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Abstract

Allogeneic stem cell transplantation (allo-SCT) is a curative therapy for different life-threatening malignant and non-malignant hematologic disorders. Acute graft-versus-host disease (aGVHD) and particularly gastro-intestinal aGVHD remains a major source of morbidity and mortality following allo-SCT, which limits the use of this treatment in a broader spectrum of patients. Better understanding of aGVHD pathophysiology is indispensable to identify new therapeutic targets for aGVHD prevention and therapy. Growing amount of data suggest a role for Th17 cells in aGVHD pathophysiology. In this review, we will discuss the current knowledge in this area in animal models and in humans. Based on it, we will then describe new potential treatments for aGVHD along the Th17 axis.

Introduction

Allogeneic stem cell transplantation (allo-SCT) is a curative therapy for different life-threatening hematologic malignancies. The therapeutic efficacy of allo-SCT relies on the combination of the cytoreductive effect of the conditioning chemotherapy and/or radiotherapy and of the graft-versus-tumor (GVT) effect mediated by the donor's immunocompetent cells (CD8+ and CD4+ T cells, natural killer cells and dendritic cells). However, the beneficial effect of graft-versus-tumor effect is counterbalance by the immunological recognition and destruction of host tissues by the donor's immune effectors, termed graft-versus-host disease (GVHD). GVHD remains a major source of morbidity and mortality following allo-SCT. Gooley et al.[1] recently reported a substantial reduction in death related to allo-SCT and an increased long-term survival. Similarly, we reported[2] a significant reduction of non-relapse mortality significantly and improvement of overall survival in the 2001-2010 period, compared to 1983-2000 period, while the incidence of acute GVHD (aGVHD) remained stable, and the incidence of extensive chronic GVHD increased, during the same period[2]. Therefore, it is essential to improve GVHD management.

Consequently, there have been several attempts to develop biological biomarkers to predict GVHD onset or responsiveness to treatment[3, 4]. This would allow a more stringent monitoring and intensified prophylaxis or curative treatment of GVHD in those patients. Furthermore, recent progress in medical imaging test and endoscopic techniques, such as contrast-enhanced ultrasound (CEUS) or probe-based confocal endomicroscopy (pCLE), may allow an earlier and more specific diagnosis of GVHD, particularly for gastrointestinal aGVHD (reviewed in 5). Finally, identification of new therapeutic targets and development of new immunosuppressive therapy are indispensables to further improve GVHD management.

The pathophysiology of aGVHD is a multistep process[8, 9]. In the first step, the conditioning regimen (chemotherapy and/or total body irradiation) leads to host tissue damage, release of proinflammatory cytokines, and increased expression of major (MHC) and minor histocompatibility antigens and co-stimulatory molecules on host antigen presenting cells (APC). In the second step, donor-derived CD4+ and CD8+ T cells are activated by host APC and migrate into

GVHD target tissues (gastrointestinal tract, skin and liver). In the third step, cellular mediators (such as cytotoxic T lymphocytes, activated macrophages and natural killer cells) and inflammatory cytokines act synergistically to enhance target tissue destruction[8, 9]. For a long time, we considered that a particular subset of CD4+ T helper (Th) cells, Th1 cells, was at play during the effector phase of aGVHD[10, 11]. However, the identification of a new Th subset, Th17 cells, raised the question of their role in aGVHD. Therefore, in this review we will discuss the most recent data on the contribution of these Th17 cells and Th17-related cytokines in aGVHD pathophysiology.

Th17 CELLS

Th17 cells differentiation

In 2005, two seminal studies[12, 13] showed in a mouse model that the development of Th17 cells from naïve precursors was independent of Th1 and Th2 specific transcription factors (T-bet and Gata-3), leading to the establishment of the Th17 lineage as independent and distinct from the Th1 and Th2 lineages[12, 13]. Another group reported that mouse Th17 cells uniquely expressed a transcription factor termed retinoid acid-related orphan receptor (ROR)γt (encoded by the gene *Rorc*)[14].

Besides RORγt, STAT3 is the second transcription factor required for Th17 cells differentiation. STAT3 has pleiotropic functions as a transcriptional activator for *Rorc*, *Il17, Il17F, IL23R* and others genes implied in Th17 cell differentiation or survival in murine models[15]. Several cytokines play a role in Th17 cell differentiation, upon the control of the RORγt and STAT3 transcription factors.

In murine models, IL-6 has an essential role in this process by activating STAT3[16], which directly drives the transcription of Th17 lineage specific genes[15] and suppresses TGFβ-induced forkhead box P3 (FOXP3) expression, thereby inhibiting regulatory T cell (Treg) development[17]. IL-6 also induces the expression of IL-1R1 by mouse Th17 cells[18]. Il-1β, through its receptor IL-1R1, promotes the transcription factor interferon-regulatory factor 4 (IRF4), which reinforces the expression of RORγt, and enhances Th17 proliferation in experimental models[18]. Therefore, these data suggest that if IL-6 directly drives the differentiation of Th17 cells, IL-1β enhances the expansion of these cells.

Alternatively, IL-21 selectively induces the phosphorylation of STAT3 and replacement of IL-6 with IL-21 in combination with TGFβ, IFNγ and Il-4, was very effective to induce high level of IL-17 producing cells in a mouse model[19]. Furthermore, IL-21 is also produced by Th17 cells, promoting self-maintenance of Th17 cells[20]. Regarding TGFβ, although the data suggest that TGFβ is required for Th17 cells differentiation in mouse models[21, 22], it probably does not act as a direct Th17 cell-inducing factor, but rather allows Th17 cells differentiation indirectly by suppressing alternative cell fates[23, 24].

In humans, while the role of IL-6 and IL-1β is well established on *in vitro* cultures of human T cells[25-27], the contribution of TGFβ to Th17 cells differentiation remains a matter of debate. While some groups have shown that TGFβ is necessary for *in* vitro Th17 cell differentiation[28, 29], others found that Th17 cells could differentiate without TGFβ, upon stimulation with a cocktail of IL-6, IL-1β and IL-23[25-27]. However, these are *in vitro* data, and it is difficult to draw definitive conclusion regarding the role of TGFβ for Th17 cells differentiation under *in vivo* inflammatory conditions in human. Besides, IL-21, produced by a number of T cells and the NK cell subset, is also involved in human Th17 cells differentiation via STAT3 signaling [29].

IL-23 is another key cytokine for Th17 cells commitment. Therefore, despite the combination of IL-6 and TGFβ being sufficient to drive Th17 cells differentiation, the cells generate by this combination fail to induce pathogenicity in mouse models[30]. However, IL-6 and TGFβ, induce IL-23R expression[16], and subsequent exposure to IL-23 stabilizes the phenotype[31] and expands the pathogenicity of Th17 cells[27, 30, 32].

Th17 cell plasticity

Despite initial thoughts that CD4+ naïve T cells differentiate into terminal phenotypes in a rigid process, it is now quite well accepted that, depending on the cytokine milieu, certain Th subset can adopt a mixed phenotype or switch entirely to the transcription and cytokine profile of another lineage. Given that TGFβ suppresses Th1 and Th2 differentiation in mouse[23, 24], both Th17 and induced Treg (iTreg) development is favoured in its presence. Therefore, after TCR engagement, CD4+ T cell differentiation into Th17 or iTreg in the presence of TGFβ will depend on the cytokine environment: in the absence of pro-

inflammatory signals, naïve T cells will differentiate into iTregs, whereas IL-6 will promote Th17 cell development both in mouse models and *in vivo* in human[23, 24, 33]. The presence of all-*trans* retinoid acid in the microenvironment seems to inhibit Th17 and promote iTreg development in mouse, in part, at least, by antagonizing the effect of IL-6[34-37]. Therefore, Yang et al. demonstrated in a mouse model that upon IL-6 stimulation, both natural Treg and iTreg repress Foxp3 and produce IL-17[38], suggesting that fully differentiated Treg could be converted into Th17-like cells. In human, Koenen et al. have shown *in vivo* that circulating Foxp3+ Treg can differentiate into IL-17-producing cells RORγt+, given that APC, in particular monocyte, and the cytokine IL-2 or Il-15 are present[39]. Of note, this differentiation process was enhanced by exogenous IL-1β, Il-23 and IL-21, whereas IL-6 or TGFβ did not affect the emergence of Il-17 producing cells[39]. The *in vivo* existence of hybrid Treg/Th17 cells in human has been established in inflamed intestinal mucosa of patients with Crohn disease[40]. These cells express Foxp3 and RORγt and produce IL-17, however unlike conventional Th17 cells, they functionally retain their suppressive activity *in vitro*[40]. A similar TCR β chain variable region between Treg/Th17 and Treg cells suggest that those Treg/Th17 cells arise form Treg cell when exposed to the inflammatory signals present in inflamed Crohn disease tissue[40]. Similarly, Voo et al. reported that human peripheral blood and lymphoid tissue contain a subpopulation of Foxp3+ Treg cells that coexpress RORγt and have the capacity to produce Il-17 upon activation[41]. In contrast, the conversion of Th17 cells into Treg has not been reported so far.

