Connexin therapeutics: blocking connexin hemichannel pores is distinct from blocking pannexin channels or gap junctions

Introduction
Connexin proteins form cell-to-cell gap junction channels for intercellular communication and signaling, but also cytoplasmic membrane pores called hemichannels, with both channel types emerging as key therapeutic targets for diverse diseases including cancer, retinopathies, neurodegenerative diseases and skin wounds (Vicario et al., 2017; Mugisho et al., 2019). Connexin protein channels contribute to the maintenance of cell integrity, and the coordination in space and time of vital communication signals. Pannexin proteins also form membrane channels of biological importance, and although generally agreed that they do not form cell-to-cell channels, recent reports show pannexins may in some circumstances form intercellular channels. Targeting pannexin and connexin protein channels and blocking the function of the pores they form minimizes the signaling burden of the biological process in which they participate. However, there are distinct differences between connexin hemichannel pores and gap junction channels, especially with regard to the action and roles of connexin and pannexin channels with important long-term consequences when blocking any one of these three distinct channel types. For example, both pannexins and connexins form ATP-release channels for paracrine signaling but pannexin channels are vital for cell homeostasis and long-term blockage of these pores causes dysregulated inflammation (Chen et al., 2019). On the contrary, connexin pores are transiently active in response to injury and are quite distinct from pannexin or gap junction channels through being formed and opening under human relevant pathological conditions (Bernstein and Fishman, 2016). There is vast literature of the therapeutic benefit of blocking hemichannels in pathological conditions and in this review, we address some of the more important features of pannexin and connexin channels that assist interpretation of the true mode of action of current anti-connexin therapeutics.

A systematic review of articles published from January 2000 through December 2019 was conducted using terms such as gap junction, connexin, pannexin, tonabersat, probenecid using the PubMed database. A few selected seminal publications expanding 1981–1998 were used to substantiate key statements. The final version of the review includes citations recommended by reviewers. Many important recent as well as seminal articles were not included in this review but have been cited by Leybaert et al. (2017).

An Overview of Connexins
The amino acid sequence of the connexin protein has biological implications for therapeutic treatments, and it is pertinent to review its basic structure first. Each connexin has four membrane spanning domains, two extracellular loops, one intracellular loop and cytoplasmic carboxyl (C) and amino (N) termini. There are 21 connexin isotypes known, named according to their molecular weight as predicted from their primary amino acid sequence as proposed by Beyer et al. (1987). This diversity of connexins is determined by the different amino acid sequences primarily of the cytoplasmic loop and the C terminus (Laird, 2006). For a review of the different connexins in human and animal models in health and disease, see recent publications (Danesh-Meyer et al., 2016; Delvaeye et al., 2018; Laird and Lampe, 2018).

Six connexin proteins come together in the endoplasmic...
reticulum to form a connexon or hemichannel. The connexons are transported to the plasma membrane and migrate around the membrane until they meet a connexon on a neighbouring cell whereby the extracellular loops dock to form a gap junction channel. At this point the channel opens and the two cells can communicate. The connexon or hemichannel prior to docking with a connexon from a neighbouring cell needs to remain primarily closed or a large, non-specific membrane pore is formed (this is discussed further later in the review).

It has been noted that one of the attributes of therapeutic intervention is that the connexin protein half-life is short, demonstrated mainly in the heart and the liver (Fallon and Goodenough, 1981; Laird et al., 1991; Beardslee et al., 1998). There are functionally associated proteins that regulate the connexin half-life within a few hours of being formed by contributing to their internalization and degradation (Laird et al., 1991). Further regulating the life cycle of connexins, as shown for example for Connexin43 (Cx43), is therapeutic, but, as discussed by Laird (2006), it is possible that these interactions are not affecting the protein unit itself but could be regulating the gating properties of the channels.

