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1 **JAK-STAT targeting offers novel therapeutic opportunities in sepsis**

2

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14

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16

17 Running title: JAK-STAT signaling in sepsis

18

19 **Abstract**

20 Sepsis is a life-threatening condition caused by exaggerated host responses to infections taking place in  
21 two phases: (1) a systemic (hyper) inflammatory response syndrome (SIRS), participating in multiple  
22 organ failure, a major complication of septic shock, followed by (2) a compensatory anti-inflammatory  
23 response syndrome (CARS), leading to sepsis-induced immunosuppression and resulting in late  
24 infections and long-term mortality. The JAK-STAT-dependent signaling pathway is involved in both  
25 manifestations, hence playing a key role during sepsis. It is also involved in emergency myelopoiesis,  
26 which participates in host defense. The aim of this review is to highlight and refine the recent  
27 implications of this signaling pathway in sepsis and illustrate why its central position makes it a  
28 potential biomarker and therapeutic target.

29

30 Key words: sepsis, Janus kinase (JAK), signal transducers and activator of transcription (STAT),  
31 immunosuppression, multiple organ failure (MOF)

32

1 **The JAK-STAT pathway, an essential player in sepsis**

2 The **Janus kinases** (See Glossary) and **Signal Transducers and Activators of Transcription (JAKs-**  
3 **STATs)** are amongst the most important signaling components downstream of cytokine receptors [1].  
4 They are indeed involved in most pathological conditions, such as cancers [2], immunological [3] and  
5 infectious diseases [4]. **Sepsis** is a deregulated host response to infection leading to organ dysfunction  
6 [5, 6], affecting about 30 million patients each year with a mortality rate of 30 to 40% [7, 8]. The  
7 deregulated host response includes an exacerbated **systemic inflammatory response syndrome (SIRS)**  
8 [9, 10] to fight off invading pathogens and a **compensatory anti-inflammatory response syndrome**  
9 **(CARS)** [9] which should balance pro- and anti-inflammatory cytokine secretion, but ultimately often  
10 leads to a secondary immunoparalysis [11] (see Clinician’s Corner). The two leading causes of death  
11 identified during sepsis are SIRS-induced multiple organ failure [12] (Figure 1) and sepsis-induced  
12 immunoparalysis responsible for late infections [13], both of which rely of JAK-STAT pathways. SIRS  
13 is mainly mediated by signal transducer and activator of transcription 1 (STAT1) [14] and STAT4 [15],  
14 whereas CARS is predominantly mediated by STAT3 [16] and STAT6 [15], with the involvement of  
15 JAK1-2 and Tyk2 (tyrosine kinase 2) for both cases [14]. Furthermore, the functions of the JAK-STAT  
16 pathway in cell proliferation and apoptosis [17] advocate for its involvement in sepsis-triggered  
17 emergency hematopoiesis [18] and sepsis-induced organ dysfunction [19].  
18 Targeting JAK-STAT signaling in sepsis could therefore reduce sepsis-induced multiple organ failure  
19 and sepsis-induced immunosuppression. The recent successful development of JAK-STAT-targeted  
20 therapies in oncology and hematology [20] provides novel opportunities to cure sepsis patients.

21

22 **Molecular aspects: the JAK-STAT pathway**

1 JAKs are tyrosine kinases that are activated upon cytokine binding to their cognate receptors and  
2 phosphorylate the receptor intracellular domain. In most cases, this phosphorylation triggers STATs  
3 binding, phosphorylation and dimerization [17]. Of note, some STATs are activated by JAKs without  
4 requiring tyrosine phosphorylation of the receptor [21]. Following their phosphorylation and  
5 dimerization, STATs translocate to the nucleus, where they act as transcription factors. There are four  
6 different JAKs (JAK1-3, TYK2) and seven STATs (STAT1-4, 5A, 5B and 6). Different cytokine  
7 receptors utilize various combinations, resulting in diverse biological responses. Among the most  
8 important examples, STAT1 and STAT2 are phosphorylated by JAK1 and TYK2 kinases following type  
9 I interferon receptor engagement. Phosphorylated STAT1 and STAT2 bind to interferon regulatory  
10 factor-9 (IRF-9) to form a heterotrimeric transcription factor complex called interferon-stimulated gene  
11 factor 3 (ISGF-3), which induces the expression of hundreds of interferon-stimulated genes (e.g IRF1, 3,  
12 7) that participate in the clearance of the invading pathogens [22].

13 JAK-STAT pathways are tightly regulated by inhibitors preventing immune response deregulation.  
14 Three main inhibitors have been described: protein inhibitors of activated STAT (PIAS), suppressors of  
15 cytokine signaling (SOCS) and protein tyrosine phosphatases (PTPases) [1]. PIAS are regulators of  
16 STATs, acting through transcriptional regulation and protein stability [23]. SOCS family members  
17 prevent STAT phosphorylation and also have important roles in inducing degradation of cytokine  
18 receptor complexes [24]. PTPases inactivate JAK and STAT family members by triggering their  
19 dephosphorylation [25]. The role of these inhibitors has been shown in physiological and pathological  
20 contexts. Inspired by their mode of action, pharmacological blockers of JAKs (**JAKinibs**) have been  
21 designed and are used to treat a broad spectrum of immune-related disorders ranging from  
22 rheumatologic diseases to hematological malignancies [26].

23

## 1 **JAKs, STATs and immunity during sepsis**

### 2 *Role of JAK-STAT signaling pathways during sepsis*

3 JAKs and STATs play complex roles during sepsis, through host defense against pathogens, and  
4 immunity regulation (Table 1). Indeed, following the **pathogen associated molecular patterns**  
5 **(PAMPs)** recognition by **pathogen recognition receptors (PRRs)**, which activate the innate  
6 immune system, many of the most important receptors involved in the immune response during  
7 sepsis need the JAK-STAT signaling pathways : these include the receptors for **granulocyte-colony**  
8 **stimulating factor (G-CSF)**, macrophage inflammatory protein (MIP)-2 (also called C-X-C motif  
9 chemokine ligand 2, CXCL2), type I cytokines, including interleukins (IL-4, IL-6, IL-10, IL-12, IL-  
10 13), and type II cytokines , including **interferons** (IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ ). JAKs and STATs are thus  
11 involved both in pro-inflammatory (IFN- $\gamma$ , IL-12, IL-27) and anti-inflammatory (IL-4, IL-10, IL-  
12 13) cytokine signaling. This reveals the capacity of various STAT family members to differentially  
13 regulate the **Th1/Th2** balance.

14 The presence of STATs in various cell types enables a partially cell-type-restricted signaling in  
15 response to cytokines. Thus, macrophage activation is mostly regulated by cytokines using STAT1,  
16 participating in inducible nitric oxide synthase (iNOS) expression and STAT3 signaling pathways,  
17 whereas neutrophils require STAT2, which enhances superoxide dismutase activity. Lymphocytes  
18 are mostly regulated by STAT4 [27] and STAT6 [28] for pro-inflammatory Th1 and  
19 immunoregulatory Th2 responses [28] respectively. STAT4 is also required for IL-12-mediated  
20 responses [29] of natural killer cells and dendritic cells [30]. Although the JAK-STAT pathways are  
21 of critical importance for innate immunity, they are also involved in the hyper-inflammation and  
22 immunosuppression following sepsis.

23

1 *Role of JAK-STAT in hyper-inflammation contributing to mortality*

2 The JAK-STAT signaling pathways are involved in the production of pro-inflammatory molecules,  
3 which require especially STAT1, STAT4, Tyk2 and JAK2, as well as in emergency myelopoiesis  
4 (Box 1), a fundamental process accompanying the immune response during sepsis. The resulting  
5 hyper-inflammatory state (Figure 2, Key Figure) contributes to death during sepsis, as demonstrated  
6 by knock-out mice models (Table 1). *Stat1*<sup>-/-</sup> mice are resistant to **lipopolysaccharide (LPS)**-  
7 induced endotoxemia [31] and **cecal ligation and puncture (CLP)**-induced **septic shock** [14].  
8 *Stat4*<sup>-/-</sup> mice display increased resistance to CLP-induced lethality [32]. This enhanced survival has  
9 been attributed to diminished Th1 and augmented Th 2 immune responses, leading to reduced  
10 inflammation and organ injuries in the liver and kidneys. Consistently, Tyk2, phosphorylating  
11 STAT1 and STAT 4, was shown to promote sepsis-induced lethality. Indeed, *Tyk2*<sup>-/-</sup> mice were  
12 resistant to LPS-induced endotoxin shock and to CLP-triggered polymicrobial sepsis by reducing  
13 the production of pro-inflammatory IL-27 [33]. Finally, hematopoiesis defects in *Jak2*<sup>-/-</sup> mice and  
14 lethality prevent their analysis in infectious settings. It could be shown, however, that JAK2  
15 inhibitors increase survival after CLP in mice and rats by decreasing the expression of pro-  
16 inflammatory mediators such as TNF (tumor necrosis factor), IL-6 and high mobility group box 1  
17 (HMGB-1) [34]. Of note, HMGB-1 can be induced by LPS through the Toll-like receptor 4  
18 (TLR4)/JAK/STAT1 pathway leading to deleterious consequences, like pyroptosis [35]. HMGB-1  
19 is an example of the pleiotropic consequences of JAK-STAT signaling. Indeed, HMGB-1  
20 expression by macrophages can be suppressed *in vitro* [36] by AG490 (a JAK2 inhibitor, see  
21 below), or fludarabine (a STAT1 inhibitor). Macrophages also undergo pyroptosis promoted by  
22 STAT1-mediated caspase 11 activation [37]. These pathways participate in multiple organ failure

1 (MOF) also by activating apoptosis of non-immune cells, such as hepatocytes, cardiac myocytes,  
2 and fibroblasts. Furthermore, STAT1 plays a well-known role in stress-induced apoptosis.