Similarly, the Th1 and the Th17 pathways share a common point: a critical event in the late development of both pathways is the induction of a receptor for an IL-12 cytokine family member: IL-12 for Th1 and IL-23 for Th17. These receptors share a common subunit, the IL-12Rβ1, associated with the IL-12Rβ2 to form the IL-12 receptor[42], and with the IL-23R to form the IL-23 receptor. Similarly to the IL-23R up regulation during Th17 cell differentiation[16], IL-12Rβ2 is up regulated during Th1 cell differentiation[43]. However, during their differentiation, Th17 cells also weakly express the IL-12Rβ1. Therefore, both *in vivo* mouse data and *in vitro* human studies have shown that depending on the balance between the cytokines present in the milieu, IL-12 can induce the conversion of Th17 cells

into interferon γ (IFNγ)-producing Th1 like cells[44, 45]. These cells maintain their IL-17 memory upon subsequent culture[44, 45]. Finally, *in vivo* existence in Crohn disease patients of a Th1/Th17 hybrid subset that arises from the modulation of Th17 cells by Il-12 has been established[45].

Regarding Th2 subset, *in vitro* culture of mouse T cells under mixed Th1 and Th2 conditions resulted in a continuum of mixed phenotypes with subpopulations of cells expressing only IFNγ, only Il-4 or both cytokines, correlating with expression level of Tbet and Gata-3[46]. The *in vivo* existence of Th1/Th2 hybrid subset has been confirmed in a mouse model; during infection with *Heligmosomoides polygyrus,* a parasite that triggers a strong Th2 response, Th1/Th2 hybrid cells, that express simultaneously Tbet and Gata-3, have been observed[47]. Hegazy et al. demonstrated in a murine model that injection of Th1 cell-promoting lymphocytic choriomeningitis virus reprogrammed otherwise stably committed Gata-3+ Th2 cells to adopt a Gata-3+ Tbet+ and IL-4+ IFNγ+ "Th1/Th2" phenotype that was maintained *in vivo* for months, [48]. Moreover, Th2 cell reprogramming into hybrid Th1/Th2 subset required TCR stimulation and concerted type I and II IFN and Il-12 signals[48]. Finally, since the presence of IL-4, during *in vitro* naive T cell activation, inhibits RORγt expression and Il-17 production, hybrid Th2/Th17 were thought not to exist. However, Califano et al. have recently shown in a mouse model of autoimmune encephalomyelitis that Th17 deficient in the transcription factor BCL11B upregulate the Th2 associated proteins Gata-3 and IL-4 without decreasing RORγt and IL-17 level[49]. So far no data has been reported on the existence of Th1/Th2 or Th2/Th17 hybrid subset in human.

Th17 role

Th17 cells produce several cytokines, which are not typically produced by Th1 and Th2 cells. These cytokines include IL-17A, IL-17F, IL-17A/F, IL-21, IL-22, GM-CSF or human IL-26, and many other factors[50]. Th17 are usually present in the *lamina propria* of the small intestine[14] and can be rapidly induced in other mucosal sites during infection[51-53]. Therefore, Th17 cells and Th17 related cytokine contribute to the host defence against a wide variety of pathogens, predominantly extracellular bacteria and fungal pathogens, in the epithelial

barrier of gut, skin and lung[50, 54]. Thus, once released, Il-17 and IL-22 act synergistically to enhance mucosal site defences by the production of antimicrobial peptide such as βdefensine-2 or S100 proteins[55].

Finally, Th17 responses also contribute to the pathogenesis of some diseases[50]. Therefore, their contribution to the pathophysiology of several autoimmune and autoinflammatory diseases affecting epithelial barrier, such as psoriasis or inflammatory bowel disease, is well established[50]. Given that aGVHD involves mostly the gastrointestinal tract, the skin and the liver, which contain epithelial barrier, Th17 contribution has been explored in aGVHD pathophysiology.

Th17 CELLS IN aGVHD

Studies in mouse models of aGVHD

The contribution of Th17 cells in aGVHD pathophysiology has been demonstrated in several mouse models. Lu et al. found that phosphorylation of STAT3, a transcriptional factor involved in Th17 cell differentiation[15], was important during T cells alloactivation during aGVHD and that interference with STAT-3 phosphorylation can inhibit T cell activation and proliferation *in vitro* and aGVHD *in vivo*, suggesting a role for Th17 cells in aGVHD[56]. Thereafter, Carlson et al. and Iclozan et al. have shown that adoptive transfer of *in vitro* differentiated Th17 cells mediate IL17-dependent lethal aGVHD with severe tissue lesions [57, 58]. In a mouse model of aGVHD using IL-17-/- donor CD4+T cells, Kappel et al.[59] found that aGVHD development was significantly delayed compared to recipients of WT CD4+ T cells, although the overall GVHD mortality remained unaffected. They concluded that despite Il-17 being dispensable for aGVHD, it contributes to its early development[59]. In contrast, Yi et al.[60] reported on a similar model that transplantation of IL-17 -/- donor CD4+ T cells induced exacerbated aGVHD compared to WT CD4+ T cells, while administration of recombinant IL-17 and neutralizing IFNγ to the recipients given IL-17 -/- donor cells ameliorated aGVHD. Their conclusion was that donor Th17 cells ameliorate aGVHD through down-regulation of Th1 cell differentiation[60]. Nevertheless, given the plasticity between Th17 and Th1 cells[44, 45], this result could be explained by an enhanced differentiation of Th1 cells in recipients given IL-17 -/- donor cells, and does not contradict a pathological role for Th17 cells in aGVHD.

Therefore, Gartlan et al. recently identified a population of inflammatory CD8+ cytotoxic T (Tc) 17 cells (iTc17) that develops rapidly after allo-SCT and contribute to GVHD but failed to maintain lineage fidelity[61].

Yi et al.[62] also showed that administration of donor CD4+ T cells depleted for both IFNγ and IL-4 (a Th2 related cytokine) resulted in augmented Th17 differentiation, and preferential, though not exclusive, aGVHD damage to the skin. Fulton et al.[63] have shown, in a major mismatch murine model, that deletion of *Rorc* in both CD4+ and CD8+ donor T cells attenuated aGVHD and decreased tissue pathology in the colon, liver and lung. Hill et al. have shown that use of granulocyte colony-stimulating factor (G-CSF) for stem cell mobilization invoke Th17 responses rather than Th1/Th2 differentiation[64]. Therefore, while transplantation of G-CSF-mobilized graft from WT or IL-17A-/- B6 donors resulted in identical GVHD outcome in models of aGVHD, transplantation of graft from IL-17A-/- BALB/c donors resulted in attenuated GVHD, suggesting a role for IL-17A in GVHD. However in both recipients of B6 and BALB/c donor grafts, IL-17A promoted cutaneous GVHD with increased levels of both inflammation and fibrosis in the skin of WT grafts, suggesting that use of G-CSF mobilized grafts promoted sclerodermatous chronic GVHD. In a more relevant haploidentical murine transplantation model, *Rorc-/-* CD4+ T cells alone diminished the severity and the lethality of aGVHD[63]. Finally, Uryu et al.[65] recently reported that α-Mannan, a major component of fungal cell wall, induced donor T cell polarization toward Th17, leading to exacerbated Th17pulmonary aGVHD in mice.

