*Inflammation & Cell Signaling* 2014; 1: e149. doi: 10.14800/ics.149; © 2014 by Chiara Caselli.
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REVIEW

# Inflammation in cardiac disease: focus on Interleukin-33/ST2 pathway

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Received: April 10, 2014
Published online: June 14, 2014

**Several studies have identified the importance of pro-inflammatory mediators in the development and progression of cardiac disease such as heart failure (HF). Recently, a number of studies from basic research have used gene expression, array screening, cloning, and other techniques to identify new cardiokines and cardiokine networks that are regulated during cardiac stress. IL-33, an IL-1 family member, binds to a ST2L, which is a member of the Toll-like receptor (TLR)/IL1R superfamily. Besides ST2L, the ST2 gene can encode two other isoforms by alternative splicing, including a secreted soluble ST2 (sST2) form that could act as a decoy receptor for IL-33. Studies in animal models suggest that IL-33/ST2 is involved in cardiovascular disease and plays an important role in protection of cardiac muscle. Furthermore, sST2 is a promising biomarker predictive of worse outcome in several cardiovascular diseases. Although manipulation of IL-33/ST2 system is still in its infancy, it may be a unique opportunity to quench the inflammatory response after cardiac injury.**

***Keywords:*** inflammation; cardiac disease; Interleukin-33/ST2 pathway

**Abbreviations:** HF, heart failure; TLR, Toll-like receptor; sST2, secreted soluble ST2; LV, left ventricle; IL, Interleukin; NF-HEV, nuclear factor from high endothelial venules; MAPK, mitogen-activated protein kinase; NF-κB, nucler factor-κB; IAP, inhibitor of apoptosis proteins; LVAD, left ventricle assist device; AP-1, adaptor protein 1; ERK, extracellular signal-regulated kinase.

**To cite this article:** Casell C. Inflammation in cardiac disease: focus on Interleukin-33/ST2 pathway. *Inflamm Cell Signal* 2014; 1: e149. doi: 10.14800/ics.149.

## Introduction

Inflammation has emerged as a crucial process that plays a role in cardiovascular disease [1]. Several studies have identified the importance of pro-inflammatory mediators in the development and progression of cardiac disease such as heart failure (HF) [2-4]. These factors can induce myocardial remodeling either by promoting the recruitment of inflammatory cells or by producing maladaptive effects in the heart, such as left ventricle (LV) remodeling and endothelial dysfunction, thus facilitating hypertrophy and fibrosis [5]. However, anti-inflammatory therapeutic strategies tested so far have been largely disappointing, due to either neutral results either worsening of HF. These findings have triggered important considerations, including the relevance of looking for novel targets [6].

Recently, a number of studies from basic research have used gene expression, array screening, cloning, and other techniques to identify new cardiokines and cardiokine networks that are regulated during cardiac stress [4]. With genetic animal models, many of these newly identified molecules have been shown to have functional roles in cardiac remodeling.

In this review we will focus on recent research related to the cardiovascular role of the IL-33/ST2 pathway, including the translational aspect. The potential of using IL-33 or its receptor ST2 for therapeutic intervention of cardiovascular disease will also be discussed. Finally, the