3 Altogether, the overwhelming inflammation mediated by JAKs-STATs during septic shock  
4 contributes to mortality through tissue damage and multiple organ failure syndrome [19], forcing  
5 the host to mount anti-inflammatory compensation mechanisms.

6

### 7 *Compensatory anti-inflammatory response syndrome and post-sepsis immunosuppression*

8 During sepsis, the systemic inflammatory response syndrome (SIRS) is rapidly followed by a down-  
9 regulation called compensatory anti-inflammatory response syndrome (CARS) (Figure 2) [9, 38],  
10 involving predominantly STAT3 and STAT6. CARS starts from the onset of septic shock [9] and its  
11 suspected physiological function is to protect cells and tissues from an excessive inflammatory  
12 immune response during septic shock. Several anti-inflammatory cytokine receptors also use the  
13 JAK-STAT pathways: IL-10 downregulates proinflammatory interferon-induced genes by  
14 suppressing STAT1 phosphorylation and initiates the STAT3-mediated anti-inflammatory Th2  
15 response [39]. Indeed, activated STAT3 limits the production of TNF and IL-6 in murine models of  
16 LPS-induced sepsis, particularly in monocytes and macrophages. The STAT3 anti-inflammatory  
17 role is confirmed by the hyperinflammation and subsequent lethality following peritonitis in mice  
18 lacking STAT3 selectively in macrophages and neutrophils [40, 41], likewise during LPS-induced  
19 endotoxin shock [42]. Similarly, conditional STAT5-deficient mice in dendritic cells and  
20 macrophages, using CD11c-cre mediated deletion, exhibit exaggerated LPS-induced inflammatory  
21 responses and vascular leak [43], which can be explained by the need of STAT5 to promote a Th2  
22 response [44].

1 IL-4 and IL-13 are two other immunoregulatory cytokines requiring STAT6 for signal transduction  
2 [28] and participating in Th2 response. Accordingly, *Stat6*<sup>-/-</sup> mice exhibit increased Th1 responses  
3 and produce higher levels of TNF and IL-6 during endotoxemia [15]. Of note, increased Th1  
4 response and enhanced bacterial clearance improved survival of *Stat6*<sup>-/-</sup> mice following CLP (Table  
5 1) [32].

6 In addition, the JAK-STAT-dependent inflammation is negatively regulated by the three main  
7 families of inhibitors : SOCS [45], PIAS [46] and PTPases [47]. Activated STATs induce the  
8 transcription of *Socs* genes, which are part of a retro control mechanism. SOCS proteins target  
9 STATs or JAKs by facilitating their ubiquitin-mediated degradation and preventing their  
10 phosphorylation, thereby down-regulating proinflammatory cytokine production during sepsis.  
11 Thus, SOCS3 contributes to the control of hyperinflammation by inhibiting STAT1, STAT4 and  
12 STAT5, while SOCS1 interacts with Tyk2 and STAT1. SOCS action on JAK-STAT can be cell  
13 specific, like macrophages following LPS stimulation or phagocytes upon CLP [48, 49]. PIAS  
14 family members are also involved in these negative feedback loops: PIAS1 and PIASx inhibit  
15 STAT1 and STAT4, respectively [50, 51]. Among PTPases, SHP1 (Src homology 2 (SH2) domain-  
16 containing protein tyrosine phosphatase 1), SHP2, and PTP1B show important immunomodulatory  
17 roles in response to LPS in mice [52-55], SHP1 being the best example. Indeed, mice lacking SHP-  
18 1 (motheaten mice) exhibit hyper-responsive macrophages after stimulation by LPS and IFN- $\gamma$ , as  
19 attested by increased TNF secretion and iNOS protein accumulation [52]. Moreover, Cre-Lox mice  
20 with hepatocyte-specific SHP1 deficiency show higher mortality following LPS stimulation and  
21 exhibit increased levels of pro-inflammatory cytokines [53], which indicate an  
22 immunoregulatory role of SHP1 through STAT1, STAT3, STAT5 and STAT6  
23 dephosphorylation [56]. PTP1B-deficient mice display higher survival and bacterial clearance



1 during *Pseudomonas aeruginosa* infection [55], in line with the role of PTP1B on STAT1- and  
2 STAT3-mediated signaling [57].

3 These anti-inflammatory processes may last for weeks and lead to post-sepsis immunosuppression.  
4 Following CARS, immunosuppression induced by sepsis [58] is the second leading cause of death  
5 after multiple organ failure [13]. Sepsis-induced immunosuppression includes apoptosis of immune  
6 cells, immunoregulatory mechanisms and cellular exhaustion [58]. The JAK-STAT pathway is a  
7 potentially interesting therapeutic target because of its critical importance in sepsis-induced  
8 immunosuppression.

9 Importantly, STAT1 and 5 regulate FOXP3 gene expression in Humans, which is constitutively  
10 expressed by the regulatory T lymphocytes (Tregs) involved in post-sepsis immunoparalysis [59].  
11 Lymphocytes apoptosis, which is another important component of immunosuppression following  
12 sepsis, is partially regulated by STAT3-activated caspase 3 and by STAT5 activating Bcl-2.  
13 Lymphocyte proliferation is controlled by STAT6 regulating the cell-cycle inhibitor p27Kip1 and  
14 by SOCS3 inhibiting STAT5 phosphorylation. Myeloid-derived suppressor cells (MDSCs), which  
15 also contribute to immunosuppression, require the NF- $\kappa$ B/C/EBP $\beta$ /STAT3 axis, and their survival is  
16 promoted by STAT5 [60]. Finally, STAT3 is involved in the reduced expression of major  
17 histocompatibility complex (MHC) class II genes leading to limited antigen-presentation [61].  
18 Hence, reduced HLA-DR expression [62] on monocytes quantified by flow cytometry is a classical  
19 biomarker of sepsis-induced immunosuppression which correlates with intensive care unit (ICU)-  
20 acquired infection [63] and high mortality [64]. Altogether, these examples highlight the therapeutic  
21 potential of targeting JAK-STAT signaling to counter sepsis-induced immunosuppression.

22

23 **JAK-STAT and organ failure during sepsis**

1 *Multiple organ failure*

2 Sepsis and septic shock are responsible for multiple organ failure. The involvement of the JAK-  
3 STAT pathways during sepsis is a matter of debate and seems to depend on the experimental  
4 settings. Indeed, CLP-induced peritonitis generates a local (peritoneal) pro-inflammatory Th1  
5 response and a systemic immunoregulatory Th2 response, while LPS-induced endotoxemia is  
6 responsible for a generalized systemic Th1 hyper-inflammatory response [15]. In this section, we  
7 discuss the relationships between JAK-STAT signaling and experimental sepsis-related organ  
8 dysfunction (Figure 1).

9

10 *Liver failure*

11 During LPS-induced endotoxemia, *Stat6*<sup>-/-</sup> mice exhibit leukocyte infiltration in the liver and  
12 hepatocyte necrosis, along with up regulation of NF-κB, increased pro-inflammatory cytokines  
13 (MIP-1α, MIP-2, interferon-γ-induced protein 10 (IP-10), and monocyte chemoattractant protein 1  
14 (MCP-1)) expression and significant hepatocellular injury attested by increased aspartate and  
15 alanine transaminase (AST and ALT) production [15]. These observations suggest that STAT6  
16 prevents liver failure, possibly by inducing a Th2 response regulating systemic inflammation.