Some studies have also explored the role of cytokines implicated in Th17 cell differentiation. Therefore, inhibition of the Il-6 signaling pathway that drives Th17 cell differentiation by way of antibody-mediated blockade of the IL-6 receptor (IL-6R) markedly reduces pathologic damage attributable to GVHD[66]. This effect is accompanied by a significant reduction of Th1 and Th17 cells infiltrating aGVHD target tissues and a significant increase of Treg[66]. Similarly, Tawara et al.[67] reported that transplantation of *IL-6-/-* donor T cells or total inhibition of Il-6 with anti-Il-6R monoclonal antibody lead to a marked decrease in aGVHD severity and prolonged survival. However, they failed to demonstrate a role of donor T cells in this effect[67]. Other authors focused on IL-23, a cytokine

that stabilizes Th17 cell phenotype[31] and expands their pathogenicity[27, 30, 32]. Das et al.[68] have shown that donor antigen-presenting cells derived IL-23 drive gastro-intestinal aGVHD. The proinflammatory effect of Il-23 was reported to be dependent upon donor-derived secretion of interferon-γ and not IL-17, despite IL-17 being significantly decreased in *IL-23-/-* compared to wild-type donors[68]. Furthermore, they have shown that under IL-23 blockade, the graft-versus-leukemia (GVL) effect was preserved[69]. Thompson et al.[70] have confirmed that absence of IL-23 in donor grafts reduced the severity of aGVHD and was associated with a decrease of IL-17. Th17 cells produce IL-21, involved in their differentiation[20, 29], promoting therefore their self-maintenance[20]. Transplantation with *IL-21R-/-* donor T cells resulted in less severe aGVHD, while sparing the GVL effect[71-74]. Furthermore, IL-21 blockade using a monoclonal antibody also decreased aGVHD[75]. In these studies, the protective effect of IL-21 signaling pathway blockade on aGVHD was associated with an expansion of Tregs, and no effect was observed on the IL-17 axis[74, 75]. In a xenogeneic GVHD model, IL-21 blockade also significantly reduced aGVHD[76]; nevertheless, this reduction was associated with an increase in Tregs and a decrease of IL-17 producing cells[76].

Several studies have explored the contribution of another Th17-related cytokine, IL-22, in aGVHD pathophysiology. IL-22 is structurally related to the IL-10 family, secreted by Th17 cells, but also by others αβT cells (Th1, Th22 and CD8+ αβT cells), γδT cells, natural killer T cells, and innate lymphoid cells (ILC)[77]. IL-22 has been reported to exert both protective and inflammatory functions, most likely depending on the cytokine microenvironment and the tissue and/or the cell type involved[78]. Thus, while IL-22 has been shown to be protective in inflammatory bowel disease[79], it is pathogenic in psoriasis[80] and rheumatoid arthritis[81]. In aGVHD, we have recently reported that IL-22 deficiency in donor T cells can decrease the severity of aGVHD while sparing the GVL effect[82]. Furthermore, once weekly administration of IL-22, starting on day 0, aggravates aGVHD in animal models[83]. In contrast, Hanash et al.[84] showed that IL-22 produced by recipient ILC decreased aGVHD tissue damage by protecting intestinal stem cells. Therefore, according to the cell source (donor or patient),

IL-22 may have an either protective or inflammatory effect in aGVHD. The IL-22 axis remains to be further explored to decipher its exact role in aGVHD.

Overall, results from aGVHD mouse models, suggest that Th17 cells may play a role in aGVHD pathophysiology.

Studies in allo-SCT patients

The role of Th17 cells has also been investigated in human aGVHD pathophysiology. Three studies evaluated the relation between the presence of the single-nucleotide polymorphism (SNP) rs11209026 (1142G>A) in *IL-23R* and aGVHD[85-87]. In two studies,[85, 86] there was a significant reduction of aGVHD incidence in patients who where transplanted from a donor with the *IL-23R* SNP, while there was no effect when it was in the recipient, and the third study fail to identify any effect of the polymorphism[87]. In healthy donors, the presence of the *IL-23r* SNP promotes the expression of soluble IL-23R[88] and, consequently, diminished IL-23 signaling, leading to a decreased IL-23-dependent IL-17 and IL-22 production and STAT3 phosphorylation[89, 90]. These data suggest that protective effects of the *IL-23R* polymorphism on aGVHD are mediated through selective attenuation of IL-23 induced-Th17 effector function.

Dander et al.[91] and Liu et al.[92] have reported that Th17 cells and IL-17 serum level were significantly increased in the blood of patients at aGVHD onset, compared to allo-SCT patients without aGVHD. Furthermore, in both studies, the increased number of circulating Th17 cells was accompanied by a decrease in circulating Tregs[91, 92].

Early studies failed to identify Th17 cells infiltrating aGVHD target tissues. Thus, Broady et al.[93] reported that only Th1 and not Th17 cells infiltrate the skin of patients with cutaneous aGVHD. Similarly, Ratajczak at al.[94] did not find Th17 cells in skin and gut biopsies of patients with cutaneous or gastrointestinal aGVHD. Identification was based on detection of IL-17+ cells directly by immunohistochemistry in patients' biopsies in both studies[93, 94], or by flow cytometry after *in vitro* culture of dermal cells in the study by Broady et al.[93] However, Th17 cells could convert into interferon γ producing Th1 like cells[44, 45]. Given Th17 cells plasticity, IL-17 is probably not the most reliable marker. Therefore, using CD161 and CCR6, two surface marker, of Th17 cells[45, 95, 96] and

RORγt, the key transcription factor that orchestrate Th17 cell differentiation[14], we have shown that the number of Th17 was significantly increased in the intestinal mucosa and the skin of patients with gastro-intestinal or cutaneous aGVHD, compared with allo-SCT patients who did not developed aGVHD[97, 98]. Similarly, using the same two markers, van der Waart et al.[99] reported that Th17 cells infiltrate aGVHD-affected tissues (intestinal mucosae and skin) whilst being decreased in the peripheral blood during aGVHD. Recent data have shown that circulating Th17 cells may be increased early after allo-SCT in patients who will develop aGVHD. Thus, Lee et al.[100] showed that a high ratio of CD4+ CD161+ to CD8+ CD161+, and an increased level of serum Il-17 at engraftment were associated with subsequent development of aGVHD, and that those CD4+ CD161+ cells expressed high levels of RORγt. Similarly, Betts et al.[101] reported that at day 21 after allo-SCT, pSTAT3, a transcription factor that directly drives the transcription of Th17 lineage specific genes[15], was significantly increased in CD4+ T cells among patients who will subsequently develop aGVHD. Furthermore they confirmed that the number of CD3+ RORγt+ Th17 cells was significantly increased in aGVHD target tissues[101]. Recently, a novel CD146+CCR5+ T cell population was identified, this population was significantly increased at gastro-intestinal aGVHD onset, and proven to be Th17-related (Li W, Liu L, Gomez A, Zhang Q, Zhang J, Ramadan A *et al.* unpublished data,). Moreover, at day 19 post allo-SCT, those cells were significantly increased before aGVHD onset in patients who subsequently developed gastro-intestinal aGVHD, suggesting that this CD146+CCR5+ T cells population could be used as an early biomarker of intestinal aGVHD. Finally, Reinhardt et al.[102] demonstrated that peripheral monocytes isolated from patients with skin and/or gastro-intestinal induce significantly increased level of Th17 cell compared with patients without aGVHD, highlighting that activated monocytes could drive peripheral Th17 cells in aGVHD.

The role of the Th17-related cytokine, IL-22, as in murine models, seems to be dependent upon the cell source. Recently, Munneke et al. have shown that appearance of donor origin natural cytotoxicity receptor (NCR)-positive ILC, an important innate source of IL-22, correlated with a decreased incidence of aGVHD[103].

Overall, these results suggest that circulating Th17 cells are increased early after allo-SCT in patients who develop aGVHD, and that, at its onset, circulating Th17 decrease in the peripheral because they migrate into the aGVHD target tissue, where they trigger its damage (**Figure 1**).

