BioTechniques
The International Journal of Life Science Methods

Advances in autoimmune disease therapeutics

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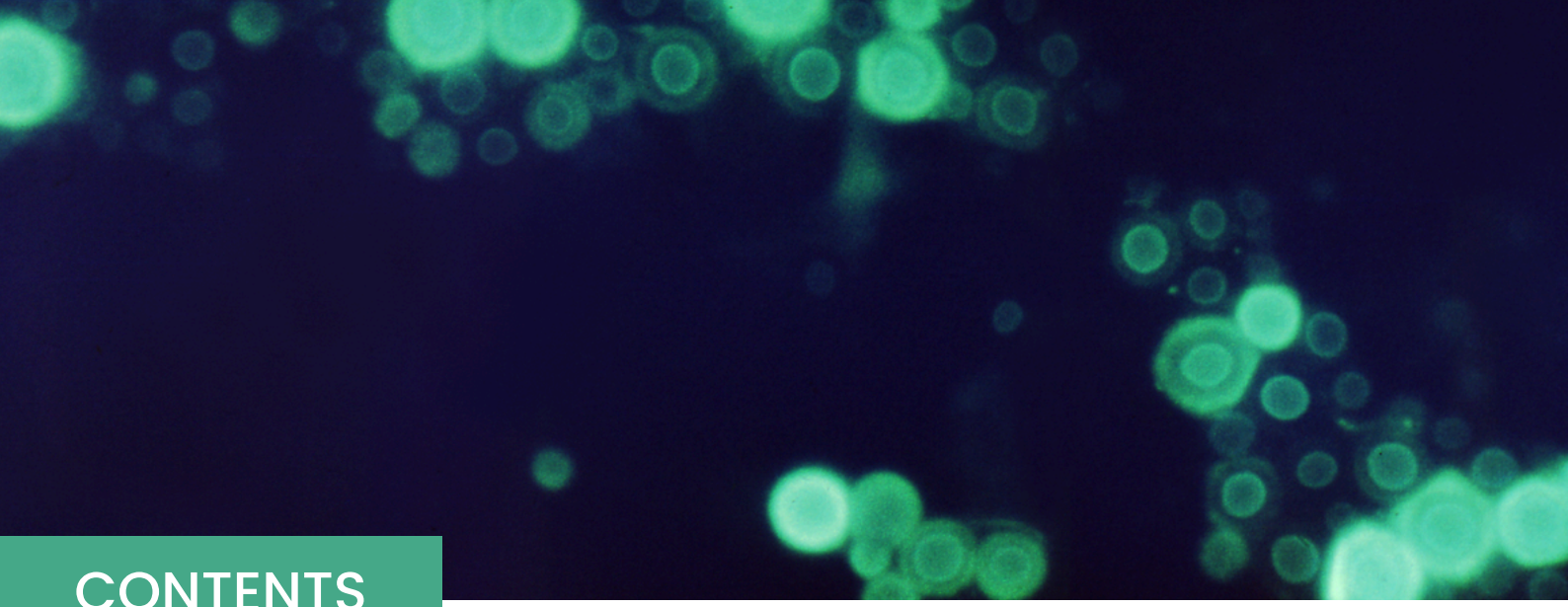

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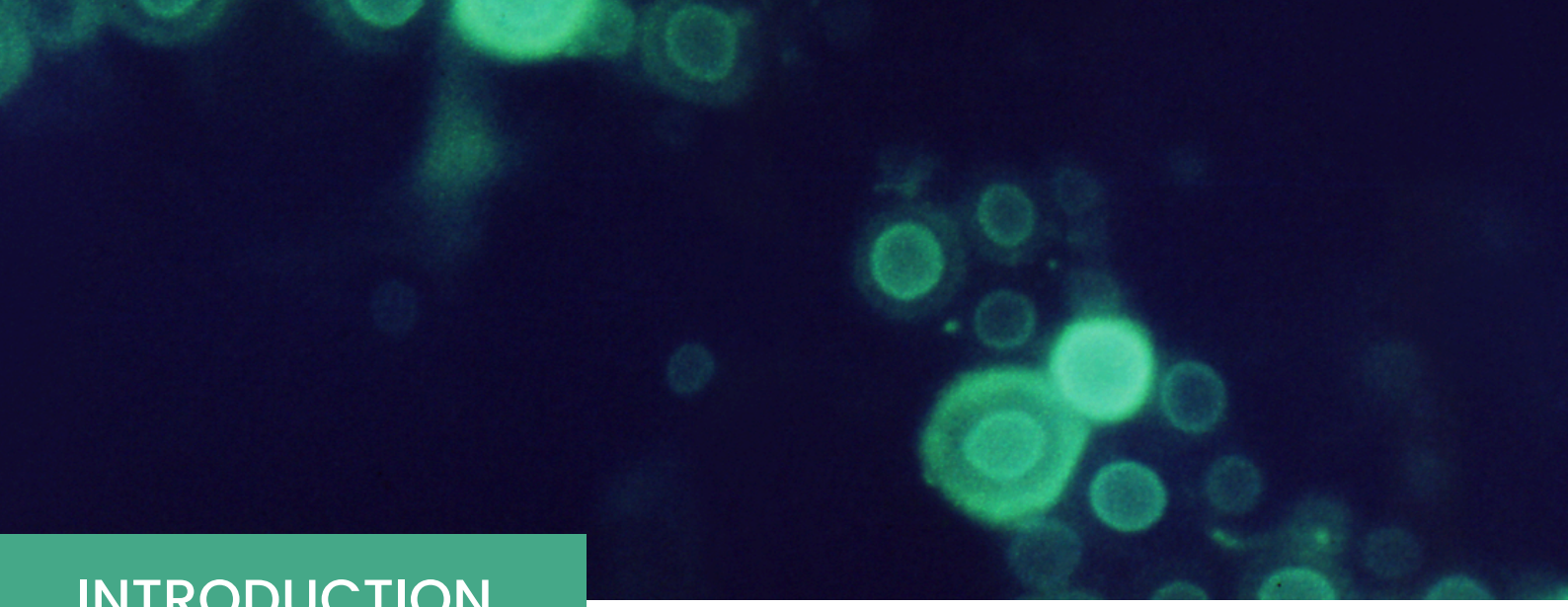

INTRODUCTION

Autoimmune diseases are a group of disorders that cause the immune system to mistakenly attack healthy cells, tissues or organs. Central to the process are cytokines, which exhibit dual functionality in autoimmune diseases as regulators of immune homeostasis and as drivers of disease. Therapeutic approaches that target cytokines, such as monoclonal antibodies and biologic therapies, have been developed and have since become established approaches for treating autoimmune diseases. More recently, emerging research on HLA-DR$^+$ regulatory T cells, a distinct class of Tregs with unique biological properties, and CAR-T cells, a promising immunotherapy, has shown their potential as therapeutic strategies for autoimmune diseases.

In this eBook, we explore both established and emerging therapeutic approaches, offering insights into new areas of research and innovative strategies for autoimmune disease drug development.

In Focus: Autoimmune disease research solutions

Video: Autoimmune disease research solutions

Leading our In Focus on research solutions for autoimmune diseases, this video gives an introduction to different types of autoimmune diseases, how they are detected and how they can be targeted with monoclonal antibodies and small molecule drugs.

Animation by James Harvie.

Talking Techniques | Cytokines networks in autoimmune diseases

In this episode of Talking Techniques, Ritwika Biswas, Field Application Scientist at Sino Biological US Inc. (PA, USA), discusses the role of cytokines in autoimmune diseases, the techniques used to examine them and some emerging therapeutic innovations beginning to change the way we approach the treatment of autoimmune diseases.

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BioTechniques In Focus

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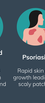
Autoimmune diseases:
the era of targeted treatments

Autoimmune diseases are diverse and complex diseases involving **immune cells**, **cytokines** and **protein kinases**. In recent years, significant progress has been made in drug development and **targeted therapies** for autoimmune diseases, especially biologic drugs. These drugs have primarily focused on **monoclonal antibodies** and **small molecule inhibitors**. This infographic provides a comprehensive overview of autoimmune diseases, covering common types, therapeutic targets, drug development, biomarkers, and their applications, while also highlighting Sino Biological's solutions in this field.

Common autoimmune diseases

Rheumatoid Arthritis
Chronic joint inflammation causing pain and deformity



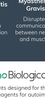
Psoriasis
Rapid skin cell growth leading to scaly patches

Multiple Sclerosis
Damage to the protective covering of nerves, disrupting signals

Ulcerative Colitis
Chronic inflammation and ulcers in the colon and rectum

Type 1 Diabetes
Destruction of insulin-producing cells in the pancreas

Lupus Nephritis
Kidney inflammation impairing filtration function



Myasthenia Gravis
Disrupted communication between nerves and muscles

Interstitial Lung Disease
Inflammation and scarring of lung tissue, affecting breathing

Crohn's Disease
Chronic inflammation that can affect any part of the digestive tract

Systemic Lupus Erythematosus
Widespread inflammation affecting multiple systems

Sino Biological's top tip!

Use reagents designed for the study of your disease of interest. We offer a wide range of research reagents for autoimmune diseases, including each of these examples.

Key therapeutic targets

Immune cell proteins

Certain immune cell proteins play a crucial role in the initiation of aberrant immune responses. Targeting these proteins can modulate the behavior of immune cells to suppress excessive immune responses, reduce inflammation and tissue damage, thereby controlling disease progression.

CD20

- Surface marker on B cells
- Targeting CD20 clears B cells, reducing autoantibody production
- Clinically approved example: The monoclonal antibody ocrelizumab has been approved for multiple sclerosis

CD19

- Co-stimulatory molecule on B cells
- Targeting CD19 clears B cells and reduces autoimmune responses
- Clinically approved example: The monoclonal antibody inebilizumab has been approved for neuromyelitis optica

CTLA-4

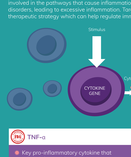
- Inhibitory receptor on T cells
- By binding to CD80/CD86, it suppresses T cell activation and prevents the immune system overactivation.
- Clinically approved example: The fusion protein abatacept has been approved for rheumatoid arthritis

CD 3

- Key component of the T cell receptor complex
- Targeting CD3 modulates T cell function and suppresses excessive immune responses
- Clinically approved example: The monoclonal antibody teplizumab has been approved for type 1 diabetes

Cytokines

Cytokines are proteins secreted by a wide range of immune and non-immune cells, which are involved in the pathways that cause inflammation. They are often overexpressed in autoimmune disorders, leading to excessive inflammation. Targeting cytokines has become an effective therapeutic strategy which can help regulate immune responses and alleviate disease symptoms.



TNF-α

- Key pro-inflammatory cytokine that drives inflammation and tissue damage
- Clinically approved example: The monoclonal antibody adalimumab approved for rheumatoid arthritis

IL-17

- Pro-inflammatory cytokine produced by Th17 cells, driving chronic inflammation and tissue damage
- Clinically approved example: The monoclonal antibody ixekizumab has been approved for plaque psoriasis

BAFF

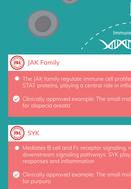
- Key factor for B cell survival and activation, promoting autoantibody production
- Clinically approved example: The monoclonal antibody belimumab has been approved for systemic lupus erythematosus

IL-23

- Key cytokine for Th17 cell differentiation and maintenance, driving chronic inflammation
- Clinically approved example: The monoclonal antibody guselkumab has been approved for plaque psoriasis

Protein Kinases

Kinases play a pivotal role in a number of cellular processes, and through their modulation of inflammatory mediator production, they help regulate immune cell activation, inflammatory responses and signaling pathways. In autoimmune diseases, the function of these kinases can become disrupted, leading to unregulated inflammatory stimulation. Small molecule inhibitors targeting these kinases help control excessive immune activation and reduce inflammation.



JAK Family

- The JAK family regulate immune cell proliferation, differentiation and function by activating STAT proteins, playing a central role in inflammation and immune regulation
- Clinically approved example: The small molecule inhibitor deuruxolitinib has been approved for alopecia areata

SYK

- Mediates B cell and Fc receptor signaling, regulating immune cell function by activating downstream signaling pathways. SYK plays a central role in antibody-mediated immune responses and inflammation
- Clinically approved example: The small molecule inhibitor fostamatinib has been approved for purpura

BTK

- Essential for B cell receptor signaling, promoting B cell activation and antibody production, contributing to autoantibody generation and inflammatory responses.
- Clinically studied example: The small molecule inhibitor tolebrutinib has been studied for treating multiple sclerosis

Sino Biological's top tip!

Skip target isolation and production! Sino Biological offer a range of recombinant targets, cytokines and protein kinases to support autoimmune disease research and drug development.

Biomarkers and Applications

The primary aim of researching biomarkers in autoimmune diseases is to discover markers that change in response to disease progression but then normalize after effective treatment. They play a critical role in diagnosing diseases, predicting outcomes and monitoring treatment responses. They include cytokines, antibodies, and cellular markers, which reflect the immune system's abnormal activity.

Antinuclear Antibodies, ANA

Targets nuclear components, forming immune complexes that activate the immune system and cause inflammation and tissue damage.