17 In contrast, *Stat4*<sup>-/-</sup> mice display an increased resistance to CLP-induced lethality [65] as well as  
18 reduced liver failure, attested by significantly lower plasma levels of transaminases (AST, ALT).  
19 This correlates with fewer neutrophil infiltrate in the liver and reduced levels of neutrophil  
20 chemokines MIP-2 (CXCL2) and KC (CXCL1), whereas IL-13 and IL-10 expression was increased  
21 [32], arguing for a deleterious pro-inflammatory Th1 response mediated by STAT4 [65].

22

23 *Kidney failure*

1 *Stat4*<sup>-/-</sup> mice are resistant to CLP-induced acute kidney failure, as assessed by their significantly  
2 lower plasma levels of urea and creatinine, reduced renal levels of MIP-2 and KC [32]. *Stat6*<sup>-/-</sup> mice  
3 also displayed reduced CLP-induced kidney injury and lower MIP-2 and KC levels. This suggests  
4 that the more effective bacterial clearance observed in these mice after CLP could reduce the  
5 severity of sepsis and subsequent organ failure.

6

### 7 *Lung inflammation*

8 During LPS endotoxemia, *Stat6*<sup>-/-</sup> mice display increased inflammation in the lungs, attested by up  
9 regulation of NF-κB, increased pro-inflammatory cytokines expression and granulocytes and  
10 infiltration of mononuclear cells, especially lymphocytes [15]. *Stat4*<sup>-/-</sup> mice displayed an increased  
11 myeloperoxidase (MPO) activity in the lungs, evocative of neutrophil accumulation. Of note, these  
12 observations were not supported by lung dysfunction after 24 hours. This contrasts with CLP-  
13 induced sepsis, during which the levels of MIP-2 and KC in the lung of *Stat4*<sup>-/-</sup> mice were  
14 significantly decreased [32].

15

### 16 *Endothelial dysfunction and implication of endothelial cells during sepsis*

17 Sepsis is characterized by an endothelial dysfunction [66], participating in vasomotor paralysis,  
18 coagulopathy [67], capillary leak and multiple organ failure. JAK3 is expressed in vascular  
19 endothelial cells, especially when stimulated by LPS, TNF, IL-1β and IFN-γ. Through STAT3  
20 phosphorylation and NF-κB activation, JAK3 drives overexpression of the endothelial adhesion  
21 molecules ICAM-1, VCAM-1 and PECAM-1, enabling the attachment of leukocytes and platelets  
22 to the **endothelium** and their transmigration into peripheral tissues [68]. While STAT3-mediated  
23 expression of PECAM-1 was shown to maintain endothelial integrity, ICAM1 promotes STAT3-

1 mediated leukocyte transmigration and increases the overall microvascular permeability, which is  
2 the onset of sepsis-induced capillary leak. This overexpression of cell adhesion molecules can be  
3 prevented by the JAK3 inhibitor Janex-1 or by the platelet-activating factor (PAF) that attenuates  
4 LPS-mediated interactions between neutrophils and endothelial cells [69]. STAT2 controls  
5 excessive ICAM-1-mediated cellular egress and *Stat2*<sup>-/-</sup> mice exhibit higher lethality by a  
6 mechanism centered on enhanced endothelial dysfunction, in line with ICAM-1 overexpression and  
7 subsequent cellular transmigration [70].

8 Sepsis also promotes atherosclerosis through IFN- $\gamma$ -induced activation of JAK2/STAT1 in  
9 endothelial cells, an inflammatory response partially prevented by salvianolic acid.

10

#### 11 *Heart failure*

12 Sepsis induces an acute myocardial dysfunction after LPS-induced endotoxemia [71] as well as in  
13 CLP models. In both cases, altered cardiac functions, increased myocardial inflammatory cytokines  
14 production, cardiomyocyte apoptosis [72, 73] and neutrophil infiltration are observed. STAT1 was  
15 shown to mediate apoptosis during sepsis-induced myocardial dysfunction. Endothelial dysfunction  
16 during sepsis contributes to myocardial dysfunction through JAK3/STAT3-mediated LPS-induced  
17 myocardial vascular leakage, which can be prevented by the JAK3 inhibitor Janex-1. IL-6 also  
18 induces cardiac dysfunction during LPS-induced endotoxemia [74]. Of note, exchange protein  
19 activated by cAMP (Epac-1) activation [75] or over-expression could prevent IL-6-induced  
20 cardiomyocyte dysfunction as well as sepsis-induced arrhythmia through decreased STAT3  
21 phosphorylation and subsequent inhibition of iNOS expression. Interestingly, Epac-1 is a recently  
22 described target of cAMP signaling, suggesting a new STAT-mediated mechanism of  
23 catecholamine action on sepsis-induced myocardial dysfunction [76].

1

## 2 **Therapeutic targets for experimental and clinical research**

### 3 *Jakinibs*

4 JAK inhibitors, also called *JAKinibs*, are a growing recent drug class, whose most representative  
5 examples are listed in Table 2, with focus on their use in clinical trials. These molecules inhibit one or  
6 more Janus kinases, thereby preventing the phosphorylation and activation of the corresponding STAT  
7 proteins. So far, most of the drugs targeting the JAK-STAT pathways have been used for their  
8 immunosuppressive or antitumoral properties. Among them, the most studied currently are ruxolitinib  
9 [77], tofacitinib [78-80], and baricitinib [81]. Ruxolitinib, which was the first non-selective JAK1/2  
10 inhibitor to be approved, was developed for the treatment of myeloproliferative neoplasms [82].  
11 Tofacitinib, a non-selective JAK1/2/3 inhibitor, was then approved for the treatment of rheumatoid  
12 arthritis. In spite of the overall high number of clinical trials involving JAK inhibitors, none is scheduled  
13 in the field of sepsis.

14

### 15 *Direct inhibitors of the JAK-STAT pathways in experimental research*

16 As demonstrated above, the JAK-STAT pathways play heterogeneous roles during sepsis. Because they  
17 can regulate either the Th1/Th2 balance, but also emergency myelopoiesis or endothelial activation,  
18 modulation of JAK-STAT pathways for therapeutic purposes in sepsis is of major interest. Experimental  
19 data confirm this hypothesis. Ruxolitinib, a JAK1/JAK2 inhibitor, is able to improve the prognosis of  
20 *Candida* sepsis in mice [83]. Of note, results obtained with ruxolitinib during murine sepsis illustrate the  
21 importance of the timing of this immunomodulatory medication: when started one day before sepsis,  
22 ruxolitinib acts as an immunosuppressor and reduces survival, while providing ruxolitinib one day after  
23 infection onset improves survival, depending on the dose. The best survival is obtained for a dose of

1 6.25 mg/kg/d, for which the hyperinflammation is down-regulated without increasing the pathogen load  
2 [84].

3 Other experimental studies have shown the effectiveness of JAK2 inhibitors and confirm that the best  
4 time of administration is about 24 hours after the onset of sepsis. Indeed, JAK2 inhibitors increase  
5 survival when administered 24 hours after the onset of CLP-induced polymicrobial sepsis in mice and  
6 rats by decreasing the expression of proinflammatory mediators such as TNF, IL-6, and HMGB-1. This  
7 effect is obtained through JAK2-inactivation by the jakinib AG490, which acts as a STAT3 inhibitor by  
8 preventing tyrosine phosphorylation. This concentration-dependent inactivation specifically precludes  
9 activation of the canonical NF- $\kappa$ B pathway but does not prevent the alternative pathway, which allows  
10 maintaining host antimicrobial defense. Of note, AG490 is not available for clinical use but has the same  
11 mode of action as WP1066 (listed in Table 2), which entered clinical trials.

12 At last, inhibitors of the JAK-STAT pathways could avoid post-sepsis immunosuppression. Indeed, the  
13 two STAT3 inhibitors sunitinib and axitinib were reported to inhibit the recruitment of MDSCs into  
14 renal carcinoma tumors in mice [85]; this is in line with STAT3 roles in the expansion and survival of  
15 MDSCs [86] involved in immune escape. MDSCs are among the most promising therapeutic targets to  
16 treat post-sepsis immunosuppression [87], providing a central position for STAT3 inhibitors during the  
17 late phase of sepsis.

18 Altogether, these inhibitors offer novel possibilities to target the JAK-STAT signaling pathways in  
19 experimental and clinical research on sepsis.