Th17 CELLS: A NEW TARGET FOR aGVHD PREVENTION AND TREATMENT

So far, the most widely used immunosuppressive drugs for aGVHD prevention and therapy increase the infection risk, and present side effects other than those related to their immunosuppressive properties. Thus there is a need for more specific and less toxic approaches. Given growing evidence suggesting that Th17 cells play a role in aGVHD, they represent a promising therapeutic target towards which to design new approaches for aGVHD treatment, but also for prevention or pre-emptive therapy, since circulating Th17 are increased before aGVHD onset (**Table 1**, **Figure 2**).

Several monoclonal antibodies anti-IL-17A (ixekizumab, secukinumab) or anti-Il-17R (brodalumab) have proven to be effective in psoriasis, an IL-17 related auto-inflammatory skin diseases[104-106]. However, these results do not guarantee the effectiveness of these monoclonal antibodies in aGVHD. In fact, brodalumab and secukinumab were ineffective for Crohn's disease treatment[107, 108], while Il-17 was reported to drive Crohn's disease[108]. For the IL-22/IL-22R axis, further exploration to delineate its inflammatory versus protective effects in aGVHD is indispensable, before considering targeting it.

Given IL-1β and IL-6 drive Th17 cell differentiation, therapy targeting Il-1β and IL-6 has been evaluated in clinical trials. Blocking IL-1β using recombinant human IL-1R antagonist was proven to be ineffective for aGVHD prevention[109], while tocilizumab, an anti-IL-6R monoclonal antibody, has shown promising results for aGVHD prophylaxis in a phase 1/2 trial[110], and several phase 2 trial are ongoing. Since IL-23 expands the pathogenicity of Th17 cells[27, 30, 32], it appears to be also a promising therapeutic target. Therefore, ustekinumab, a monoclonal antibody that binds the p40 subunit shared by IL-12 and IL-23, approved for psoriasis and effective in Crohn's disease[111], has demonstrated efficacy in one case report of glucocorticoid-refractory aGVHD[112]. Ustekinumab is currently evaluated for aGVHD prevention in combination with tacrolimus and

sirolimus (NCT01713400). Several monoclonal antibodies targeting the IL-23p19 are also being evaluated in phase I, II or III trials for psoriasis and rheumatoid arthritis; raising the possibility to evaluate them for aGVHD treatment. Finally, several monoclonal antibodies targeting Il-21, a cytokine that promotes Th17 cell self-maintenance[20], are under development for rheumatoid arthritis, Crohn's disease and systemic lupus erythematosus[113], and represent a potential therapeutic strategy for aGVHD.

Some inhibitory molecules directly target Th17 cells. Thus, pharmacological inhibition with KD025 of Rho-associated kinase 2 significantly diminished STAT3 phosphorylation and binding to *IL-17* and *IL-21* promoters in mouse models[114]. Also, the Janus family kinase (JAK) inhibitors, Tofacitinib, a JAK1/3 inhibitor and Ruxolitinib, a JAK1/2 inhibitor, block STAT3 phosphorylation, resulting in the suppression of Th17 cell differentiation[115]. Tofacitinib and Ruxolitinib have proven to be effective for psoriasis treatment in human in phase III and II trials respectively. Zeiser et al. recently reported a retrospective study evaluating Ruxolitinib for corticosteroid refractory aGVHD in 54 patients[116]. The overall response rate was 81.5%, including 25 complete responses (46.3%), highlighting the therapeutic potential of JAK-inhibitors for the treatment of aGVHD[116]. Prospective studies evaluating Ruxolitinib and Tofacitinib for aGVHD prevention or treatment are expected. Finally, several additional molecules that could block the Th17 pathway are at a preclinical development stage, such as inhibitors of RORγt[117] or retinoid acid receptor α agonist[118].

Evaluation of Tofacitinib and

CONCLUSION AND PERSPECTIVE

Significant achievements have been made in the understanding of Th17 cells pathophysiology. Recent data showing an increased Th17 cell population during or preceding aGVHD are of particular interest, highlighting that these cells could be targeted not only for aGVHD treatment, but also earlier for its prevention. The increased number of monoclonal antibodies and inhibitory molecules targeting the Th17 pathway hold promise for identification of more effective treatment for aGVHD prevention and treatment. Efforts must be pursued to evaluate those forms of treatment in aGVHD.

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References.

1. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M *et al.* Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med* 2010; **363**(22)**:** 2091-2101.

2. Malard F, Chevallier P, Guillaume T, Delaunay J, Rialland F, Harousseau JL *et al.* Continuous Reduced Nonrelapse Mortality after Allogeneic Hematopoietic Stem Cell Transplantation: A Single-Institution's Three Decade Experience. *Biol Blood Marrow Transplant* 2014.

3. Paczesny S. Discovery and validation of graft-versus-host disease biomarkers. *Blood* 2013; **121**(4)**:** 585-594.

4. Paczesny S, Levine JE, Braun TM, Ferrara JL. Plasma biomarkers in graft-versus-host disease: a new era? *Biol Blood Marrow Transplant* 2009; **15**(1 Suppl)**:** 33-38.

5. Malard F, Mohty M. New insight for the diagnosis of gastrointestinal acute graft-versus-host disease. *Mediators Inflamm* 2014; **2014:** 701013.

6. Coron E, Laurent V, Malard F, Le Rhun M, Chevallier P, Guillaume T *et al.* Early detection of acute graft-versus-host disease by wireless capsule endoscopy and probe-based confocal laser endomicroscopy: results of a pilot study. *United European Gastroenterol J* 2014; **2**(3)**:** 206-215.

7. Bodet-Milin C, Lacombe M, Malard F, Lestang E, Cahu X, Chevallier P *et al.* F-FDG PET/CT for the assessment of gastrointestinal GVHD: results of a pilot study. *Bone Marrow Transplant* 2013.

8. Mohty M, Gaugler B. Inflammatory cytokines and dendritic cells in acute graft-versus-host disease after allogeneic stem cell transplantation. *Cytokine Growth Factor Rev* 2008; **19**(1)**:** 53-63.

9. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet* 2009; **373**(9674)**:** 1550-1561.

10. Krenger W, Ferrara JL. Graft-versus-host disease and the Th1/Th2 paradigm. *Immunol Res* 1996; **15**(1)**:** 50-73.

11. Holler E, Kolb HJ, Mittermuller J, Kaul M, Ledderose G, Duell T *et al.* Modulation of acute graft-versus-host-disease after allogeneic bone marrow transplantation by tumor necrosis factor alpha (TNF alpha) release in the course of pretransplant conditioning: role of conditioning regimens and prophylactic application of a monoclonal antibody neutralizing human TNF alpha (MAK 195F). *Blood* 1995; **86**(3)**:** 890-899.

12. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM *et al.* Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; **6**(11)**:** 1123-1132.

13. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; **6**(11)**:** 1133-1141.

14. Ivanov, II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ *et al.* The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006; **126**(6)**:** 1121-1133.

15. Durant L, Watford WT, Ramos HL, Laurence A, Vahedi G, Wei L *et al.* Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity* 2010; **32**(5)**:** 605-615.

16. Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS *et al.* STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem* 2007; **282**(13)**:** 9358-9363.

17. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M *et al.* Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; **441**(7090)**:** 235-238.

18. Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS *et al.* Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity* 2009; **30**(4)**:** 576-587.

19. Wei L, Laurence A, Elias KM, O'Shea JJ. IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. *J Biol Chem* 2007; **282**(48)**:** 34605-34610.

20. Zhou L, Ivanov, II, Spolski R, Min R, Shenderov K, Egawa T *et al.* IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 2007; **8**(9)**:** 967-974.

21. Veldhoen M, Hocking RJ, Flavell RA, Stockinger B. Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat Immunol* 2006; **7**(11)**:** 1151-1156.

22. Gutcher I, Donkor MK, Ma Q, Rudensky AY, Flavell RA, Li MO. Autocrine transforming growth factor-beta1 promotes in vivo Th17 cell differentiation. *Immunity* 2011; **34**(3)**:** 396-408.

