE, et al. 2005. Expression and characterization of the intracellular vanilloid receptor (TRPV1) in bronchi from patients with chronic cough. *Exp Lung Res* 31: 295–306. [PubMed: 15962710]
117. Zhang G, Lin RL, Wiggers M, et al. 2008. Altered expression of TRPV1 and sensitivity to capsaicin in pulmonary myelinated afferents following chronic airway inflammation in the rat. *J Physiol* 586: 5771–5786. [PubMed: 18832423]
118. Bevan S & Andersson DA 2009. TRP channel antagonists for pain--opportunities beyond TRPV1. *Curr Opin Investig Drugs*. 10: 655–663.
119. Gomtsyan A & Brederson J-D 2015. "Clinical and Preclinical Experience with TRPV1 Antagonists as Potential Analgesic Agents". In *TRP Channels As Therapeutic Targets : From Basic Science to Clinical Use*. Szallasi A, Ed.: 129–144. Academic Press.
120. ClinicalTrials.Gov. "TRPV1" in clinical trials. 07/21/2020. <https://clinicaltrials.gov/ct2/results?cond=TRPV1&term=&cntry=&state=&city=&dist=>.
121. Choi JY, Lee HY, Hur J, et al. 2018. TRPV1 Blocking Alleviates Airway Inflammation and Remodeling in a Chronic Asthma Murine Model. *Allergy Asthma Immunol Res* 10: 216–224. [PubMed: 29676068]
122. Helyes Z, Elekes K, Nemeth J, et al. 2007. Role of transient receptor potential vanilloid 1 receptors in endotoxin-induced airway inflammation in the mouse. *Am J Physiol Lung Cell Mol Physiol* 292: L1173–1181. [PubMed: 17237150]
123. Blum CA, Caldwell T, Zheng X, et al. 2010. Discovery of novel 6,6-heterocycles as transient receptor potential vanilloid (TRPV1) antagonists. *J Med Chem* 53: 3330–3348. [PubMed: 20307063]
124. Gunthorpe MJ, Hannan SL, Smart D, et al. 2007. Characterization of SB-705498, a potent and selective vanilloid receptor-1 (VR1/TRPV1) antagonist that inhibits the capsaicin-, acid-, and heat-mediated activation of the receptor. *J Pharmacol Exp Ther* 321: 1183–1192. [PubMed: 17392405]
125. Khalid S, Murdoch R, Newlands A, et al. 2014. Transient receptor potential vanilloid 1 (TRPV1) antagonism in patients with refractory chronic cough: a double-blind randomized controlled trial. *J Allergy Clin Immunol* 134: 56–62. [PubMed: 24666696]
126. Deng Z, Paknejad N, Makshev G, et al. 2018. Cryo-EM and X-ray structures of TRPV4 reveal insight into ion permeation and gating mechanisms. *Nat Struct Mol Biol* 25: 252–260. [PubMed: 29483651]
127. Shigematsu H, Sokabe T, Danev R, et al. 2010. A 3.5-nm structure of rat TRPV4 cation channel revealed by Zernike phase-contrast cryoelectron microscopy. *J Biol Chem* 285: 11210–11218. [PubMed: 20044482]
128. Everaerts W, Nilius B & Owsianik G 2010. The vanilloid transient receptor potential channel TRPV4: from structure to disease. *Prog Biophys Mol Biol* 103: 2–17. [PubMed: 19835908]
129. White JP, Cibelli M, Urban L, et al. 2016. TRPV4: Molecular Conductor of a Diverse Orchestra. *Physiol Rev* 96: 911–973. [PubMed: 27252279]
130. Sonkusare SK, Bonev AD, Ledoux J, et al. 2012. Elementary Ca²⁺ signals through endothelial TRPV4 channels regulate vascular function. *Science*. 336: 597–601. [PubMed: 22556255]
131. Lorenzo IM, Liedtke W, Sanderson MJ, et al. 2008. TRPV4 channel participates in receptor-operated calcium entry and ciliary beat frequency regulation in mouse airway epithelial cells. *Proc Natl Acad Sci U S A* 105: 12611–12616. [PubMed: 18719094]
132. Thorneloe KS, Cheung M, Bao W, et al. 2012. An orally active TRPV4 channel blocker prevents and resolves pulmonary edema induced by heart failure. *Science translational medicine*. 4: 159ra148.
133. Michalick L & Kuebler WM 2020. TRPV4-A Missing Link Between Mechanosensation and Immunity. *Front Immunol* 11: 413. [PubMed: 32210976]