Main Diseases

- Systemic Lupus Erythematosus
- Systemic Sclerosis
- Sjogren's Syndrome

C-Reactive Protein, CRP

Acute-phase protein synthesized by the liver in response to inflammation or tissue damage. CRP binds to pathogens or damaged cells, activating the complement system and promoting inflammation and immune clearance.

Main Diseases

- Rheumatoid Arthritis
- Systemic Lupus Erythematosus
- Psoriasis

Interleukin-6, IL-6

Produced by immune cells, IL-6 binds to IL-6 receptors, activating the JAK-STAT pathway and promoting inflammation, immune cell differentiation and acute-phase protein production.

Main Diseases

- Rheumatoid Arthritis
- Castleman disease
- Psoriasis

Complement C3/C4

Central to the complement system, C3 participates in the classical, alternative and lectin pathways, while C4 is involved in the classical and lectin pathways. Upon, they are cleaved into fragments, mediating inflammation and immune clearance.

Main Diseases

- Systemic Lupus Erythematosus
- Lupus Nephritis
- IgA Nephropathy

Sino Biological's top tip!

Improve the reproducibility of your biomarker investigations with our high-quality tools for biomarker analysis and drug discovery, featuring high activity, purity and lot-to-lot consistency.

Techniques for detection

Immunofluorescent antinuclear antibody test

This test involves mixing a blood sample with cells, often HEp-2 cells, whose nuclei are exposed. If ANAs in the sample are present, they will bind to nuclear antigens on the cells. A secondary antibody tagged with fluorescent dye is then added, allowing the ANAs to be viewed through a microscope.

This test is often used to detect ANAs as it is a sensitive test that allows for visualization of present ANAs.

Immunoturbidimetry or nephelometry

These techniques can quantify levels of CRP in a blood sample by measuring the attenuation of light with antigen-antibody complexes.

Either technique can be used for reliable and accurate detection of CRP. Immunoturbidimetry is simple to perform, while nephelometry is more sensitive.

ELISA kit

This technique uses an enzyme-linked antibody to detect the presence of specific biomarkers, such as IL-6 and C3/C4.

This test is often used to detect key biomarkers due to its sensitivity and specificity.

Sino Biological's top tip!

Don't reinvent the wheel! We offer a range of antibodies and ELIZA kits for biomarker studies.

What solutions are available?

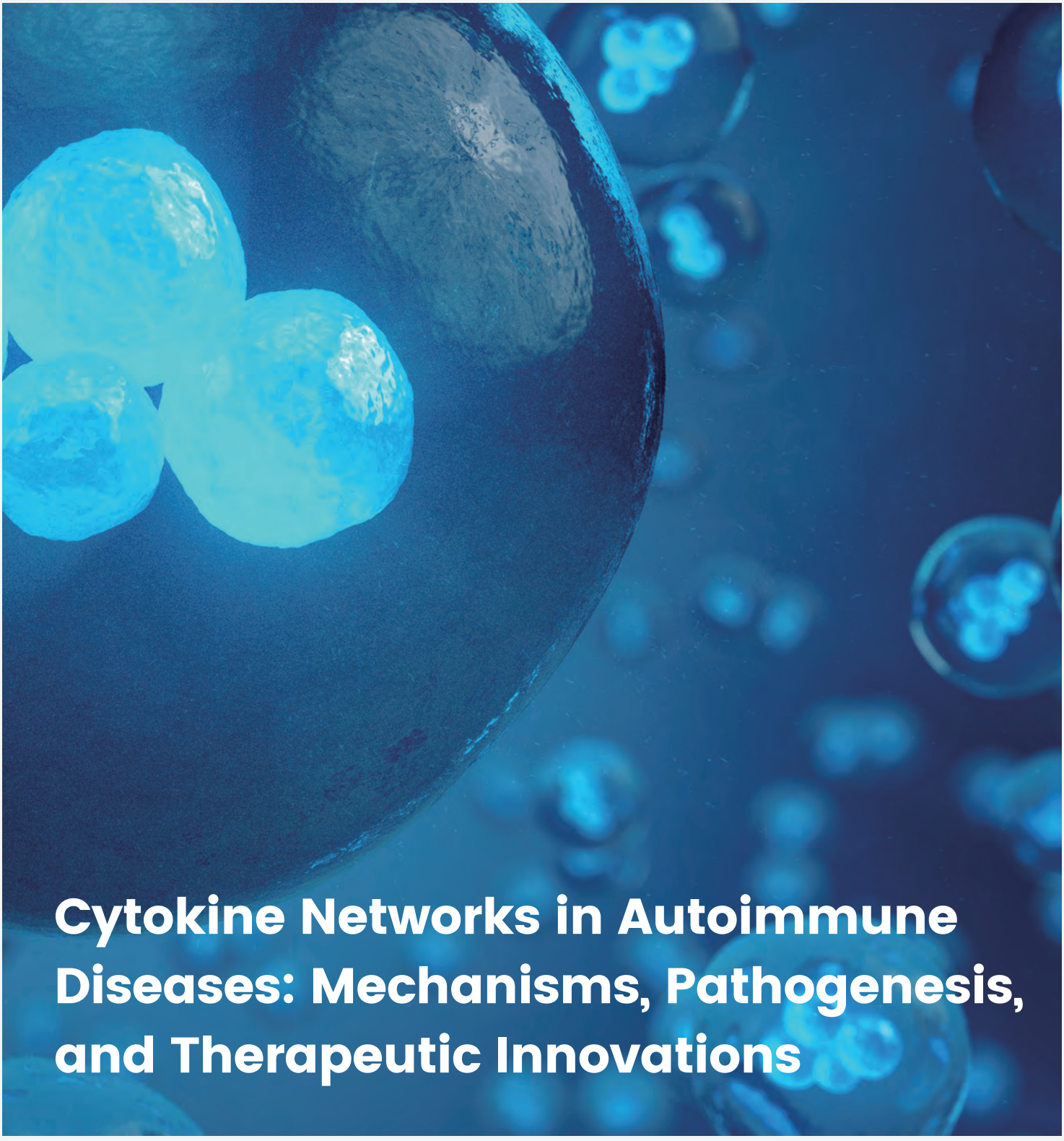
Solutions are available to assist with autoimmune disease research and drug discovery. Sino Biological, for instance, offers comprehensive reagents for autoimmune disease research, such as recombinant target proteins, cytokines and kinases, supporting pathogenesis study, biomarker analysis, targeted drug discovery and diagnostic kit development.

Click here to find out more

This infographic was created as part of a BioTechniques In Focus sponsored by Sino Biological.

BioTechniques In Focus

Sino Biological



Cytokine Networks in Autoimmune Diseases: Mechanisms, Pathogenesis, and Therapeutic Innovations

Autoimmune diseases arise from a complex interplay of immune dysregulation, characterized by the loss of self-tolerance and chronic inflammation. Central to these processes are cytokines - soluble signaling proteins that orchestrate immune responses by mediating communication between cells. Cytokine networks exhibit dual functionality: they are essential regulators of immune homeostasis, yet paradoxically act as pathogenic drivers in diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), psoriasis, and inflammatory myopathies. Key findings highlight the therapeutic potential of cytokine-targeted biologics, Janus kinase (JAK) inhibitors, and emerging strategies such as miRNA modulation and engineered cytokines.

Mechanism of Autoimmunity

Autoimmunity results from complex disruptions in immune signaling pathways where genetic predisposition and environmental factors trigger the activation of self-reactive lymphocytes, excessive cytokine production, and autoantibody release that collectively damage normal tissues. Resolution of autoimmune processes, when possible, depends on the restoration of regulatory mechanisms through regulatory T cells (Tregs) and regulatory B cells (Bregs) that produce immunosuppressive cytokines like transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10), which help repair tissue damage and suppress inflammatory pathways activated during initiation and propagation phases.

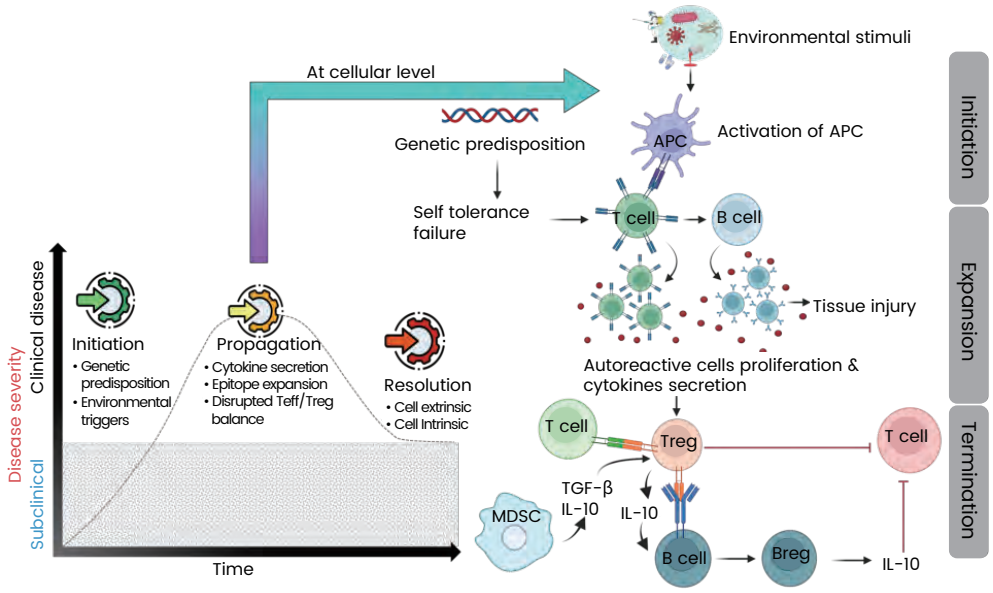


Figure 1. Mechanism of autoimmune disease progression[1].
Figure source : https://doi.org/10.3390/ijms25147666

Cytokine Networks in Autoimmune Pathogenesis

Pro-Inflammatory Cytokine Dominance

The pathogenesis of autoimmune diseases is frequently driven by a hyperactive pro-inflammatory cytokine environment. Tumor necrosis factor-alpha (TNF-α), IL-6, and IL-17 are pivotal mediators in conditions like RA and psoriasis. The IL-1 family, including IL-1β and IL-18, further exacerbates tissue damage by activating the NLRP3 inflammasome, a mechanism implicated in SLE[2,3]. These cytokines not only drive local inflammation but also contribute to systemic manifestations, such as fever and fatigue, through their actions on the hypothalamic-pituitary-adrenal axis[4].

Table 1. Disease-specific cytokines

Disease	Cytokines Involved	References
Rheumatoid Arthritis	TNF-α, IL-6, GM-CSF, IL-23/IL-17 axis, IL-7 and IL-21	Kondo, N., *et al*, 2021; Leung, S. *et al*, 2010
Systemic Lupus Erythematosus	IFN-α/β, IL-2, IL-1β, IL-18 and IL-12	Leung, S., *et al*, 2010; Kotyla, P., *et al*, 2022
Psoriasis and Psoriatic Arthritis	IL-23/IL-17 axis (IL-17A, IL-17F), IL-22, TNF-α and IL-6	Wojas-pelc, A., *et al*, 2006
Inflammatory Myopathies	IFN-α/β, IFN-γ, CXCL9, CXCL10, and IL-1β	De Paepe, B., *et al*, 2015

GM-CSF: granulocyte-macrophage colony-stimulating factor
IFN-α/β: type I interferons

Regulatory Cytokine Deficiencies

Counterbalancing pro-inflammatory signals are immunosuppressive cytokines such as IL-10 and TGF-β. IL-10, produced by Bregs and Tregs, inhibits antigen presentation and suppresses Th1/Th17 responses[5,6]. In SLE, reduced IL-10 production by Bregs correlates with disease flares, highlighting its protective role[6]. TGF-β, conversely, maintains peripheral tolerance by inducing Treg differentiation and suppressing effector T cell proliferation[3]. Dysregulation of these regulatory pathways creates a permissive environment for autoimmunity, as seen in RA and multiple sclerosis (MS), where defective TGF-β signaling permits unchecked Th17 activity[3,7].

Cytokine Imbalance and Feedback Loops

Autoimmune diseases often feature self-reinforcing cytokine feedback loops. For instance, IL-6 enhances Th17 differentiation while inhibiting Treg development, creating a pathogenic cycle that sustains inflammation[7]. In psoriasis, IL-23 produced by dendritic cells perpetuates Th17 survival, which in turn secretes IL-17 and IL-22, further activating keratinocytes and stromal cells[8]. These loops are exacerbated by tissue-resident cells, such as synovial fibroblasts in RA, which produce chemokines like CXCL13 to recruit B cells and plasma cells, fostering ectopic lymphoid structure formation[7].

Therapeutic Targeting of Cytokine Networks

Biologic Therapies

Anti-TNF-α Agents: TNF inhibitors (e.g., infliximab, adalimumab) revolutionized RA treatment by reducing synovitis and radiographic progression[7]. However, TNF blockade may paradoxically induce psoriasiform lesions in some patients, underscoring cytokine pleiotropy[8].

IL-6 Inhibition: Tocilizumab, an IL-6 receptor antagonist, ameliorates systemic inflammation in RA and juvenile idiopathic arthritis (JIA)[7]. IL-6 blockade also shows promise in neuromyelitis optica spectrum disorder (NMOSD), reducing relapse frequency.