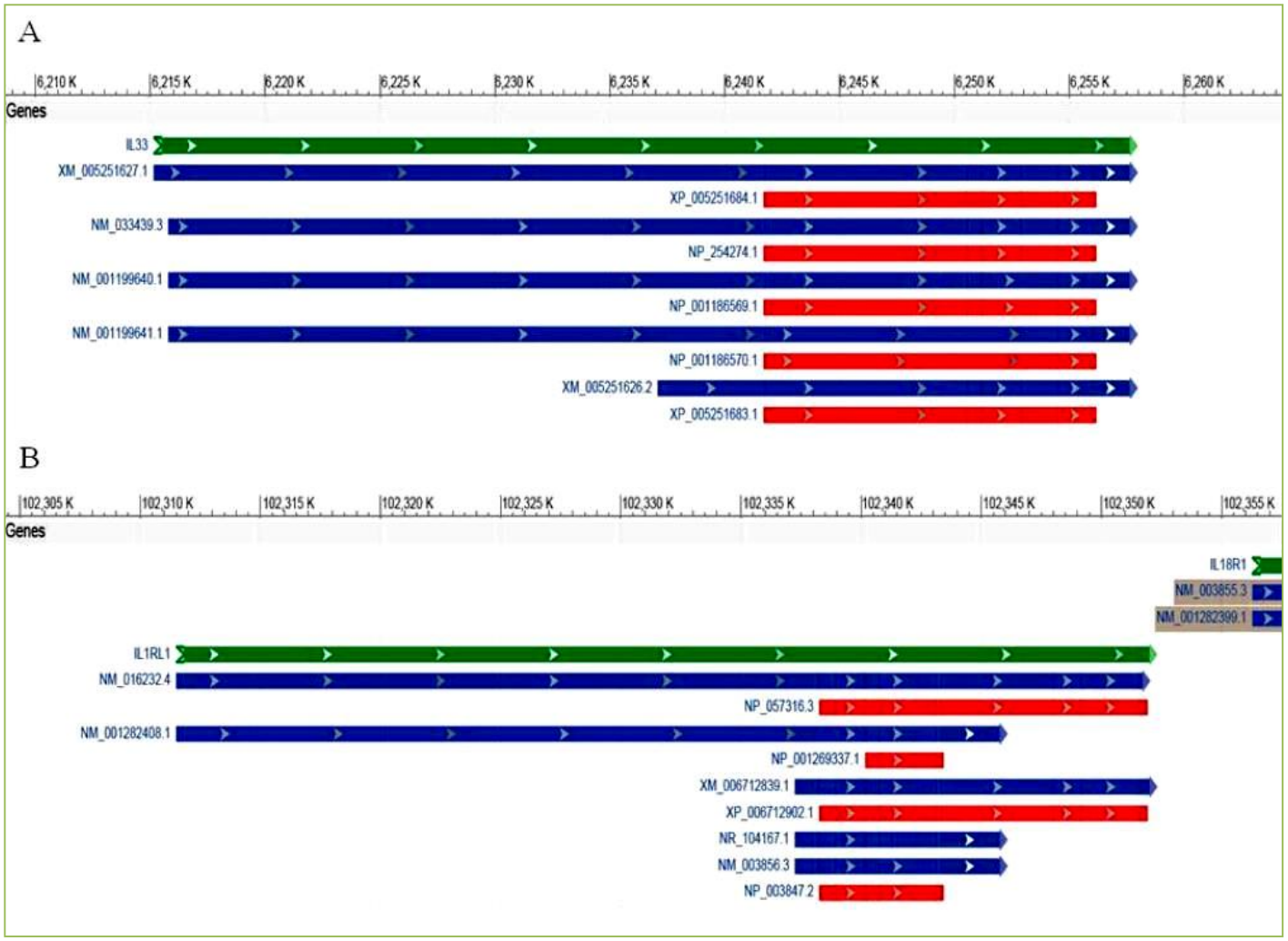


**Figure 1. Genomic regions, transcripts, and products of IL-33 (A) and ST2 (B).** Human IL-33 gene is located on chromosome 9, ST2 gene on chromosome 2. Figures were built up by Sequence Viewer 3.1 available at http://www.ncbi.nlm.nih.gov/gene/ (Green bar: gene; blue bar: RNA transcripts; red bar: coding region).

role of soluble ST2 as a potential biomarker for cardiovascular disease will be debated.

## 1. IL-33/ST2 pathway

### a. IL-33 identification, expression and activation

Interleukin (IL)-33 (also known as IL-1F11) was initially identified as DVS27, a gene up-regulated in canine cerebral vasospasm [7], and as a "nuclear factor from high endothelial venules" (NF-HEV) [8]. In 2005, analysis of computational structural databases showed that this cytokine had a high homology to IL-18, and a β-sheet trefoil fold structure characteristic of IL-1 family proteins [9].