134. Alvarez DF, King JA, Weber D, et al. 2006. Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. *Circulation research*. 99: 988–995. [PubMed: 17008604]
135. Grace MS, Bonvini SJ, Belvisi MG, et al. 2017. Modulation of the TRPV4 ion channel as a therapeutic target for disease. *Pharmacol Ther* 177: 9–22. [PubMed: 28202366]
136. Goldenberg NM, Ravindran K & Kuebler WM 2015. TRPV4: physiological role and therapeutic potential in respiratory diseases. *Naunyn Schmiedebergs Arch Pharmacol* 388: 421–436. [PubMed: 25342095]
137. Klausen TK, Pagani A, Minassi A, et al. 2009. Modulation of the transient receptor potential vanilloid channel TRPV4 by 4alpha-phorbol esters: a structure-activity study. *J Med Chem*. 52: 2933–2939. [PubMed: 19361196]
138. Thorneloe KS, Sulpizio AC, Lin Z, et al. 2008. N-((1S)-1-{[4-((2S)-2-{[(2,4-dichlorophenyl)sulfonyl]amino}-3-hydroxypropanoyl)-1-piperazinyl]carbonyl}-3-methylbutyl)-1-benzothiophene-2-carboxamide (GSK1016790A), a novel and potent transient receptor potential vanilloid 4 channel agonist induces urinary bladder contraction and hyperactivity: Part I. *J Pharmacol Exp Ther* 326: 432–442. [PubMed: 18499743]
139. Lawhorn BG, Brnardic EJ & Behm DJ 2020. Recent advances in TRPV4 agonists and antagonists. *Bioorg Med Chem Lett* 30: 127022. [PubMed: 32063431]
140. Duncton MAJ 2015. *Small Molecule Agonists and Antagonists of TRPV4* Elsevier Science & Technology. San Diego, CA.
141. Willette RN, Bao W, Nerurkar S, et al. 2008. Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. *J Pharmacol Exp Ther* 326: 443–452. [PubMed: 18499744]
142. Liedtke W & Friedman JM 2003. Abnormal osmotic regulation in *trpv4*^{-/-} mice. *Proc Natl Acad Sci U S A* 100: 13698–13703. [PubMed: 14581612]
143. Mizuno A, Matsumoto N, Imai M, et al. 2003. Impaired osmotic sensation in mice lacking TRPV4. *American journal of physiology. Cell physiology*. 285: C96–101. [PubMed: 12777254]
144. Yin J, Michalick L, Tang C, et al. 2016. Role of Transient Receptor Potential Vanilloid 4 in Neutrophil Activation and Acute Lung Injury. *Am J Respir Cell Mol Biol* 54: 370–383. [PubMed: 26222277]
145. Goyal N, Skrdla P, Schroyer R, et al. 2019. Clinical Pharmacokinetics, Safety, and Tolerability of a Novel, First-in-Class TRPV4 Ion Channel Inhibitor, GSK2798745, in Healthy and Heart Failure Subjects. *Am J Cardiovasc Drugs*. 19: 335–342. [PubMed: 30637626]
146. Brooks CA, Barton LS, Behm DJ, et al. 2019. Discovery of GSK2798745: A Clinical Candidate for Inhibition of Transient Receptor Potential Vanilloid 4 (TRPV4). *ACS Med Chem Lett* 10: 1228–1233. [PubMed: 31413810]
147. [NCT02497937](https://clinicaltrials.gov/ct2/show/NCT02497937?term=trpv4&draw=2&rank=1), C.g.I. & GlaxoSmithKline. 2018. A Study to Evaluate the Effect of the Transient Receptor Potential Vanilloid 4 (TRPV4) Channel Blocker, GSK2798745, on Pulmonary Gas Transfer and Respiration in Patients With Congestive Heart Failure. Accessed 05/03/2020, 2020. <https://clinicaltrials.gov/ct2/show/NCT02497937?term=trpv4&draw=2&rank=1>.
148. [NCT02119260](https://clinicaltrials.gov/ct2/show/NCT02119260?term=trpv4&draw=2&rank=6), C.g.I. & GlaxoSmithKline. 2018. A First Time in Human Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of GSK2798745 in Healthy Subjects and Stable Heart Failure Patients. Accessed 05/03/2020, 2020. <https://clinicaltrials.gov/ct2/show/NCT02119260?term=trpv4&draw=2&rank=6>.
149. Stewart GM, Johnson BD, Sprecher DL, et al. 2020. Targeting pulmonary capillary permeability to reduce lung congestion in heart failure: a randomized, controlled pilot trial. *Eur J Heart Fail*
150. [NCT03511105](https://clinicaltrials.gov/ct2/show/NCT03511105?term=trpv4&draw=2&rank=5), C.g.I. & GlaxoSmithKline. 2020. Effects of GSK2798745 on Alveolar Barrier Disruption in a Segmental Lipopolysaccharide (LPS) Challenge Model Accessed 05/03/2020, 2020. <https://clinicaltrials.gov/ct2/show/NCT03511105?term=trpv4&draw=2&rank=5>.
151. [NCT03372603](https://clinicaltrials.gov/ct2/show/NCT03372603?term=trpv4&draw=2&rank=4), C.g.I. & GlaxoSmithKline. 2019. A Study to Assess the Effectiveness and Side Effects of GSK2798745 in Participants With Chronic Cough. Accessed 05/03/2020, 2020. <https://clinicaltrials.gov/ct2/show/NCT03372603?term=trpv4&draw=2&rank=4>.

