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Targeting Tregs in Juvenile Idiopathic Arthritis and Juvenile Dermatomyositis—Insights From Other Diseases

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Regulatory T cells (Tregs) are believed to be dysfunctional in autoimmunity. Juvenile idiopathic arthritis (JIA) and juvenile dermatomyositis (JDM) result from a loss of normal immune regulation in specific tissues such as joints or muscle and skin, respectively. Here, we discuss recent findings in regard to Treg biology in oligo-/polyarticular JIA and JDM, as well as what we can learn about Treg-related disease mechanism, treatment and biomarkers in JIA/JDM from studies of other diseases. We explore the potential use of Treg immunoregulatory markers and gene signatures as biomarkers for disease course and/or treatment success. Further, we discuss how Tregs are affected by several treatment strategies already employed in the therapy of JIA and JDM and by alternative immunotherapies such as anti-cytokine or co-receptor targeting. Finally, we review recent successes in using Tregs as a treatment target with low-dose IL-2 or cellular immunotherapy. Thus, this mini review will highlight our current understanding and identify open questions in regard to Treg biology, and how recent findings may advance biomarkers and new therapies for JIA and JDM.

Keywords: regulatory T cells, juvenile idiopathic arthritis, juvenile dermatomyositis, biomarker, therapy

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INTRODUCTION

$CD4^+FOXP3^+$ regulatory T cells (Tregs) are a subset of $CD4^+$ T helper cells present in lymphoid and non-lymphoid tissues, and are crucial for mediating tolerance to self, preventing allergies and controlling immune reactions after infections (1). They develop in the thymus or are induced in the periphery and exhibit contact-dependent and -independent mechanisms of action (1). Inactivating mutations in FOXP3 lead to multi-organ autoimmune disease [immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX)], highlighting the importance of Tregs (2). Importantly, it is becoming clear that the local microenvironment affects the phenotype and function of tissue-localized Tregs, which also have additional roles in repair and regeneration (3).

Treg–tissue interaction might be particular important in autoimmunity with tissue-specific presentation, such as juvenile idiopathic arthritis (JIA) and juvenile dermatomyositis (JDM). While JIA is the most common inflammatory rheumatic disease in children, JDM is rare. JIA is characterized by persistent arthritis and subtype-dependent symptoms [reviewed in (4)]. Here, we focus on polyarticular and oligoarticular JIA, which present without involvement of systemic

organs or skin. JDM is characterized by inflammation of muscles and skin, resulting in muscle weakness and rashes [reviewed in (5)]. Interestingly, for both conditions researchers may take advantage of clinical sample collection from the site of inflammation: synovial fluid (SF) drained during therapeutic joint injection (JIA) and biopsies (mostly muscle, JDM). While some patients respond to therapy, others do not and studying the underlying differences may lead to better understanding and treatments.

Here, we discuss recent advances in the understanding of Treg biology in oligo-/ polyarticular JIA and JDM, and what we can learn about Treg-related disease mechanisms, treatments and biomarkers from other diseases.

ALTERED TREGS IN JIA AND JDM

The phenotype of $CD4^+FOXP3^+$ Tregs in JIA has been considerably characterized in the past (6) with the molecular roles of FOXP3 in JIA reviewed by Copland and Bending in this special collection (7). It is now clear that the Treg TCR (T cell receptor) repertoire is highly restricted in JIA, both at the site of inflammation (8–11) and in circulation (10, 12). Interestingly, in blood only Tregs but not conventional $CD4^+$ non-Treg cells (Tconv) are more clonal (10, 12). Some suggest that the TCR repertoires of Tregs from SF and peripheral blood (PB) significantly overlap (8), while others only found a very small overlap (9, 11). These differences might be explained by different sequencing depth and analysis strategies and/or by different Treg subsets studied: total (11) or effector Tregs defined by HLA-DR (8) or CD161 expression (9). Further, one study found that SF Tregs, but not Tconv, share specificity at an amino acid sequence level among different patients (10), suggesting disease-associated Treg clones might foster JIA.