The human and mouse sequences for IL-33 have been localized to chromosomes 9 (9p24.1) and 19 (19qc1), coding proteins of 270 and 266 amino acids, respectively (Fig. 1). The 30 kDa molecule has high homology to IL-18 (Fig. 1) [9]. IL-33 is a protein with a double role, acting as a traditional cytokine as well as a nuclear factor with transcriptional properties, although its physiological role is not fully clear [10].

IL-33 is present in many tissues, but its expression is greatest in stomach, lung, spinal cord, brain, and skin and low in lymph tissue, spleen, pancreas, kidney, and heart [9].

Some controversy exists regarding IL-33 biologically active form. During necrosis, the full-length IL-33, considered the biologically active form, may be released from injured cells. Conversely during apoptosis, IL-33 is cleaved by caspases-3/7 producing an inhibition of its pro-inflammatory effects. These data suggest that full-length IL-33 may act as an endogenous danger signal or alarmin, while inactivation of IL-33 may be needed as a fail-safe control mechanism to avoid further impairment of host

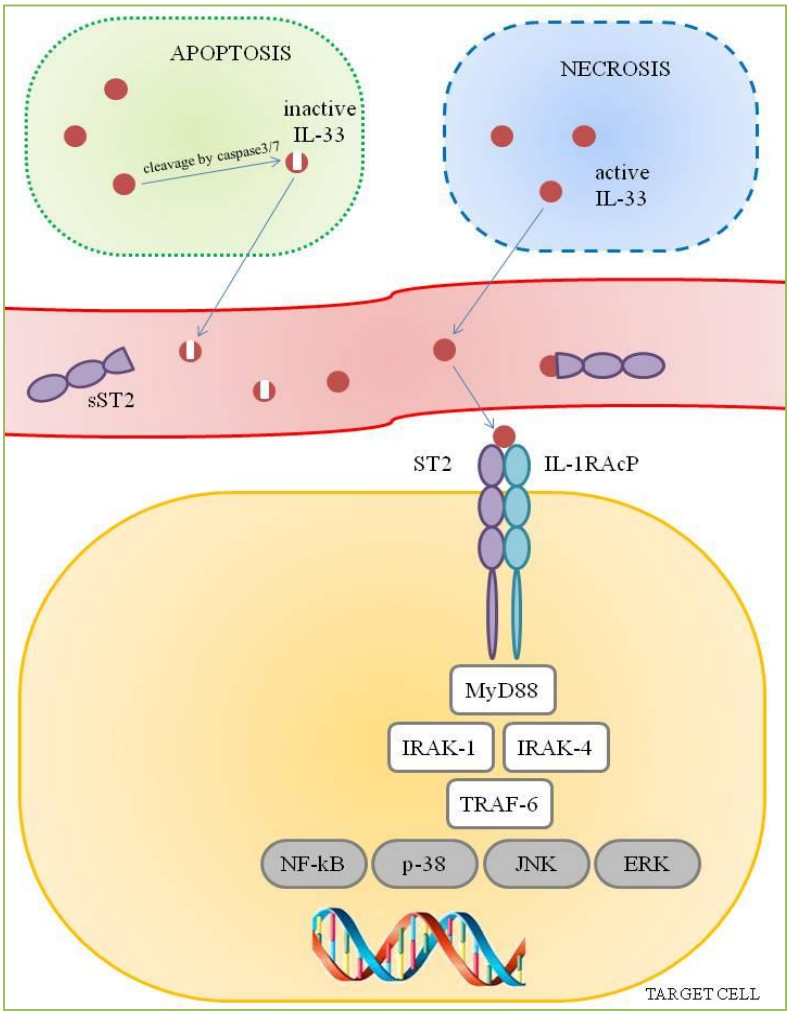


**Figure 2. Production and signaling of IL-33/ST2 pathway.** The full length IL-33, considered the biologically active form, may be produced during necrosis. Conversely, IL-33 is cleaved by caspases-3/7 producing an inactive form during apoptosis. Active IL-33 can stimulate the formation of the heterodimeric ST2L/IL-1RAcP complex on the target cells or can be inhibited by sST2 that acts as a decoy receptor. Upon the activation of this complex, the signaling is induced. The MyD88, IRAK1/4 and TRAF-6 are localized to the receptor complex, leading to activation of transcription factors as NF-kB, p38 and JNK, as well as ERK (directly by MyD88). This leads to transcription of inflammatory genes.