20  
21 *Modulators of the JAK-STAT pathways and applicability in sepsis*

22 **JAK-STAT-based therapies and the two sides of sepsis-induced dysimmunity.** As seen above,  
23 sepsis is a progressive disease, involving many tissues and cell types, initiated by a hyperinflammatory

1 response followed by immune cells exhaustion, both of which being responsible for the high mortality  
2 rate of this condition (Figure 2). Importantly, the common feature between these cell types and immune  
3 dysfunctions is their requirement of the JAK-STAT pathway. This has led us to consider the potential  
4 interest of JAK inhibitors to modulate sepsis using time- and organ-oriented therapies.

5

6 **Early phase of sepsis and hyperinflammation.** During the acute hyperinflammatory phase of sepsis,  
7 using the immunosuppressive properties of JAK-STAT inhibitors might be tricky. Although ruxolitinib  
8 is able to improve the survival during *Candida* sepsis in mice, a 2.6%-risk of infections was observed in  
9 Humans [88]. As stated above, ruxolitinib doses and timing of administration greatly influence the  
10 immune state, the subsequent pathogen clearance and survival [84]. Besides, a withdrawal syndrome is  
11 described when patients cease ruxolitinib treatment, which is termed “ruxolitinib discontinuation  
12 syndrome” and can lead to a cytokine storm that could be highly detrimental in a context of sepsis [89].  
13 All this makes the systemic delivery double-edged in human sepsis, in which the real onset time of the  
14 infection may be unknown, with greater variability of the offending pathogen, of its initial load, and of  
15 the immune response. A recent meta-analysis showed a low rate of serious infections with jakinibs [90];  
16 such potential immunosuppressive effects make their systemic delivery risky, leading us to suggest  
17 alternative strategies.

18 A more specific, cell- or tissue-targeted strategy could be an alternative of choice and is currently one of  
19 the best research focus to improve inflammatory diseases treatment. This targeting strategy is based on a  
20 nanoparticle encapsulation of therapeutic molecules [91]. This long-dreamed strategy to avoid the  
21 systemic side effects of drugs of interest is now technically possible and several options have now been  
22 developed (Figure 3), as evidenced by the newly designed nano-encapsulated JAKinibs baricitinib in  
23 2019 [92]. In 2017, ruxolitinib-conjugated gold nanoparticles were designated for topical administration,

1 with a reduced systemic absorption into the blood flow [93]. Nanoparticles have a ligand that allow  
2 targeting of specific cells or tissue, ranging from a given organ [94] to immune cells subtypes such as  
3 macrophages [95], B cells [96] and T cells [97]. This targeting allows two major strategies during the  
4 hyperinflammatory phase of septic shock (Figure 2 and Figure 4). The first one is to target a tissue  
5 highly implicated in the pathophysiology of septic shock, such as the endothelium, in which  
6 dysfunctional JAK3 and STAT3 are involved, with repercussion on heart dysfunction (see above). The  
7 existence of nanoparticles targeting the endothelium *via* CD31 [98] makes it possible to integrate an  
8 anti-STAT3 or anti-JAK3 (Decernotinib) to reduce the endothelial dysfunction. The second strategy is to  
9 target a specific immune cell type involved in hyperinflammation, such as macrophages (Figure 3B and  
10 Figure 4) with CD206-targeted nanoparticles [95].

11 This targeted strategy aims at a macrophage reprogramming towards M2 polarization by JAK1  
12 inhibitors, such as Tofacitinib [99], in order to modulate inflammation at the acute phase of sepsis  
13 without killing the immune cells. Interestingly, this cell-targeted strategy may also apply to post-sepsis  
14 immunosuppression, according to the targeted cells.

15

16 **Post-sepsis immunosuppression.** Despite the absence of clinical trials directly targeting JAK-STAT  
17 during post-sepsis immunosuppression until now, this track must be kept in view. Indeed, treatments  
18 currently in therapeutic trials in the field of sepsis impact on the JAK-STAT pathways to mediate their  
19 effect. IL-7, which aims at restoring post-sepsis immunity [100] by promoting CD4+ T cells survival,  
20 and which entered phase 2 clinical studies in this perspective (e.g. NCT03821038)<sup>†</sup>, upregulates Bcl-2  
21 by phosphorylating STAT5. As the IL-7 signaling pathway involves STAT5, phosphorylated STAT5  
22 could be a convenient theranostic marker to follow and evaluate IL-7 effects, particularly in T  
23 lymphocytes [101] to monitor the treatment of sepsis-induced immunosuppression. Moreover,



1 nanoparticles can target T cells (Figure 3B) *via* the anti-PD1 pembrolizumab [97], which also acts as  
2 anti-apoptotic agent on T cells. Such T cell-targeted nanoparticles could be loaded with IL-7 (Figure 4).  
3 During sepsis-induced immunosuppression, cellular exhaustion also involves macrophages, and CD206-  
4 targeted NPs could be proposed for M1 reprogramming [95], which enables to use phosphorylated  
5 STAT3 as a theranostic marker [102] (Figure 2). In addition to using the status (phosphorylation) of  
6 JAK-STAT proteins as biomarkers, JAK-STAT inhibitors may be an adjuvant treatment for  
7 immunomodulators, especially during post-sepsis immunoparalysis (Figure 2). This strategy is inspired  
8 by recent therapeutic trials in oncology, in which STAT3 inhibitors such as sunitinib or axitinib are  
9 combined with the anti-PD1 nivolumab in phase 1 and 2 studies (NCT03277924<sup>II</sup>, NCT03172754<sup>III</sup>) to  
10 prevent MDSC-mediated immune escape [103]. Indeed, some therapeutic targets in post-sepsis  
11 immunosuppression are common with oncology, especially the immune checkpoint molecules PD1 and  
12 CTLA4: anti-PD1 and anti-CTLA4 both improve survival in Humans with malignant diseases; they also  
13 increase survival in mice during post-sepsis immunosuppression [104]. Moreover, the anti-PD1  
14 nivolumab is already being tested for its capacity to relieve sepsis-induced immunosuppression in a  
15 phase 1 study (NCT02960854)<sup>IV</sup>. The demonstrated role of MDSCs in the resistance to immunotherapy  
16 in cancer patients [103] suggests that an association of nivolumab with STAT3 inhibitors could be  
17 relevant in future trials to control sepsis-induced immunosuppression. Similarly with the cell-targeted  
18 strategies considered for the hyperinflammatory phase, MDSCs could be targeted by nanoparticles,  
19 which would minimize the side-effects of JAK/STAT inhibitors.

20

## 21 **Concluding remarks**

22 Targeting JAKs and/or STATs might prove to become an interesting approach to reduce mortality in  
23 septic shock patients because these proteins are clearly involved in immunological dysfunctions and

1 multiple organ failure. Original JAK/STAT-based therapies should be designed to treat sepsis-induced  
2 immunosuppression, and could reach new targets in this field, such as MDSCs. Moreover,  
3 phosphorylated STATs may offer creative theranostic markers to drive such treatments. Of note, the  
4 large number of animal studies on JAK-STAT pathways and their well-known effects on sepsis  
5 strikingly contrast with the lack of clinical trials, which, up to now, mainly concern the fields of  
6 autoimmune diseases and onco-hematology. This may be explained by the pleiotropic effects (and  
7 sometimes opposing, such as proinflammatory or immunoregulatory) of the various JAK-STAT family  
8 members, depending on the cell-type in which they act and the signal that triggers their activation.  
9 Therefore, development of innovative JAK/STAT-based therapies in sepsis should probably integrate a  
10 tissue-or cell-targeted approach (See Outstanding Questions), which can be achieved through the design  
11 of innovative nanoparticles. From this perspective, JAK-STAT-based therapies could reduce  
12 hyperinflammation in a selected organ, or in a key tissue such as the endothelium, thereby allowing the  
13 improvement of organ dysfunctions during sepsis.

14 We have thus highlighted tactical approaches (Figure 4) which combine recent existing therapeutic  
15 means at different stages of clinical use, most of them in pathologies other than sepsis until now. These  
16 strategies provide technically feasible treatments to counteract the two main causes of mortality during  
17 sepsis, namely the multiple organ failure syndrome and the immunosuppression following sepsis. Thus,  
18 the development of JAK/STAT-based clinical trials will be the next exciting step towards improving the  
19 prognosis of sepsis patients.