Besides a restricted TCR repertoire, Tregs from the JIA inflammatory sites show unstable FOXP3 and CD25 (13), altered homing markers (9), cytokine production (6, 9), deficiency in specific chemokine production (14), and low responsiveness to IL-2 (13)—indicating impaired Treg function in JIA. Nevertheless, many reports found that JIA SF and PB Tregs are fully demethylated (8, 13), thus committed to the Treg-lineage, and suppressive *in vitro* (6, 8, 9, 13, 15). Hence, JIA Tregs are likely functioning inappropriately or insufficiently in the context of the inflammatory microenvironment. Interestingly, adding SF to *in vitro* cultures can both increase/stabilize Treg FOXP3 expression (11, 16) and *in situ* induce effector T cells to be resistant to Treg-mediated suppression *ex vivo* (17, 18). Thus, more research is needed to decipher the effects of the inflammatory microenvironment on Treg function.

In comparison, we know little about the contribution of Tregs to JDM pathogenesis. Similar to JIA, the Treg repertoire is restricted with a lack of diversity (12). $FOXP3^+$ Tregs were found to be enriched in JDM muscle compared to muscle tissue from patients with Duchenne muscular dystrophy (19). Since the latter is already enriched in Tregs compared to normal muscle (20), this suggests a hyper-enrichment in JDM in response to autoimmune inflammation. PB Tregs of active JDM also appear less suppressive *in vitro* with decreased expression of CTLA4 (19). Adult DM/ polymyositis muscle biopsies are also enriched with Tregs (21). Interestingly, both Treg and effector T cell numbers decreased post immunosuppressive therapy in adult myositis, suggesting that Treg enrichment is a response to inflammation. However, juvenile and adult DM have different clinical presentation (22) and JDM PB express more Th17-type and FOXP3 transcripts (23). JDM and other myopathies are characterized by a type 1 IFN signature (24–26) and interferons may be a potential therapeutic target (27), but their effects on Tregs remain to be investigated.

Tregs are crucial in resolving muscle injury in animal studies (28) and Treg-deficient mice develop more severe myopathies in response to antigen, while adoptive Treg transfer prevents inflammation (29, 30). Thorough immune-profiling recently revealed pan-tissue and tissue-specific signatures and enhancers of murine Tregs (31). The muscle Treg signature was highly enriched in cell cycle genes, showed a dynamic response to injury and was more similar to circulating Treg signatures than to other tissue Tregs (31), indicating that muscle Tregs might acutely infiltrate muscle and are not necessarily long-term resident cells. While myopathy is a defining characteristic of JDM, skin inflammation and rash are other symptoms (5). Skin-resident Tregs are crucial for immune homeostasis (3) and have been characterized in health and various disease settings (32). However, studies on JDM-affected skin are lacking, and more work is needed to characterize JDM skin-resident Tregs.

TREGS AS A BIOMARKER?

JIA and JDM can exhibit an unpredictable disease course. While mounting evidence indicates that an early aggressive treatment is best for severe disease (4, 27, 33, 34), the disease course is unpredictable at presentation. Additionally, due to potential short- and long-term side effects children should not be exposed to unnecessary medication. Unfortunately, once a patient appears to be in clinical remission (on or off medications), disease may flare without any notice or obvious trigger (**Figure 1A**). Indeed, among JIA patients who are in clinical remission, 30–50% experience flares (35, 36).

Hence, reliable biomarkers need to predict (i) the future disease course, (ii) treatment response, and (iii) the safety for medication withdrawal during clinical remission (**Figure 1B**).

Inflammation markers in the serum can indicate disease activity and potentially treatment response in JIA [reviewed in (36)]. In JDM, histology of biopsies and myositis-specific auto-antibodies can indicate future disease severity or complications [reviewed in (27)].

Only a few putative biomarkers probe the immunoregulatory balance in autoimmune arthritis and myopathies. The frequency of inflammation-associated Tregs ($HLA\text{-}DR^+$) in PB was proposed as a biomarker for disease activity in arthritis (8). TCR sequence overlap of these PB $HLA\text{-}DR^+$ Tregs with SF Tregs in JIA, and an increase of $HLA\text{-}DR^+$ Tregs in active rheumatoid arthritis (RA) were found. Low expression of the immunoregulatory receptor CD39 has been suggested as an