tissues by the IL-33 pro-inflammatory effect during apoptosis [10-11] (Fig. 2).

**b.IL-33 signaling by ST2**

The gene called ST2 (also known as T1, IL1RL1, or Fit1) was discovered in 1989 and is mapped on chromosome 2q12 together with the wider interleukin 1 (IL-1) gene cluster [12]. Alternative splicing of gene promoter and 3' processing of the same mRNA produce four transcriptional products. Of these, two are the most important isoforms: IL1RL1-β or ST2L, a membrane receptor member of the interleukin-1 receptor family, and IL1RL1-α or sST2, a truncated soluble receptor that could be measured in peripheral circulation (Fig. 1B). ST2 gene has a proximal and a distal promoter, which could modify its transcriptional regulation [13]. ST2L is composed of three extracellular immunoglobulin G domains, a single trans-membrane domain, and an intracellular domain [9, 12, 14]. The sST2 lacks the trans-membrane and intracellular domains and it moves freely through the peripheral circulation.

IL-33 has been recognized as a functional ligand of ST2L [9, 14] and it binds ST2L on inflammatory cell membranes. This binding activates mitogen-activated protein kinase (MAPK)-kinases as well as several

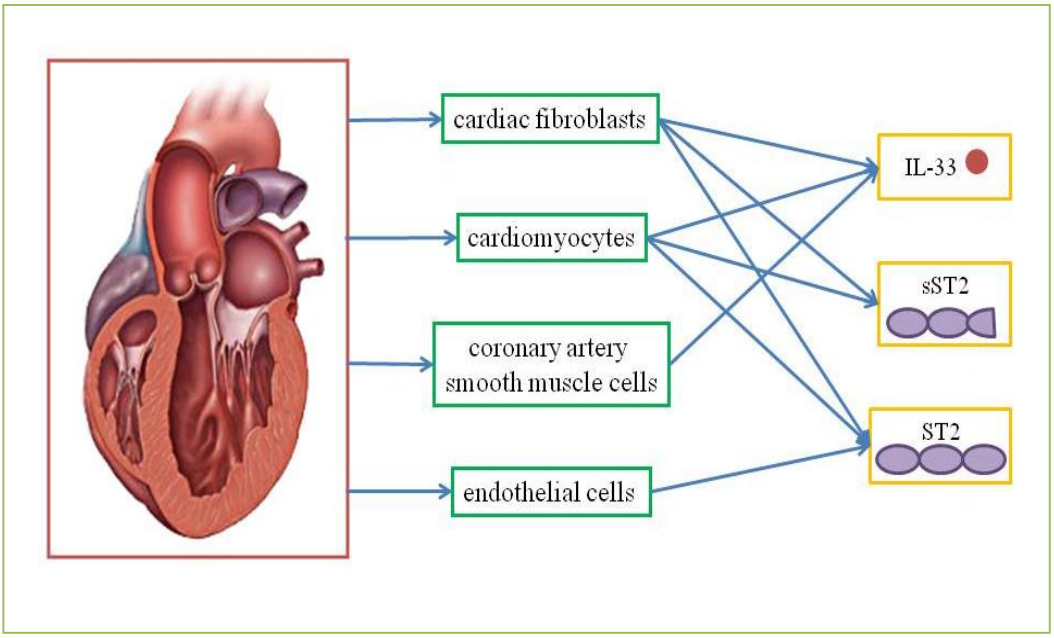


**Figure 3. Specific production of IL-33, ST2 and sST2 by the different cellular types of the cardiovascular compartment.** IL-33 is expressed by human adult cardiac myocytes and fibroblasts and by human coronary artery smooth muscle cells. The receptor ST2 is predominantly expressed by endothelial cells of the cardiac vasculature. Both sST2 and ST2L are induced in cardiomyocytes and fibroblasts after biomechanical stress.