23. Das J, Ren G, Zhang L, Roberts AI, Zhao X, Bothwell AL *et al.* Transforming growth factor beta is dispensable for the molecular orchestration of Th17 cell differentiation. *J Exp Med* 2009; **206**(11)**:** 2407-2416.

24. Qin H, Wang L, Feng T, Elson CO, Niyongere SA, Lee SJ *et al.* TGF-beta promotes Th17 cell development through inhibition of SOCS3. *J Immunol* 2009; **183**(1)**:** 97-105.

25. Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD *et al.* Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* 2007; **8**(9)**:** 950-957.

26. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 2007; **8**(9)**:** 942-949.

27. Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE *et al.* Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature* 2010; **467**(7318)**:** 967-971.

28. Volpe E, Servant N, Zollinger R, Bogiatzi SI, Hupe P, Barillot E *et al.* A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. *Nat Immunol* 2008; **9**(6)**:** 650-657.

29. Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. *Nat Immunol* 2008; **9**(6)**:** 641-649.

30. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T *et al.* TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol* 2007; **8**(12)**:** 1390-1397.

31. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM *et al.* The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat Immunol* 2009; **10**(3)**:** 314-324.

32. Haines CJ, Chen Y, Blumenschein WM, Jain R, Chang C, Joyce-Shaikh B *et al.* Autoimmune memory T helper 17 cell function and expansion are dependent on interleukin-23. *Cell reports* 2013; **3**(5)**:** 1378-1388.

33. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 2006; **24**(6)**:** 677-688.

34. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M *et al.* Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007; **317**(5835)**:** 256-260.

35. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y *et al.* A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007; **204**(8)**:** 1757-1764.

36. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J Exp Med* 2007; **204**(8)**:** 1765-1774.

37. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR *et al.* Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* 2007; **204**(8)**:** 1775-1785.

38. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP *et al.* Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. *Immunity* 2008; **29**(1)**:** 44-56.

39. Koenen HJ, Smeets RL, Vink PM, van Rijssen E, Boots AM, Joosten I. Human CD25highFoxp3pos regulatory T cells differentiate into IL-17-producing cells. *Blood* 2008; **112**(6)**:** 2340-2352.

40. Hovhannisyan Z, Treatman J, Littman DR, Mayer L. Characterization of interleukin-17-producing regulatory T cells in inflamed intestinal mucosa from patients with inflammatory bowel diseases. *Gastroenterology* 2011; **140**(3)**:** 957-965.

41. Voo KS, Wang YH, Santori FR, Boggiano C, Wang YH, Arima K *et al.* Identification of IL-17-producing FOXP3+ regulatory T cells in humans. *Proc Natl Acad Sci U S A* 2009; **106**(12)**:** 4793-4798.

42. Presky DH, Yang H, Minetti LJ, Chua AO, Nabavi N, Wu CY *et al.* A functional interleukin 12 receptor complex is composed of two beta-type cytokine receptor subunits. *Proc Natl Acad Sci U S A* 1996; **93**(24)**:** 14002-14007.

43. Rogge L, Barberis-Maino L, Biffi M, Passini N, Presky DH, Gubler U *et al.* Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J Exp Med* 1997; **185**(5)**:** 825-831.

44. Lexberg MH, Taubner A, Forster A, Albrecht I, Richter A, Kamradt T *et al.* Th memory for interleukin-17 expression is stable in vivo. *Eur J Immunol* 2008; **38**(10)**:** 2654-2664.

45. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B *et al.* Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007; **204**(8)**:** 1849-1861.

46. Antebi YE, Reich-Zeliger S, Hart Y, Mayo A, Eizenberg I, Rimer J *et al.* Mapping differentiation under mixed culture conditions reveals a tunable continuum of T cell fates. *PLoS Biol* 2013; **11**(7)**:** e1001616.

47. Peine M, Rausch S, Helmstetter C, Frohlich A, Hegazy AN, Kuhl AA *et al.* Stable T-bet(+)GATA-3(+) Th1/Th2 hybrid cells arise in vivo, can develop directly from naive precursors, and limit immunopathologic inflammation. *PLoS Biol* 2013; **11**(8)**:** e1001633.

48. Hegazy AN, Peine M, Helmstetter C, Panse I, Frohlich A, Bergthaler A *et al.* Interferons direct Th2 cell reprogramming to generate a stable GATA-3(+)T-bet(+) cell subset with combined Th2 and Th1 cell functions. *Immunity* 2010; **32**(1)**:** 116-128.

49. Califano D, Sweeney KJ, Le H, VanValkenburgh J, Yager E, O'Connor W, Jr. *et al.* Diverting T helper cell trafficking through increased plasticity attenuates autoimmune encephalomyelitis. *J Clin Invest* 2014; **124**(1)**:** 174-187.

50. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; **27:** 485-517.

51. Ivanov, II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009; **139**(3)**:** 485-498.

52. Pepper M, Linehan JL, Pagan AJ, Zell T, Dileepan T, Cleary PP *et al.* Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. *Nat Immunol* 2010; **11**(1)**:** 83-89.

53. Chen K, McAleer JP, Lin Y, Paterson DL, Zheng M, Alcorn JF *et al.* Th17 cells mediate clade-specific, serotype-independent mucosal immunity. *Immunity* 2011; **35**(6)**:** 997-1009.

54. Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal immunology* 2009; **2**(5)**:** 403-411.

55. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M *et al.* Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006; **203**(10)**:** 2271-2279.

56. Lu SX, Alpdogan O, Lin J, Balderas R, Campos-Gonzalez R, Wang X *et al.* STAT-3 and ERK 1/2 phosphorylation are critical for T-cell alloactivation and graft-versus-host disease. *Blood* 2008; **112**(13)**:** 5254-5258.

57. Carlson MJ, West ML, Coghill JM, Panoskaltsis-Mortari A, Blazar BR, Serody JS. In vitro-differentiated TH17 cells mediate lethal acute graft-versus-host disease with severe cutaneous and pulmonary pathologic manifestations. *Blood* 2009; **113**(6)**:** 1365-1374.

58. Iclozan C, Yu Y, Liu C, Liang Y, Yi T, Anasetti C *et al.* T helper17 cells are sufficient but not necessary to induce acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2010; **16**(2)**:** 170-178.

59. Kappel LW, Goldberg GL, King CG, Suh DY, Smith OM, Ligh C *et al.* IL-17 contributes to CD4-mediated graft-versus-host disease. *Blood* 2009; **113**(4)**:** 945-952.

60. Yi T, Zhao D, Lin CL, Zhang C, Chen Y, Todorov I *et al.* Absence of donor Th17 leads to augmented Th1 differentiation and exacerbated acute graft-versus-host disease. *Blood* 2008; **112**(5)**:** 2101-2110.

61. Gartlan KH, Markey KA, Varelias A, Bunting MD, Koyama M, Kuns RD *et al.* Tc17 cells are a pro-inflammatory, plastic lineage of pathogenic CD8+ T-cells that induce GVHD without anti-leukemic effects. *Blood* 2015.

62. Yi T, Chen Y, Wang L, Du G, Huang D, Zhao D *et al.* Reciprocal differentiation and tissue-specific pathogenesis of Th1, Th2, and Th17 cells in graft-versus-host disease. *Blood* 2009; **114**(14)**:** 3101-3112.

63. Fulton LM, Carlson MJ, Coghill JM, Ott LE, West ML, Panoskaltsis-Mortari A *et al.* Attenuation of acute graft-versus-host disease in the absence of the transcription factor RORgammat. *J Immunol* 2012; **189**(4)**:** 1765-1772.

64. Hill GR, Olver SD, Kuns RD, Varelias A, Raffelt NC, Don AL *et al.* Stem cell mobilization with G-CSF induces type 17 differentiation and promotes scleroderma. *Blood* 2010; **116**(5)**:** 819-828.

65. Uryu H, Hashimoto D, Kato K, Hayase E, Matsuoka S, Ogasawara R *et al.* alpha-Mannan induces Th17-mediated pulmonary graft-versus-host disease in mice. *Blood* 2015.

66. Chen X, Das R, Komorowski R, Beres A, Hessner MJ, Mihara M *et al.* Blockade of interleukin-6 signaling augments regulatory T-cell reconstitution and attenuates the severity of graft-versus-host disease. *Blood* 2009; **114**(4)**:** 891-900.