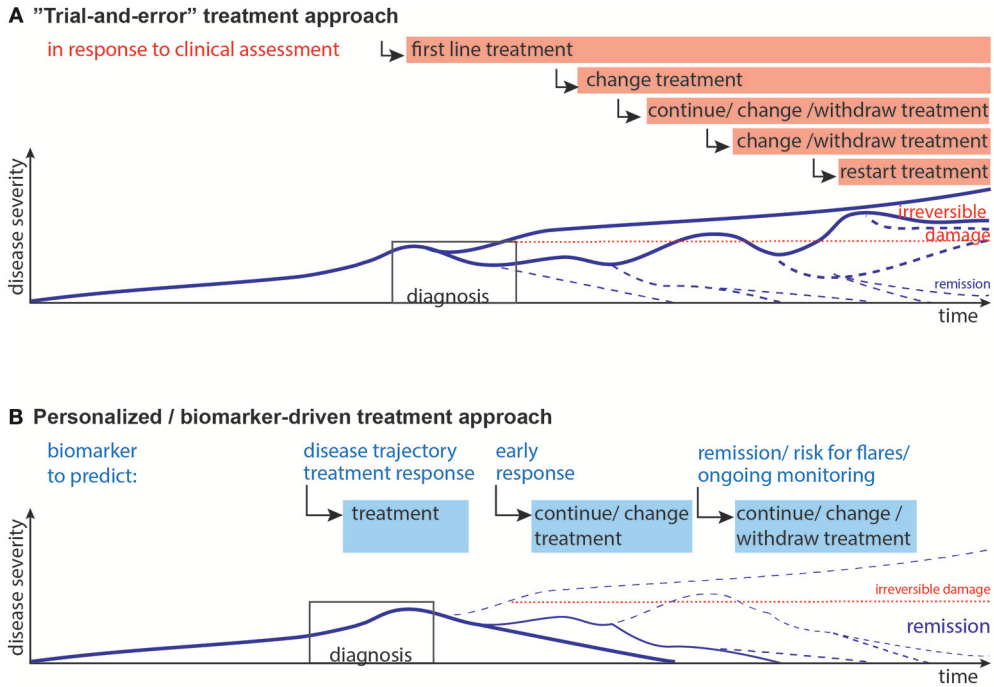

FIGURE 1 | Current and desired disease progression models. **(A)** Current "Trial-and-error" model: Upon diagnosis the first line of treatment is started, which may lead to remission, or partial remission. Often, a second, third or fourth treatment strategy needs to be implemented when the previous treatments are not effective. Choice of treatments is guided by previous experience, e.g., upon presentation non-steroidal anti-inflammatory drugs with glucocorticoids are used, after a couple of month many patients are switched to methotrexate as first disease modifying therapy, often followed by anti-TNF-α agents with/without methotrexate (4). This disease progression and subsequent staggering of therapy can result in irreversible damage and long-term therapy. **(B)** Desired personalized/biomarker-driven model: Biomarkers could be used for prediction and aid decisions at the following stages: disease trajectory, selection of treatment, early response to therapy, remission/minimal disease activity and the risk of flare upon withdrawal of therapy, ongoing monitoring of immune activity and risk of flares. Biomarkers might thus contribute to more efficient therapy, pre-empt flares, and minimize short- and long-term effects of flares and reduce long-term damage. Lines represent models of disease progression, with line thickness representing frequency estimates.

indicator of methotrexate resistance in RA (37). Also, response to the TNF-α blocker adalimumab could be predicted by a Treg increase in PBMCs from RA patients cultured with adalimumab prior to treatment (38). Finally, the soluble form of the high affinity IL-2 receptor α chain (CD25), crucial for Treg phenotype and function, might be a biomarker for adult myositis disease activity (39).

In the recent past, gene signatures have been defined as multi-parameter biomarkers. Thus, far, efforts to define JIA immune-based gene biomarkers have focused on whole genome expression profiling (40–42) and epigenomic signatures (43, 44). JIA displays an altered immune signature, which changes during remission, but does not return to a state comparable to healthy controls (41, 42). Myositis is characterized by type 1 IFN signatures (27). While interesting and highlighting potential disease mechanisms, whole-genome/exome expression profiling is not feasible for routine clinical practice due to cost, logistics and data interpretation. We have recently developed a Treg gene signature associated with Treg competency using the clinically-applicable multiplex platform nanoString (45). NanoString is fast and fewer than 10,000 lysed cells are sufficient without the need to purify RNA. Although the proportion of Tregs that express FOXP3 was similar between type 1 diabetes (T1D) and controls, there was a significant change in their Treg signature (45). Future work will elucidate whether the Treg gene signature may also be used as a biomarker in JIA and other autoimmune conditions.

