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Approaches Targeting T Regulatory Cells in Autoimmune Diseases

The immune system has multiple mechanisms to detect and eradicate or neutralize pathogenic microorganisms. However, misguided immune response directed against self, dietary, or environmental antigens can lead to excessive inflammation or tissue damage and can potentially impair or cause loss of organ function. Regulatory T (Treg) cells, a subset of $CD4^+$ T cells ($CD4^+CD25^{high}FoxP3^+$), have potent immunosuppressive properties and can limit the negative consequences of an adaptive immune response. Treg cell-mediated suppression is a vital mechanism of negative regulation of immune-mediated inflammation and plays a prominent role in autoimmune and auto-inflammatory disorders, allergy, acute and chronic infections, and cancer. This association of Treg cells with a variety of diseases has prompted investigations into the therapeutic manipulation of these cells.

Treg cells control inflammation by employing diverse mechanisms of suppression including production of immunomodulatory cytokines (IL-10 and TGF-β), enzymatic activity of CD39 and CD73 ecto-enzymes, consumption of IL-2, expression of co-inhibitory molecules (CTLA-4 and LAG3), depletion of crucial growth factors, cytotoxicity and extra-immune functions.

Tregs and autoimmune diseases

Treg cell deficiency or dysfunction is thought to be central to the pathogenesis of diverse autoimmune diseases. Studies on the link between autoimmune diseases and Tregs have demonstrated a significantly reduced number and/or function of Tregs in the initiation and progression of type 1 diabetes mellitus, multiple sclerosis, lupus erythematosus, asthma, autoimmune uveitis, rheumatoid arthritis and refractory Crohn's disease.

However, Treg cell numbers are not uniformly reported to be reduced in patients with all autoimmune disease, and indeed Treg cells can even be found in very high numbers in some afflicted tissues. For example, an abundance of Treg cells has been reported in the synovial fluid of patients with rheumatoid arthritis (even though these cells are still very much outnumbered by effector T cells).

Types of Treg Cells

	Thymic-derived Treg (tTreg)	Peripheral Treg (pTreg)	Other Tregs
Key Characteristics	• Arise in thymus • tTreg cells are thought to mainly be responsible for preventing autoimmune diseases	• Generated outside thymus • Develop from naïve T-cell precursors upon exposure to antigenic stimulation under tolerogenic conditions • pTreg cells accumulate mostly at barrier sites (such as the gut) where they maintain immune homeostasis	• In vitro induced Treg (iTreg) cells • Activated (or 'effector') Treg cells • Human Treg cells with different levels of CD25 expression and varying capacities for suppression and FOXP3 expression • $FOXP3^+$ Treg cells reside in certain peripheral tissues
Role	• Likely to be responsible for tolerance to self-antigens	• pTreg cells restrain immune responses to "non-self," including allergens, commensal microbiota, and dietary antigens at barrier sites and paternal alloantigens	• Role not yet clearly understood

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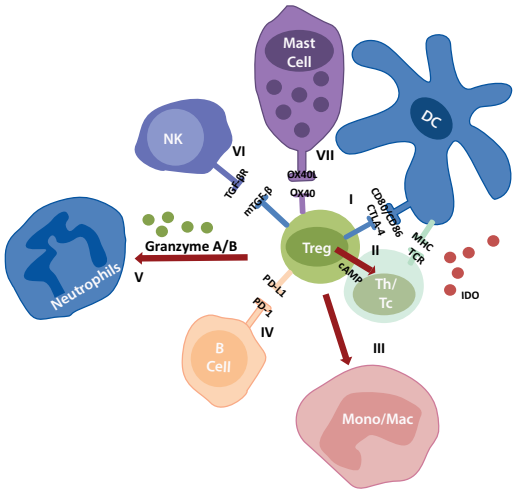