152. Liedtke W, Choe Y, Marti-Renom MA, et al. 2000. Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell*. 103: 525–535. [PubMed: 11081638]
153. Liedtke W & Simon SA 2004. A possible role for TRPV4 receptors in asthma. *Am J Physiol Lung Cell Mol Physiol* 287: L269–271. [PubMed: 15246981]
154. Tiruppathi C, Ahmmed GU, Vogel SM, et al. 2006. Ca²⁺ signaling, TRP channels, and endothelial permeability. *Microcirculation*. 13: 693–708. [PubMed: 17085428]
155. Scheraga RG, Abraham S, Grove LM, et al. 2020. TRPV4 Protects the Lung from Bacterial Pneumonia via MAPK Molecular Pathway Switching. *J Immunol* 204: 1310–1321. [PubMed: 31969384]
156. Scheraga RG, Southern BD, Grove LM, et al. 2017. The Role of Transient Receptor Potential Vanilloid 4 in Pulmonary Inflammatory Diseases. *Front Immunol* 8: 503. [PubMed: 28523001]
157. Vincent F & Duncton MA 2011. TRPV4 agonists and antagonists. *Curr Top Med Chem* 11: 2216–2226. [PubMed: 21671873]
158. Hu W, He Y, Li Q, et al. 2020. TRPV4 Channels as Therapeutic Targets in Diabetes and Diabetes-related Complications. *J Diabetes Investig*
159. Morty RE & Kuebler WM 2014. TRPV4: an exciting new target to promote alveolocapillary barrier function. *Am J Physiol Lung Cell Mol Physiol* 307: L817–821. [PubMed: 25281637]
160. Yang XR, Lin AH, Hughes JM, et al. 2012. Upregulation of osmo-mechanosensitive TRPV4 channel facilitates chronic hypoxia-induced myogenic tone and pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 302: L555–568. [PubMed: 22207590]
161. Jian MY, King JA, Al-Mehdi AB, et al. 2008. High vascular pressure-induced lung injury requires P450 epoxigenase-dependent activation of TRPV4. *Am J Respir Cell Mol Biol* 38: 386–392. [PubMed: 17962608]
162. Dalsgaard T, Sonkusare SK, Teuscher C, et al. 2016. Pharmacological inhibitors of TRPV4 channels reduce cytokine production, restore endothelial function and increase survival in septic mice. *Sci Rep* 6: 33841. [PubMed: 27653046]
163. Ulasli M, Gurses SA, Bayraktar R, et al. 2014. The effects of *Nigella sativa* (Ns), *Anthemis hyalina* (Ah) and *Citrus sinensis* (Cs) extracts on the replication of coronavirus and the expression of TRP genes family. *Mol Biol Rep* 41: 1703–1711. [PubMed: 24413991]
164. Kuebler W, Jordt S-E & Liedtke W 2020. TRPV4: An Underappreciated Target to Control Alveolar Lung Edema in Severe SARS-CoV-2 Infections *SSRN*.
165. CDC & N.C.f.I.a.R.D.N. Division of Viral Diseases. 2020. Cleaning and Disinfecting Your Facility. Accessed 04/08/2020, 2020. https://www.cdc.gov/coronavirus/2019-ncov/community/disinfecting-building-facility.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fprepare%2Fdisinfecting-building-facility.html.
166. Brown DF, Dunn WE & Policastro AJ 2000. A National Risk Assessment for Selected Hazardous Materials in Transportation. Accessed 04/08/2020, 2020. <https://publications.anl.gov/anlpubs/2001/01/38251.pdf>.
167. Balte PP, Clark KA, Mohr LC, et al. 2013. The Immediate Pulmonary Disease Pattern following Exposure to High Concentrations of Chlorine Gas. *Pulm Med* 2013: 325869. [PubMed: 24386566]
168. CDC, M.a.M.W.R. 2005. “Public Health Consequences from Hazardous Substances Acutely Released During Rail Transit --- South Carolina, 2005; Selected States, 1999–2004 (<https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5403a2.htm>)”. In, Vol. 54: 64–67. Morbidity and Mortality Weekly Report (MMWR), Centers for Disease Control and Prevention. [PubMed: 15674184]
169. Jani DD, Reed D, Feigley CE, et al. 2016. Modeling an irritant gas plume for epidemiologic study. *Int J Environ Health Res* 26: 58–74. [PubMed: 25772143]
170. [GlobalSecurity.org](https://www.globalsecurity.org/security/library/report/2004/hsc-planning-scenarios-jul04_08.htm). 2004. Chemical Attack - Chlorine Tank Explosion. Accessed 04/08/2020, 2020. https://www.globalsecurity.org/security/library/report/2004/hsc-planning-scenarios-jul04_08.htm.

171. Barrett AM 2009. Mathematical Modeling and Decision Analysis for Terrorism Defense: Assessing Chlorine Truck Attack Consequence and Countermeasure Cost Effectiveness Carnegie Mellon University, Pittsburgh, Pennsylvania
172. Branscomb L, Fagan M, Auerswald PE, et al. 2010. Rail Transportation of Toxic Inhalation Hazards: Policy Responses to the Safety and Security Externality.
173. Runkle JR, Zhang H, Karmaus W, et al. 2013. Long-term impact of environmental public health disaster on health system performance: experiences from the Graniteville, South Carolina chlorine spill. *South Med J* 106: 74–81. [PubMed: 23263318]
174. White CW & Martin JG 2010. Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models. *Proc Am Thorac Soc* 7: 257–263. [PubMed: 20601629]
175. Das R & Blanc PD 1993. Chlorine gas exposure and the lung: a review. *Toxicol Ind Health*. 9: 439–455. [PubMed: 8367885]
176. Van Sickle D, Wenck MA, Belflower A, et al. 2009. Acute health effects after exposure to chlorine gas released after a train derailment. *The American journal of emergency medicine*. 27: 1–7. [PubMed: 19041527]
177. Abara W, Wilson S, Vena J, et al. 2014. Engaging a chemical disaster community: lessons from Graniteville. *Int J Environ Res Public Health*. 11: 5684–5697. [PubMed: 24871259]
178. Leustik M, Doran S, Bracher A, et al. 2008. Mitigation of chlorine-induced lung injury by low-molecular-weight antioxidants. *Am J Physiol Lung Cell Mol Physiol* 295: L733–743. [PubMed: 18708632]
179. Stanley MW, Henry-Stanley MJ, Gajl-Peczalska KJ, et al. 1992. Hyperplasia of type II pneumocytes in acute lung injury. Cytologic findings of sequential bronchoalveolar lavage. *Am J Clin Pathol* 97: 669–677. [PubMed: 1575213]
180. Honavar J, Samal AA, Bradley KM, et al. 2011. Chlorine gas exposure causes systemic endothelial dysfunction by inhibiting endothelial nitric oxide synthase-dependent signaling. *Am J Respir Cell Mol Biol* 45: 419–425. [PubMed: 21131444]
181. Poole DP, Amadesi S, Veldhuis NA, et al. 2013. Protease-activated receptor 2 (PAR2) protein and transient receptor potential vanilloid 4 (TRPV4) protein coupling is required for sustained inflammatory signaling. *J Biol Chem* 288: 5790–5802. [PubMed: 23288842]
182. McGovern TK, Goldberger M, Allard B, et al. 2015. Neutrophils mediate airway hyperresponsiveness after chlorine-induced airway injury in the mouse. *Am J Respir Cell Mol Biol* 52: 513–522. [PubMed: 25192041]
183. Hickey MJ 2011. MPO and neutrophils: a magnetic attraction. *Blood*. 117: 1103–1104. [PubMed: 21273327]
184. Grommes J & Soehnlein O 2011. Contribution of neutrophils to acute lung injury. *Molecular medicine (Cambridge, Mass.)* 17: 293–307.
185. Aratani Y 2018. Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. *Arch Biochem Biophys* 640: 47–52. [PubMed: 29336940]
186. Bradshaw HB, Raboune S & Hollis JL 2013. Opportunistic activation of TRP receptors by endogenous lipids: exploiting lipidomics to understand TRP receptor cellular communication. *Life Sci* 92: 404–409. [PubMed: 23178153]
187. Saghatelian A, McKinney MK, Bandell M, et al. 2006. A FAAH-regulated class of N-acyl taurines that activates TRP ion channels. *Biochemistry*. 45: 9007–9015. [PubMed: 16866345]
188. Musah S, Chen J & Hoyle GW 2012. Repair of tracheal epithelium by basal cells after chlorine-induced injury. *Respir Res* 13: 107. [PubMed: 23170909]
189. Mo Y, Chen J, Humphrey DM Jr., et al. 2015. Abnormal epithelial structure and chronic lung inflammation after repair of chlorine-induced airway injury. *Am J Physiol Lung Cell Mol Physiol* 308: L168–178. [PubMed: 25398987]
190. Jonasson S, Koch B & Bucht A 2013. Inhalation of chlorine causes long-standing lung inflammation and airway hyperresponsiveness in a murine model of chemical-induced lung injury. *Toxicology*. 303C: 34–42.
191. O’Koren EG, Hogan BL & Gunn MD 2013. Loss of basal cells precedes bronchiolitis obliterans-like pathological changes in a murine model of chlorine gas inhalation. *Am J Respir Cell Mol Biol* 49: 788–797. [PubMed: 23742075]