Pannexins Form Membrane-Based Hemichannels

Pannexins were more recently discovered to form unopposed channels structurally similar to connexin hemichannels but different from them in many ways. At least one of the three known proteins, Pannexin1 (Panx1), Pannexin2 (Panx2) and Pannexin3 (Panx3), has been found in every organ in mammals (Bennett et al., 2012). In the retina, their location in neurons suggests a role in normal metabolic and electrical cell function (Penuela et al., 2014b). While Panx1 is important during the early stages of development, it needs to be down-regulated in mature tissues to avoid negative effects of its expression on cell function (Penuela et al., 2014b).

One of the main functions ascribed to pannexins which also suggests these channels as a therapeutic target, is their role in ATP release, leading to the proposal that connexin hemichannels and pannexin channels may have similar functions. However, more recent evidence indicates that this may not be the case (Figure 1). One major difference from connexin hemichannels is that Panx1 hemichannel half-life at the cell surface is long and when removed from the plasma membrane it is internalized by a classical membrane protein recycling pathway (Gehi et al., 2011). The mechanism for closing and opening the channel is unclear; however, cysteine and tyrosine residues in the intracellular loop play a major role in Panx1 function (Begandt et al., 2017), suggesting that there is an established mechanism for its removal.

Both types of channels release ATP but there is a strong suggestion that Panx1 ATP release activates purinergic receptors as a physiological pathway for cellular communication (Lohman and Isakson, 2014), rather than in the pathological conditions that connexin hemichannels do (Figure 1). Pannexin channels seem to release ATP under physiological conditions in response to activation of purinergic receptors, bradykinin B2 receptors, histamine receptors and N-methyl-D-aspartate receptors among others (Lohman and Isakson, 2014). The function of these channels seems to be tightly regulated to allow them to act in connection with other cellular pathways (Esseltine and Laird, 2016). For example, pannexins release ATP but are also closed by extracellular ATP and are therefore self-regulating. The physiological role of pannexins is supported by experiments showing that Panx1 channels are active at physiological Ca2+ levels (Barbe et al., 2006). The experimental evidence implicates the role of these channels in intracellular Ca2+ flux, as they were found in the plasma membrane and the endoplasmic reticulum, thus contributing to sustained modulation of Ca2+ (Vanden Abeele et al., 2006; Thompson et al., 2008).

As for connexin channels, pannexin mutations have been linked to human disease including oocyte death and system dysfunction (hearing loss, skeletal defects and primary ovarian failure) (Shao et al., 2016; Sang et al., 2019), and pannexin channels open in response to experimental mechanical stress or ischemia suggesting a possible role in pathology. Penuela et al. (2014a) has reviewed the evidence for contrasting roles of pannexins in diseases, showing that their activation leads to cell death in experimental brain ischemia/stroke. In their review, they note that spinal astrocytes and neurons in culture release ATP via Cx43 hemichannels for the activation of Panx1, triggering cell death in experimental neurotoxic conditions (Orellana et al., 2010, 2011; Bennett et al., 2012). Nevertheless, there is also a suggestion that the mechanism of ATP release is the other way around. Kim et al. (2018) showed that about one-third of ATP release was from pannexins during ischemia (and two-thirds from connexin hemichannels), but the entire ATP was released from connexin hemichannels upon reperfusion. This, together with the knowledge that pannexin channels are located close to purinergic receptors suggest a key role for pannexin channels in ATP release. Opening Panx1 channels releases ATP leading to autocrine activation of P2X7 receptors (Alberto et al., 2013). P2X7 then activates the NOD-like receptor pyrin domain-containing protein 3 inflammasome and enhances the inflammation effect. This activation can be either harmful or helpful depending on the degree of severity and length of exposure to insult (Silverman et al., 2009). The relative role of each channel type and the interrelationship between them are yet to be fully determined. However, since pannexin channels are also regulated by extracellular ATP, and it is likely that whilst pannexin release of ATP may trigger inflammation, it is perpetuated in chronic disease by connexin hemichannels (Mugisho et al., 2019); and discussed further in the final section of this review. This means that activation of the inflammasome (Figure 1) leads to further induction of cytokine release; in turn opening hemichannels, increasing Cx43 expression and resulting in more ATP release. The effect crosses to other cells, as it has been shown that
Connexins Form Membrane Channels – The Physiological Roles of Opening the Gap, but the Therapeutic Target of Closing the Pathologic Pore