In summary, some progress has been made, but more biomarkers are needed for biological disease activity, prognosis, treatment success, and risk of flares. Further, a consensus of criteria to describe active/inactive disease is needed to better estimate the currently widely variable incidence of clinically inactive JIA disease (46). For JDM, a comprehensive set of criteria to assess disease activity and damage has been proposed (47).

TREGS AS THERAPEUTIC TARGET/TOOL?

Convincing evidence demonstrates that functioning Tregs are crucial to prevent autoimmunity and our understanding of how different immunotherapies affect Tregs has improved.

(Unforeseen) Treg Effects of Immuno-Therapy

High levels of TNF-α in the inflamed JIA joint (32, 48) offer a clear rationale for anti-TNF therapy in JIA with marked success (4, 49). Anti-TNF therapy has also been used in refractory JDM (50), but with mixed evidence for its effectiveness (27, 51–53).

Blocking TNF-α can, however, also elicit further autoimmune responses, particular in the skin and muscle (54–57). TNF-α itself can have both positive and negative effects on Tregs (32, 58, 59). Interestingly, the negative effects are found especially in inflamed joints (32, 58, 60), whereas positive effects of TNF-α on Treg function were reported using healthy human cells or in mice (58, 61–64). TNF-α has two receptors CD120a (TNFR1) and CD120b (TNFR2) (58). CD120b may mediate the pro-Treg functions of TNF-α, including Treg proliferation, stabilizing Tregs, and preventing disease in mouse models (58, 62–64). Little is known about the effects of ligation of CD120a in Tregs, but some research suggests targeting CD120a while sparing CD120b-TNF-interaction can alleviate collagen-induced arthritis (65). In RA, adalimumab has been shown to enhance Treg frequency and potency via CD120b (38, 66, 67). Etanercept, a soluble CD120b as TNF-α blocker, instead might predominantly affect effector T cells, by reversing their resistance to suppression in JIA (68). Unfortunately, a considerable group of JIA/JDM patients do not respond to anti-TNF therapy (27, 49, 53) and anti-TNF agents are immunogenic (69), with 50% of patients developing anti-drug antibodies leading to resistance to therapy and disease progression.

Ustekinumab is another potentially attractive anti-cytokine therapy which targets the p40 subunit of IL-12 and IL-23, key cytokines driving Th1, Th17, and Th17.1, (ex-)Th17 cells with a Th1-like phenotype, function (70–74). Ustekinumab is well-tolerated in adult and pediatric patients for treating psoriasis, psoriatic arthritis, systemic lupus erythematosus (SLE), and Crohn's disease (70, 71, 75–77), and has shown lower immunogenicity compared to most anti-TNF agents (69). Th17.1 are enriched in JIA (72, 73), and ustekinumab therapy had some success in enthesitis-related JIA (78), psoriatic arthritis (69, 79) and is in trial for various rheumatological diseases (80). While no imbalance in IL-17 has been established in JDM (19), Th17.1 have not been investigated. Ustekinumab has been suggested as a potential therapy for JDM, and a case of JDM with psoriasis was treated successfully with ustekinumab (81). Due to the reciprocal relationship between Th17 and Tregs (82), Tregs might also be affected by ustekinumab therapy, and this was indeed suggested in a case report of giant cell arteritis (83) and in T1D treated with ustekinumab (NCT02117765; Pesenacker et al.).

IL-6 also drives inflammatory environments, including skewing the Treg/Th17 balance toward Th17 (71). Anti-IL-6 receptor therapy (tocilizumab) increases Treg frequency and numbers in RA (71). IL-6 has also been implicated in JIA and JDM (11, 17, 84, 85) and is used in polyarticular, extended oligoarticular, systemic JIA (49), and refractory JDM (50), but mechanistic studies in pediatric disease are lacking.

Whether drugs such as ustekinumab and tocilizumab act on Tregs directly or through changing the microenvironment is unclear. Human Tregs can express the receptors for IL-6 (86), IL-12 (87), and IL-23 (88), but evidence for direct drug action on Tregs is lacking.