IL-17/IL-23 Axis Targeting: Secukinumab (anti-IL-17A) and ustekinumab (anti-IL-12/23p40) achieve rapid skin clearance in psoriasis by disrupting Th17 signaling[8]. Brodalumab, targeting the IL-17 receptor, demonstrates efficacy in psoriatic arthritis[8].

JAK/STAT Inhibition

JAK inhibitors (jakinibs) modulate cytokine signaling by blocking downstream STAT phosphorylation. Tofacitinib (JAK1/3 inhibitor) and baricitinib (JAK1/2 inhibitor) are approved for RA, suppressing IFN-γ, IL-6, and GM-CSF pathways[9]. In SLE, JAK inhibitors reduce IFN-α signature and ameliorate nephritis, offering an alternative to broad immunosuppression[9].

miRNA Modulation

miRNAs regulate cytokine production post-transcriptionally. miR-155 promotes TNF-α and IL-6 in RA synovium, while miR-146a feedback inhibits NF-κB signaling[12]. Antagomirs targeting miR-155 reduce disease severity in experimental autoimmune encephalomyelitis (EAE), highlighting the potential of miRNA-based therapies[12].

IL-2-Based Immunotherapies

Low-dose IL-2 expands Tregs, restoring immune tolerance in SLE and type 1 diabetes[10]. Engineered IL-2 variants with enhanced Treg specificity (e.g., IL-2-anti-IL-2 complexes) show promise in preclinical models, though clinical trials report variable efficacy[10,11].

Engineered Cytokines

Cytokine engineering aims to enhance therapeutic specificity. PEGylated IL-10 (AM0010) prolongs half-life and suppresses colitis in preclinical models[11]. Similarly, IL-4 fusion proteins bias macrophage polarization toward an anti-inflammatory phenotype, offering novel strategies for fibrosis-prone diseases like systemic sclerosis[11].

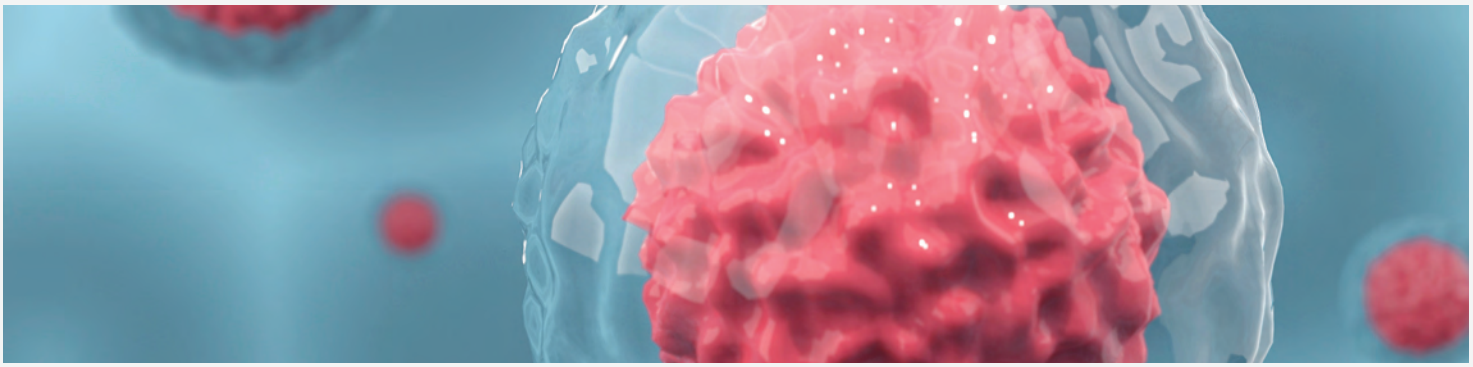


Table 2. Representative FDA-approved drugs for autoimmune diseases targeting cytokines

Drug Name	Target Cytokine	Brand Name	FDA Approval	Indications
Etanercept	TNF-α	Enbrel	1998	Rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), ankylosing spondylitis (AS), psoriasis, psoriatic arthritis (PsA)
Infliximab	TNF-α	Remicade	1998	RA, AS, psoriasis, PsA, ulcerative colitis (UC), Crohn's disease (CD)
Adalimumab	TNF-α	Humira	2002	RA, JIA, AS, psoriasis, PsA, UC, CD, hidradenitis suppurativa, uveitis
Golimumab	TNF-α	Simponi	2009	RA, AS, PsA, UC
Certolizumab	TNF-α	Cimzia	2008	RA, AS, psoriasis, PsA, CD
Anakinra	IL-1	Kineret	2001	RA, Cryopyrin-associated periodic syndromes (CAPS)
Tocilizumab	IL-6R	Actemra	2010	RA, juvenile idiopathic arthritis (JIA), adult-onset Still's disease (AOSD), giant cell arteritis, cytokine release syndrome
Sarilumab	IL-6R	Kevzara	2017	RA
Siltuximab	IL-6	Sylvant	2014	Multicentric Castleman's disease
Satralizumab	IL-6R	Enspryng	2020	Neuromyelitis optica spectrum disorder (NMOSD)
Secukinumab	IL-17A	Cosentyx	2015	AS, psoriasis, PsA
Ixekizumab	IL-17A	Taltz	2016	Psoriasis, PsA, AS
Brodalumab	IL-17 receptor	Siliq	2017	Psoriasis
Ustekinumab	IL-12, IL-23	Stelara	2009	Psoriasis, PsA, CD, UC
Canakinumab	IL-1β	Ilaris	2009	CAPS, systemic juvenile idiopathic arthritis (sJIA), TRAPS, HIDS/MKD, familial Mediterranean fever (FMF)
Satralizumab	IL-6R	Enspryng	2020	Neuromyelitis optica spectrum disorder (NMOSD)
Tofacitinib	JAK1, JAK3	Xeljanz	2018	RA, PsA, UC, AS, JIA

Summarized based on Jung S and Kim W's paper[13].

Abbreviations:
RA: Rheumatoid Arthritis
AS: Ankylosing Spondylitis
PsA: Psoriatic Arthritis
UC: Ulcerative Colitis
CD: Crohn's Disease
JIA: Juvenile Idiopathic Arthritis
pJIA: Polyarticular Juvenile Idiopathic Arthritis
FMF: Familial Mediterranean Fever
NMOSD: Neuromyelitis Optica Spectrum Disorder

Conclusion

Advances in cytokine biology have unraveled disease-specific signatures, enabling precision therapies that target key nodes within these networks. Despite successes, challenges remain, including cytokine redundancy, pleiotropy, and interpatient heterogeneity. Future directions include microbiome-directed interventions, personalized cytokine profiling, and engineered biologics with enhanced cell-type specificity. By integrating mechanistic insights with innovative therapeutics, the next frontier in autoimmunity lies in harnessing the cytokine network to restore immune equilibrium.

To support targeted research and drug development for autoimmune diseases, Sino Biological provides a range of high-quality cytokine products. Our products undergo stringent quality control to ensure high purity, bioactivity, stability, and low endotoxin levels, with options available across multiple species, including human, mouse, monkey, and rat. Additionally, Sino Biological provides comprehensive solutions for autoimmune diseases , offering a wide range of research reagents for nearly 50 diseases. Our portfolio includes autoimmune disease target reagents such as target proteins, cytokines, and kinases, as well as research reagents for biomarker studies. By providing high-quality tools for biomarker analysis and drug discovery, Sino Biological plays a crucial role in advancing early detection and targeted therapy development of autoimmune diseases.

Featured Products for Autoimmune Research

Cat#	Molecule	Species	Expression Host	SEC-HPLC Purity	Activity
GMP-11848-HNAE	IL-2	Human	*E. coli*	≥ 95%	Active
GMP-11846-HNAE	IL-4	Human	*E. coli*	≥ 95%	Active
GMP-10360-HNAE	IL-15	Human	*E. coli*	≥ 95%	Active
GMP-CT011-H08H	IL-12	Human	HEK293 Cells	≥ 95%	Active
51112-MNAH	M-CSF/CSF1	Mouse	HEK293 Cells	≥ 95%	Active
10602-HNAE	TNF-alpha	Human	*E. coli*	≥ 95%	Active
10804-HNAC	TGF beta 1	Human, Rhesus, Cynomolgus, Canine	CHO Stable Cells	> 95% (SDS-PAGE)	Active
10395-HNAE	IL-6	Human	*E. coli*	≥ 95% (SDS-PAGE)	Active
11821-HNAE	IL-7	Human	*E. coli*	≥ 95%	Active
50245-MNAE	IL-10	Mouse	*E. coli*	≥ 90% (SDS-PAGE)	Active
12047-HNAE	IL-17	Human	*E. coli*	≥ 95% (SDS-PAGE)	Active
CT048-H08H	IL-23	Human	HEK293 Cells	> 90% (SDS-PAGE)	Active
10119-HNCE	IL-18	Human	*E. coli*	≥ 95%	Active
17049-H07H2	TL1A/TNFSF15	Human	HEK293 Cells	≥ 95%	Active

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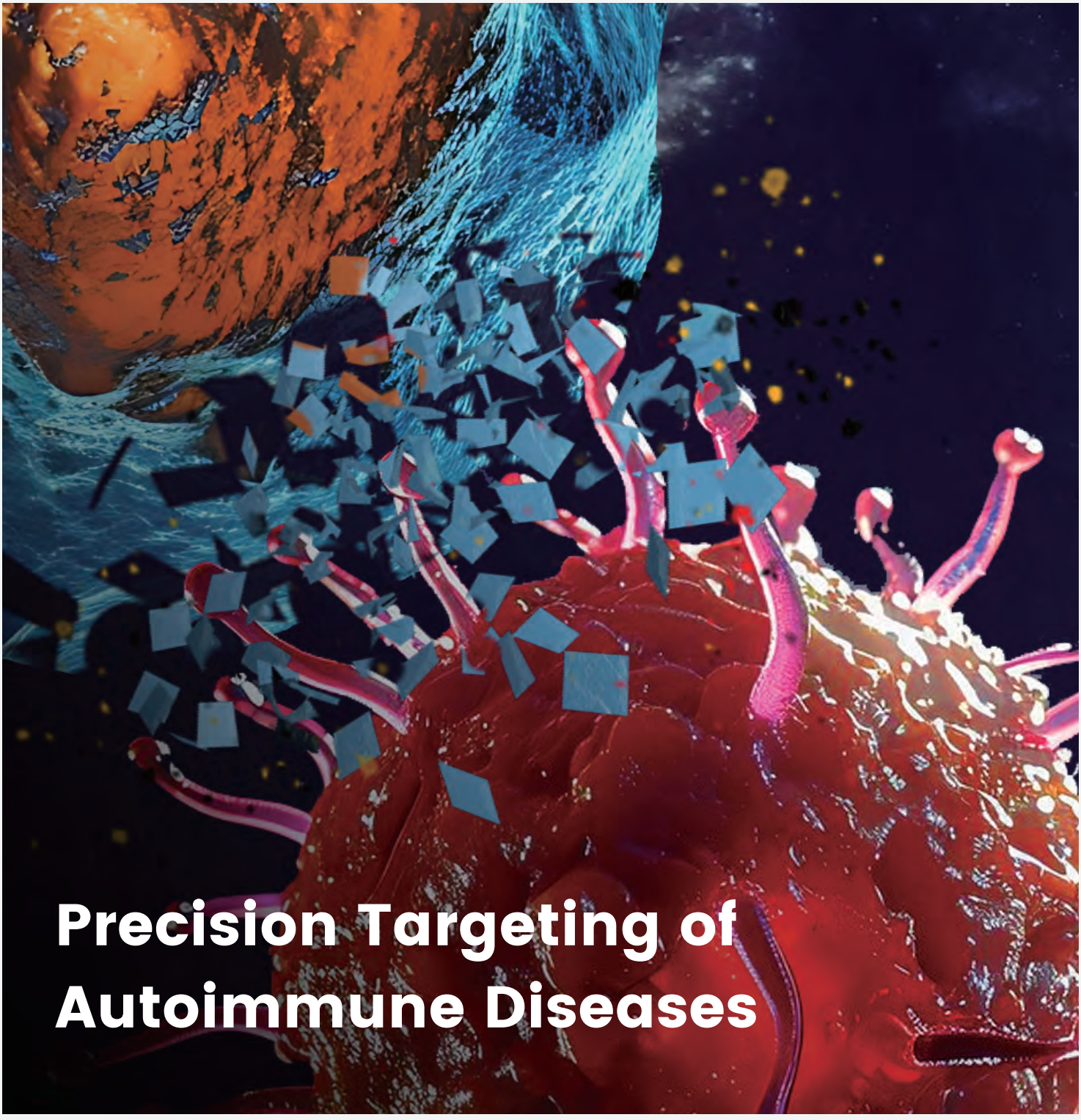

Precision Targeting of Autoimmune Diseases

Autoimmune diseases represent a wide range of disorders affecting specific organs or the entire body, all characterized by the immune system's misrecognition of self-antigens. These conditions arise from a combination of genetic and environmental factors. The immune system in these disorders fails to properly distinguish between foreign pathogens and the body's own structures, leading to sustained attacks on healthy cells, tissues and organs.[1] This pathological process results in chronic inflammation, progressive tissue damage, fibrosis, and eventual organ dysfunction. To date, researchers have identified around 150 autoimmune diseases, with several validated drug targets in approved therapies (Table 1). Common autoimmune diseases include rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus (SLE), type 1 diabetes (T1DM), and inflammatory bowel disease (IBD)[2,3,4].