Connexin protein channels serve many roles in our body from cell proliferation and patterning to electrical coupling and in organ functions such as coordinated heart muscle contraction; however, they also form pathologic membrane pores in injury and disease. The specific location of the channels in neurons, glia and endothelial cells highlights their role in normal physiological functions and in pathologic conditions may impact upon neurodegenerative diseases (Orellana et al., 2016). Connexins contribute to two distinct structures at the plasma membrane: gap junctions and connexin hemichannels (connexons). The connexon is closed until docking with another connexon. Once they dock, these double membrane channels tightly couple the cytoplasm of connecting cells and allow for coordinated cellular metabolism and signaling functions. Gap junctions have transient communication between cells. Once docked they stay open but they do have a short half-life, which may be associated with their quick response to physiological requirements resulting in up- or down-regulation of cell-to-cell coupling.

From a few to several hundred gap junction channels in close proximity to each other, linking adjacent cells, have been seen in electron microscopy and negative stain studies with this arrangement known as gap junction plaques (Kumar and Gilula, 1996). Within the plaque, there is a highly coordinated removal of older channels from the centre and continual accrual of new gap junctions to the periphery of the plaque arrangement (Gaietta et al., 2002). The turnover process involves intracellular recycling via lysosome or proteolytic pathways. As each gap junction channel allows passive diffusion of molecules up to 1000 Da (Evans and Martin, 2002), including nutrients, metabolites, second messengers, cAMP, Ca++, adenosine, ATP and ADP (Goldberg et al., 2004), the plaque is a hot spot for intracellular exchanges (Decrock et al., 2009; Danesh-Meyer et al., 2016). Plaques show different properties to the dynamics of single, isolated gap junction channels.

Conductance through connexin channels is different for each connexin isotype, with dye transfer experiments showing different permeability depending also upon the perfused molecule charge, size and shape (Kanaporis et al., 2008). A gap junction also allows electrical coupling, with dual patch clamp experiments showing synchronized and rapid responses of the connecting cells. The active process of communication can be regulated by fast acting mechanisms, such as protein phosphorylation. Phosphorylation alters the gating activity of connexins by changing the surface charge of the pore lining as well as the pore size, as shown for the regulation of Cx43 (Lampe and Lau, 2000). The passage of ions can also be regulated by an increase in extracellular alkalization that increases intracellular Ca++ resulting in an increased gating mechanism (Bennett et al., 1991). In some organs, such as the heart, cell depolarization is fundamentally linked to the electrical communication function of gap junctions. Heart contraction employs differential expression of connexin isoatypes depending upon specific functional requirements. The working myocardial cells, for example, are linked by Cx43 channels, but the fast conducting pathway utilizes larger Cx40 channels and the atrioventricular node has lower conductance Cx45 channels to induce a slight delay between atrial and ventricular contraction. In another example, Goligier and Paul (1994) identified that an absence of intercellular gap junctions may stimulate a cancerous phenotype in cells.

Much of the early literature, however, has attributed roles to gap junctions that are now being revised in light of the increasing understanding of connexin hemichannels. For example, tissue inflammation roles were often extrapolated from migration and contraction, or wound healing studies. While in pathological conditions where gap junctions were thought to be responsible for cellular alterations in inflammation, it is now understood that inflammation is more likely mediated by the opening of unopposed Cx43 hemichannels (Bennett et al., 2012; Davidson et al., 2014; Willebrods et al., 2016; Kim et al., 2017). The connexin hemichannel appears to be a fundamental channel forming a pathologic membrane pore in injury and disease, proving a prime therapeutic target, especially for chronic disease conditions (Orellana et al., 2016; Laird and Lampe, 2018). This will be discussed more fully in the following sections.