67. Tawara I, Koyama M, Liu C, Toubai T, Thomas D, Evers R *et al.* Interleukin-6 modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation. *Clin Cancer Res* 2011; **17**(1)**:** 77-88.

68. Das R, Chen X, Komorowski R, Hessner MJ, Drobyski WR. Interleukin-23 secretion by donor antigen-presenting cells is critical for organ-specific pathology in graft-versus-host disease. *Blood* 2009; **113**(10)**:** 2352-2362.

69. Das R, Komorowski R, Hessner MJ, Subramanian H, Huettner CS, Cua D *et al.* Blockade of interleukin-23 signaling results in targeted protection of the colon and allows for separation of graft-versus-host and graft-versus-leukemia responses. *Blood* 2010; **115**(25)**:** 5249-5258.

70. Thompson JS, Chu Y, Glass JF, Brown SA. Absence of IL-23p19 in donor allogeneic cells reduces mortality from acute GVHD. *Bone Marrow Transplant* 2010; **45**(4)**:** 712-722.

71. Meguro A, Ozaki K, Oh I, Hatanaka K, Matsu H, Tatara R *et al.* IL-21 is critical for GVHD in a mouse model. *Bone Marrow Transplant* 2010; **45**(4)**:** 723-729.

72. Oh I, Ozaki K, Meguro A, Hatanaka K, Kadowaki M, Matsu H *et al.* Altered effector CD4+ T cell function in IL-21R-/- CD4+ T cell-mediated graft-versus-host disease. *J Immunol* 2010; **185**(3)**:** 1920-1926.

73. Meguro A, Ozaki K, Hatanaka K, Oh I, Sudo K, Ohmori T *et al.* Lack of IL-21 signal attenuates graft-versus-leukemia effect in the absence of CD8 T-cells. *Bone Marrow Transplant* 2011; **46**(12)**:** 1557-1565.

74. Hanash AM, Kappel LW, Yim NL, Nejat RA, Goldberg GL, Smith OM *et al.* Abrogation of donor T-cell IL-21 signaling leads to tissue-specific modulation of immunity and separation of GVHD from GVL. *Blood* 2011; **118**(2)**:** 446-455.

75. Bucher C, Koch L, Vogtenhuber C, Goren E, Munger M, Panoskaltsis-Mortari A *et al.* IL-21 blockade reduces graft-versus-host disease mortality by supporting inducible T regulatory cell generation. *Blood* 2009; **114**(26)**:** 5375-5384.

76. Hippen KL, Bucher C, Schirm DK, Bearl AM, Brender T, Mink KA *et al.* Blocking IL-21 signaling ameliorates xenogeneic GVHD induced by human lymphocytes. *Blood* 2012; **119**(2)**:** 619-628.

77. Dudakov JA, Hanash AM, van den Brink MR. Interleukin-22: immunobiology and pathology. *Annu Rev Immunol* 2015; **33:** 747-785.

78. Zenewicz LA, Flavell RA. Recent advances in IL-22 biology. *Int Immunol* 2011; **23**(3)**:** 159-163.

79. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 2008; **29**(6)**:** 947-957.

80. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. *Immunity* 2004; **21**(2)**:** 241-254.

81. Geboes L, Dumoutier L, Kelchtermans H, Schurgers E, Mitera T, Renauld JC *et al.* Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. *Arthritis Rheum* 2009; **60**(2)**:** 390-395.

82. Couturier M, Lamarthee B, Arbez J, Renauld JC, Bossard C, Malard F *et al.* IL-22 deficiency in donor T cells attenuates murine acute graft-versus-host disease mortality while sparing the graft-versus-leukemia effect. *Leukemia* 2013.

83. Zhao K, Zhao D, Huang D, Yin L, Chen C, Pan B *et al.* Interleukin-22 aggravates murine acute graft-versus-host disease by expanding effector T cell and reducing regulatory T cell. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* 2014; **34**(9)**:** 707-715.

84. Hanash AM, Dudakov JA, Hua G, O'Connor MH, Young LF, Singer NV *et al.* Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. *Immunity* 2012; **37**(2)**:** 339-350.

85. Elmaagacli AH, Koldehoff M, Landt O, Beelen DW. Relation of an interleukin-23 receptor gene polymorphism to graft-versus-host disease after hematopoietic-cell transplantation. *Bone Marrow Transplant* 2008; **41**(9)**:** 821-826.

86. Gruhn B, Intek J, Pfaffendorf N, Zell R, Corbacioglu S, Zintl F *et al.* Polymorphism of interleukin-23 receptor gene but not of NOD2/CARD15 is associated with graft-versus-host disease after hematopoietic stem cell transplantation in children. *Biol Blood Marrow Transplant* 2009; **15**(12)**:** 1571-1577.

87. Nguyen Y, Al-Lehibi A, Gorbe E, Li E, Haagenson M, Wang T *et al.* Insufficient evidence for association of NOD2/CARD15 or other inflammatory bowel disease-associated markers on GVHD incidence or other adverse outcomes in T-replete, unrelated donor transplantation. *Blood* 2010; **115**(17)**:** 3625-3631.

88. Yu RY, Brazaitis J, Gallagher G. The Human IL-23 Receptor rs11209026 A Allele Promotes the Expression of a Soluble IL-23R-Encoding mRNA Species. *J Immunol* 2015; **194**(3)**:** 1062-1068.

89. Di Meglio P, Di Cesare A, Laggner U, Chu CC, Napolitano L, Villanova F *et al.* The IL23R R381Q gene variant protects against immune-mediated diseases by impairing IL-23-induced Th17 effector response in humans. *PLoS One* 2011; **6**(2)**:** e17160.

90. Sarin R, Wu X, Abraham C. Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses. *Proc Natl Acad Sci U S A* 2011; **108**(23)**:** 9560-9565.

91. Dander E, Balduzzi A, Zappa G, Lucchini G, Perseghin P, Andre V *et al.* Interleukin-17-producing T-helper cells as new potential player mediating graft-versus-host disease in patients undergoing allogeneic stem-cell transplantation. *Transplantation* 2009; **88**(11)**:** 1261-1272.

92. Liu Y, Cai Y, Dai L, Chen G, Ma X, Wang Y *et al.* The Expression of Th17-Associated Cytokines in Human Acute Graft-versus-Host Disease. *Biol Blood Marrow Transplant* 2013; **19**(10)**:** 1421-1429.

93. Broady R, Yu J, Chow V, Tantiworawit A, Kang C, Berg K *et al.* Cutaneous GVHD is associated with the expansion of tissue localised Th1 and not Th17 cells. *Blood* 2010.

94. Ratajczak P, Janin A, Peffault de Latour R, Leboeuf C, Desveaux A, Keyvanfar K *et al.* Th17/Treg ratio in human graft-versus-host disease. *Blood* 2010; **116**(7)**:** 1165-1171.

95. Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A *et al.* Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol* 2007; **8**(6)**:** 639-646.

96. Kleinschek MA, Boniface K, Sadekova S, Grein J, Murphy EE, Turner SP *et al.* Circulating and gut-resident human Th17 cells express CD161 and promote intestinal inflammation. *J Exp Med* 2009; **206**(3)**:** 525-534.

97. Bossard C, Malard F, Arbez J, Chevallier P, Guillaume T, Delaunay J *et al.* Plasmacytoid dendritic cells and Th17 immune response contribution in gastrointestinal acute graft-versus-host disease. *Leukemia* 2012.

98. Malard F, Bossard C, Brissot E, Chevallier P, Guillaume T, Delaunay J *et al.* Increased plasmacytoid dendritic cells and RORgammat-expressing immune effectors in cutaneous acute graft-versus-host disease. *J Leukoc Biol* 2013.

99. van der Waart AB, van der Velden WJ, van Halteren AG, Leenders MJ, Feuth T, Blijlevens NM *et al.* Decreased Levels of Circulating IL17-Producing CD161(+)CCR6(+) T Cells Are Associated with Graft-versus-

Host Disease after Allogeneic Stem Cell Transplantation. *PLoS One* 2012; **7**(12)**:** e50896.