Alternatively, co-receptors can be targeted to manipulate the immunoregulatory balance. Initially established for cancer therapy (checkpoint blockade), mimicking checkpoints such as CTLA4 (CTLA4-Ig, abatacept, belatacept) is used as treatment for autoimmunity. Abatacept has been shown to be safe and effective in oligo- and polyarticular JIA (49, 89–92), adult DM/polymyositis (34, 53), a case report of steroid-sparing abatacept in complex JDM (93) and a trial in JDM is underway (27). A reduction of the T cell activation state is the main reported effect of abatacept (90, 94–96). Surprisingly, the majority of studies found abatacept decreases the frequency of Tregs (90, 95, 97–99), with some studies showing an increase in function (99). On the other hand, increased Treg frequency, but decreased activity after abatacept therapy in RA was demonstrated (100). In muscle tissue of adult DM/polymyositis, more Tregs were found following abatacept (34), suggesting that abatacept treatment could change Treg localization. Other co-receptor targeting therapies are in use/development for malignancies (e.g., anti-PD1, anti-TIM3, anti-TIGIT, etc.) and these pathways might be useful targets in autoimmunity.

Treg (-Targeted) Therapy

Adoptive transfer of Tregs has been shown to be safe and possibly effective at reducing inflammation, inducing transplant tolerance, preventing graft-vs.-host disease (GVHD) and treating autoimmunity [reviewed in (101)].

Important considerations for Treg-therapy currently under investigation are the source of therapeutic cells, antigen-specificity and possibly tailoring homing characteristics for improved activity. Isolating and expanding sufficient numbers of Tregs from patients awaiting transplantation, under immunosuppression or with autoimmune disease is feasible and can restore their function (101–103), although achieving clinically relevant Treg numbers from pediatric JIA and JDM patients might prove challenging. Third-party Tregs from umbilical cord blood have been found safe and possibly effective as GVHD prophylaxis in adults (104, 105) and pediatric thymus—routinely removed during pediatric cardiac surgery—might be a plentiful source for highly functional therapeutic Tregs (106, 107). Antigen-specific Tregs are more effective than polyclonal Tregs for therapy and with recent successes of chimeric antigen receptor (CAR) T effector therapies for cancer, there has been a surge to adapt this technology to generate CAR-Tregs [reviewed in (108)]. While generation of antigen-specific Tregs recognizing allogeneic HLA-molecules is relatively straightforward in transplantation, generation of CAR-Tregs for autoimmunity without known antigen (i.e., JIA) might be difficult. Still, CAR-Tregs reacting with antigen found at the site of inflammation (i.e., JIA joints or JDM muscle) could activate Tregs locally. Alternatively, Tregs could be conditioned *in vitro* to home to specific sites (107) or Tregs could be injected locally, as shown with intra-dermal injection of Tregs to inhibit murine allograft skin inflammation (109).

Since Treg cell therapies are challenging and expensive, targeting Treg expansion *in vivo* might be more feasible for conditions such as JIA and JDM. The most promising advances of non-cellular therapies targeting Tregs have been low-dose IL-2, IL-2 complexes, or IL-2 bio-similars (110–112). While high doses of IL-2 stimulate mainly effector cells, low-dose IL-2 [0.3–3 $\times$ 10^6 units/day (112)] skews the response toward Tregs. Low-dose IL-2 increases the frequency of activated, functional