Connexins Form Hemichannels That Are Therapeutic Targets

A hemichannel is a connexon that remains uncoupled. The open or closed status of hemichannels depends on the requirements of cells, such as the activity of Ca++ ion channels and glutamate receptors in protecting against apoptosis. There is evidence that within the nervous system, the opening of glial cell hemichannels under physiological conditions may be crucial for some biological processes and it is important
to keep this possibility in mind when targeting connexin hemichannels therapeutically (Huckstepp et al., 2010; Orellana et al., 2012a; Chever et al., 2014; Meunier et al., 2017). However, the extent to which brain slices with associated lesion spread and neuronal death, or in vitro models, may reflect normal physiological conditions is uncertain. In clinical trials, tonabersat, now known to be a hemichannel blocker, showed no adverse neuronal effects in over 1000 patients with once daily dosing for up to 12 weeks (Bialer et al., 2009).

In normal physiological conditions, hemichannels would seem to remain primarily closed (Contreras et al., 2002; Giaume et al., 2013) and Cx43 channels only open at a very low rate under physiological conditions (Contreras et al., 2003; Decrock et al., 2009). However, the undocked hemichannel may take on a prolonged or more frequently open state following an insult, whether it be mechanical stress, ischemia including deprivation of oxygen or glucose, metabolic inhibition, inflammation, ethanol or high extracellular Ca2+ (Contreras et al., 2003; Goodenough and Paul, 2003; Decrock et al., 2009; Froger et al., 2010; Guo et al., 2014; Gomez et al., 2018; Kim et al., 2018; Saez et al., 2018). When open in an undocked state, these hemichannels provide a pathway for the release of paracrine and autocrine signals in a relatively uncontrolled manner. Uncontrolled activation of hemichannels disrupts tissue homeostasis through the passage of molecules and ions between the cell cytoplasm and the extracellular milieu, and by preventing docking of hemichannels and so later also affecting gap junction communication in certain cell types (Abudara et al., 2014; Orellana et al., 2016).

Whilst some studies have indicated that injury, such as that causing chronic inflammation, may alter connexin expression in tissues, in particular Cx26, Cx32 and Cx43, most report that hemichannel numbers increase in response to injury or inflammation (Willebrords et al., 2016). Furthermore, failure of gap junction communication as well as hemichannel activation can be attributed to cumulative oxidative stress, as has been suggested for age-related macular degeneration (Danesh-Meyer et al., 2016). Increased Cx43 expression in vascular endothelium and astrocytes has also been reported in association with human central nervous system injuries (O’Carroll et al., 2008; Danesh-Meyer et al., 2012; Davidson et al., 2015; Mugisho et al., 2018; O’Carroll et al., 2008). The current evidence for connexin hemichannel-mediated ATP release in particular points toward a role for these channels during disease conditions such as ischemia and inflammation, pathologies that are often associated with a decrease in extracellular Ca2+ levels and large fluctuations in membrane potential, but with the inflammasome pathway being activated.

Pathologies of the central nervous system add a layer of complexity to the mechanism of connexin hemichannel action, as it involves not only neurons but also astrocytes in the release of cytokines and gliotransmitters. Upon calcium increase, communication between astrocytes and neurons enhance the feedback mechanisms that lead to neuronal injury, as reviewed in (Bennett et al., 2012; Chever et al., 2014; Freitas-Andrade and Naus, 2016; Meunier et al., 2017; Mayorquin et al., 2018).

The most interesting and widely reported role of Cx43 hemichannels in pathology seems to be associated with purinergic receptors and the NOD-like receptor pyrin domain-containing protein 3 inflammasome pathway of the innate immune system with opening of Cx43 hemichannels being an early event in the inflammatory process. Sustained hemichannel opening and ATP release, in conjunction with released inflammatory cytokines, provide a mechanism for the amplification and perpetuation of the inflammasome response as described in cell cultures by Mugisho et al. (2018).