192. Rahaman SO, Grove LM, Paruchuri S, et al. 2014. TRPV4 mediates myofibroblast differentiation and pulmonary fibrosis in mice. *J Clin Invest* 124: 5225–5238. [PubMed: 25365224]
193. Office HP 2017. HHS leverages potential respiratory drug as chemical weapon antidote. Accessed 05/04/2020, 2020. <https://www.phe.gov/Preparedness/news/Pages/gsk-chem.aspx>.
194. Accessed 05/04/2020, 2020. <https://govtribe.com/award/federal-idv-award/indefinite-delivery-contract-hhso100201700009c>.
195. Aktan AO 2013. Tear gas is a chemical weapon, and Turkey should not use it to torture civilians. *BMJ* 346: f3801. [PubMed: 23757759]
196. Arbak P, Baser I, Kumbasar OO, et al. 2014. Long term effects of tear gases on respiratory system: analysis of 93 cases. *TheScientificWorldJournal* 2014: 963638.
197. Park S & Giammona ST 1972. Toxic effects of tear gas on an infant following prolonged exposure. *Am J Dis Child* 123: 245–246. [PubMed: 4554617]
198. Hout JJ, White DW, Stevens M, et al. 2014. Evaluation of an Intervention to Reduce Tear Gas Exposures and Associated Acute Respiratory Illnesses in a US Army Basic Combat Training Cohort. *Open Epidemiology Journal*. 7: 34–45.
199. Steffee CH, Lantz PE, Flannagan LM, et al. 1995. Oleoresin capsicum (pepper) spray and “in-custody deaths”. *Am J Forensic Med Pathol* 16: 185–192. [PubMed: 7495257]
200. Bessac BF, Sivula M, von Hehn CA, et al. 2009. Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases. *FASEB J* 23: 1102–1114. [PubMed: 19036859]
201. Lindsay CD, Green C, Bird M, et al. 2015. Potency of irritation by benzylidenemalononitriles in humans correlates with TRPA1 ion channel activation. *Royal Society open science*. 2: 140160. [PubMed: 26064575]
202. Karaman E, Erturan S, Duman C, et al. 2009. Acute laryngeal and bronchial obstruction after CS (o-chlorobenzylidenemalononitrile) gas inhalation. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies*. 266: 301–304.
203. Karagama YG, Newton JR & Newbegin CJ 2003. Short-term and long-term physical effects of exposure to CS spray. *Journal of the Royal Society of Medicine*. 96: 172–174. [PubMed: 12668703]
204. Deering-Rice CE, Shapiro D, Romero EG, et al. 2015. Activation of Transient Receptor Potential Ankyrin-1 by Insoluble Particulate Material and Association with Asthma. *Am J Respir Cell Mol Biol* 53: 893–901. [PubMed: 26039217]
205. Morris JB, Stanek J & Gianutsos G 1999. Sensory nerve-mediated immediate nasal responses to inspired acrolein. *Journal of applied physiology*. 87: 1877–1886. [PubMed: 10562632]
206. Kurhanewicz N, McIntosh-Kastrinsky R, Tong H, et al. 2017. TRPA1 mediates changes in heart rate variability and cardiac mechanical function in mice exposed to acrolein. *Toxicol Appl Pharmacol* 324: 51–60. [PubMed: 27746315]
207. Escalera J, von Hehn CA, Bessac BF, et al. 2008. TRPA1 mediates the noxious effects of natural sesquiterpene deterrents. *J Biol Chem* 283: 24136–24144. [PubMed: 18550530]
208. Andre E, Campi B, Materazzi S, et al. 2008. Cigarette smoke-induced neurogenic inflammation is mediated by alpha,beta-unsaturated aldehydes and the TRPA1 receptor in rodents. *J Clin Invest* 118: 2574–2582. [PubMed: 18568077]
209. Mehta PS, Mehta AS, Mehta SJ, et al. 1990. Bhopal tragedy’s health effects. A review of methyl isocyanate toxicity. *JAMA* 264: 2781–2787. [PubMed: 2232065]
210. Cain WS, Dourson ML, Kohrman-Vincent MJ, et al. 2010. Human chemosensory perception of methyl isothiocyanate: chemesthesis and odor. *Regul Toxicol Pharmacol* 58: 173–180. [PubMed: 20633589]
211. Taylor-Clark TE, Kiros F, Carr MJ, et al. 2009. Transient receptor potential ankyrin 1 mediates toluene diisocyanate-evoked respiratory irritation. *Am J Respir Cell Mol Biol* 40: 756–762. [PubMed: 19059884]
212. 1899. Phosgene Gas. *Hospital (Lond)* 1886. 26: 227.
213. Holmes WW, Keyser BM, Paradiso DC, et al. 2016. Conceptual approaches for treatment of phosgene inhalation-induced lung injury. *Toxicol Lett* 244: 8–20. [PubMed: 26562770]