Table 1. Approved Therapies for Autoimmune Disease and Their Targets

Target	Drug Name	Indications	Approved Year
FCRN/FCGRT	Rozanolixizumab	Myasthenia Gravis	2023/6/26
CD20	Ublituximab	Multiple Sclerosis Relapse	2022/12/28
CD3	Teplizumab	Type 1 Diabetes	2022/11/17
IL1RL2	Spesolimab	Generalized Pustular Psoriasis	2022/9/1
IFNAR1	Anifrolumab-FNIA	Systemic Lupus Erythematosus	2021/7/30
IGF1R	Teprotumumab-TRBW	Graves Ophthalmopathy	2020/1/21
CD19	Inebilizumab-cdon	Neuromyelitis Optica	2020/6/11
CD20	Ocrelizumab	Multiple Sclerosis	2017/3/28

Despite their clinical manifestations, they share the same core pathology: dysregulated T and B cell activity. B cells contribute to disease progression by producing autoantibodies, secreting pro-inflammatory cytokines, and serving as antigen-presenting cells (APCs) that activate other components of the immune system.[5] Meanwhile, CD4⁺ T lymphocytes coordinate immune responses by secreting cytokines and activating various immune cell populations. When dysregulated, they generate autoreactive T cells, leading to chronic inflammation and tissue damage (Figure 1)[6].

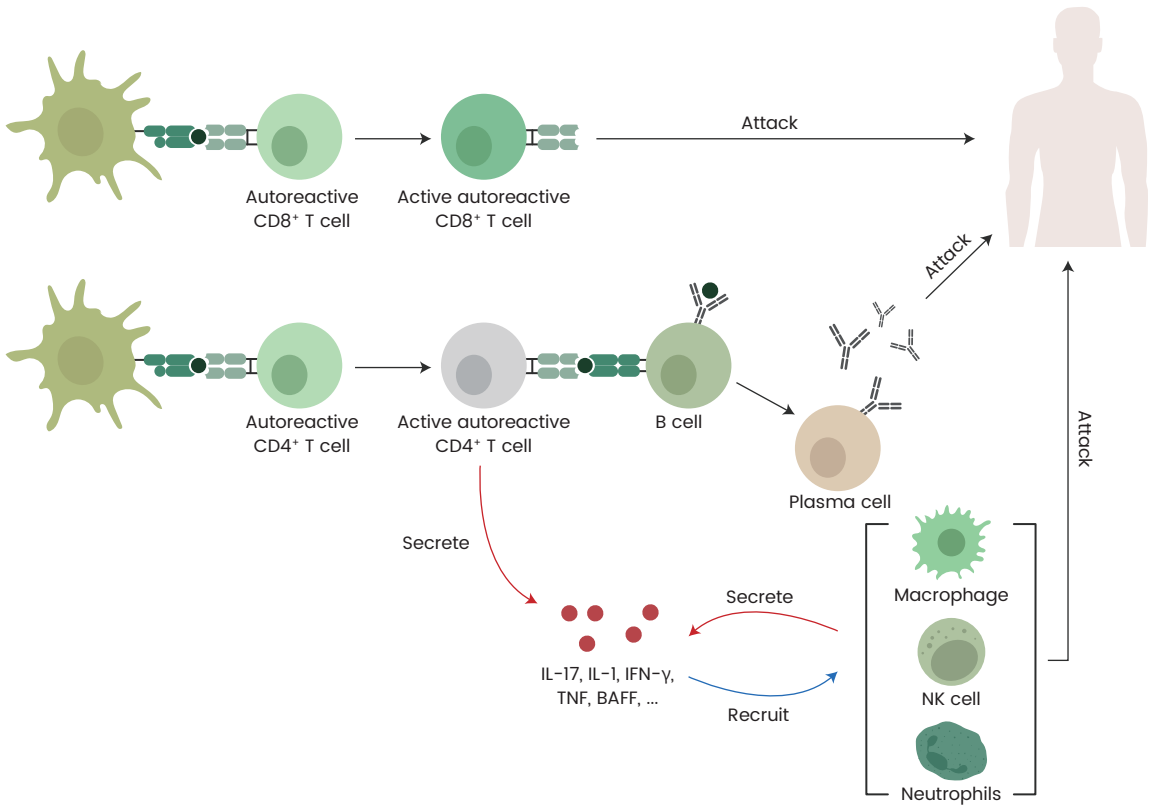

Figure 1. Autoreactive T cells, B cells, macrophages, NK cells, neutrophils, and cytokines (IL-17, IL-1, IFN-γ, TNF, BAFF) contribute to autoimmune responses and inflammation.[7]

The rising prevalence and complexity of autoimmune diseases present significant challenges for healthcare and the pharmaceutical industry. Current treatments often lack specificity, broadly targeting the immune system and leading to side effects like increased infection risk and allergic reactions[8]. However, promising strategies with higher target specificity—such as chimeric antigen receptor T (CAR-T) cell therapy and antibody-drug conjugates (ADC)—are now under investigation.[6, 9]

CAR-T Cell Therapy: A Targeted Approach for Autoimmune Diseases

Originally designed for cancer treatment, CAR-T therapy is now being explored for autoimmune diseases. Since many of these conditions arise from autoreactive B and T cells, selectively eliminating them offers a novel therapeutic strategy. Research is focused on engineering CAR-T cells to recognize and remove cells expressing specific surface markers. In B cell–mediated autoimmune diseases, targets like CD19, CD20, CD38, and BCMA have been studied (Table 2), while CD7 and CD70 are being investigated in T cell–driven disorders[4].

Among all CAR-T therapy targets, CD19 appears to be the most promising and well-studied. Clinical trials investigating CD19 CAR-T cells for severe SLE, MS, systemic sclerosis (SSc), and lupus nephritis (LN) have shown effective disease control with favorable safety profiles[10-11].

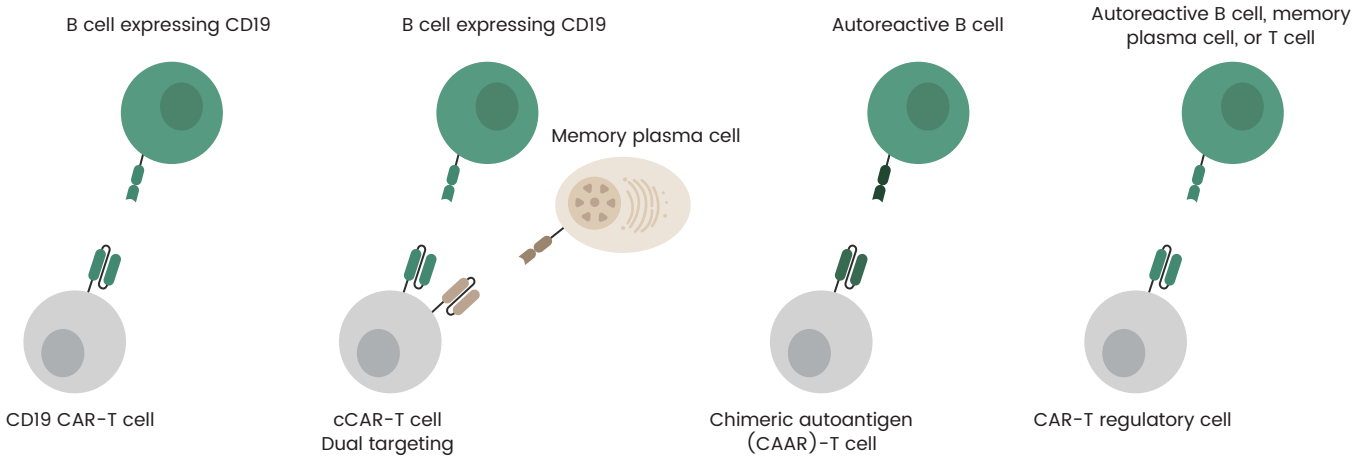

Figure 2. Overview of CAR-T cell engineering strategies for autoimmune diseases, including broad B cell targeting, dual targeting, selective depletion of autoreactive B cells, and T regulatory cell engineering.[6]

A recent case of severe SLE treated with autologous CD19 CAR-T cells showed complete and sustained depletion of circulating B cells, leading to the disappearance of dsDNA autoantibodies. No adverse events were reported during the treatment.[10] A separate study involving five patients with SLE treated with CD19 CAR-T cells further supports these findings. All patients achieved sustained clinical remission one year after treatment. Naïve B cells began to re-emerge three months post-therapy, without recurrence of SLE symptoms. This suggests an effective reset of the immune system, promoting long-term disease control.[11]

Table 2. Representative clinical trials for CAR-T cell therapy in autoimmune diseases.[11]

Target	Condition	NTC	Phase	Sponsor
CD19	Severe, refractory systemic lupus erythematosus	NCT05869955	Phase 1	Bristol-Myers Squibb
CD19	Systemic lupus erythematosus	NCT05765006	Phase 1	Shanghai Ming Ju Biotechnology Co., Ltd.
CD19	Refractory lupus nephritis	NCT05938725	Phase 1	Kyvema Therapeutics
CD19	Systemic lupus erythematosus/lupus nephritis	NCT05798117	Phase 1	Novartis Pharmaceuticals
CD19	Refractory/Moderate-to-severe systemic lupus erythematosus	NCT06106096	Phase 1/2	Wuhan Union Hospital, China
CD19/BCMA	Relapsed/refractory systemic lupus erythematosus	NCT05474885	Phase 1	iCell Gene Therapeutics
CD19/CD20	Refractory systemic lupus erythematosus	NCT06153095	Phase 1	ImmPACT Bio
CD19	Relapsing/Progressive multiple sclerosis	NCT06222001	Phase 1	Juno Therapeutics
CD19	Refractory myasthenia gravis	NCT05828225	Phase 2	Zhejiang University
BCMA	Generalized myasthenia gravis	NCT04146051	Phase 1	Cartesian Therapeutics

Despite promising results in SLE, challenges remain in applying CAR-T therapy to other autoimmune diseases. In some cases, autoantibodies are produced by plasmablasts or plasma cells that lack CD19 expression, allowing them to evade CD19-targeted therapies.[12] Further research is needed to refine and expand CAR-T cell strategies, adapting them to the specific immunopathology of various autoimmune diseases.

ADC: Leveraging Cancer Therapy Success for Autoimmune Treatment

Antibody-drug conjugates (ADCs), which combine the precision of monoclonal antibodies with the potent cytotoxicity of cytotoxic payloads, are well-established in cancer therapies but now show promise for autoimmune diseases. Given the limitations of antibody-based treatments (e.g., anti-TNF monoclonal antibody) for conditions like rheumatoid arthritis (RA), ADCs offer a more targeted approach by selectively eliminating pathogenic immune cell subsets, such as autoreactive T or B cells, while sparing protective immunity. Several studies have shown promising results with ADCs targeting TNF,[13] CD74,[14] CD30,[15] CD163[16] and CD6[17]. By delivering immunomodulatory or cytotoxic payloads directly to pathogenic cells, ADCs could overcome limitations of systemic immunosuppression, reducing off-target toxicity and improving therapeutic precision in autoimmune conditions.

For instance, D30, a TNF receptor family member, is elevated in RA serum and joint fluid, making it a potential therapeutic target. Brentuximab vedotin (BV), a CD30-targeting antibody-drug conjugate (ADC) combining brentuximab with antimitotic MMAE, is currently being investigated for RA treatment. In CAIA mouse models, BV significantly reduced arthritis severity at a higher dose (70 mg/kg), while the lower dose (30 mg/kg), equivalent to the human clinical dose, showed no significant effect[16].

While ADCs have shown promise for autoimmune diseases, their adaptation for presents various challenges. Autoimmune targets like CD30 may be expressed on both pathogenic and protective immune cell populations, increasing the risk of unintended immunosuppression. Additionally, ADC designs should enhance safety profiles, particularly in linker stability, to prevent off-target payload release and long-term immune cell depletion. Further research is needed to refine target selection and optimize payload efficacy to develop next-generation ADCs featuring tunable cytotoxicity suitable for prolonged autoimmune disease therapy.

Conclusion and Future Outlook

Precision-targeted therapies are transforming autoimmune disease management, addressing the limitations of conventional immunosuppressants. CAR-T cell therapy offers a promising approach by selectively eliminating autoreactive B and T cells, with CD19-targeted therapies demonstrating sustained disease remission in conditions like systemic lupus erythematosus (SLE). Meanwhile, ADCs leverage antibody specificity to deliver cytotoxic payloads, refining immune modulation strategies for autoimmune diseases. As research advances, optimizing these therapies for broader applications will be crucial. Future investigations should focus on refining CAR-T targets beyond CD19, adapting ADC payloads for autoimmune specificity, and mitigating off-target effects. Continued exploration of immunotherapy will pave the way for more precise, long-lasting treatments[18,19].

Sino Biological's Efforts on Autoimmune Disease Research

Sino Biological offers comprehensive solutions for autoimmune disease research, providing reagents for nearly 50 diseases. Our portfolio includes target proteins, cytokines, and kinases, as well as biomarker research tools. By delivering high-quality reagents for biomarker analysis and drug discovery, Sino Biological supports early detection and the development of targeted therapies. Additionally, we also provide CAR-T therapy development solutions and ADC development solutions. Our solutions are dedicated to providing high-quality reagents and technical support for global drug R&D companies and life science research institutions.