**Therapeutic Approaches in Clinical Trials Designed to Target Connexin and Pannexin Channels**

It has been reported that therapeutic effects could be achieved at four levels: the pannexin, the connexin gap junction, or the connexin hemichannel in the plasma membrane, as well as in mitochondrial hemichannels (Naus and Giaume, 2016). While some therapeutics have been specifically design to block
these channels, a vast number are also re-purposed drugs (Additional Table 1). For example, probenecid was a widely used drug for the treatment of gout and is employed clinically to augment the efficacy of antibiotics, chemotherapeutics and other drugs. The proposed mechanism of action is similar to that of carbonoxolone. However, carbonoxolone is unrestrained, affecting both connexin and pannexin channels whereas probenecid interacts with the first extracellular loop of Panx1 and specifically inhibits Panx1 channels at a concentration that does not inhibit connexin hemichannels (Silverman et al., 2008). The antiviral drug tenofvir, used in the treatment of viral hepatitis, reversing hepatic fibrosis/cirrhosis in patients with chronic hepatitis B, is an inhibitor of Panx1-mediated ATP release, by downregulating adenine levels in the liver and sinus (Feig et al., 2017). Feig et al. (2017) also demonstrated in human cell cultures that the effect is due to block of unstimulated ATP release through Panx1 channels, but not Panx2 hemichannels. The therapeutic effect is achieved by blocking the function of the pannexin hemichannel, closing it permanently or transitorily and this strategy, widely used for blocking connexin channels has now started to be investigated systemically for pannexins.

Drugs that act on connexins can be differentiated between those acting on gap junctions for therapeutic use to maintain the gap junction open, contrary to the desired action on hemichannels (Wang et al., 2013). An example of a clinically used therapeutic that acts through modulation of Cx43 gap junctions is the peptide α-connexin carboxyl terminal (ACT1) that maintains Cx43 at gap junction sites at cell-cell membrane borders of breast cancer cells and augments gap junction activity in functional assays (Grek et al., 2015). The increase in Cx43 gap junctional activity achieved by ACT1 impairs proliferation and survival of breast cancer cells. This peptide also re-establishes a normal wound repair cascade when applied on cutaneous wounds, as ACT1 is said to generate gap junctions that were lost in diabetic-related injured tissues (Ghatnekar et al., 2015). However, it is more likely that ACT1 is acting as a hemichannel blocker in an identical manner to Gap19, which mimics the cytoplasmic loop to inhibit loop-C terminus interactions (Jiang et al., 2019). Other gap junction activators that have entered clinical trials include rotigaptide for the treatment of cardiac arrhythmia, a peptide that increases gap junction electrical conductance (Maca et al., 2011). Meclofenamate is clinically used to treat moderate pain and to reduce swelling, and is a gap junction inhibitor. It has also been trialed for the inhibition of tumour growth (Gleisner et al., 2017).

For blocking connexin hemichannels, a range of monoclonal antibodies and synthetic molecules including antisense oligonucleotide approaches and peptidomimetic strategies are now available, the latter building on the first peptides recognising the connexin extracellular loop sequences, a very significant contribution to the field by the late W. Howard Evans (Evans, 2015). [Howard died in 2019 and was a key player in the field, the first to isolate gap junctions and the first to coat connexin peptide antibodies onto glass; he was the first to describe functional mimetic peptides]. A Cx43 antisense oligodeoxynucleotide was first applied in a series of compassionate use cases to treat severe ocular surface burns (Ormonde et al., 2012). Originally taken into clinical trials by CoDaTherapeutics, Inc, USA, it was subsumed into OcuNexus Therapeutics Inc. and has been licensed to Eyevance Pharmaceuticals, USA. It is currently in stage 3 clinical trials for ocular surface indications.