20

1 **Box 1. JAK-STAT and hematopoiesis during sepsis.**

2 Pro- and anti-inflammatory molecular mechanisms are accompanied by a hematopoietic cellular  
3 response. Indeed, fighting against bacterial pathogens requires a rapid and massive production of  
4 immune cells, a process termed “emergency myelopoiesis” or “emergency granulopoiesis” [105],  
5 mainly driven by G-CSF and MIP-2 (CXCL2). G-CSF is produced by hematopoietic stem and  
6 progenitor cells (HSPC) and endothelial cells. Granulocyte-colony stimulating factor receptor (G-  
7 CSFR) activation induces STAT3 phosphorylation in bone marrow hematopoietic stem cells,  
8 resulting their proliferation and expansion [106]. STAT3 is phosphorylated by JAK1-2 and Tyk2  
9 during emergency myelopoiesis and plays a major role in this process. It directly controls the  
10 expression of CCAAT-enhancer-binding protein  $\beta$  (C/EBP $\beta$ ), a key factor in the emergency  
11 myelopoiesis response, upregulating c-Myc and activating hematopoietic cells proliferation and  
12 Fanconi C (FANCC) gene, a DNA repair pathway maintaining myeloid cells viable during this  
13 emergency proliferation [107]. In a positive feedback loop, phospho-STAT3 upregulates the  
14 transcription of the genes encoding MIP-2 (CXCL2) and its receptor (C-X-C motif chemokine  
15 receptor, CXCR2) [108, 109].

16

1 **Box 2. Clinician's Corner**

2 From the clinician's point of view, the prognosis of sepsis patients is greatly influenced by the two  
3 leading causes of mortality following disease onset: the SIRS-induced multiple organ failure syndrome  
4 and the sepsis-induced immunosuppression resulting in late infections and long-term mortality. The  
5 balance between proinflammatory and anti-inflammatory immune responses is therefore the core of  
6 these two issues that physicians have to deal with, and the JAK-STAT pathways are fully involved in  
7 both sides of these responses. The challenge is to highlight and put into practice the tremendous  
8 potential of JAK-STAT-based therapies in clinical research on sepsis.

9

10 Several JAK-STAT-based therapeutic approaches have been identified, like the *jakinibs*. The  
11 comprehension of JAK-STAT involvement in each tissue and cell type has become essential, because  
12 each STAT may exhibit different, cell-specific roles. Therefore, the most appropriate approach to use  
13 these molecules in sepsis likely requires cell- or tissue-targeted strategies, which are made possible by  
14 the use of cell-targeted nanoparticles, thereby providing an exciting new area of clinical research in  
15 sepsis.

16

17 SIRS relies on STAT1, 2 and 4, which are involved in liver failure, lung inflammation and acute kidney  
18 injury. In addition, STAT3 mediates endothelial activation and dysfunction and subsequent  
19 cardiovascular dysfunction. Endothelial dysfunction plays a key role during septic shock, as it is  
20 responsible for vasoplegia participating in hemodynamic failure, coagulopathy resulting in  
21 **disseminated intravascular coagulation (DIC)** and capillary leak that worsens all organ damages.  
22 Moreover, STAT1 and 3 are involved in sepsis-induced myocardial failure, which is an aggravating  
23 component of the hemodynamic failure. These recent discoveries may indicate a path towards

1 developing a tissue-targeted inhibition of the JAK/STAT pathways. An endothelium-targeted inhibition  
2 of STAT3 would be of major interest in future experimental and clinical research.

3

4 Following hyperinflammation, CARS, which requires STAT3 and 6, can lead to a long-lasting sepsis-  
5 induced immunosuppression. Therefore, STAT3 inhibitors such as sunitinib or axitinib are drugs of  
6 choice to counteract the anti-inflammatory immune response mediated by several types of  
7 immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs). Moreover, using  
8 nanoparticles coupled to specific receptors, JAK/STAT-based therapies could target Th2 cells, M2  
9 macrophages or MDSCs, and would be a highly innovative therapeutic track.

10

1 **Table 1: Respective roles of JAK and STAT family members in the host response against bacterial sepsis.**

STATs	Upstream cytokine	JAK	Downstream genes <sup>#</sup>	Effector cells	Response to bacterial infections	Phenotype of STAT <sup>-/-</sup> mice		
						CLP-Resistant	LPS-Resistant	[Ref]
<b>STAT1</b>	IFN- $\gamma$ IFN- $\alpha/\beta$ IL-27, IFN- $\lambda$	JAK1, JAK2 JAK1, Tyk2	Interferon-Stimulated Genes*	Monocytes Macrophages	Proinflammatory Th1	Yes	Yes	[14, 31]
<b>STAT2</b>	IFN- $\alpha/\beta$	JAK1, Tyk2	Interferon-Stimulated Genes*	Monocytes Macrophages Neutrophils	Proinflammatory Th1	None Published	No (more susceptible)	[70]
<b>STAT3</b>	IL-6 IL-10 G-CSF	JAK1-2, Tyk2 JAK1, Tyk2 JAK1-2, Tyk2	Immunoregulatory genes (e.g. TGF- $\beta$ ) Apoptosis regulation (e.g. BCL-XL) Granulopoiesis (e.g. C/EBP $\beta$ , c-Myc)	Monocytes Macrophages MDSC	Proinflammatory Th17 (IL-6) Immunoregulatory Th2 (IL-10) Emergency granulopoiesis	No (more susceptible) <sup>†</sup>	No (more susceptible) <sup>†</sup>	[41, 42]
<b>STAT4</b>	IL-12	JAK2, Tyk2	IFN- $\gamma$ , AP-1 (IFN- $\gamma$ promoter) IRF-1 IL-12R (positive feedback loop)	Lymphocytes Natural killers Dendritic cells	Proinflammatory Th1	Yes	No (more susceptible)	[15, 32]
<b>STAT5a</b>	IL-2, IL-3	JAK2	Foxp3	Lymphocytes	Immunoregulatory Th2 (IL-2,3)	None Published	No (more susceptible) <sup>‡</sup>	[43]
<b>STAT5b</b>	IL-7 GM-CSF	JAK1,3 JAK1,2	Bcl-2 Granulopoiesis (e.g. C/EBP $\alpha/\beta/\delta$ )	(CD4 T cells) Myeloid cells	T cell survival (IL-7) Emergency granulopoiesis			
<b>STAT6</b>	IL-4 IL-13	JAK1, JAK3 JAK1-2, Tyk2	IL-4, IL-13 (positive feedback loop) IL-5, IL-9 Gata3	Lymphocytes (B & T cells)	Immunoregulatory Th2	Yes	No (more susceptible)	[15, 32]

2 **Footnotes:**

3 \*mediated by the Interferon-Stimulated Gene Factor 3 (ISGF-3), heterotrimeric transcription factor complex associating STAT1,  
4 STAT2 and IRF-9. Interferon-Stimulated Genes include hundreds of genes aiming at eliminating the invading pathogen (eg. IRF1,3,7).

5 <sup>#</sup>Each STAT protein may activate a plethora of genes in response to cytokine signaling. Only some of the most representative  
6 activated genes during sepsis are mentioned above

7 <sup>†</sup>conditional deficient mice in neutrophils and macrophages (LysM-cre mediated deletion), as Stat3<sup>-/-</sup> mice are not viable.

8 <sup>‡</sup>conditional deficient mice in dendritic cells and macrophages (CD11c-cre mediated deletion), as Stat5<sup>-/-</sup> mice exhibit a high  
9 perinatal lethality.

10 Abbreviations: AP-1, activator protein-1; CLP, cecal ligation and puncture; G-CSF, Granulocyte-Colony Stimulating Factor;  
11 interferon; IL, interleukin; IRF, interferon regulatory factor; JAK, Janus kinase; LPS, lipopolysaccharides; LysM, lysozyme M; STAT,  
12 signal transducers and activator of transcription; TGF- $\beta$ , Transforming Growth Factor- $\beta$ ; Tyk, tyrosine kinase.