	Target Immune Cells	Treg Actions
I	Dendritic Cells (DCs)	Suppression of antigen presentation • Induction of expression of IDO (indoleamine 2,3-dioxygenase) via the CTLA-4
II	T helper (Th) and Cytotoxic T Cells	Inhibition of activation through: • Cell-to-cell interactions • Extracellularly produced adenosine via CD39 and CD73 receptors • Transferred cAMP and consumption of IL-2
III	Monocytes/Macrophages	Induction of apoptosis
IV	B-cells	Inhibition of proliferation and induction of apoptosis via PD-1
V	Neutrophils	Induction of apoptosis
VI	Natural Killer (NK) Cells	Inhibition of function and proliferation
VII	Mast Cells	Inhibition of degranulation

It is therefore likely that the following mechanisms are involved in the failure of Treg cells:

a) Functional or phenotypic abnormalities
b) The relative balance of Treg cells and effector T cells
c) Elements in the inflammatory microenvironment

Treg-based therapies in autoimmune, inflammatory and allergic diseases

The emerging modalities of Treg cell based therapies include augmenting the numbers and/or increasing the functional activity of Tregs. The current research on Tregs is multi-pronged and includes adoptive Treg cell therapies, biologics, microbial cocktails and small molecule based approaches to boost Treg cell numbers for treatment of autoimmune and inflammatory diseases and prevent transplant rejection.

a) The relative balance of Treg cells and effector T cells

- Augmenting the Treg cell pool by administering additional, functionally suppressive Treg cells as a cellular therapy represents an exciting strategy for treating autoimmune diseases by increasing both Treg cell numbers and their frequency relative to potentially pathological effector leukocytes.
  - ➤ The infusion of ex vivo expanded Treg cell population is currently being explored as a means to alleviate organ transplant rejection and induce tolerance in graft recipients, and has shown some success.
  - ➤ Similar approaches may prove useful in the treatment of some autoimmune diseases.
    - – Preclinical studies using mouse models of autoimmunity and graft-versus-host disease have shown that the adoptive transfer of Treg cells can either prevent or ameliorate severe inflammatory pathologies.
    - – Recent clinical trials in adult and juvenile diabetes (Phase I) and in patients with Crohn's disease (Phase I/IIa) are also yielding promising results.
  - ➤ Other approaches under evaluation include stimulation of in vivo formation and expansion of Tregs using nanoparticles coated with disease-relevant peptide-major histocompatibility complexes (pMHCs).

b) Targeting elements in the inflammatory micro-environment

- Inflammatory cytokines are likely to restrict Treg cell function in patients with autoimmune diseases.
- Interleukin-1β (IL-1β), IL-6 and tumour necrosis factor (TNF) can antagonize FOXP3 expression or function.
- By contrast, Treg cell dysfunction may arise due to a paucity of stabilizing cues such as IL-2.
  - ➤ In support of this, the concentrations of plasma transforming growth factor-β and the activity of signal transducer and activator of transcription 5 have been reported to be decreased in patients with systemic lupus erythematosus and patients with type 1 diabetes, respectively. There are multiple assets under development that target elements in inflammatory microenvironment (e.g. IL-2 agonist and IL-10 agonist).

c) Targeting FOXP3

FOXP3, the transcriptional anchor of the major population of Treg cells responsible for enforcing immune homeostasis, can be exploited and may lead to an unprecedented level of therapeutic control over immune tolerance, especially in autoimmune disease and cancer. Currently, there are no assets targeting FOXP3 in clinical development for autoimmune diseases.

Pipeline Assets Based on Treg Approaches for Management of Autoimmune, Inflammatory and Allergic Diseases