biochemical pathways that lead the activation of the inhibitor of nucler factor-κB (NF-κB) kinase (IKK) complex, which activates NF-kB to exert its pro-inflammatory actions [15]. Moreover, sST2 appears to act as a decoy-receptor for IL-33: it binds IL-33, consequently removing this protein from its possible binding with ST2L. sST2 binding with IL-33 could limit the expression and activation of NF-kB, thus reducing the inflammatory response (Fig. 2). IL-33 has been supposed to regulate the ST2L and sST2 mRNA transcription by itself [15, 16].

## 2.Cardiac role of IL-33/ST2 pathway

### a.Cellular models

The involvement of ST2 in cardiac compartment was initially suggested by Weinberg *et al*. in a screen of gene transcripts expressed by mechanically stressed cardiomyocytes in an *in vitro* model [17]. They found that both sST2 and ST2L are induced in cardiomyocytes and fibroblasts after biomechanical stress [17, 18].

IL-33 and its receptor ST2 show distinct expression patterns in the heart. IL-33 is expressed by human adult cardiac myocytes and fibroblasts and by human coronary artery smooth muscle cells, while ST2 is predominantly expressed by endothelial cells of the cardiac vasculature. IL-33 is upregulated by TNF-α, IFN-γ and IL-1β and is released during necrosis of human cardiac and smooth muscle cells [19] (Fig. 3).

### b.Animal models

The discovery of IL-33 as a ligand for ST2 has led to exploration of the role of IL-33/ST2 signaling in the myocardium. Thus, following its binding with ST2L, IL-33 has been shown to have anti-hypertrophic and antifibrotic effects in the heart. In an *in vitro* rodent model of cardiomyocytes undergoing stretching, a direct relationship between duration of biomechanical strain and IL-33 and ST2 expression was observed [17]. Furthermore, administration of sST2, the soluble form, blocked the positive anti-hypertrophic actions of IL-33 in a dose-dependent manner, suggesting that sST2 may act as a "decoy receptor" for circulating IL-33. In an *in vivo* model of pressure overload, ST2 knockout mice showed- higher myocyte hypertrophy and fibrosis and lower fractional shortening than wild-type mice after 4 weeks of aortic banding. IL-33 administration preserved wild-type mice from the hypertrophic phenotype, but this action was not observed in $ST2^{-/-}$ mice, suggesting that IL-33/ST2 signaling protects against adverse cardiac remodeling in vivo [19-20].

A possible mechanism by which the alteration in ST2 signaling may lead to tissue fibrosis has been identified by Seki and co-workers. IL-33 inhibits cardiomyocyte