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Innovative strategies for the discovery of new drugs against alopecia areata: taking aim at the immune system

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REVIEW

Innovative strategies for the discovery of new drugs against alopecia areata: taking aim at the immune system

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ABSTRACT

Introduction: The autoimmune hair loss condition alopecia areata (AA) exacts a substantial psychological and socioeconomic toll on patients. Biotechnology companies, dermatology clinics, and research institutions are dedicated to understanding AA pathogenesis and developing new therapeutic approaches. Despite recent efforts, many knowledge gaps persist, and multiple treatment development avenues remain unexplored.

Areas covered: This review summarizes key AA disease mechanisms, current therapeutic methods, and emerging treatments, including Janus Kinase (JAK) inhibitors. The authors determine that innovative drug discovery strategies for AA are still needed due to continued unmet medical needs and the limited efficacy of current and emerging therapeutics. For prospective AA treatment developers, the authors identify the pre-clinical disease models available, their advantages, and limitations. Further, they outline treatment development opportunities that remain largely unmapped.

Expert opinion: While recent advancements in AA therapeutics are promising, challenges remain, including the lack of consistent treatment efficacy, long-term use and safety issues, drug costs, and patient compliance. Future drug development research should focus on patient stratification utilizing robust biomarkers of AA disease activity and improved quantification of treatment response. Investigating superior modes of drug application and developing combination therapies may further improve outcomes. Spirited innovation will be needed to advance more effective treatments for AA.

PLAIN LANGUAGE SUMMARY

Alopecia areata (AA) is an autoimmune condition that causes hair loss. It significantly affects a patient's emotional well-being and quality of life. Companies, clinics, and researchers are working hard to understand AA and create better treatments. Despite these efforts, there are still many unanswered questions, and new treatment methods still need to be explored.

This review summarizes how AA develops, current treatment options, and new therapies like Janus Kinase (JAK) inhibitor drugs. JAK inhibitors show promise, but they are not fully effective for everyone. We emphasize that there is still a need for new and innovative drug discovery strategies to meet the medical needs of AA patients, as current treatments often fall short.

For researchers and developers of AA treatments, we discuss the available pre-clinical models used to test new drugs, highlighting their strengths and weaknesses. We also point out new areas for treatment development that have not been thoroughly investigated.

Although recent advancements in AA treatments are encouraging, several challenges remain. These include inconsistent effectiveness of treatments, safety concerns with long-term use, high drug costs, and issues with patient adherence to treatment programs. We believe future research should focus on identifying biomarkers that can help tailor treatments to individual patients and improving measurements of treatment success. Additionally, exploring better ways to apply drugs and combining different therapies together may enhance treatment outcomes.

Ultimately, innovative approaches and spirited efforts will be required to develop more effective treatments for AA to improve the lives of those affected by this challenging condition.

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Alopecia areata; autoimmune disease; disease pathogenesis; JAK inhibitors; disease models; drug development; hair follicles; immune privilege; hair regrowth

1. Introduction

Alopecia areata (AA) is a complex autoimmune disease characterized by unpredictable non-scarring hair loss which can be observed on the scalp or any other hairy surface on the body [1]. AA can either resolve on its own, progress to cycles of repeated relapse and recovery, or it may persist over a prolonged period of time [2,3]. Though the first onset of AA can occur at any age, current data shows only around 20% of the patients are over 40 years old, making it an issue of particular concern for young adults [4]. The sudden onset of AA can significantly diminish a patient's self-confidence and quality of life (QoL), and it imposes a substantial psychological burden on affected individuals [5–7].

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Article highlights

- Alopecia areata (AA) is primarily driven by an autoimmune response where cytotoxic CD8+ T cells attack hair follicles, leading to a non-scarring, potentially reversible, form of hair loss.
- Existing AA treatments, specifically corticosteroids and contact sensitizers, offer limited and variable efficacy; whereas JAK inhibitors have shown promise in recent clinical trials, their long-term safety and efficacy remain uncertain.
- Innovative drug discovery strategies are still required to address the unmet medical needs of AA patients, focusing on improving efficacy for more patients, reducing long-term safety issues, lowering costs, and increasing patient compliance.
- Several *in vitro* and *in vivo* pre-clinical models are available for AA research and therapeutics development, each with specific advantages and limitations.
- The drug discovery process for AA is hindered by the heterogeneity of the condition, our still relatively poor understanding of the AA disease mechanisms, a lack of validated biomarkers, and limitations around clinical trial designs for AA.
- Future research should focus on patient stratification using robust biomarkers to tailor treatments and improve the accuracy of treatment response assessments.
- Exploration of alternative molecular pathways, such as hair follicle immune privilege promotion and interference with hair follicle antigen presentation, could provide other avenues for AA treatment development beyond systemic immunosuppressive drugs.
- Improving drug delivery methods, systematic optimization of new and existing treatments, and developing adjunctive combinatory therapies may enhance treatment outcomes and improve patient adherence.

1.1. Alopecia areata clinical diagnosis

AA can occur in any hair-bearing area, but mostly affects the scalp. The clinical presentation varies in the extent and configuration of hair loss involving patches, ophiasis (occipital scalp), ophiasis inversus or sisapho (top and vertex), diffuse AA, alopecia totalis (whole scalp), and alopecia universalis (whole scalp and body hair) [8,9]. Most cases are straightforward to diagnose, especially patchy AA which is the most common presentation. In contrast, diffuse AA can be difficult to differentiate from telogen effluvium [3,10]. If necessary, a biopsy can be done; the acute disease phase characteristically shows a lymphocytic infiltrate surrounding dystrophic anagen hair follicles (HFs) in a 'swarm of bees' pattern. As the disease progresses to a chronic state, there is an increase in catagen/telogen stage HFs and development of miniaturized hairs [11].

1.2. Alopecia areata disease pathogenesis

Both cellular and humoral immunity are active during AA onset, though cellular immunity plays the primary pathogenic role [12,13]. Multiple studies have provided data consistent with a cytotoxic $CD8^+$ T lymphocyte cell (CTL) mediated attack on HFs that leads to hair loss [14–18]. A few studies suggest $CD8^+$ CTLs are assisted in their HF attack by $CD4^+$ T cells in their classic 'helper' role [17,19–23]. Some of the potential autoantigen targets for autoreactive CTLs have been identified, but most remain unknown at this time. The most salient endogenous antigens investigated thus far include hair-follicle keratinocyte-expressed trichohyalin and keratin 16 and melanocyte-related proteins such as tyrosinase-related protein 2 [24].

1.3. Hair follicle immune privilege

Anagen stage HFs exhibit immune privilege (IP) [15,25]. The essential function of IP is to protect and preserve high-frequency stem cells for HF regeneration and cycling. Several factors are considered to be important in providing HF IP, including physical barriers that inhibit lymphocyte infiltration [26], the downregulation of major histocompatibility complex I (MHC-I) [27], the expression of immunosuppressive factors in the local environment [15], the maintenance and distribution of immunoregulatory cells [28], low NKG2D ligand expression, and the downregulation of NKG2D receptors on local NK cells [29]. Numerous immunosuppressive factors are produced by HFs, including transforming growth factor beta (TGFβ), alpha-melanocyte-stimulating hormone (αMSH), interleukin (IL)10, macrophage migration inhibitory factor (MIF), somatostatin, vasoactive intestinal peptide (VIP), and programmed death-ligand 1 (PDL1) among others [15].

1.4. Hair follicle immune privilege collapse in alopecia areata

A popular explanation for the pathogenesis of AA focuses on the collapse of HF IP [15]. Under skin-localized inflammatory and/or stress stimulations, including psychological and environmental stressors, HFs in individuals carrying susceptibility genes for AA can respond with aberrant expression of MHC-I molecules and IP collapse [29]. As part of the physiological stress response, the affected HFs express pro-inflammatory chemokines and receptors [18,30], toll-like receptors (TLRs) [31], and cell adhesion molecules [32,33]. The initial HF damage and upregulation of danger signals activate variable combinations of local antigen-presenting cells (APCs) [18], skin resident lymphocytes [34], NK cells [35], and mast cells [36].

1.5. Cytotoxic CD8 T cells mediate the attack on hair follicles with CD4+ T cells assistance

While such a scenario begins in a highly localized fashion, migration of APCs to skin draining lymph nodes may promote a much larger lymphocytic response from autoreactive CTLs. Activated circulating $CD8^+$ cells, likely the primary executors of overt AA, migrate to danger signal-expressing HF peripheries [34], where they recognize exposed autoantigens in IP collapsed HFs [15]. $CD8^+$ lymphocytes mediate an immune response against HF-specific antigen epitopes primarily characterized by a Th1 cytokine response mode [37]. Notably, however, there is also evidence of a significant Th2 response in at least a subset of AA patients, consistent with a supporting role for $CD4^+$ cells [38]. Targeting and damaging anagen growth phase HFs by pathogenic $CD8^+$ T cells leads to acute AA development, whereas secondary humoral immunity might play a role in chronic subacute AA development and alopecia universalis [13].

1.6. The significant role of IFNγ and associated signals in AA

The increased MHC-I expression facilitates the $CD8^+$ T cell attack against exposed HF antigens. The associated IFNγ

secretion from infiltrating lymphocytes can further contribute to IP collapse [15] and trigger local CTL reactivities leading to the upregulation of NKG2D ligands like MICA and ULBP3/6 [29], proinflammatory cytokines [37], and chemokines [39], in adjacent HFs. Consequently, HF IP collapse ripples out in a wave-like manner to produce expanding lesions of hair loss [40]. Depending on the intensity of the inflammatory insult, HFs may remain in a dystrophic anagen state, unable to produce meaningful hair fiber, or alternatively, are pushed into a premature telogen state [11]. The presence of lymphocytes around HFs may prevent them from reentering a subsequent growth phase as normal. While the inflammatory infiltrate dissipates during telogen, any attempt by HFs to return to anagen growth is met with rapid lymphocytic reinfiltration [9].

While there are holes in this hypothesis of AA pathogenesis, the cumulative research evidence thus far generally fits the scenario. Regardless of the initial disease development cascade, there is clear evidence of $CD8^+$ CTLs being the drivers of AA, with $CD4^+$ cells in a supportive role. As such, most current treatments focus on modulating inflammatory cell infiltrate activity.

2. Current alopecia areata treatments

The course of AA is unpredictable, but in general, treatment of extensive AA is less likely to be successful than for patients with limited AA. First-line therapies for patchy AA are topical and intralesional corticosteroids [41]. Contact immunotherapy, such as diphenylcyclopropenone (DPCP) or squaric acid dibutyl ester (SADBE) sensitization, can be effective in more extensive AA [42]. Immunosuppressive agents such as cyclosporine, methotrexate, and azathioprine also demonstrate hair regrowth in some AA patient subsets [43]. Biological agents, particularly tumor necrosis factor (TNF) inhibitors, have been tried in AA, but the results have not been encouraging [44]. Recently, some JAK inhibitor drugs have been approved as AA treatments, while still more are undergoing clinical trial evaluation [45,46]. Each drug has its own advantages and disadvantages (Table 1), none are curative, and no treatment has been identified that is universally effective.

2.1. Corticosteroids for alopecia areata

Corticosteroid drug treatments for AA can be administered topically, intralesionally, or systemically to reduce local inflammation and suppress the immune attack on HFs. The effectiveness of topical corticosteroids in treating AA can be limited given follicular inflammation around HF bulbs may be 4–7 mm deep in the skin [47]. Intralesional corticosteroids, directly injected into AA lesions to enable high local concentrations of the drug, produce a more potent immunosuppressive effect at the site of hair loss [42]. Intralesional injections are often used for limited and localized areas of AA. Systemic corticosteroid administration provides widespread immunosuppression, which is effective in promoting hair regrowth in AA patients, but side effect risks are significant (Table 1) [41].

2.2. Immunomodulatory drugs

Contact-sensitizing chemicals such as DPCP, SADBE, and the irritant anthralin are employed in the treatment of AA [41]. The agents seem to leverage localized immune modulation to counteract the autoimmune mechanisms underlying the AA disease. They induce an allergic contact dermatitis or irritant dermatitis, and this inflammatory response is hypothesized to redirect the immune attack away from HFs [42]. The approach has better efficacy results for patchy AA as compared to more extensive AA [48], but there are high relapse rates and side

Table 1. Current standard treatments for alopecia areata.