214. Andres D, Keyser B, Benton B, et al. 2015. Transient receptor potential (TRP) channels as a therapeutic target for intervention of respiratory effects and lethality from phosgene. *Toxicol Lett*
215. (2009) Acid spill forces hundreds from Denver hospital, s <http://edition.cnn.com/2009/US/03/09/hospital.hazmat/index.html?iref=allsearch>.
216. Holzer P 2009. Acid-sensitive ion channels and receptors. *Handb Exp Pharmacol* 283–332. [PubMed: 19655111]
217. Buckley LA, Jiang XZ, James RA, et al. 1984. Respiratory tract lesions induced by sensory irritants at the RD50 concentration. *Toxicol Appl Pharmacol* 74: 417–429. [PubMed: 6740688]
218. Trevisani M, Patacchini R, Nicoletti P, et al. 2005. Hydrogen sulfide causes vanilloid receptor 1-mediated neurogenic inflammation in the airways. *Br J Pharmacol* 145: 1123–1131. [PubMed: 15937520]
219. Ang SF, Mochhala SM & Bhatia M 2010. Hydrogen sulfide promotes transient receptor potential vanilloid 1-mediated neurogenic inflammation in polymicrobial sepsis. *Critical care medicine*. 38: 619–628. [PubMed: 19851090]
220. Takahashi K & Ohta T 2013. Inflammatory acidic pH enhances hydrogen sulfide-induced transient receptor potential ankyrin 1 activation in RIN-14B cells. *J Neurosci Res* 91: 1322–1327. [PubMed: 23873754]
221. Ogawa H, Takahashi K, Miura S, et al. 2012. H₂S functions as a nociceptive messenger through transient receptor potential ankyrin 1 (TRPA1) activation. *Neuroscience*. 218: 335–343. [PubMed: 22641084]
222. Poursaleh Z, Harandi AA, Vahedi E, et al. 2012. Treatment for sulfur mustard lung injuries; new therapeutic approaches from acute to chronic phase. *Daru* 20: 27. [PubMed: 23351279]
223. Stenger B, Zehfuss F, Muckter H, et al. 2015. Activation of the chemosensing transient receptor potential channel A1 (TRPA1) by alkylating agents. *Arch Toxicol* 89: 1631–1643. [PubMed: 25395009]
224. Stenger B, Popp T, John H, et al. 2017. N-Acetyl-L-cysteine inhibits sulfur mustard-induced and TRPA1-dependent calcium influx. *Arch Toxicol* 91: 2179–2189. [PubMed: 27738742]
225. Dhaka A, Uzzell V, Dubin AE, et al. 2009. TRPV1 is activated by both acidic and basic pH. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 29: 153–158. [PubMed: 19129393]
226. 21CFR314.600. <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=314&showFR=1&subpartNode=21:5.0.1.1.4.9>.
227. Grace MS, Dubuis E, Birrell MA, et al. 2012. TRP channel antagonists as potential antitussives. *Lung* 190: 11–15. [PubMed: 21964632]
228. McGaraughty S, Chu KL, Perner RJ, et al. 2010. TRPA1 modulation of spontaneous and mechanically evoked firing of spinal neurons in uninjured, osteoarthritic, and inflamed rats. *Molecular pain*. 6: 14. [PubMed: 20205719]
229. Schenkel LB, Olivieri PR, Boezio AA, et al. 2016. Optimization of a Novel Quinazolinone-Based Series of Transient Receptor Potential A1 (TRPA1) Antagonists Demonstrating Potent in Vivo Activity. *J Med Chem* 59: 2794–2809. [PubMed: 26942860]
230. Lehto SG, Weyer AD, Youngblood BD, et al. 2016. Selective antagonism of TRPA1 produces limited efficacy in models of inflammatory- and neuropathic-induced mechanical hypersensitivity in rats. *Molecular pain*. 12.
231. Bevan S, Hothi S, Hughes G, et al. 1992. Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. *Br J Pharmacol* 107: 544–552. [PubMed: 1422598]
232. Surowy CS, Neelands TR, Bianchi BR, et al. 2008. (R)-(5-tert-butyl-2,3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)-urea (ABT-102) blocks polymodal activation of transient receptor potential vanilloid 1 receptors in vitro and heat-evoked firing of spinal dorsal horn neurons in vivo. *J Pharmacol Exp Ther* 326: 879–888. [PubMed: 18515644]
233. CID=56603682, P.D. 2012. “<https://pubchem.ncbi.nlm.nih.gov/compound/56603682> “. In. National Center for Biotechnology Information.
234. Quiding H, Jonzon B, Svensson O, et al. 2013. TRPV1 antagonistic analgesic effect: a randomized study of AZD1386 in pain after third molar extraction. *Pain* 154: 808–812. [PubMed: 23541425]