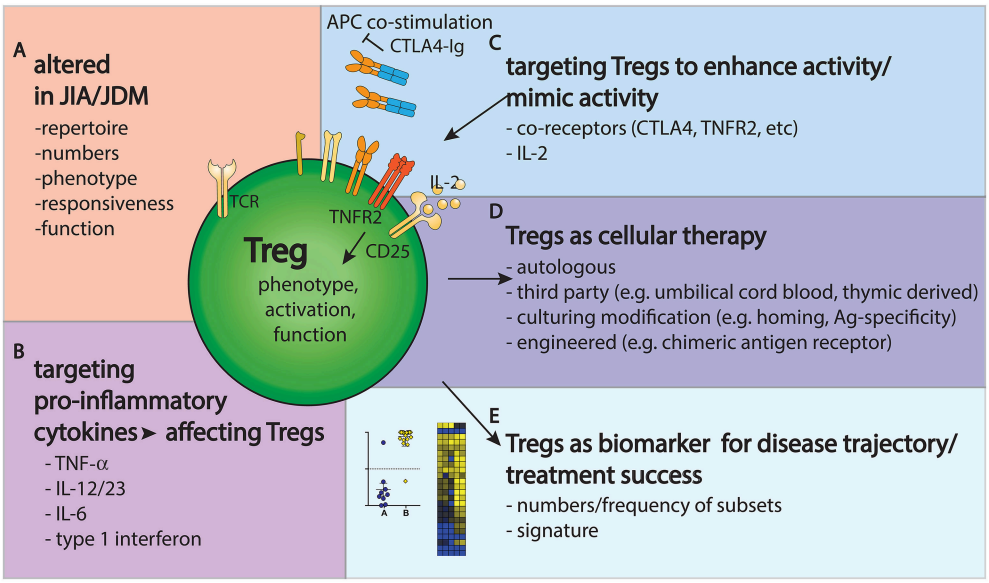

FIGURE 2 | Tregs provide challenges and opportunities in JIA/JDM pathogenesis, treatment and monitoring. **(A)** Tregs are altered in repertoire, phenotype and frequency in JIA/JDM, particularly at the site of inflammation. **(B–D)** Treatment options to restore the immunoregulatory balance include targeting pro-inflammatory cytokines **(B)**, targeting Tregs to enhance their activity **(C)** or using Tregs as a cellular therapy **(D)**. **(E)** Further, changes in Treg gene signatures could aid as biomarkers to measure or predict disease/treatment response.

and fully demethylated $CD25^{+}FOXP3^{+}$ Tregs (113–115) and induces STAT5 phosphorylation *in vivo* (114, 115). Low-dose IL-2 therapy has been deemed safe and successful in the treatment of T1D (112, 114, 115), GVHD (116, 117), and SLE (113). Indeed, low-dose IL-2 therapy rescued Tregs with low levels of CD25 in SLE (113), indicating that it might also rescue JIA Tregs with low CD25 expression (13). To further fine-tune specificity or increase the half-life of IL-2, IL-2 complexes, and bio-similars are in development (110, 111, 118); these expand Tregs and induce phosphorylated STAT5 *in vitro*, *in vivo*, and prevent disease in animal models (118–121), including resolution of muscular dystrophy (20). Covalently linking IL-2 to anti-IL-2 (122), to non-FcRγ-binding human IgG1 (123) or CD25 (124) may enhance potential clinical application by mitigating the risk of *in vivo* dissociation of complexes.

However, increasing Treg numbers alone might not be sufficient to overcome the highly inflammatory environment and effector cell resistance. Thus, to achieve sustained remission combination-therapy might be necessary to reduce the inflammatory milieu paralleled with boosting Tregs to maintain a renewed tolerance.

CONCLUDING REMARKS

Taken together, it is clear that Tregs present challenges and opportunities in JIA and JDM research and clinical management (**Figure 2**). Their phenotype and function are clearly altered in JIA and JDM, targeting them might improve disease outcome and Tregs could be used as biomarkers to gage the state and progress of disease.

The role of the microenvironment on Treg function and phenotype in JIA- and JDM-affected tissues remains to be explored further. Researchers should take advantage of biopsies taken for clinical diagnosis (JDM) and SF aspirated during therapeutic joint injections (JIA). Novel techniques, such as single cell sequencing, multidimensional mass/flow cytometry and microscopy, will aid using clinical samples to their full potential (125–127). Additionally, co-culture with SF or muscle-derived cells could highlight how the microenvironment affects Tregs. Since JDM in particular is a rare disease, collaborations between groups are crucial to increase sample size for fundamental research, biomarker-finding and -validation studies and controlled treatment trials. This could be achieved by consortiums similar to juvenile diabetes research foundation (JDRF) biomarker working group for T1D (114) and the immune tolerance network trials (128).

While there is progress toward unified measures of disease activity (46, 47), these will need to be tested and verified, followed by development of feasible, reliable and cost-effective biomarkers to predict disease activity, risk of flare and ideal treatment strategies. The ultimate goal, aided by biomarkers, is to go from a trial-and-error treatment approach toward a more efficient and personalized medicine approach with more patients achieving drug-free remission without major long-term disabilities (**Figure 1**).

Success of various agents affecting the immunoregulatory balance in other diseases point to potential uses in JIA and JDM. Any (new) therapy will need to be considered

in regards to both effector cells AND Tregs, since some therapies might have unexpected effects on Tregs. Thus, it is important to continuously build our understanding of how various agents affect the immunoregulatory balance.

In conclusion, important recent advances might lead to valid future contributions to the widened arsenal of treatment options available to restore the immunoregulatory balance in a heterogeneous disease spectrum.

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