Drug treatment	Advantages	Disadvantages
Topical corticosteroids (betamethasone valerate; clobetasol propionate; mometasone; etc.)	(1) Cheap and readily available (2) Easy to apply, can be applied at home (3) Minimal systemic side effect risk at low dose (4) Can be used on children	(1) Limited efficacy for AA (2) Skin penetration can be limited (3) Risk of localized skin atrophy, striae, telangiectasia, and/or folliculitis (4) Possible risk of osteoporosis with high cumulative dose [162]
Intralesional corticosteroids (triamcinolone acetonide; etc.)	(1) Relatively easy to apply by injection (2) Often efficacious for limited and localized patches of AA (3) Can be used as an adjunctive therapy for extensive disease	(1) Must be applied in a clinic setting (2) Can be discomfort during injection procedure (3) Risk of localized skin atrophy (4) Upper maximal exposure limit per patient that restricts the use of intralesional corticosteroids over larger areas of hair loss
Systemic corticosteroids (prednisone; etc.)	(1) Systemic administration provides widespread immunosuppression (2) Can be provided as pills for use at home with monitoring (3) Can be used in a pulse-dose or tapered-dose regimen to limit side effect risk	(1) Risk of adrenal suppression (2) Risk of hyperglycemia (3) Risk of stomach upset (4) Risk of fluid buildup and lower leg swelling (5) Risk of weight gain (6) Risk of hypertension (7) Risk of osteoporosis (8) Risk of adverse effects on bone growth
Contact sensitizing/irritating agents (DPCP and SADBE, anthralin)	(1) Topical method of application (2) Can be used to treat large areas of hair loss, including alopecia totalis (3) Can be effective, even for patients with multi-year chronic AA	(1) Dosage needs to be adjusted for each patient (2) Relatively labor-intensive treatment process in clinic (3) Risk of dermatitis and erythema (4) Risk of severe blistering (5) Risk of regional lymphadenopathy (6) Patients with atopy are contraindicated as poor responders [156]

effect risks such as persistent dermatitis, painful cervical lymphadenopathy, generalized eczema, and urticaria (Table 1).

3. Emerging treatments for alopecia areata

Many candidates that previously received attention for their potential as AA therapeutics have fallen by the wayside and do not seem to be currently under active investigation (Table 2). In part, this may be due to a lack of attention and funding as JAK (Janus Kinase) inhibitor drugs have claimed the spotlight in recent years.

3.1. JAK inhibitors

Janus kinases are a class of tyrosine kinases that play a pivotal role in cytokine signaling, particularly in regulating the proliferation, differentiation, and function of immune cells. JAK inhibitors, through blocking the JAK/Signal Transducer and Activator of Transcription (STAT) signaling pathway, reduce the production and signal reception of cytokines associated with AA, including IFNγ, thereby alleviating the inflammatory response against HFs [49–51]. These proinflammatory cytokines are required for immune cell signaling, but they may also directly inhibit hair growth [15]. In addition, JAK inhibitors may also directly help to restore the normal HF growth cycle by advancing HFs into anagen [52]. The development of JAK inhibitors for AA is still very active with several clinical trials currently ongoing (Table 3). While clinical study results look very promising, their safety remains an area of ongoing investigation [46]. It is too early in the development of JAK inhibitors to fully identify the side effect risk profile for AA patients, but the use of JAK inhibitors for other diseases can be associated with multiple adverse reactions, from increased risk of opportunistic infections to adverse impact on liver function [53].

4. The need for innovative strategies in drug discovery for alopecia areata

Despite recent advances in AA treatment, the current landscape remains suboptimal. There is still an urgent need for drug discovery and AA treatment innovation for several reasons:

4.1. High population prevalence

While past studies suggested a lifetime risk for AA of 1.7% [54], more recent assessments have revised the rate upwards to 2.1% [55]. Further, studies from the U.S.A. and U.K. suggest that AA is significantly more common in Black, Latino, and Asian ethnicities as compared to White people and it is expected that these differentials will be reflected in other countries' AA prevalence rates [56,57]. Overall, limited data suggest the global AA incidence rate is increasing [58–61].

Table 2. Therapeutic modalities recently considered for development to treat alopecia areata.

Treatment Name	Potential Mode of Action in Alopecia Areata	Alopecia Areata Related Trial Data
Secukinumab	Monoclonal antibody targeting interleukin-17A (IL-17A), primarily expressed in Th17 cells. By binding to IL-17A, Secukinumab may reduce production of chemokines involved in AA pathogenesis, slowing or halting disease progression [163]	In a double-blind, randomized pilot study involving 11 AA patients with 60% scalp involvement, no patient reached the primary endpoint of SALT50 post-treatment with secukinumab [164]. In a 2021 study on secukinumab treatment for psoriasis, a mild therapeutic response to AA was observed [165]
Dupilumab	Monoclonal antibody blocks IL-4 receptor (IL-4 Rα) and inhibits IL-4 and IL-13 which mediate Th2 immune activity [166]. Provides benefits for some patients with atopic dermatitis-associated AA [154]	By week 48, 32.5% of patients treated with dupilumab achieved a SALT30 response, 22.5% reached SALT50, and 15% attained SALT75. Patients with higher baseline IgE levels showed better response to dupilumab [167]
Tralokinumab	Inhibits proinflammatory cytokine interleukin-13 (IL-13). Data for AA is conflicted, with some suggestion that IL-13 upregulation is a component of successful treatment using DPCP [168]	In treating AD with concomitant AA, tralokinumab promoted a reduction in the AD EASI score to 8 points and a decrease in the AA SALT score to 14 within 3 months. By six months, clinical remission of AD (EASI score of 2) and nearly complete hair regrowth (SALT score of 2) was achieved [169]
Ustekinumab	Monoclonal antibody blocks the p40 subunit of IL-12 and IL-23. These cytokines play a key role in Th1 and Th17 cell-mediated responses [38]. Data on significance for AA is conflicted [170,171]	No trials of ustekinumab for adult AA treatment published to date. In a pediatric study, three AA individuals received ustekinumab treatment, all experienced hair regrowth with two achieving complete hair recovery [152]
Abatacept	Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and IgG1 fusion protein (CTLA4-Ig), selectively attenuates T cell activation. Inhibits the CD80/86:CD28 co-stimulatory pathway signaling that is required for T cell activation in AA [18,109]	In an open-label, single-arm study, 15 AA patients were administered abatacept 125 mg subcutaneously daily for 24 weeks. One patient experienced significant hair regrowth, with 91% hair regrowth by 24 weeks. Four other patients observed approximately 15%-25% hair regrowth at week 24, and another four patients showed a 3%-10% response [172]
Etrasimod	Selective sphingosine-1-phosphate (S1P) receptor modulator that exhibits high affinity for S1P1, S1P4, and S1P5. Etrasimod regulates lymphocyte trafficking, reducing circulation of lymphocytes, and impeding their ability to follow S1P gradients toward inflamed areas. This potentially may reduce immune cell attack on HFs in patients with AA [173]	Multicenter, randomized, double-blind, placebo-controlled clinical trial was launched to assess the safety and efficacy of Etrasimod in AA, however the data has not yet been published [174]
Low dose IL-2	Low-dose IL-2 selectively stimulates Treg cell proliferation, while high doses predominantly boost effector T cell expansion [175]. In AA, a reduction in Tregs may lead to the collapse of immune homeostasis. Low-dose IL-2 treatment could activate and expand Tregs, restoring the immune balance [176]	A 52-week multicenter prospective placebo-controlled study on low-dose IL-2 treatment for moderate to severe AA evaluated its clinical efficacy and impact on NK cell and Tregs. Despite significant elevation in peripheral blood Tregs during treatment, it did not substantially promote hair regrowth in severe AA patients [177].

Table 3. JAK inhibitors under investigation for treating alopecia areata.

JAK Inhibitor Name	Mode of Action	Alopecia Areata Related Trial Data
Baricitinib (INCB28050)	First JAK inhibitor approved by the FDA for AA, inhibits both JAK1 and JAK2 [178]	In two clinical trials for adults with severe AA, 40.9% and 36.8% exhibited a SALT score ≤ 20 at week 52 when receiving 4 mg baricitinib [179]
Brepocitinib (PF-06700841)	A dual inhibitor that selectively targets TYK2 and JAK1	SALT30 was achieved by 64% of patients receiving brepocitinib by 24 weeks [180]. Brepocitinib 30 mg may have the best relative effect in reducing the SALT score [64]
Deuruxolitinib (CTP-543)	A JAK1/2 inhibitor	Of patients receiving 8 mg and 12 mg deuruxolitinib, 29.6% and 41.5% achieved SALT score ≤ 20 by 24 weeks [181]. Deuruxolitinib 12 mg may have a superior relative effect in reducing the SALT score [64]
Ivarmacitinib (SHR0302)	Highly selective inhibitor of JAK1	Of 94 patients, the reduction in SALT score for 2, 4, and 8 mg ivarmacitinib groups were −30.51%, −56.11%, and −51.01%, respectively at 24 weeks [182]
Ritlecitinib (PF-06651600)	Selective JAK3/TEC kinase inhibitor drug. Inhibits TEC kinases like ITK, impairing cytolytic functions of $NKG2D^{+}CD8^{+}$ T cells [183].	SALT30 was achieved by 50% of patients receiving ritlecitinib by 24 weeks [180]. Of 105 adolescents receiving 30-50 mg ritlecitinib, by Week 48, 25%-50% of patients had a SALT score ≤ 20 [184]
Ruxolitinib	A JAK1/2 predominant inhibitor	Limited data from case reports and one open label study suggests 20 mg ruxolitinib twice daily can elicit hair regrowth in 75% of patients [185]
Tofacitinib	A selective inhibitor of JAK1 and JAK3	Of 202 patients after 18 months of treatment, 55.9%, 42.6% and 29.2% achieved 50%, 75% and 90% reductions in SALT scores respectively [186]. Of 90 patients, 58% achieved greater than 50% change in SALT score by 18 months [187]
Upadacitinib	A selective JAK1 inhibitor	Mostly case reports published thus far. Has shown therapeutic effects in patients with severe atopic dermatitis and AA, promoting hair growth in 53% of AA patients [188].

The exact causes of this increase remain unclear, but potential factors include heightened awareness of AA and improved diagnosis in dermatology clinics worldwide. Further, while genetic predisposition to AA may not change, it is possible that environmental triggers for AA onset have become more common, such as chronic stress promoting neurogenic inflammation [62] or dietary changes enabling autoimmunity [63]. A robust therapeutic arsenal to address the diverse needs of this expanding global patient cohort is required.

4.2. Unmet medical needs

The quest for treatments that offer more substantial hair growth and are effective for a greater percentage of AA-affected patients, with fewer side effects, remains an unmet medical need in AA management. Patients and clinicians alike seek therapies that provide significant hair growth, with minimal adverse effects, in a relatively simple method of application. Topical or intralesional treatments are particularly preferred due to their localized action and reduced systemic toxicity. Topical JAK inhibitor formulations have been explored, but thus far they have not demonstrated significant efficacy for AA in clinical trials [64,65]. Topical contact-sensitizing treatments are labor-intensive; the dosage and frequency of application need to be tailored to each individual to achieve maximum benefit while minimizing local side effects. Therefore, there is still a pressing demand for the development of new topical drugs that can meet these unmet treatment criteria as well as to enhance patient compliance.

4.3. Limited efficacy of current treatments with long-term use

Current treatments for AA offer variable efficacy and are often inadequate for sustained disease control. Around 33% of patients develop chronic AA [2]. In practice, current drugs, including JAK inhibitors, are unlikely to be used over many years due to the requirement for regular clinic attendance for treatment application and/or monitoring, and the cumulative risk of side effect development with long-term use. There is also the potential for some patients to develop resistance to a treatment over time, and occasionally to experience a 'rebound effect' upon discontinuation of treatment [66]. A minority of patients may also find that if they later reinitiate treatment, the degree of hair growth response is diminished [65,67,68]. While JAK inhibitors have shown promise in recent clinical trials, their long-term effectiveness is unknown [69]. Novel therapies are still needed that can provide more consistent and robust hair regrowth outcomes with long-term treatment plans.

4.4. Psychological impacts on health

Remarkably, over half of all AA cases are reportedly linked to stress-related factors [70]. Acute stress can cause overactivation of the central and peripheral nervous systems, leading to the development and progression of autoimmune diseases [71]. While stress can induce AA, equally, AA can induce stress. It is generally accepted that the visible nature of AA hair loss can lead to considerable psychological distress, affecting patients' self-esteem and mental health [72]. Studies with rodent models suggest that the link may not be purely psychological and that the inflammation associated with AA can modulate stress hormones [73]. Individuals with AA can demonstrate diminished capabilities in coping with and managing emotional and stress-related challenges [74,75]. The severity of scalp hair loss displays a strong, positive correlation with the degree to which patients' QoL is adversely affected [76]. While the mechanisms that link AA and stress together are not fully understood, effective treatments should

significantly relieve psychological burden and improve AA patients' QoL.

4.5. Potential physical impacts on health

There is also some evidence for a more direct biochemical link between chronic AA inflammation and aberrant expression of systemic disease phenotypes. Studies have shown that people who develop AA are 43% more likely to subsequently develop a new onset of an atopic condition, and 45% more likely to develop another autoimmune disease [77]. AA patients are more likely to develop thyroid disease [78], inflammatory arthritis [79], and psoriasis [80]. It is also possible that long-term AA is linked to an increased risk of cardiovascular diseases, though the data are conflicting [81–84]. Whether these links are due to a genetic pre-disposal toward inflammation and autoimmunity or whether the onset of AA directly activates other inflammatory cascades is unclear. Never-the-less, modulating inflammation and promoting hair growth with new effective treatments could potentially mitigate other health risks associated with AA.