235. Krarup AL, Ny L, Astrand M, et al. 2011. Randomised clinical trial: the efficacy of a transient receptor potential vanilloid 1 antagonist AZD1386 in human oesophageal pain. *Aliment Pharmacol Ther* 33: 1113–1122. [PubMed: 21410733]
236. Kitagawa Y, Miyai A, Usui K, et al. 2012. Pharmacological characterization of (3S)-3-(hydroxymethyl)-4-(5-methylpyridin-2-yl)-N-[6-(2,2,2-trifluoroethoxy)pyridin-3-yl]-3,4-dihydro-2H-benzo[b][1,4]oxazine-8-carboxamide (JTS-653), a novel transient receptor potential vanilloid 1 antagonist. *J Pharmacol Exp Ther* 342: 520–528. [PubMed: 22588258]
237. Manitisitkul P, Flores CM, Moyer JA, et al. 2018. A multiple-dose double-blind randomized study to evaluate the safety, pharmacokinetics, pharmacodynamics and analgesic efficacy of the TRPV1 antagonist JNJ-39439335 (mavarep). *Scand J Pain* 18: 151–164. [PubMed: 29794306]
238. Parsons WH, Calvo RR, Cheung W, et al. 2015. Benzo[d]imidazole Transient Receptor Potential Vanilloid 1 Antagonists for the Treatment of Pain: Discovery of trans-2-(2-{2-[2-(4-Trifluoromethyl-phenyl)-vinyl]-1H-benzimidazol-5-yl}-phenyl)-propan-2-ol (Mavarep). *J Med Chem* 58: 3859–3874. [PubMed: 25850459]
239. Vincent F, Acevedo A, Nguyen MT, et al. 2009. Identification and characterization of novel TRPV4 modulators. *Biochem Biophys Res Commun* 389: 490–494. [PubMed: 19737537]
240. Nilius B, Vriens J, Prenen J, et al. 2004. TRPV4 calcium entry channel: a paradigm for gating diversity. *American journal of physiology. Cell physiology*. 286: C195–205. [PubMed: 14707014]
241. Everaerts W, Zhen X, Ghosh D, et al. 2010. Inhibition of the cation channel TRPV4 improves bladder function in mice and rats with cyclophosphamide-induced cystitis. *Proc Natl Acad Sci U S A* 107: 19084–19089. [PubMed: 20956320]
242. Cheung M, Bao W, Behm DJ, et al. 2017. Discovery of GSK2193874: An Orally Active, Potent, and Selective Blocker of Transient Receptor Potential Vanilloid 4. *ACS Med Chem Lett* 8: 549–554. [PubMed: 28523109]
243. Brnardic EJ, Ye G, Brooks C, et al. 2018. Discovery of Pyrrolidine Sulfonamides as Selective and Orally Bioavailable Antagonists of Transient Receptor Potential Vanilloid-4 (TRPV4). *J Med Chem* 61: 9738–9755. [PubMed: 30335378]
244. Pero JE, Matthews JM, Behm DJ, et al. 2018. Design and Optimization of Sulfone Pyrrolidine Sulfonamide Antagonists of Transient Receptor Potential Vanilloid-4 with in Vivo Activity in a Pulmonary Edema Model. *J Med Chem* 61: 11209–11220. [PubMed: 30500190]
245. Brooks CA, Barton LS, Behm DJ, et al. 2019. Discovery of GSK3527497: A Candidate for the Inhibition of Transient Receptor Potential Vanilloid-4 (TRPV4). *J Med Chem* 62: 9270–9280. [PubMed: 31532662]

Figure 1. Transient receptor potential ankyrin repeat 1 ion channel (TRPA1).

(A) TRPA1 consists of 6 membrane-spanning subunits (S1–S6) that assemble as a tetramer to form cation-permeable pores, with a putative pore-forming helix between S5 and S6. Both the N- and C-termini are in the cytosol. The N-terminus has 16 ankyrin repeats in human TRPA1, whereas other species have a varying number. The C-terminus has a unique Zn²⁺ binding site that is important for permeating the TRPA1 pore. (B) Localization of TRPA1 ion channels in the respiratory system. The upper respiratory tract (nasal cavity, pharynx, and larynx) and lower respiratory tract (trachea, bronchi, and lungs) are heavily innervated by primary afferent C-fibers and A δ -fibers from the trigeminal ganglia (TG), jugular and nodose ganglia (JNG), vagus nerve (VG), and dorsal root ganglia (DRG). TRPA1 is expressed in sensory afferents that have cell bodies in the TG, JNG, VG, and DRG. TRPA1 is also believed to be expressed on non-neuronal cells such as airway epithelia, endothelium, fibroblasts, and mesenchymal cells. (C) The agonists of TRPA1. AITC, allyl isothiocyanate; MITC, methyl isothiocyanate; 4-HNE, 4-hydroxy-2-nonenal; ROS, reactive oxygen species; RNS; reactive nitrogen species; H₂O₂, hydrogen peroxide; PAMPs, pathogen-associated molecular patterns; DAMPs, danger-associated molecular patterns.

Figure 2. Transient receptor potential vanilloid 1 ion channel (TRPV1).

(A) General structure of TRPV1 consists of 6 membrane-spanning subunits (S1–S6) that assemble as a tetramer to form cation-permeable pores, with a putative pore-forming helix between S5 and S6. Both the N- and C-termini are in the cytosol. The N-terminus has a pre-S1 helix, 6 ankyrin repeats, a calmodulin-binding site (CaM), and a linker domain. The C-terminus has a TRP box and a calmodulin-binding site. Critical residues involved in the binding of various ligands, calmodulin, phosphatidylinositol 4,5-bisphosphate (PIP₂), adenosine triphosphate (ATP), and phosphorylation are also shown. (B) Localization of TRPV1 ion channels in the pulmonary system. TRPV1 ion channels are expressed on sensory nerve endings of trigeminal ganglia (TG), vagus nerve (VG), jugular-nodose ganglia (JNG), and dorsal root ganglia (DRG). (C) Representative endogenous and exogenous agonists of TRPV1 ion channels.