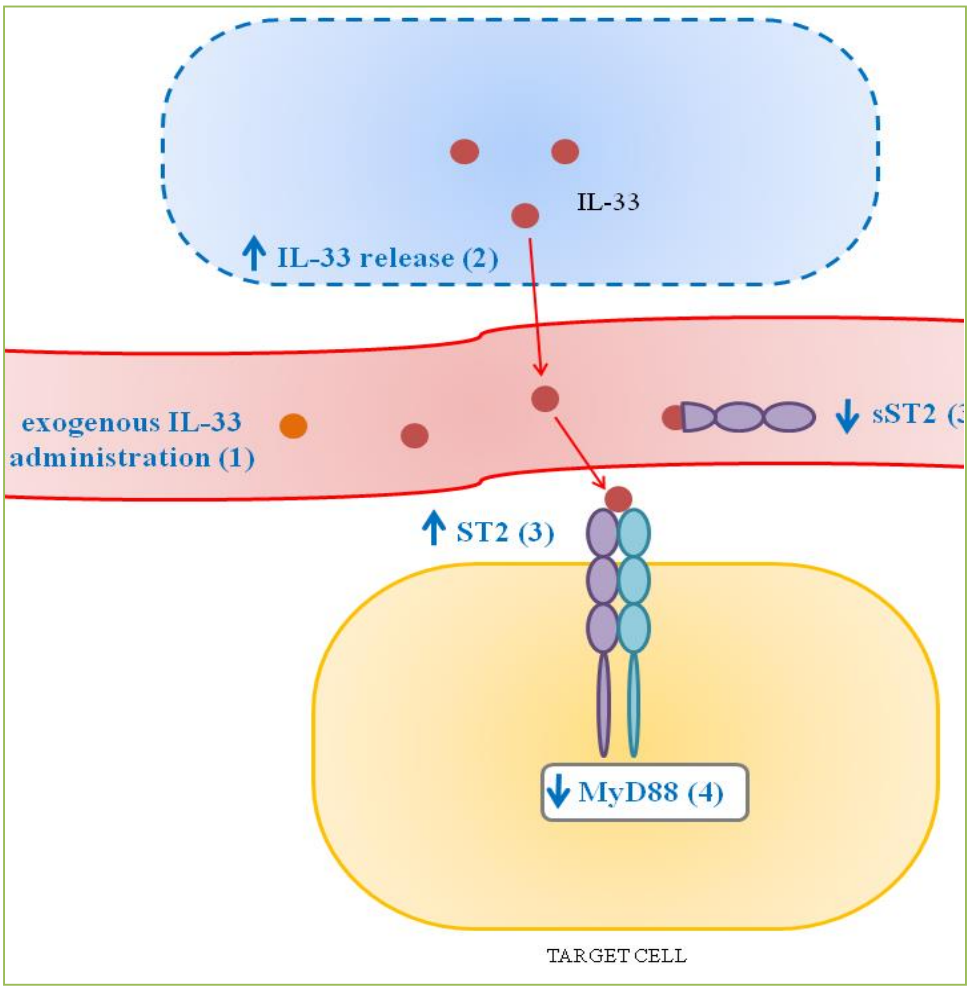


**Figure 4.Theraputic strategies targeting IL-33/ST2 pathway.** The IL-33/ST2 pathway may be triggered by exogenous administration of IL-33 (1) or by promoting IL-33 release from cardiac cells (2). IL-33/ST2 complex could be increased by inhibiting sST2 by therapeutic compounds designed to directly stimulate the ST2 (3). Modifications of the intracellular signaling, including sequestration of MyD88 by exogenous pharmaco-therapeutics, could represent a possible option (4).

apoptosis both *in vitro* and *in vivo* via suppression of caspase-3 activity and increased expression of inhibitor of apoptosis proteins (IAP), thus improving cardiac contractile function after ischemia/reperfusion myocardial injury in rats. The cardioprotective effects of IL-33 were abolished in ST2-null mice, demonstrating that IL-33 is cardioprotective through ST2 signaling [21]. Moreover, it has been demonstrated that the protective role of IL-33 may be reduced by endothelin-1, which enhanced the production of sST2 and inhibited IL-33 downstream signaling through p38 MAP Kinase [22].

### c. Human model

In a very recent paper [23], it has been shown that patients with HF presented differentially expressed levels of ST2/IL-33 as well as conventional inflammatory mediators (IL-6, IL-8 and TNFα) in both plasma and cardiac tissue, and that these modifications are corrected by mechanical unloading through left ventricle assist device (LVAD) support. Lower expression of ST2 and IL-33 was found in cardiac tissue of patients undergoing LVAD support compared to more stable patients undergoing heart transplantation on medical therapy only. These data suggested a protective effect of ST2/IL-33 pathway in the worsening of cardiac function, as previous reported [17, 20-21]. This cardioprotective action was confirmed by the increase in their levels by mechanical unloading after LVAD support, possibly due to the reverse remodeling process, which was able to restore levels comparable to those observed for the heart transplant group of patients.