1 **Table 2: JAK/STAT Inhibitors: Targets, indications and current status in clinical research.**

Types of inhibitors	Inhibitor	Targeted JAK/STAT	Indications	Phase	Therapeutic trials in progress *
Non selective	<b>Ruxolitinib</b>	JAK1, JAK2	Myelofibrosis / myeloproliferative neoplasms Hematological malignancies Auto-immune diseases	FDA-approved (2011)	223
	<b>Tofacitinib</b>	JAK1, JAK2, JAK3#	Autoimmune diseases (First FDA approval : rheumatoid arthritis)	FDA-approved (2012)	167
	<b>Baricitinib</b>	JAK1, JAK2	Autoimmune diseases	FDA-approved (2018)	76
	<b>Peficitinib</b>	JAK1, JAK2, JAK3, Tyk2#	Autoimmune diseases	Approved in Japan (2019)	34
	<b>Momelotinib</b>	JAK1, JAK2	Myelofibrosis / myeloproliferative neoplasms Advanced Solid Malignancies	III	14
	<b>PF-06700841</b>	Tyk2, JAK1	Autoimmune diseases (Systemic lupus erythematosus, Psoriasis, Inflammatory Bowel Diseases)	II	19
	<b>Cerdulatinib</b>	JAK1, JAK3, Tyk2	Lymphoid malignancies Vitiligo	II B	3
	<b>AZD-1480</b>	JAK1, JAK2	Advanced Solid Malignancies	I	3
Selective	<b>Upadacitinib</b>	JAK1	Autoimmune diseases (First FDA approval : rheumatoid arthritis)	FDA-approved (2019)	41
	<b>Filgotinib</b>	JAK1	Autoimmune diseases (rheumatoid arthritis, Crohn's disease)	III	37
	<b>BMS-986165</b>	Tyk2	Autoimmune diseases (Systemic lupus erythematosus, Psoriasis, Inflammatory Bowel Diseases)	III	36
	<b>Pacritinib</b>	JAK2	Myelofibrosis Hematological malignancies	III	23
	<b>PF-06651600</b>	JAK3	Autoimmune diseases (Alopecia areata, Inflammatory Bowel Diseases)	III	24
	<b>Fedratinib</b>	JAK2	Myelofibrosis / myeloproliferative neoplasms	FDA-approved (2019)	13
	<b>Solcitinib</b>	JAK1	Systemic lupus erythematosus	II	0
	<b>Decernotinib</b>	JAK3	Rheumatoid arthritis	II	0
Other inhibitors	<b>Sunitinib</b>	STAT3†	Renal cell carcinoma (RCC) gastrointestinal stromal tumor (GIST)	FDA-approved (2006)	606
	<b>Axitinib</b>	STAT3‡	Renal cell carcinoma (RCC)	FDA-approved (2012)	150
	<b>AZD-9150</b>	STAT3	Advanced Solid Malignancies Digestive neoplasms, ovarian cancer	II	11
	<b>WP-1066</b>	STAT3	Brain neoplasm	I	2

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**Footnotes (Table 2):**

#"pan-JAK" inhibitor. †sunitinib is a multi-targeted receptor tyrosine kinase (RTK) inhibitor, which prevents STAT3 phosphorylation. ‡axitinib is an inhibitor of VEGF-receptor, which is a receptor tyrosine kinase; axitinib prevents STAT3 phosphorylation, especially in endothelial cells. \*Number of clinical trials in progress listed in <https://clinicaltrials.gov/> as of May 20, 2020. Does not include the past trials. Abbreviations: JAK, Janus kinase; STAT, signal transducer and activator of transcription; Tyk, tyrosine kinase; VEGF, vascular endothelial growth factor.



1 **Glossary**

2

3 **Compensatory anti-inflammatory response syndrome (CARS):** Anti-inflammatory process aiming at  
4 limiting the consequences of SIRS during sepsis and restoring the immune balance

5

6 **Cecal ligation and puncture (CLP):** Mouse model for sepsis. CLP-mice undergo a laparotomy, a  
7 ligation and puncture of the caecum, which is then reintegrated in the peritoneum. Mice develop  
8 peritonitis within a few hours, resulting in sepsis.

9

10 **Disseminated intravascular coagulation (DIC):** Abnormal activation of coagulation and defective  
11 fibrinolysis, resulting in the formation of disseminated microthrombosis, along with a consumption of  
12 platelets and clotting factors, resulting in bleeding complications.

13

14 **Endothelium:** Single layer-type of epithelium lining the interior of the heart and blood vessels.  
15 Normally, the endothelial surface is impermeable, antiaggregant and anticoagulant.

16

17 **Granulocyte-Colony Stimulating Factor (G-CSF):** Cytokine acting as a growth factor, which  
18 stimulates neutrophils production. Neutrophils are white blood cells belonging to the granular lineage.

19

20 **Interferons (IFNs):** Cytokines produced by immune cells during the proinflammatory response to an  
21 infection. Interferons can be of type I ( $\alpha, \beta$ ), II ( $\gamma$ ) or III ( $\lambda$ ). Interferons stimulate the production and  
22 activation of immune cells.

23

1 **Interferon regulatory factor-9 (IRF-9):** Intracytoplasmic protein which, in association with  
2 phosphorylated forms of STAT1 and STAT2, forms the trimeric transcription factor interferon-  
3 stimulated gene factor 3 (ISGF-3).

4

5 **Janus kinase (JAK):** Intracellular tyrosine-kinases allowing the transduction of signals downstream of  
6 cytokine receptors, and phosphorylating the signal transducers and activator of transcription (STATs).

7

8 **Jakinibs:** Recent pharmaceutical class inhibiting Janus kinases (JAK).

9

10 **Lipopolysaccharide (LPS):** essential component of the outer membrane of Gram-negative bacteria.

11

12 **Pathogen associated molecular patterns (PAMPs):** Molecular motifs specific to a class of  
13 microorganisms and activating innate immunity *via* the **PRRs** (e.g LPS is a PAMP from Gram-negative  
14 bacteria which is recognized by the PRR called TLR4).

15

16 **Pathogen recognition receptors (PRRs):** Receptors expressed by innate immunity effector cells,  
17 recognizing PAMPs and activating proinflammatory response to fight off the pathogen. PRRs include  
18 Toll-like receptors (TLRs).

19

20 **Sepsis:** Dysregulated hyperinflammatory host response to an infection, resulting in organ dysfunction.

21

22 **Septic shock:** Sepsis with acute circulatory failure, defined by a low blood pressure requiring  
23 vasopressors and by hyperlactatemia, which reflects tissue hypoxia.

1

2 **Systemic inflammatory response syndrome (SIRS):** Hyperinflammatory response with release of  
3 proinflammatory cytokines, which can be triggered by an infection, and aiming at fighting off the  
4 pathogen.

5

6 **Signal transducers and activator of transcription (STAT):** Cytoplasmic transcription factors  
7 enabling, after their phosphorylation by Janus kinases (JAK), the transduction of cytokines signals from  
8 the cell membrane to the nucleus.

9

10 **Th1 response:** Proinflammatory immune response mediated by T-bet<sup>+</sup> CD4<sup>+</sup> T cells (type 1 T helper  
11 cells), producing IFN- $\gamma$ .

12

13 **Th2 response:** Immune response mediated by GATA3<sup>+</sup> CD4<sup>+</sup> T cells (type 2 T helper cells) involved in  
14 the anti-inflammatory response following Th1 and leading to the production of IL-10. Th2 is also  
15 involved in atopy and in the response against helminths *via* IL-4, -5, -13.

16

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9

10 **Resources**

- 11 I. This study is registered with ClinicalTrials.gov and available at  
12 <https://clinicaltrials.gov/ct2/show/NCT03821038>
- 13 II. This study is registered with ClinicalTrials.gov and available at  
14 <https://clinicaltrials.gov/ct2/show/NCT03277924>
- 15 III. This study is registered with ClinicalTrials.gov and available at  
16 <https://clinicaltrials.gov/ct2/show/NCT03172754>
- 17 IV. This study is registered with ClinicalTrials.gov and available at  
18 <https://clinicaltrials.gov/ct2/show/NCT02960854>

19

20 **Author's contributions**

21 All the authors participated in the writing and editing of the manuscripts. RCJ and JH reviewed the  
22 clinical trials.

23

24

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47

48



1 **Figure legends**

2

3 **Figure 1. JAK-STAT signaling in sepsis-induced organ dysfunction.** STAT1, 2, 3, 4 and 6 have  
4 been linked to experimental sepsis-related organ dysfunctions. STAT1 and 2 proinflammatory  
5 response (Th1) is mediated by the Interferon-Stimulated Gene Factor 3 (ISGF-3), which is a  
6 heterotrimeric transcription factor complex associating STAT1, STAT2 and Interferon Regulatory  
7 Factor 9 (IRF-9). STAT 4 essentially exert pathogenic effects by promoting Th1 immune responses  
8 involved in acute kidney and liver failures and lung inflammation leading to acute respiratory  
9 distress syndrome (ARDS). Conversely, STAT6 mediates Th2 response and acts by two different  
10 ways: 1) by directly restricting liver inflammation or 2) by compromising Th1-mediated bacterial  
11 clearance and exacerbating acute kidney injury and lung inflammation. The JAK3-STAT3 pathway  
12 was also associated to myocardial failure directly and indirectly by promoting endothelial  
13 dysfunction. STAT3-mediated endothelial dysfunction plays a key role in sepsis-induced multiple  
14 organ failure (MOF), because of the endothelial role in capillary leak, vasoplegia and disseminated  
15 intravascular coagulopathy (DIC), all of which worsen the tissue hypoxia observed in septic shock.  
16 Moreover, tissues injuries are responsible for the generation of damage-associated molecular  
17 patterns (DAMPs) that reinforce the proinflammatory response, forming a vicious circle. Red  
18 arrows symbolize organ insults and their mechanisms. Protective effects are indicated in green.