Some drugs that inhibit connexin channels may be non-specific, and can also inhibit pannexins and gap junctions, such as anaesthetics, fatty acids, alcoholic substances, carbonoxolone and glycyrrhetinic acid (Willebrods et al., 2017). Peptides, on the other hand recognize the primary protein specific sequence and interfere directly with the channel function. The majority of compounds with therapeutic potential are peptides and organic compounds that block the interaction between the cytoplasmic loop and the C terminus of Cx43, or interact directly with the extracellular loops. Low concentrations of some peptides can be used to specifically block Cx43 hemichannels (such as Peptide5) without impacting significantly on gap junction coupling, and at least one peptide, Gap19, mimics the cytoplasmic loop to inhibit loop-C terminus interactions. Cx43 antisense oligonucleotides and Peptide5, a Cx43 hemichannel blocker, and more recently Xentry-Gap19, a construct of Gap19 with increased efficacy at low concentrations and disease targeting potential, also appear to provide viable therapeutic options for the treatment of chronic ocular surface and retinal inflammatory diseases associated with Cx43 hemichannel opening as well as both acute and chronic inflammation. The dose of the compound is critical for the therapeutic effect as high dose systemic Peptide5 have been associated with greater brain cell swelling after hypoxic-ischemic brain injury in fetal sheep (Davidson et al., 2012a). These data suggest that higher doses of extracellular acting connexin mimetic peptides are not beneficial to treat brain ischemia, most likely due to uncoupling of gap junctions. However, for example, intravitreal injection of low doses of Peptide5, and the benefits that intracellular acting Gap19 and Xentry-Gap19 offer with their hemichannel block specific mode of action (Abudara et al., 2014), has large potential, especially for acute injuries (for example in retinal stroke such as vein or artery occlusion) where the pharmacokinetics of an oral treatment such as Tonabersat may not be ideal.

The known involvement of ATP in inflammasome activation in many diseases, and the prospect of hemichannel opening mediating the release of this ‘activation signal’ that triggers assembly of the inflammasome complex in primed cells, has increased the interest in connexin hemichannels as a drug target candidate (Laird and Lampe, 2018; Mugisho et al., 2019). Blocking hemichannels requires lower drug concentrations than blocking or uncoupling gap junctions and this has been exploited for therapeutic use. Among all the therapeutic compounds, those that block Cx43 hemichannels have proven to be most effective in preventing disease progression and in shutting down disease perpetuation. Tonabersat is one of these compounds, originally proposed for the treatment of migraine with aura, migraine prophylaxis and as a treatment for epilepsy. Tonabersat is now recognised as a connexin specific hemichannel blocker. It is a benzopyran derivate with a unique stereo-selective binding site originally reported to inhibit gap junction communication (Read et al., 2000). Other studies, however, suggested that its mode of action is as a connexin hemichannel blocker and it has been re-purposed for this use in the treatment of inflammasome-related diseases (Kim et al., 2017). It has been shown recently that tonabersat directly reduces opening of hemichannels under pathological conditions (Kim et al., 2017; Mat Nor et al., 2020), which at low concentrations effectively acts only on undocked hemichannels. Tonabersat treatment has also proven to be effective in glioma (Aasen et al., 2019; De Meulenaere et al., 2019), again indicating a review of connexin channel effects in the biology of the glioma is overdue and that the involvement of hemichannel mediated effects may be understated. Since Tonabersat has proven safe in low and medium dose toxicity studies and has been used in humans for up to twelve weeks, it cannot be acting by uncoupling gap junctions, which would have severe adverse effects. This is one of the most clinically advanced compounds, available as an oral tablet and Phase II ready compounds, available as an oral tablet and Phase II ready.
Conclusion

The mechanism of diseases and the target for therapeutic interventions has evolved from gap junctions to connexin hemichannels, with a debatable role for pannexins in ATP-mediated disease mechanisms. Further evidence implicating pannexins in disease should ideally be regarded in light of what appears to be a key link between ATP-connexin hemichannels and pannexin channel activation in disease onset. Nevertheless, therapeutic interventions for inflammatory diseases still have Cx43 hemichannels as key therapeutic targets that can prevent disease spread while allowing ATP homeostasis and purinergic signaling to remain via pannexin channels. Cx43 hemichannels are an upstream target for a range of inflammatory diseases with promising therapies currently in clinical trials evaluating the use of peptides and small compounds specific for connexin hemichannels.