4.6. Technological advancements

Technological advancements inevitably provide new opportunities for the development of innovative treatments that target novel pathways in AA disease pathogenesis. High-throughput screening, genomic and proteomic technologies, and advanced bioinformatics have facilitated the identification of new drug targets and pathogenic mechanisms for other diseases [85]. It seems reasonable to expect that similar technologies can also identify new treatments for AA. Novel therapeutic strategies, such as gene editing technologies (e.g. CRISPR-Cas9 manipulation of HF antigens), cell therapy and cell-derived products (e.g. HF stem cells and exosomes), and regenerative medicine approaches (e.g. regeneration of IP in AA-affected HFs), hold promise not just for treating the symptom of hair loss but also for addressing the underlying causes of AA. The integration of cutting-edge technologies into drug discovery pipelines could provide more effective, more durable AA treatment solutions.

5. Alopecia areata in vitro and ex vivo systems for preclinical treatment screening

The need for new and improved treatments for AA is clear, and there are a number of *in vitro* and *ex vivo* model systems specific to AA that can facilitate research and development:

5.1. In vitro disease models for alopecia areata

Leukocytes from patients with AA can provide a representative model for studying immunological aspects of the disease (Table 4). By isolating and culturing these cells, researchers can investigate the cells' behavior [23], interactions with other cells [86,87], responses to antigenic stimuli [24], and the *in vivo* effects of therapeutic agents [88]. Peripheral blood mononuclear cells (PBMCs) can also be isolated from AA patients and subjected to candidate drugs *in vitro* [89]. These PBMC populations primarily include lymphocytes (T cells, B cells, and NK cells) and monocytes. Rarely, immune cells are isolated from AA scalp biopsies for culture [90]. In any event, $CD8^+$ and $CD4^+$ T cells are of primary interest as the drivers of actual hair loss [15].

Other scientists have taken a different approach whereby HF keratinocytes [91], dermal papilla cells [92], adipose-derived stem cells [93], or immune cells from non-AA affected volunteers [94] have been induced to express an inflammatory state and then candidate AA treatments were assessed for their ability to suppress the phenotype. Such cell cultures could provide a basic platform for initial screening of treatments that inhibit leukocyte activation, cell migration, cytokine secretion, or cytotoxic activity. It is a simple approach that offers advantages, but also disadvantages, in determining a candidate drug's potential to modulate inflammation in AA pathogenesis (Table 4).

5.2. Ex vivo hair follicle inflammatory organ culture model

The HF organ culture model [95] involves maintaining dissected human HFs in a controlled culture environment to study inflammatory responses and screen therapeutic agents. Although it is possible to microdissect HFs from scalp biopsies obtained from AA patients (unpublished), more typically, HFs are obtained from patients undergoing hair transplants or face lifts. These healthy follicles are then exposed to cytokines such as IFNγ to induce a characteristic inflammatory phenotype, including the upregulation of MHC class I and II molecules and breakdown of HF IP [96]. The follicles can be evaluated in isolation, with past studies investigating the responses to calcitonin gene-related peptide (CGRP) [97], VIP [98] and IL-15 [99]. Recent studies have also co-cultured HFs with immune cells to evaluate their interaction (Table 4) [100,101].

5.3. Whole alopecia areata affected skin explant biopsies

Whole-skin explant biopsies from AA patients offer a model that includes all skin components, including epidermis, dermis, HFs, and local inflammatory infiltrate cells. This model retains the complex cellular interactions and extracellular matrix components, providing a more holistic view of the disease process and any treatment effects. Skin biopsies can be obtained from the edge of advancing AA lesions for direct culture (unpublished). More typically, however, healthy scalp biopsies are subjected to IFNγ to induce inflammation [99,102]. The biopsies are cultured on a supportive matrix in an air–liquid interface culture system. Topical treatments can then be applied to the surface of the biopsy and local responses to the treatment are evaluated; typically using RNA sequencing, cytokine expression assays, and immunohistochemistry. Cultured explant analysis provides insights into the inflammatory pathways active in AA immediately in and around affected follicles (Table 4).

6. In vivo alopecia areata disease model systems for preclinical treatment screening

Rodent models are invaluable for studying AA due to their ability to recapitulate key aspects of the disease and facilitate

Table 4. Advantages and disadvantages of *in vitro* and *ex vivo* alopecia areata models.

Disease model	Advantages	Disadvantages
Peripheral blood mononuclear cells (PBMCs) from AA patients in culture	(1) Blood samples are relatively accessible with links to dermatology clinics (2) AA patients are often enthusiastic to provide a blood donation for research (3) A large number of drugs or parameters can be evaluated in parallel	(1) Only 1–2% of the cells derived from the PBMC sample will be truly pathogenic cells (2) PBMC populations do not fully reflect the local HF inflammatory milieu (3) Does not include Langerhans cells, dendritic cells, and tissue-resident memory T cells (4) Maintaining cell activation beyond a few hours without resorting to nonspecific immune cell activators limits the time frame in which studies need to be completed (5) Cytokine milieu, extracellular matrix components, and cell–cell interactions in skin are difficult to replicate in this model
Inflammation induced healthy cells in culture (hair follicle keratinocytes; dermal papilla cells; adipose derived stem cells; etc.)	(1) Relatively easy to obtain from commercial cell suppliers; no access to AA patients needed (2) Simple methodology using common cell culture materials and equipment	(1) Induction, typically using IFNγ, and then maintenance of proinflammatory state for the duration of culture can be challenging (2) Open to question whether induced inflammatory state is an accurate reflection of AA condition (3) Cannot model the complex dynamics of multiple cell types in hair follicle units (4) Cannot model hair cycling state
Ex vivo hair follicle inflammatory organ culture model	(1) Model more closely mimics the *in vivo* conditions of hair follicles within AA lesional skin (2) The complex distribution and interactions of hair follicle cells and immune privilege factors can be assessed (3) Can evaluate hair follicle response to whole-cell populations or specific cell subsets	(1) Limited availability of human hair follicles for larger screening studies (2) Currently, the model does not allow for physical contact between immune cells and hair follicles (might be remedied by culture in alginate gel or similar matrix) (3) Hair follicles in organ culture do not undergo the natural hair cycle phases (anagen, catagen, telogen) limiting assessment of any drug hair regrowth effects (4) Hair follicles are usually from a donor who is different from the AA patient who supplied PBMCs; there is a possible genetic mismatch and corresponding allogeneic immune cell challenge to contend with
Whole AA affected skin explant biopsies in culture	(1) Ideal for evaluating the immunomodulatory efficacy of topical treatments and, to a lesser extent, systemic drugs (2) Can apply drugs directly to the explants and monitor changes in immune cell migration, cytokine production, and tissue integrity (3) Model fully represents all of the local skin components of AA	(1) Very limited availability of lesional AA scalp biopsies (2) Biopsies only last for around 7 days in culture limiting time for drug assessments (3) Does not address other contributing factors beyond the skin, such as central immune tolerance, hormonal and environmental influences (4) Model does not provide information on treatments that target the recruitment of pathogenic immune cells into skin or interference with antigen presentation in lymph nodes/spleen

the investigation of underlying mechanisms and therapeutic interventions. In older literature, the DEBR (Dundee Experimental Bald Rat) model was utilized both for understanding disease pathogenesis and for evaluating drug treatments [103]. Other animal models have also been suggested, including transgenic rodents [104], dogs, horses, and chickens [105]. These models no longer exist (DEBR), are poorly characterized (transgenics, chickens) or are not readily accessible (large animals with AA). However, two rodent models have progressed to the point where they can be used for practical screening and development of AA treatments:

6.1. C3H/HeJ mouse model of alopecia areata

The C3H/HeJ mouse strain is the most widely used model for studying AA due to its ability to spontaneously develop the disease, as well as the potential for inducing AA through grafting and cell injection techniques [106,107]. Up to 20% of C3H/HeJ mice can spontaneously develop AA, typically presenting with patchy hair loss that mirrors human AA [108]. Research shows that mouse AA involves the infiltration of $CD8^+$ T cells, $CD4^+$ cells and other immune cells around HFs, leading to follicular disruption and hair loss [17,109]. Overall, the mouse model is comparable to human AA (Table 5) [1,110–112]. Notably, there have been attempts to artificially induce AA in the C3H/HeJ strain using subcutaneous injections of IFNγ alone [113], or in combination with polyinosinic: polycytidylic acid [114], to initiate immunity. The hair loss and skin inflammation patterns look different as compared to spontaneous AA (Table 5). None-the-less, the model has been used in a recent interesting study [115].

Although only some C3H/HeJ mice spontaneously develop AA, all C3H/HeJ mice can be induced to develop AA by skin grafting [116]. In this approach, skin grafts from one donor C3H/HeJ mouse with spontaneous AA are transplanted to ~20 healthy C3H/HeJ mice. This induces AA in the recipients with clinically visible hair loss typically beginning at around 10 weeks post grafting. Graft-induced AA allows for the study of disease transmission and the identification of key immune components involved in AA pathogenesis. It also serves as a robust model for testing potential therapeutic interventions (Table 5) [117]. Notably, this model was utilized in initial

Table 5. Selected advantages and disadvantages of murine alopecia areata models.

Disease model	Advantages	Disadvantages
Spontaneous C3H/HeJ (also C3H/HeN) AA mice	(1) Widely available in academia and from commercial suppliers (2) Docile strain characteristics (3) Only $CD8^+$ cells penetrate to intra-follicular locations as with human AA (4) $CD8^+$ and $CD4^+$ cells shown to be primary drivers of hair loss as with human AA	(1) Limited numbers spontaneously develop AA (2) AA only develops in aged mice which increases maintenance costs (3) $CD4^+$ and $CD8^+$ cell skin infiltration ratios not the same as seen in humans (4) Variable/unpredictable time of AA onset makes it difficult to conduct time course studies
Skin grafted C3H/HeJ AA mouse model	(1) AA onset can be time controlled (2) Up to 20 mice with AA can be produced using one spontaneous AA donor (3) Well characterized in multiple publications (4) Previously used in several studies to evaluate drug treatments, including JAK inhibitors	(1) AA affected mice not generally commercially available (2) Requires surgical skills to produce AA affected mice (3) Labor intensive mouse monitoring and nursing required post operation (4) Long-term studies beyond ~12 months duration are not practical
Cultured lymph node cell injected C3H/HeJ mouse model	(1) AA onset can be time controlled (2) Up to 100 mice with AA can be produced using cells from one spontaneous AA donor (3) Systemic immune responses beyond the skin can be evaluated (4) Studies indicate cells can be stored in liquid nitrogen for later use to induce AA mice as/when needed [189]	(1) AA affected mice not commercially available (2) Requires good cell culture skills to produce AA affected mice [107] (3) Long-term studies beyond ~12 month duration are not practical (4) Newer model and so less well characterized compared to skin graft model
Induced C3H/HeJ mouse model (using IFNγ; Poly I:C)	(1) AA onset can be time controlled (2) Potentially many mice can be produced for large-scale screening studies (3) Model is potentially available at short notice and requires much less preparation compared to other AA mouse models (4) Relatively cheap model to produce compared to skin graft/cell injected models	(1) Induced skin inflammation is mild and diffuse in nature; does not clearly replicate the features of classic mouse AA (2) Open to question whether the induced inflammatory hair loss is an accurate reflection of AA disease/autoimmune mechanisms (3) Unclear whether significant systemic immune responses occur beyond the skin (4) Limited characterization as new models/few publications
Humanized AA skin graft – AA PBMC injection SCID mouse model	(1) Uses AA affected human scalp skin (2) Preserves the local AA scalp immune and HF environment (3) Allows the study of human-specific skin immune responses (4) Allows testing of therapies directly targeting human immune components	(1) Human donor AA skin in very limited supply (2) Requires AA PBMCs from the same scalp skin donor (otherwise an allogeneic immune response will occur) (3) Requires surgical skills to produce mice with AA grafts (4) Does not capture immune responses to treatments that may occur beyond the skin
Humanized normal skin graft – activated PBMC injection SCID mouse model	(1) Uses normal human scalp skin from face lifts or hair transplants (2) Preserves the local scalp HF environment (3) Allows the study of human-specific skin immune responses (4) Allows testing of therapies directly targeting human immune components	(1) Requires use of scalp skin and PBMCs from the same donor (otherwise an allogeneic immune response may occur) (2) Does not capture immune responses to treatments that may occur beyond the skin (3) Open to question whether activated PBMCs injected into normal human scalp explants elicit an inflammatory state equivalent to AA (4) Open to the question of whether injected activated healthy PBMCs specifically target HF antigen epitopes as seen in AA

studies to identify JAK inhibitors as new drugs suitable for AA treatment development [49].

Skin graft induced AA probably works through the transfer of pathogenic immune cells and exposed antigens present in the graft. Based on this observation, a cell injection-induced AA model was developed [118]. This involves the isolation of skin-draining lymph node lymphocytes from a single AA-affected C3H/HeJ mouse, and cell culture using a cytokine cocktail and nonspecific immune cell activation, to produce enough cells to inject up to 100 syngeneic mice to induce AA [119]. As large numbers of mice can be produced with AA at a similar time point in disease development, this model offers the potential to screen drugs, or multiple drug parameters, in parallel. The C3H/HeJ mouse model provides several advantages for AA study and treatment development; not least that systemic immune responses beyond the skin can be evaluated, and hair regrowth patterns can be quantified in response to treatments over a multi-week time course (Table 5).