100. Lee SE, Lim JY, Yoon JH, Shin SH, Cho BS, Eom KS *et al.* CD161(+) T Cells as Predictive Markers for Acute Graft-versus-Host Disease. *Biol Blood Marrow Transplant* 2015; **21**(3)**:** 421-428.

101. Betts BC, Sagatys EM, Veerapathran A, Lloyd MC, Beato F, Lawrence HR *et al.* CD4+ T cell STAT3 phosphorylation precedes acute GVHD, and subsequent Th17 tissue invasion correlates with GVHD severity and therapeutic response. *J Leukoc Biol* 2015.

102. Reinhardt K, Foell D, Vogl T, Mezger M, Wittkowski H, Fend F *et al.* Monocyte-induced development of Th17 cells and the release of S100 proteins are involved in the pathogenesis of graft-versus-host disease. *J Immunol* 2014; **193**(7)**:** 3355-3365.

103. Munneke JM, Bjorklund AT, Mjosberg JM, Garming-Legert K, Bernink JH, Blom B *et al.* Activated innate lymphoid cells are associated with a reduced susceptibility to graft-versus-host disease. *Blood* 2014; **124**(5)**:** 812-821.

104. Leonardi C, Matheson R, Zachariae C, Cameron G, Li L, Edson-Heredia E *et al.* Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. *N Engl J Med* 2012; **366**(13)**:** 1190-1199.

105. Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K *et al.* Secukinumab in plaque psoriasis--results of two phase 3 trials. *N Engl J Med* 2014; **371**(4)**:** 326-338.

106. Papp KA, Leonardi C, Menter A, Ortonne JP, Krueger JG, Kricorian G *et al.* Brodalumab, an anti-interleukin-17-receptor antibody for psoriasis. *N Engl J Med* 2012; **366**(13)**:** 1181-1189.

107. Targan SR, Feagan BG, Vermeire S, Panaccione R, Melmed GY, Blosch C *et al.* A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Efficacy of AMG 827 in Subjects With Moderate to Severe Crohn's Disease. *Gastroenterology* 2012; **143**(3)**:** e26.

108. Hueber W, Sands BE, Lewitzky S, Vandemeulebroecke M, Reinisch W, Higgins PD *et al.* Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut* 2012; **61**(12)**:** 1693-1700.

109. Antin JH, Weisdorf D, Neuberg D, Nicklow R, Clouthier S, Lee SJ *et al.* Interleukin-1 blockade does not prevent acute graft-versus-host disease: results of a randomized, double-blind, placebo-controlled trial of

interleukin-1 receptor antagonist in allogeneic bone marrow transplantation. *Blood* 2002; **100**(10)**:** 3479-3482.

110. Kennedy GA, Varelias A, Vuckovic S, Le Texier L, Gartlan KH, Zhang P *et al.* Addition of interleukin-6 inhibition with tocilizumab to standard graft-versus-host disease prophylaxis after allogeneic stem-cell transplantation: a phase 1/2 trial. *Lancet Oncol* 2014; **15**(13)**:** 1451-1459.

111. Sandborn WJ, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C *et al.* Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med* 2012; **367**(16)**:** 1519-1528.

112. Pidala J, Perez L, Beato F, Anasetti C. Ustekinumab demonstrates activity in glucocorticoid-refractory acute GVHD. *Bone Marrow Transplant* 2012; **47**(5)**:** 747-748.

113. Spolski R, Leonard WJ. Interleukin-21: a double-edged sword with therapeutic potential. *Nat Rev Drug Discov* 2014; **13**(5)**:** 379-395.

114. Zanin-Zhorov A, Flynn R, Luznik L, Panoskaltsis-Mortari A, Jing D, Goodman K *et al.* A Selective and Potent Rock 2 Inhibitor (KD025) Decreases Human STAT3-Dependent IL-21 and IL-17 Production and Experimental Chronic Graft-Versus-Host Disease (cGVHD). *Blood* 2014; **124**(21)**:** 540-540.

115. Hsu L, Armstrong AW. JAK inhibitors: treatment efficacy and safety profile in patients with psoriasis. *Journal of immunology research* 2014; **2014:** 283617.

116. Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK *et al.* Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. *Leukemia* 2015; **29**(10)**:** 2062-2068.

117. Huh JR, Littman DR. Small molecule inhibitors of RORgammat: targeting Th17 cells and other applications. *Eur J Immunol* 2012; **42**(9)**:** 2232-2237.

118. Nishimori H, Maeda Y, Teshima T, Sugiyama H, Kobayashi K, Yamasuji Y *et al.* Synthetic retinoid Am80 ameliorates chronic graft-versus-host disease by down-regulating Th1 and Th17. *Blood* 2012; **119**(1)**:** 285-295.

Figures legends

Figure 1. Kinetic of Th17 cells after allogeneic stem cell transplantation in patients who develop acute graft-versus-host disease. aGVHD indicates acute graft-versus-host disease; allo-SCT, allogeneic stem cell transplantation.

Figure 2. Potential therapeutic targets of the Th17 pathway implicated in acute graft-versus-host disease, example of the intestinal acute graft-versus-host disease. The conditioning regimen leads to host intestinal tissue damage and activation of host antigen presenting cells (APC), that will drive Th17 differentiation through IL-6, IL-1β, TGFβ and IL-23. Various therapeutic tools are available to target Th17 pathway. Cytokines driving Th17 cells differentiation could be target by monoclonal antibodies: tocilizumab target IL-6R ustekinumab target the p40 subunit share by IL-12 and IL-23 and the p19 subunit of IL-23 is targeted by Tildrakizumab, Guselkumab, AMG 139, BI 655066 and LY3074828. Th17 differentiation could also be target by inhibitors of Th17 generation, such as the JAK inhibitors Tofacitinib and Ruxolitinib that block STAT3 phosphorylation. Several monoclonal antibodies could target Th17 related cytokines: IL-17A (Ixekizumab, Secukinumab, CNTO 6785, SCH 900117 and CJM112), IL-17A and IL-17F (Bimekizumab and ALX-0761) and IL-21 (NNC0114-0005, NNC0114-0006 and ATR-107). Alternatively, IL-17R could be targeted by the monoclonal antibody Brodalumab. Finally given the contradictory data regarding the inflammatory versus protective effect of IL-22 in aGVHD, no therapeutic strategy related to IL-22 could be proposed at moment. Ab indicates monoclonal antibody; APC, antigen presenting cell.

Table 1. Potential therapeutic agents targeting the TH17 axis for acute GVHD treatment.

Target	Drug	Companies	Clinical stage in aGVHD	Clinical trial identifier and publication
Th17 differentiation				
IL-6R	Tocilizumab	Roche	Phase I/II completed[110]	ACTRN12612000726853
			Phase I/II ongoing	NCT01475162
			Phase II ongoing	NCT01757197
			Phase II ongoing	NCT02206035
IL-23-p40	Ustekinumab	Janssen	Phase II ongoing	NCT01713400
IL-23-p19	Tildrakizumab	Merk/ Sun Pharma		
	Guselkumab	Janssen		
	AMG 139	Amgen	Not evaluated in GVHD	NA
	BI 655066	Boehringer Ingelheim		
	LY3074828	Eli Lilly		
STAT3	Ruxolitinib	Novartis	Retrospective study[116]	NA
	Tofacitinib	Pfizer	Not evaluated in GVHD	
Th17 related cytokine				
IL-17A	Ixekizumab	Eli Lilly		
	Secukinumab	Novartis		
	CNTO 6785	Janssen	Not evaluated in GVHD	NA
	SCH 900117	Merk		
	CJM112	Novartis		
IL-17A and IL-17F	Bimekizumab	UCB		
	ALX-0761	Merk Serono/Ablynx		NA
IL-17R	Brodalumab	Amgen	Not evaluated in GVHD	NA
IL-21	NNC0114-0005	Novo Nordisk		
	NNC0114-0006	Novo Nordisk	Not evaluated in GVHD	NA
	ATR-107	Pfizer		

Abbreviation: NA, not available;