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Conflicts of interest: CRG has intellectual property related to the regulation of connexin channels in the treatment of ocular and other disease. CRG, IDR and FPC have filed a patent application for Xentry fusion peptides and this technology is now licensed by OcuNexus.

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Additional file: Additional Table 1: Selected therapeutic targeting connexins, pannexins and gap junctions.

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## Additional Table 1 Selected therapeutics targeting connexins, pannexins and gap junctions

| Target | Drug effect | Drug (formula) | Description | Condition | References |
|--------|-------------|----------------|-------------|-----------|------------|
| Connexins, pannexins | Blocks connexins and pannexins | Carbenoxolone (C34H50O7) Glycyrrhetinic acid derivative with a steroid-like structure | Lip sores, mouth ulcers | Willebrords et al. (2017) |
| Connexins, pannexins | Several pharmacological targets, including blocking pannexins | Probenecid (C13H19NO4S) 4 -Dipropylsulfamoyl benzoic acid derivative | Chronic gout, gouty arthritis | Silverman et al. (2008) |
| Pannexins | Inhibitor of pannexin channel function | Tenofovir (C9H14N5O4P) Acyclic nucleotide analogue of adenosine | Chronic hepatitis virus infection | Feig et al. (2017) |
| Connexins | Gap junction blocker | Meclofenamate (C14H10Cl2NNaO2) 2-(2,6-Dichloro-3-methylanilino) benzoate | Osteoarthritis | Gleisner et al. (2017) |
| Cx43 | Cx43 hemichannel blocker | Tonabersat (Xiflam™) (C20H19ClFNO4) Benzopyran derivative | Retinal ischemic injury, inflammation disease | Kim et al. (2017) |
| Cx43 | Decreases Cx43 levels by inhibiting protein translation | Cx43 oligonucleotide (Octagam®) (5′-GTA-ATT-GCG-GCA-GGA-GGA-ATT-GTT-TCT-GTC-3′) Cx43 antisense oligodeoxynucleotide | Corneal/skin wounds | Ormonde et al. (2012) |
| Cx43 | Increases Cx43 gap junction function | Rotigaptide (YPXGAG) Antiarrhythmic peptide analogue | Ischemic injury of the heart | Macia et al. (2011) |
| Cx43 | Decreases Cx43 gap junction formation and decreases Cx43 hemichannel formation | α-Connexin carboxyl terminal peptide (ACT1) (RQPKIWFPNRRKPKWKK - RPRPDDLEI) Peptide incorporating the zonula occludens-1 (ZO-1)-binding domain of Cx43 | Skin scars | Ghatnekar et al. (2015); Jiang et al. (2019) |
| Cx43 | Decrease Cx43 levels | Peptide5 (Peptagon™) (VDCFLSRPRTEKT) | Cx43 extracellular loop 2 mimetic peptide | Retinal diabetic injury | O’Carroll et al. (2008) |
| Cx43 | Cx43 hemichannel blocker | Gap19 (YGRKKRRQRRR-KQIEIKKF) Gap Junctional Communication in Astrocytes | Gap Junctional Communication in Astrocytes | Abudara et al. (2014) |
| Cx43 | Cx43 hemichannel blocker | Xentry–Gap 19 LCLRPVGG -KQIEIKKF | Cx43 intracellular loop mimetic peptide | Choroidal neovascularisation (wet AMD model) | Coutinho et al. (2019) |