Figure 3. Transient Receptor Potential Vanilloid 4 ion channel (TRPV4).

(A) TRPV4 consists of 6 membrane-spanning subunits (S1–S6) that assemble as a tetramer to form cation-permeable pores, with a putative pore-forming helix between S5 and S6. Both N- and C-termini are in the cytosol. The N-terminus has 6 ankyrin repeats and a proline-rich domain (PRD). The C-terminus has a TRP box, a calmodulin-binding site (CaM), and a PDZ (PSD95/SAP90-Discs-large-Zonula-occludentes-1) domain. (B) Localization of TRPV4 ion channels in the pulmonary system. TRPV4 ion channels are present on sensory nerve endings of trigeminal ganglia (TG), vagus nerve (VG), and dorsal root ganglia (DRG); immune cells such as T-cells, neutrophils, and alveolar macrophages; airway epithelia; vascular endothelium; airway and vascular smooth muscles; and alveolar–capillary barrier system. (C) Representative agonists of TRPV4 ion channels. Human TRPV4 EC₅₀ values are indicated in parentheses for the following agonists: 4α-PDD (0.37 μM); 5,6-epoxyeicosatrienoic acid (0.15 μM); RN-1747 (0.77 μM); GSK1016790A (5 nM).

TRPV4 modulates alveolar-capillary barrier

Figure 4. TRPV4 modulates the alveolar–capillary barrier.

Upon exposure to TIHs, TRPV4 ion channels on epithelia, endothelia, macrophages, neutrophils, and A δ sensory nerve endings are activated. Activation of TRPV4 leads to acute lung injury through a series of events. TRPV4-mediated Ca^{2+} influx into endothelial cells and the release of proinflammatory neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), result in contraction of endothelial cells, vasodilation, transmigration of granulocytes, and protein and fluid leakage into the alveolus. Activated neutrophils and macrophages contribute to the release of reactive oxygen and nitrogen species, which exacerbates production of proinflammatory cytokines and chemokines. The proteases released from the degranulating neutrophils cause necrosis of epithelial cells. Accumulation of protein-rich fibrinous edematous fluid hampers oxygen gas exchange across the alveolar–capillary barrier. All of these events contribute to the loss of integrity of the alveolar–capillary barrier and the classic cardinal signs of acute lung injury.

TRP ion channels mediate chemical threat agents-induced pulmonary injuries

Figure 5. Role of TRPs in respiratory physiology and pathophysiology of pulmonary chemical threat agents.

Exposure to chemical threat agents leads to acute lung injury (ALI) mediated by TRP channels in a complex cascade of inflammatory pathways. Coughing and sneezing, the first mechanisms of defense, are triggered by activation of TRPA1 and TRPV1 channels in trigeminal (TG) and vagal sensory nerve endings. Activated goblet cells produce mucin. Mucociliary clearance is hindered by activation of TRPV4 channels in the bronchial system. ALI is a result of the massive influx of proinflammatory cells, overexpression of proinflammatory mediators, and formation of alveolar exudate due to failure of the alveolar-capillary barrier. Proinflammatory cells migrate from the blood vessels into the alveolar space and secrete interleukins. Activation of TRP channels in lung capillary endothelial cells leads to increased vascular permeability and in turn, promotes protein and fluid leakage. Activation of TRP channels in proinflammatory cells leads to the generation of reactive oxygen species. In a chronic situation, growth factors are secreted, inducing repair and remodeling of injured tissue through myofibroblast generation.

Table 1.

Representative small molecule TRPA1 antagonists

TRPA1 antagonists						
Name	Structure	Activity		IC ₅₀ values	Remarks	Refs.
		Species	Selectivity			
HC-030031 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl)acetamide		Mouse, rat, human	Selective	6.2 μM (human); 7.6 μM (rat)	Xanthine derivative; developed by Hydra Biosciences	97
API8 4-(4-chlorophenyl)-3-methyl-3-buten-2-one oxime		Mouse, rat, human	Selective	3.1 μM (human); 8.8 μM (rat); 4.5 μM (mouse)	Reversible TRPA1 inhibitor; developed by Novartis	80
Chembridge-5861528 N-(4-butan-2-ylphenyl)-2-(1,3-dimethyl-2,6-dioxopurin-7-yl)acetamide		Rat, human	Selective	4.9 μM (human); 0.3 μM (rat)	Xanthine derivative; developed by Orion Pharma	98
A-967079 (1 <i>E</i> :3 <i>E</i>)-1-(4-fluorophenyl)-2-methyl-1-pentene-3-one oxime		Mouse, rat, human	Selective	0.067 μM (human); 0.289 μM (rat)	Oxime derivative; developed by Abbott	228
GRC17536	Structure/CAS number was not disclosed	Human, guinea pigs	Selective	<10 nM (human)	Completed Phase II clinical trials; developed by Glenmark Pharmaceuticals.	67, 82
AMG-0902		Rat, human	Selective	0.071 μM (rat); 0.131 μM (human)	Developed by Amgen.	229, 230
B101305834	Structure not yet disclosed	Guinea pig, human	Selective	40 nM (guinea pig and human)	Developed by Boehringer Ingelheim	87

Table 2.