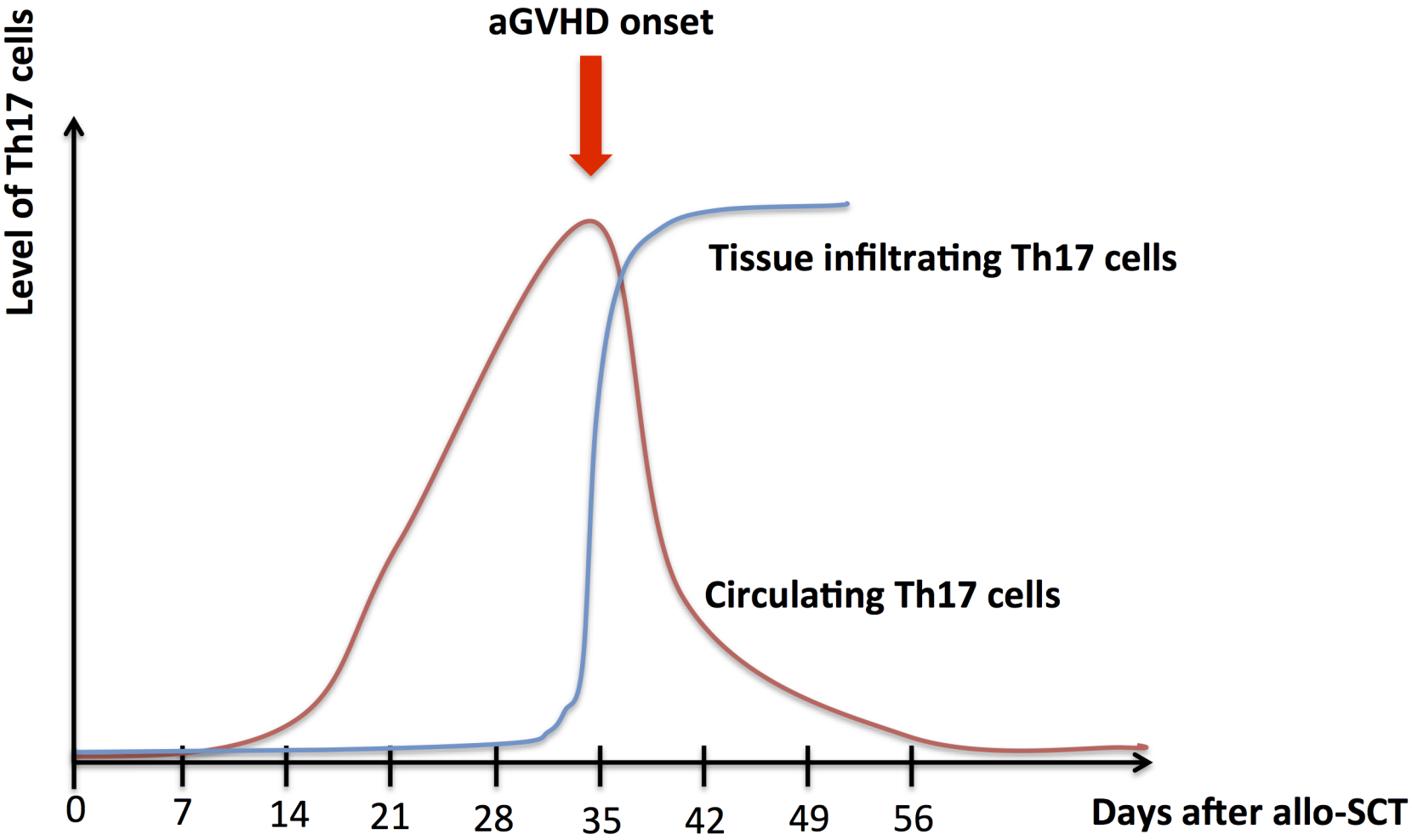
aGVHD onset
Level of Th17 cells
Tissue infiltrating Th17 cells
Circulating Th17 cells
0
7
14
21
28
35
42
49
56
Days after allo-SCT

Figure 1

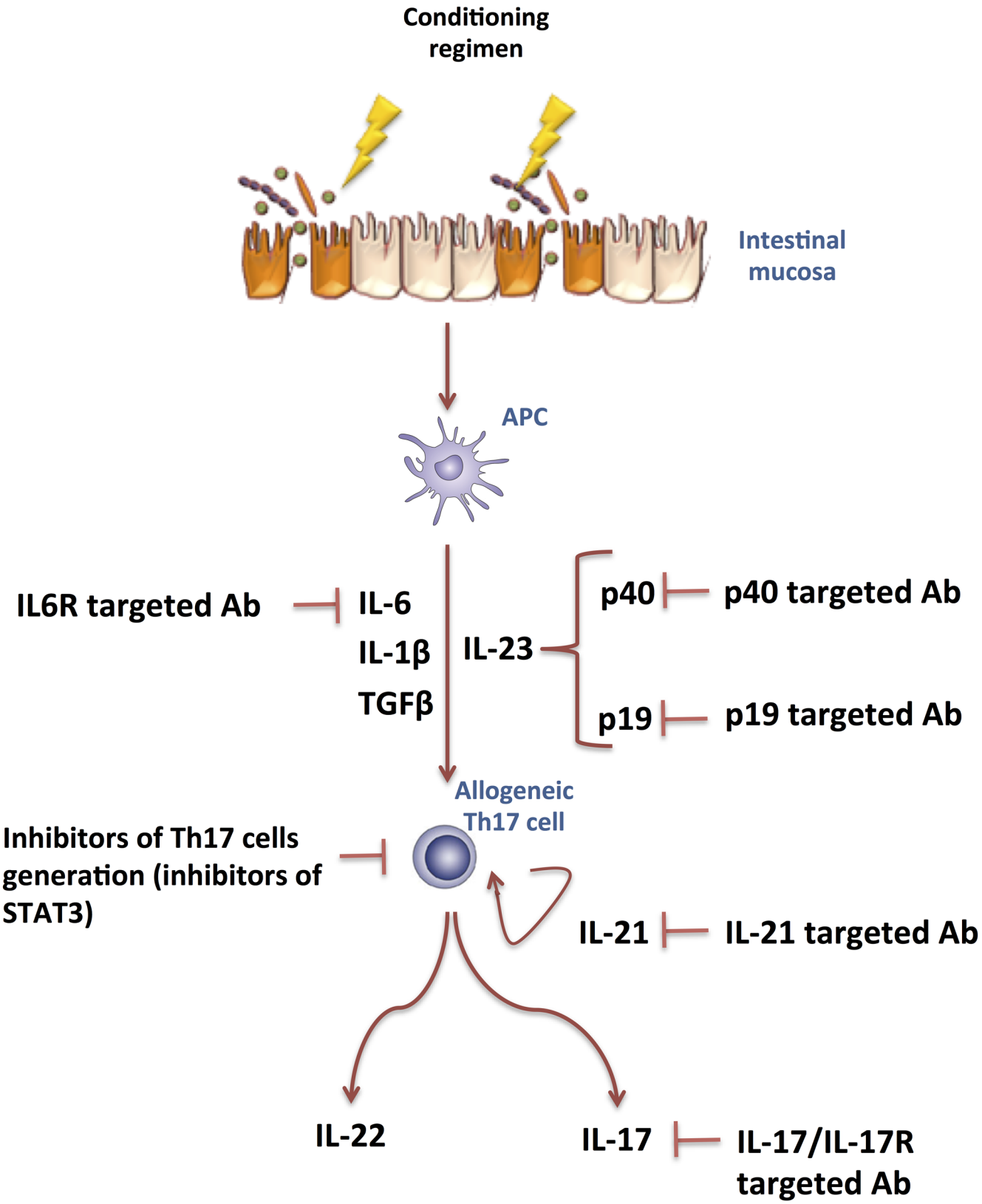
Conditioning regimen
Intestinal mucosa
APC
IL6R targeted Ab
IL-6
IL-1β
TGFβ
IL-23
p40
p40 targeted Ab
p19
p19 targeted Ab
Allogeneic Th17 cell
Inhibitors of Th17 cells generation (inhibitors of STAT3)
IL-21
IL-21 targeted Ab
IL-22
IL-17
IL-17/IL-17R targeted Ab

Figure 2