Asset	Company	Highest Phase	Disease
Relative balance of Treg cells and effector T cells			
CLBS03 (autologous)	Caladrius Biosciences	Phase II	Type 1 diabetes mellitus
TR004 (autologous)	Miltenyi Biotec	Phase I/II	Crohn's disease
OvaSave (autologous)	TxCell/Sangamo	Phase I/IIa	Crohn's disease
CK0801 (cord blood)	Cellenkos	Phase I	Graft-versus-host disease, aplastic anemia
TregCel (autologous)	TRACT Therapeutics	Phase I	Transplant rejection, Crohn's disease and autoimmune hepatitis
TREG (autologous)	PolTREG	Preclinical	Type 1 diabetes mellitus
TX200 (CAR engineered autologous Treg cells)	TxCell/Sangamo	Preclinical	Transplant rejection
Navacim (nanoparticles coated with disease-relevant peptide-major histocompatibility complexes)	Novartis/Parvus Therapeutics	Preclinical	Type 1 diabetes mellitus
Targeting elements in the inflammatory microenvironment			
ILT-101 (IL-2 agonist)	Iltoo Pharma (Servier has exclusive license option agreement)	Phase II	SLE, recently diagnosed type 1 diabetes mellitus
AMG 592 (IL-2 agonist)	Amgen	Phase I/II	SLE, graft-versus-host disease, rheumatoid arthritis
PF-06687234 (IL-10 agonist)	Pfizer/Philogen	Phase II	Rheumatoid arthritis, ulcerative colitis
RGI-2001	REGiMMUNE	Phase II	Graft-versus-host disease
NKTR 358 (IL-2 agonist)	Nektar/Eli Lilly	Phase I	SLE
RG 7835 (IgG1-IL2 FP)	Roche	Phase I	Autoimmune diseases
DEL106 (IL-2 agonist)	Celgene	Preclinical	Autoimmune diseases

Note: The list of assets is only for illustrative purpose and not exhaustive

Challenges for Treg-based Therapies

Manufacturing (Adoptive Treg Therapies)	Use in Clinical Practice
• Lack of consensus on the most optimal approach for isolation of Tregs • FDA mandate of documenting sterility, identity, purity, and potency of cell therapy products ➤ While sterility and identity are relatively easy to demonstrate, there is a need to arrive at an acceptable level of non-Treg contamination and disease-specific Treg potency testing systems • Tregs without FOXP3 transcription factor cannot be identified • Difficult to obtain adequate number of cells from patients with pre-existing diminished Treg cells • Manufacturing performance for autologous Tregs can be highly variable and may be influenced by patient demographics, disease status, and medications • In vitro expansion associated with risk of contamination and a decline in functional capacity	• Limited evidence from clinical trials indicates that Tregs are not a 'magic bullet' for all immunopathologies, and good efficacy is seen only in some diseases • Prophylaxis or Treatment - Use in early phase of disease appears optimal as in later phase of disease, Tregs are likely to be used in addition to immunosuppressants, which may alter Treg function • Functional stability - A small population of Foxp3+ cells loses Foxp3 expression over time and is associated with: ➤ A pro-inflammatory microenvironment and switching to an effector T cell phenotype • Therapies targeting elements in the inflammatory microenvironment (e.g. IL-2 agonists) may be associated with the risk of off-cell effect (i.e. activation of conventional T cells)

Conclusion

Treg-based therapeutic approaches represent a novel approach to manage autoimmune diseases by regulating disrupted immune system. Multiple approaches such as adoptive Treg cell therapies as well as targeting mediators that can enhance action of endogenous Treg cells are under evaluation, both in academia and industry. Of particular interest are the Treg-based approaches that can induce antigen-specific immunosuppression and thus have potential to avoid the pitfalls of generalized immunosuppression. Interest in Treg-based approaches by companies such as Celgene, Eli Lilly, Novartis and Pfizer can be taken as an early indicator of the potential of this approach. The focus of pipeline assets is concentrated on diseases such as type 1 diabetes mellitus, SLE, GvHD, transplant rejection and IBD.

Most pipeline assets are in early development, and proof of concept is likely to be available in the next two or three years. The potential of these therapies is likely to be in the prophylactic setting and treatment of newly diagnosed as well as patients refractory to currently available treatment approaches. Pricing, market access and logistic considerations will have a bearing on success of adoptive Treg cell therapies.

Key Questions for Treg-based Therapies

1. Will Treg-based approaches deliver the promise of efficacy and safety, and meet physician and payor thresholds for success?
2. What will be the place in therapy for Treg-based approaches - adoptive Treg cell therapies and therapies targeting elements in the inflammatory microenvironment?
3. What are the likely challenges for adoption of adoptive Treg cell therapies, especially in market access and logistics?
4. What learnings can be drawn from the marketed CAR-T therapies, to understand implications for the commercial opportunity of adoptive Treg cell therapies?
5. What is the true commercial opportunity for Treg-based therapies considering target indications, place in therapy and future competition?

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