19

20 **Figure 2, Key figure. JAK-STAT-targeted therapies in sepsis.** Therapeutic strategies vary  
21 according to the phase of sepsis and the cells that are involved. During the early phase of sepsis, the  
22 systemic inflammatory response syndrome (SIRS) triggers organ damage through an exacerbated  
23 cellular and molecular inflammatory response, part of which is JAK-STAT-mediated. Nanoparticles

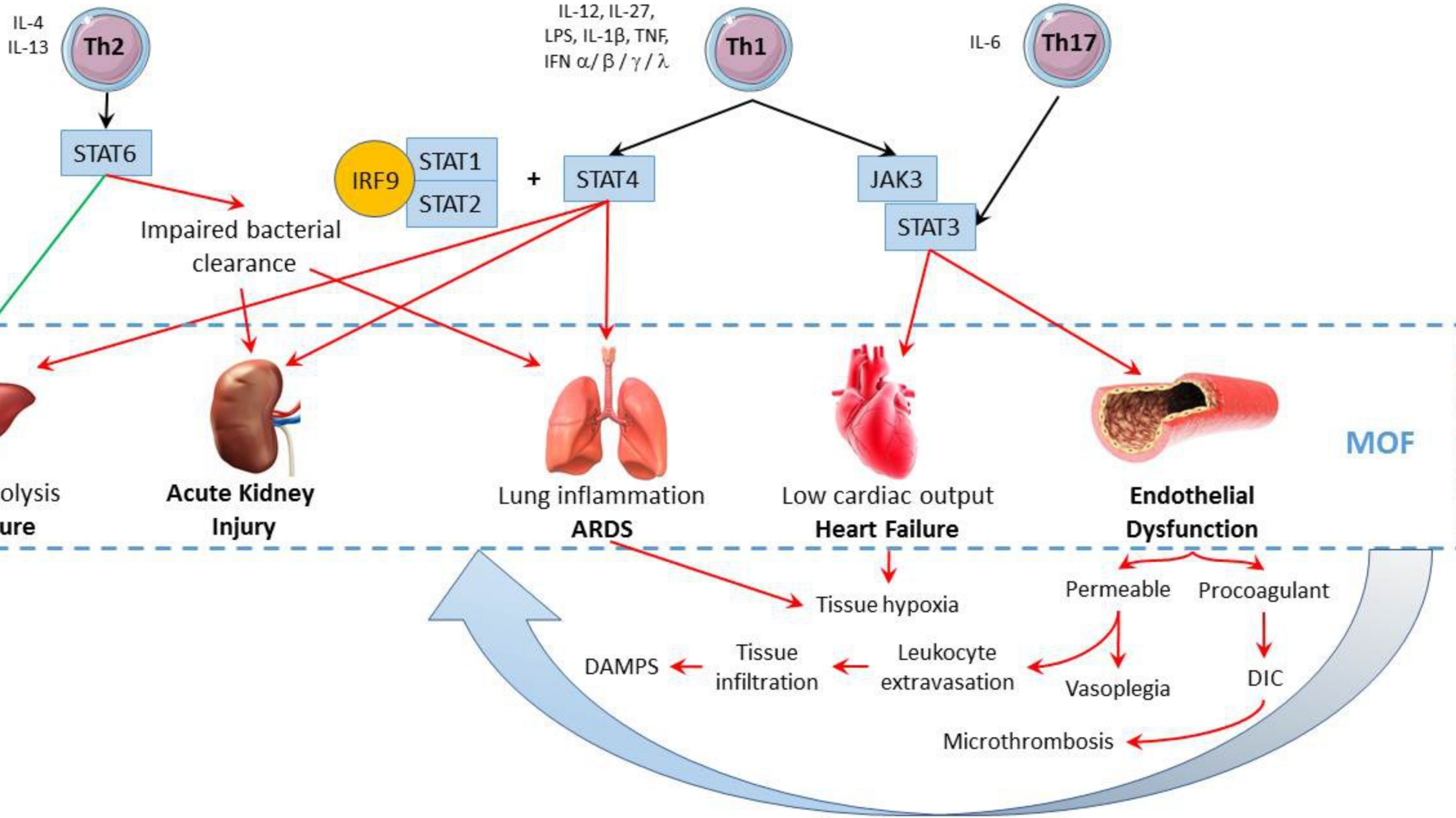
1 (NPs) allow targeted JAK-STAT inhibition on cells of interest. Using this cell-targeted strategy, the  
2 following therapies are proposed: 1) Macrophage-targeted tofacitinib to perform a M2  
3 reprogramming, which leads to an immunoregulatory phenotype; 2) Endothelium-targeted  
4 decernotonib (Jak3 inhibitor) or axitinib (inhibiting STAT3) to reduce endothelial dysfunction and  
5 subsequent multiple organ failure; 3) Kidney- or liver-targeted tofacitinib to reduce the deleterious  
6 consequences of STAT1/4-mediated Th1 response on these organs. Of note, targeting the  
7 endothelium allows to target both the liver and kidneys, which are rich in blood vessels. During the  
8 late phase of sepsis, the compensatory anti-inflammatory response syndrome (CARS) leads to  
9 sepsis-induced immunosuppression and results in late infections and long-term mortality. CARS  
10 also uses the JAK-STAT pathways. Therefore, the following strategies are considered: 4) Using  
11 sunitinib (STAT3 inhibitor) as an adjuvant treatment with boosting immunotherapies (nivolumab,  
12 ipilimumab) to prevent the immune escape mediated by myeloid-derived suppressor cells (MDSCs);  
13 5) Interleukin-7 (IL-7) stimulating STAT5-mediated T cells survival, which allows to use  
14 phosphorylated STAT5 as a theranostic marker; 6) Macrophages-targeted NPs containing mRNA  
15 aiming at M1 reprogramming, which enables to use phosphorylated STAT3 as a theranostic marker.  
16

17 **Figure 3. Existing strategies for JAK-STAT-based therapies of sepsis.** (a) Already existing  
18 jakinib-coupled nanoparticles (NPs): 1) Ruxolitinib-conjugated gold NPs (Boca *et al.* [93])  
19 developed to treat alopecia topically while limiting systemic absorption; 2) Nano-encapsulated  
20 baricitinib (Ansari *et al.* [92]) in poly lactic-co-glycolic acid (PLGA); PLGA-NPs allow to increase  
21 oral bioavailability. (b) Cell-specific targeted strategies: 1) Human endothelial CD31-targeted  
22 poly(lactic acid)-poly(ethylene)glycol-nanoparticles (PLA-PEG-NPs) developed to administer  
23 therapeutics directly to the graft before kidney transplantation (Tietjen *et al.* [98]); 2) Macrophage-

1 targeted polyglutamic acid-NPs (Zhang *et al.* [95]) using CD206 as a receptor (mannose receptor),  
2 and containing mRNA encoding M1-polarization; 3) B-cell-targeted paclitaxel loaded albumin-NPs,  
3 aiming at treating CD20+ B-cell lymphoma (Nevala *et al.* [96]); 4) T-cell-targeted PEG-PLGA-NPs  
4 binding to PD-1 (Schmid *et al.* [97]).

5

6 **Figure 4: Innovative human cell- and tissue-targeted therapeutics acting on JAK-STAT**  
7 **during sepsis: (a)** CD31-targeted PEG-PLGA-NPs delivering decernotinib (anti-JAK3) to  
8 endothelial cells to reduce endothelial dysfunction and subsequent multiple organ failure during  
9 systemic inflammatory response syndrome (SIRS); **(b)** CD206-targeted PEG-PLGA-NPs delivering  
10 tofacitinib (JAK1-3 inhibitor) selectively to macrophages during SIRS for M2-reprogramming to  
11 reduce hyperinflammation without killing macrophages; **(c)** PD-1-targeted immunostimulation of T-  
12 cells by IL-7 during sepsis-induced immunosuppression, resulting in STAT5-mediated T-cell  
13 survival. T-cells phosphorylated STAT5 is proposed as a theranostic marker.