Results from this study also provided further insight into the role of classic inflammatory mediators in HF [23]. As with the IL-33/ST2 pathway, IL-6, IL-8 and TNF-a were

**Table 1. Cardiac diseases in which sST2 circulating levels were measured**

| Cardiac disease | References |
|---|---|
| Acute myocardial infarction | 29-33, 50 |
| Acute heart failure | 35-37, 44, 48, 50 |
| Chronic heart failure | 38-43, 52-54 |
| Pleural cardiac effusion | 55 |
| Aortic stenosis | 56 |
| Diastolic dysfunction and diabetes | 57 |
| Coronary bypass and heart surgery | 58, 59 |
| Acute cardiac allograft rejection | 60 |
| Acute Kawasaki disease | 61 |

low in less stable HF patients and were higher after LVAD support up to a level comparable to that of patients directly undergoing heart transplantation with only medical therapy. In spite of their well-documented role as pro-inflammatory cytokines, these molecules showed a negative role in HF progression and were positively involved in the reverse remodeling process by LVAD, eventually suggesting a compensatory effect to the adverse remodeling process of HF. Recent studies hypothesized that temporally regulated activation and suppression of inflammation may be critical for achieving effective cardiac repair, indicating a paradoxical role of inflammation in cardiac repair [24].

A very recent paper demonstrated that in human myocardial tissue from hearts of patients undergoing heart transplantation, endothelial cells are the main cell type expressing both IL-33 as well as its receptor ST2 and that IL-33 expression correlates positively with that TNF-α and IFN-γ, respectively [19].

## 3. ST2 as therapeutic target

The results from experimental and clinical studies suggest that modulation of the IL-33/ST2 system could exert cardioprotective activity in the context of heart disease. Thus, strategies that chronically target IL-33/ST2 signaling should be considered to have potential adverse cardiovascular consequences. Moreover, manipulation of the IL-33/ST2 pathway is a promising new therapeutic approach for treating or preventing various disorders in which inflammation is a critical process. To date, several approaches have been proved to modulate IL-33/ST2 signaling, addressing its cardioprotective activity [12] (Fig. 4). The IL-33/ST2 pathway may be triggered by exogenous administration of IL-33 or by promoting IL-33 release from cardiac cells. Moreover, IL-33/ST2 complex could be increased by inhibiting sST2, the IL-33 decoy receptor, by therapeutic compounds designed to directly stimulate the ST2. Alternatively, modification of intracellular signaling could be a possible option: the cardioprotective effects of IL-33 may be reproduced by sequestration of MyD88 by exogenous pharmacotherapeutics. Moreover, further study is needed in order to explain the possible causal relationships between the molecules involved in this signaling, such as nuclear factor-kB (NF-kB), adaptor protein 1 (AP-1) or extracellular signal-regulated kinase (ERK). These clarifications might be an important step in the IL/33/ST2 signaling that is accessible to manipulation.

However, due to the involvement of the IL-33/ST2 system in a variety of processes, its manipulation may also have negative consequences, resulting in exacerbation of inflammatory conditions. Conversely, inhibition of this system to regulate these inflammatory conditions could result in a worsening of cardiovascular disease.

## 4. ST2 as circulating biomarker

The possibility of using sST2 as a potential biomarker for cardiac disease was originally raised in 2002 when it was found that sST2 levels were transiently increased in peripheral circulation of mice after myocardial infarction [17]. Later, it was shown that blood concentrations of sST2 increase in heart disease and are taken into account as a possible prognostic marker [25-27].