Representative small molecule TRPV1 antagonists

TRPV1 antagonists						
Name	Structure	Activity		IC ₅₀ values	Remarks	Refs.
		Species	Selectivity			
Capsazepine N-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2H-2-benzazepine-2-carbothioamide		Rat, human	Selective	420–562 nM (rat) 58 nM (human)	Developed by Novartis	231
SB-705498 N-(2-bromophenyl)-N'-[(3R)-1-[5-(trifluoromethyl)-2-pyridinyl]-3-pyrrolidinyl]-urea		mouse, rat, human	Selective	0.9 nM (rat); 3–6 nM (human)	The first TRPV1 antagonist tested in humans; developed by GlaxoSmithKline	124
AMG-517 N-[4-[6-[4-(trifluoromethyl)phenyl]-4-pyrimidinyl]oxy]-2-benzothiazolyl]-acetamide		Mouse, rat, dog, monkey, and human	Selective	0.5 nM (rat); 0.9 nM (human)	Developed by Amgen; completed clinical trials; hyperthermia was one of the side effects.	123
ABT-102 (R)-1-(5-tert-butyl-2,3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)urea		Rat, human	Selective	1–16 nM (rat); 5–7 nM (human)	Developed by AbbVie; completed clinical trials	232
MK-2295/NGD8243 N-[4-(trifluoromethyl)phenyl]-7-[4-(trifluoromethyl)pyridin-3-yl]quinazolin-4-amine		Mouse, rat, human	Selective	0.3–6 nM (human)	Developed by Merck/Neurogen	233
AZD-1386 (S)-N-(1-(4-(tert-butyl)phenyl)ethyl)-2-(6,7-difluoro-1H-benzodimidazol-1-yl)acetamide		Human	Selective	25 nM	Developed by AstraZeneca; completed multiple clinical trials with no adverse events.	234, 235

TRPV1 antagonists						
Name	Structure	Activity		IC ₅₀ values	Remarks	Refs.
		Species	Selectivity			
JTS-653 (3S)-3-(4-hydroxymethyl)-4-(5-methylpyridin-2-yl)-N-[6-(2,2,2-trifluoroethoxy)pyridin-3-yl]-3,4-dihydro-2H-benzo[b][1,4]oxazine-8-carboxamide		Mouse, rat, human	Selective	0.347 nM (rat); 0.32 nM (human)	Orally active; developed by Japan Tobacco	236
Mavatrep (JNJ-39439335) trans-2-(2-{2-[2-(4-trifluoromethyl-phenyl)-vinyl]-1H-benzimidazol-5-yl}xx-phenyl)-propan-2-ol		Human	Selective	4.6 nM (human)	Developed by Janssen Research and Development, LLC	237, 238
GRC-6211 (R)-1-(6-fluorospiro[chromane-2,1'-cyclobutan]-4-yl)-3-(isoquinolin-5-yl)urea		Human	Selective	3.8 nM	Developed by Lilly/Glenmark	

Table 3.

Representative small molecule TRPV4 antagonists

Name	Structure	Activity			Remarks	Refs.
		Species	Selectivity	Human TRPV4 IC ₅₀ value		
Ruthenium red		Rat, human, porcine	Non-selective	14 nM	A non-specific metal-derived Ca ²⁺ ion channel blocker; known to act as a pore blocker of TRPV4 and 12 other ion channels	239, 240
HC-067047 (2-methyl-1-[3-(4-morpholinyl)propyl]-5-phenyl- <i>N</i> -[3-(trifluoromethyl)phenyl]-1 <i>H</i> -pyrrole-3-carboxamide)		Rat, mouse	Selective (?)	48 nM	Developed by Hydra Biosciences	239, 241
RN-1734 (2,4-dichloro- <i>N</i> -isopropyl- <i>N</i> -(2-isopropylaminoethyl)benzene sulfonamide)		Human, rat, mouse	Selective	2.3 μM	Developed by Renovis, Inc.	239
GSK2193874 (3-(1,4'-bipiperidin-1'-ylmethyl)-7-bromo- <i>N</i> -(1-phenyl(cyclopropyl)-2-[3-(trifluoromethyl)phenyl]-4-quinolinecarboxamide)		Human, mouse, rat, canine	Selective	40 nM	Quinoline antagonist; orally bioavailable; efficacy was demonstrated in mitigating hydrochloric acid-induced acute lung injury; developed by GlaxoSmithKline (GSK)	132, 144, 242
GSK2220691		Human, rat, mouse, dog, monkey	Selective	2.5 nM	Ameliorated Cl ₂ - and hydrochloric acid-induced ALI in rodent models; developed by GSK	21
GSK2337429A		Human, rat, mouse, dog, monkey	Selective	6.3 nM	Ameliorated chlorine gas- and hydrochloric acid-induced ALI in rodent models; developed by GSK	21

Name	Structure	Activity			Remarks	Refs.
		Species	Selectivity	Human TRPV4 IC ₅₀ value		
GSK2798745 (1-(((5S,7S)-3-(5-(2-hydroxypropan-2-yl)pyrazin-2-yl)-7-methyl-2-oxo-1-oxa-3-azaspiro[4.5]decan-7-yl)methyl)-1Hbenzo[d]imidazole-6-carbonitrile)		Human, rat, mouse, dog, monkey	Selective	1.8 nM	Spirocarbamate antagonist of TRPV4; first TRPV4 antagonist to reach clinical trials; Currently under BARDA-funded contract to develop as a medical countermeasure against Cl ₂ -induced ARDS; developed by GSK	145–148, 150, 151, 193
GSK3395879 (4-(((3S,4R)-1-(2-cyano-4-(trifluoromethyl)phenyl)sulfonyl)-4-hydroxy-4-(hydroxymethyl)pyrrolidin-3-yl)oxy)-2-fluorobenzonitrile)		Human, rat	Selective	1 nM	Pyrrolidine sulfonamide derivative; reduced pulmonary edema in a rat model; exhibited good oral bioavailability; developed by GSK	243
GSK3491943		Human, rat	Selective	3.2 nM	Pyrrolidinesulfonamide derivative; developed by GSK	244
GSK3527497		Human, rat	Selective	12 nM	Currently under BARDA-funded contract to develop as a medical countermeasure against Cl ₂ -induced ARDS; developed by GSK	193, 245
GSK2263095		Human, mouse, rat, canine	Selective	3 nM	Analog of GSK2193874; developed by GSK	132