Hepatic cytolysis  
**Liver Failure**

**Acute Kidney Injury**

Lung inflammation  
**ARDS**

Low cardiac output  
**Heart Failure**

**Endothelial Dysfunction**

**MOF**

DAMPs

Tissue infiltration

Leukocyte extravasation

Vasoplegia

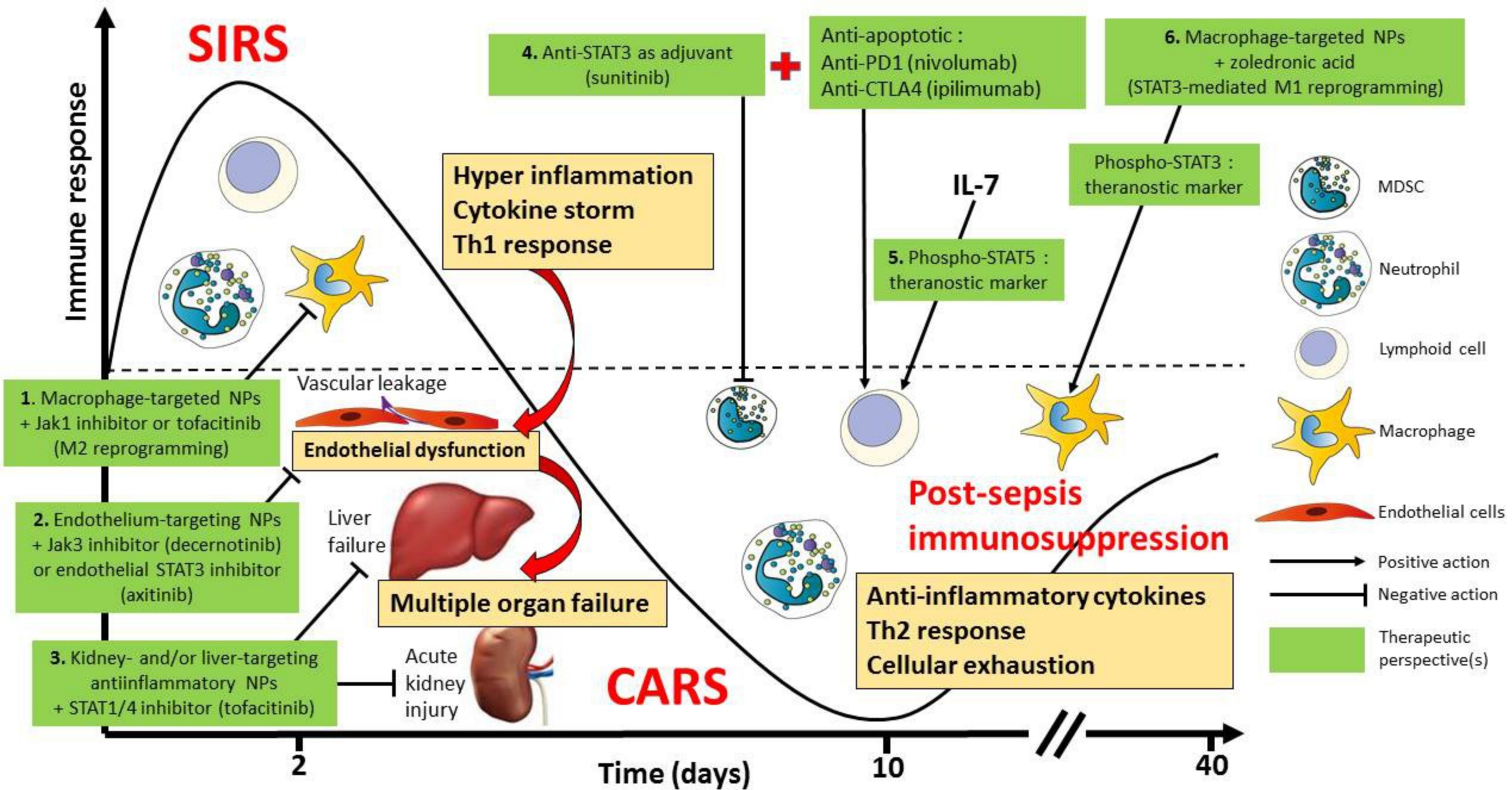
DIC

Microthrombosis

Permeable

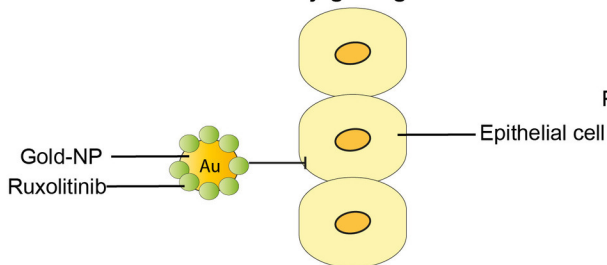
Procoagulant

Tissue hypoxia

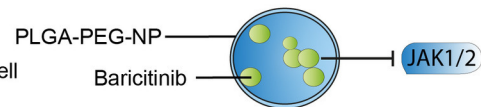


## (a) Already existing jakinin-coupled NPs

### 1. Ruxolitinib-conjugated gold NPs

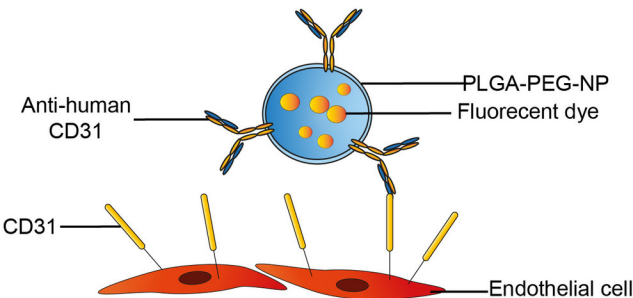


### 2. Nano-encapsulated baricitinib

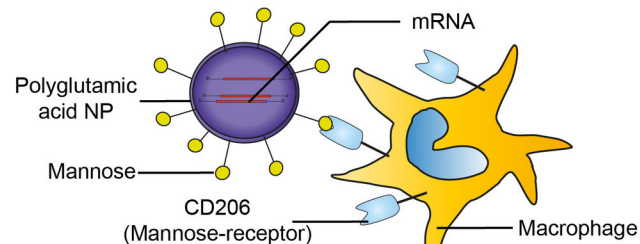


## (b) Cell-specific targeting strategies

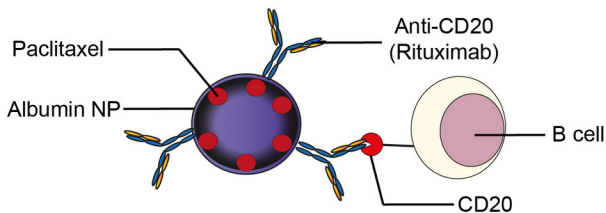
### 1. Endothelium-targeted NPs



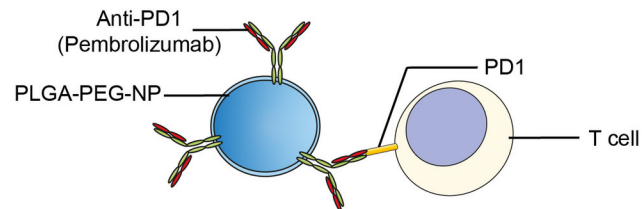
### 2. Macrophage-targeted NPs



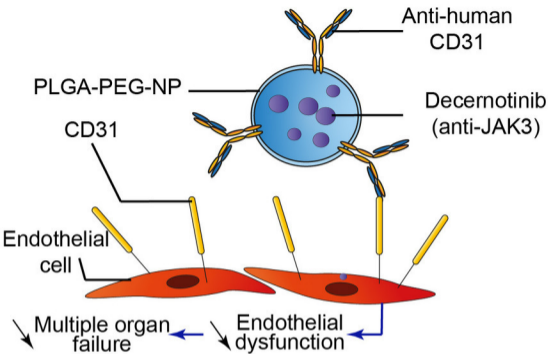
### 3. B cell-targeted targeted NPs



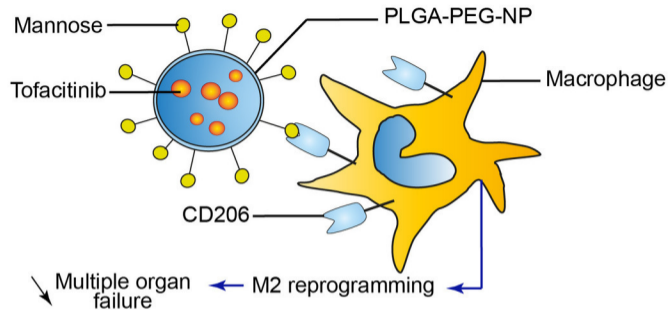
### 4. T cell targeted NPs



**(a) Endothelium-targeted anti-JAK3**



**(b) Macrophage-targeted M2 reprogramming**



**(c) T cell-targeted immunostimulation**