### a. sST2 Assay

The first ELISA for evaluating circulating sST2 in serum/plasma was developed in 2000 [25]. To date, three main assays have been tested: the MBL ST2 ELISA kit (Medical & Biological Laboratories, MA, USA), the Human ST2/IL-1 R4 DuoSet® (R&D Systems, MN, USA) and the Presage ST2 Assay (Critical Diagnostics, CA, USA) [26]. The MBL ST2 assay and the R&D ST2 assay are research assays. In 2011, the Presage ST2 Assay received the Conformit è Europ èenne (CE) Mark and the US FDA approved the Presage ST2 Assay for use in assessing the prognosis of HF patients [27].

sST2 concentrations obtained by these three commercially available assays are not equal to each other, probably due to the different methodological conditions, including standards, antibodies, and also reagents and buffers [26, 28]. Thus, the direct comparison of the results obtained with the three methods is not feasible and the superiority of one out of the three assays has yet to be demonstrated. Moreover, the three methods should be standardized because many methodological aspects should be clarified. It is not clear if any of the three methods has a calibrator that is correctly quantified and which epitopes are detected by the antibodies against sST2 used for the three methods. Therefore, it is not known whether primary, secondary or tertiary structures of the sST2 protein are specifically recognized by the different antibodies used in the three assays. Another important issue is related to the analytical sensitivity of each methods [26, 28].

**b. Clinical relevance**

Several clinical studies in patients with acute myocardial infarction or acute coronary syndrome [29-34], in acute and chronic HF [35- 43], showed that high sST2 levels are related to adverse outcome. Moreover, in HF serial determination of sST2 has a prognostic role and could show value in biomarker-directed therapy [27, 43, 44]. Conversely, determination of sST2 was not useful for the diagnosis of acute myocardial infarction or acute coronary syndrome [44-46] and HF in patients with acute dyspnea [47, 48].

Compared with cardiac Natriuretic Peptides (ANP, BNP or NT-proBNP) that specifically mirror the pathophysiological conditions of cardiac stretch, sST2 does not completely show this specificity, thus lacking the prerequisite for diagnostic purposes. sST2 does not merely reflect the condition of cardiac stretch but is also involved in other non-cardiac conditions such as inflammation. In fact, inflammation is a process simultaneously present in a large proportion of patients with heart disease, making sST2 a poor diagnostic marker in such a setting. On the contrary, as a consequence of the non-specificity of sST2, it seems to be a reliable prognostic marker in various diseases (Table 1).

Accordingly, sST2 could be a good prognostic marker in patients with negative outcome presenting simultaneously HF and inflammatory diseases [27, 49]. Because sST2 appears to be associated with both inflammation and cardiac stretch, it could be a strong and independent outcome predictor in this setting. Of note, it is becoming evident that sST2 is not only an independent prognostic biomarker, but it is also able to provide incremental prognostic value outperforming clinical variables and other biomarkers. This is a very relevant issue in clinical practice, because clinicians currently use diverse clinical information, several scoring systems and established biomarkers such as cardiac Troponins or Natriuretic Peptides for evaluation of patient outcome and management [27].

It was recently shown that baseline cardiac ST2 positively correlated with its soluble isoform and did not show any modification after 1 month of LVAD support [23]. These data might confirm the cardiac production of soluble sST2, and considering the role of ST2 as a soluble decoy receptor for IL-33, could explain the negative prognostic value of this biomarker in individuals with HF. Conversely, before LVAD implant cardiac IL- 33 was negatively related with its plasma concentration and resulted significantly decreased after 1 month- compared to its values before LVAD support, suggesting a different regulatory mechanism for IL-33 [23].

**Conclusions**

IL-33/ST2 pathway plays an important role in protection of cardiac muscle. Furthermore, sST2 is a promising biomarker predictive of worse outcome in several cardiovascular diseases. Although modulation of the IL-33/ST2 system is still in its infancy, it may be a unique opportunity to quench the inflammatory response after cardiac injury. It remains to better understand many aspects of IL-33/ST2 downstream intracellular signaling.

**Conflict of interest**

The author declares that she has no conflicting interests.

**Acknowledgments**

This study was supported partially by grants from the projects SensorART-A Remote Controlled Sensorized ARTificial Heart Enabling Patients Empowerment and New Therapy Approaches (FP7-ICT-2009 project, grant agreement 24863).

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