6.2. Humanized alopecia areata skin graft SCID mouse model

The humanized AA skin graft SCID (severe combined immunodeficiency) mouse model represents a sophisticated approach to studying AA with a more human context [120]. There are two variations of this system. Originally, the model involved grafting the AA-affected human scalp skin onto SCID mice and injecting activated PBMCs from patients to maintain the AA phenotype [121]. However, in more recent studies, healthy non-AA scalp explants are grafted and later injected with healthy donor PBMCs enriched for $NKG2D^+$ and $CD56^+$ cells to induce an AA-like inflammatory hair loss [122]. This model preserves the local scalp environment, potentially providing a more accurate representation of human HF inflammation dynamics compared to rodent models. It allows for the study of human-specific immune responses and the testing of therapies directly targeting human immune components [99,123]. As some drugs are developed specifically against

human targets, they may not always be functional in other mammals. Consequently, this human skin graft model may be the only practical *in vivo* screening tool applicable in certain situations. However, this model and its variations suffer from a number of limitations, not least a lack of easy scalability (Table 5).

7. Current and potential alopecia areata disease pathogenesis targets for therapeutic intervention

Thus far, AA treatments have focussed on either suppressing the inflammatory cell infiltrate or modulating it using contact sensitizers and irritant chemicals. These treatments have broad effects acting on multiple immune cell types. Targeting the activities of pathogenic cells with new drugs is entirely logical. There are, however, other potential intervention points in the disease pathogenesis mechanism that could be targeted. As alternatives to concentrating on pathogenic cell suppression, therapeutic strategies could focus on other aspects of AA pathobiology, aiming to reestablish immune tolerance, suppress immune activation, and/or protect HFs.

7.1. Targeting pathogenic lymphocytes

Currently emerging JAK inhibitor treatments for AA modulate specific signaling pathways and cellular interactions related to the immune system response in AA [45]. Concerted efforts to investigate other biologics for AA seem to have largely fallen by the wayside with several clinical studies registered, but either incomplete or showing suboptimal results in initial evaluations (Table 3). Returning to some of these candidates and improved versions for a more systematic analysis of their application to AA might be appropriate.

7.2. Targeting immune tolerance mechanisms

One of the primary challenges in AA is the loss of immune tolerance against HF autoantigens, which enables the infiltration of autoreactive lymphocytes. To address this, therapies might aim to reestablish tolerance to HF autoantigens. Approaches could include promotion of tolerogenic regulatory T cells (Tregs) and dendritic cells to 'educate' autoreactive cells [124–126]. Promoting these tolerogenic cells *in situ* may be possible using agents such as low-dose IL-2 or aspirin, for example [127,128]. Additionally, mesenchymal cells or exosomes, known for their inherent IP properties, might be used to re-establish HF antigen tolerance [92,129,130].

7.3. Targeting immune privilege restoration or enhancement

Inducers of IP collapse, produced in response to HF distress signals, are a key factor in AA pathogenesis. Therapies that aim to block the receipt of these stimulatory signals and suppress the production of IP collapse inducers may be effective. Techniques include modifying or blocking signal–ligand interactions within HFs, particularly those involving NKG2D, MICA, and ULBP3 [131,132]. Neutralizing IP collapse inducers, such as IFNγ [133], with monoclonal antibodies, and/or applying substance P receptor antagonists [134], Kv1.3 channel inhibitors [135], and PDE4 inhibitors [136] could be additional strategies to prevent HF attack by immune cells. Alternatively, enhancing the expression of IP-conferring factors such as Indoleamine 2,3-dioxygenase (IDO) [137] may prevent AA. Gene therapy holds potential for increasing the expression of weak IP factors, or potentially introducing new protective components not normally seen in HFs. Direct application of factors such as αMSH and analogs [27,138,139], CGRP receptor agonists [97], and VIP analogs [98] could strengthen HF IP.

7.4. Targeting immune cell migration

Research has shown that the CTL infiltrates in AA lesions are comprised of skin resident cells and also circulating cells [34]. As such, another treatment strategy could be to impede the migration of APCs from the skin, as observed with contact sensitization in AA mouse models [140]. This might then prevent activation and recruitment of circulating autoreactive cells from draining lymph nodes. Post CTL activation, interfering with T-cell chemoattractant signaling, such as preventing HF expression or T-cell reception of chemokines, also shows promise and may be adaptable for treating AA [141,142].

7.5. Targeting antigen recognition

Suppressing MHC class I and II expression in HFs, blocking the binding of MHC plus antigen epitopes to TCRs on autoreactive T cells, modulating co-stimulatory signaling, and/or blocking antigen recognition responses by infiltrating T cells and APCs could be effective strategies against AA. Therapies might include using MHC class I down-regulating drugs like tacrolimus [27], suppressing MHC class II with agents such as IL-10 [143] or Red/IK [144], and neutralizing co-stimulatory factors using CTLA4 and its ligands CD80 and CD86 [18,145]. Hypothetically, modifying the glycosylation of autoantigens can reduce their immunogenic properties and control their uptake, proteolytic processing, and presentation by MHC [146]. Additionally, interfering with TCR recognition of autoantigen-MHC class I complexes using antibody-TCR mimics could prevent the activation of autoreactive T cells [147].

7.6. Targeting supporting ancillary cells

Appropriate supporting signals from various cell types, including $CD4^+$ cells, mast cells, and macrophages, likely play a significant role in sustaining the AA autoimmune response. Suppressing these cells, blocking their activation, and inhibiting their signaling, could be therapeutic objectives for new drug development. Blocking the migration of supporting cells to the skin using monoclonal antibodies against skin homing receptors [148–150], inhibiting pro-inflammatory cytokine signaling [151,152], and potentially using anti-histamines or desensitization therapy [153] to block mast cell support could represent effective strategies. Dupilumab is one example of a drug that interferes with the role of supporting cells in AA. Recent studies indicate that Th2-skewing may play a key pathogenic role in AA in

addition to the IFNγ-induced Th1 immune pathway. As a monoclonal antibody blocking interleukin 4 and interleukin 13 cell signaling, dupilumab provides hair regrowth improvement in patients with atopic dermatitis-associated AA [154].

7.7. Targeting direct hair growth promotion

Finally, while there have been some attempts to cultivate the use of direct hair growth promoters for treating AA, the results have been disappointing. Minoxidil is used for AA, but typically only in an adjunctive fashion alongside an immunosuppressive agent. In a recent reassessment of multiple studies, 57% of patients receiving adjunctive minoxidil treatment experienced hair regrowth. Successful response increased significantly to 85% when patients received combination oral minoxidil and oral JAK inhibitor treatment [155]. The intensity of inflammation in AA is quite significant such that direct hair growth promoter drugs are rendered largely ineffective on their own. Consequently, an effective drug development strategy for AA probably lies in focusing on some aspect of the immune system and/or its interaction with (mal)functioning anagen stage HFs. Nonetheless, direct hair growth promotion may still be very useful as an adjunctive AA treatment (see below).

8. Challenges in alopecia areata drug discovery

Those intent on discovery and development of new effective drugs for AA are confronted with numerous obstacles that reflect the complex nature of the disease. Considering each of these potential issues may at least partly help in mitigating the challenges to address:

8.1. Heterogeneity of alopecia areata

AA presents in various forms, from patchy to alopecia universalis, including diffuse and ophiasis AA, each potentially requiring different treatment approaches. This heterogeneity complicates drug discovery as different types of AA may respond variably to the same drug treatment. The mode of application, whether topical, intralesional, or systemic, also influences results with the same drug. Moreover, the duration of AA likely affects treatment responses. Additionally, distinctions among patients, due to the presence of atopy for example, may define poor-responders [156]. Clinical trials often exclude AA patients with less than 50% hair loss, or with greater than 95% scalp hair loss, and those with more unusual AA forms, as an attempt to increase consistency in clinical appraisal. Many clinical trials also deliberately exclude patients with AA who have not experienced any hair regrowth within the past 7 or 10 years, and as such, these patients' responses to treatment are essentially unknown [157]. However, at some point in drug development these clinical variations will need to be addressed.

8.2. Complexity of disease mechanisms

The pathophysiology of AA is complex, with localized disease phenotypes often masking the underlying systemic immune activity. This complexity is further compounded by the variability in genetic and environmental inputs across individuals. Some patients exhibit strong genetic predispositions, while others may develop AA primarily due to environmental factors such as stress. It is generally accepted that over time, the immune input in AA can change, with variations in epitope targeting and memory T cell populations. In addition, the stage of disease, characterized by inflammation intensity and the dystrophic state of HFs, could influence therapeutic efficacy. For example, long-term AA patients may require interventions that not only address immune dysregulation but also stimulate HFs out of their chronic telogen state.

8.3. Issues with current clinical models and methods

The lack of effective patient stratification mechanisms makes it difficult to predict treatment responses and tailor treatment plans based on specific disease presentations. Furthermore, the absence of standardized measures for assessing disease severity and treatment response hinders the comparability of clinical trial results. While the Severity of Alopecia Tool (SALT) score is commonly used to quantify the extent of hair loss in most modern clinical trials [158], different studies may define treatment 'success' based on different end point parameters such as hair regrowth achieving 70% net scalp density or 40% improvement in hair growth from baseline, etc (Table 3). The lack of sensitive clinical outcome measures to assess disease severity and treatment efficacy complicates the determination of what constitutes a truly successful treatment response. Further, comparison of results between clinical trials for different drugs is problematic due to the absence of standardized endpoints.

8.4. Lack of biomarker identification and validation

Identifying and validating biomarkers for disease activity and treatment response in AA remains a significant gap in our knowledge. Understanding how a treatment interacts with the underlying disease mechanisms at a molecular level could lead to additional therapeutic strategy optimization in the near term. In the longer term, better biomarkers could enable the development of more novel interventions. Clinical studies often incorporate evaluation of PBMC samples for pro-inflammatory cytokine levels and/or leukocyte cell analysis of their activation state. However, these biomarkers only reflect what is happening around HFs in a very limited way. Scalp biopsies would give a much better insight, but patients are usually reluctant to permit skin sampling. More effective biomarkers are needed for assessing treatment safety and monitoring disease progression/stability/regression. Their current inadequacies limit the precision of clinical assessments and the development of therapies optimized for particular subsets of AA patients. By stratifying patients based on their unique AA disease pathogenesis mechanisms, clinicians could potentially tailor treatments more effectively to enhance overall therapeutic outcomes. Furthermore, better biomarkers could help in identifying patients who may be at risk of adverse side

effects with particular treatments, thereby improving safety profiles and patient management.

8.5. In vitro and in vivo disease model fidelity

The fidelity of current animal and *in vitro* systems in modeling human AA is a challenge to preclinical research and development. While the models provide valuable insights, they do not fully replicate the immune responses, HF cycling, and the genetic/environmental factors involved in human AA. Translating findings from these models to human trials can be difficult due to inherent differences in disease pathology and immune system functioning between species, and the artificial environment *in vitro*. In the longer term, this translational gap will necessitate the development of more accurate models that can better predict human responses to potential treatments.

9. Conclusions

Significant progress has been made in AA research, though much deeper exploration is needed to fully understand the signaling pathways active in AA pathogenesis. Current AA treatments have been developed and used since the 1950–1970s [103], but have limited efficacy and carry the risk of potential adverse reactions. This has led to several companies developing new AA treatments, most often focusing on systemic immunosuppression modalities. Beyond the current excitement around JAK inhibitors, the results from clinical trials thus far have been mixed. Some patients achieve significant hair regrowth, but by no means do all attain good scalp coverage.

Overall, the drug discovery process for AA is hindered by the heterogeneity of the condition, our relatively poor understanding of the disease mechanisms involved, limitations with current research models, the lack of validated biomarkers, and limitations around clinical trial designs for AA. Addressing these challenges will require a multifaceted approach; integrating a better understanding of personalized medicine accommodating both variations in clinical presentation and AA disease status, standardized assessment protocols for hair growth and appropriate trial end points to define treatment efficacy, development of robust biomarkers that provide a very sensitive and accurate readout of the AA disease state, and improved disease model systems to accelerate the discovery of effective therapies for AA.

10. Expert opinion

Developing novel anti-AA therapy schemes is urgent given current treatment modalities' shortcomings. However, novel AA therapy research is still mostly in the early exploratory stages due to a limited understanding of disease pathogenesis mechanisms. Why AA can be self-limiting, allowing some patients to recover untreated, while others transition to chronic recurrent hair loss, remains to be elucidated. Some patients have tried new treatments without yielding expected significant results. In certain cases, new therapy efficacy does not compare favorably with traditional treatments. All the forgoing leads to some viewpoints on the future of drug development for AA. Inevitably, these opinions are not necessarily held by the majority of experts:

10.1. There is a growing market for AA treatments

While the lifetime risk for AA has been calculated at about 2.1%, suggesting 150 million people worldwide are affected during their lives, the value of the AA treatment market is much larger than indicated solely by the numbers affected. AA patients are more motivated to obtain treatment given the extent of hair loss can be significant, first onset is most common in late teens to early twenties, and the AA can affect women and children as much as men. Thanks to patient advocacy groups, AA is now regarded as a medical condition and private health insurance and public health systems may cover treatment costs. Going forward, the market size is likely to increase significantly with the advent of new drug treatments superior to those currently available, increased awareness from patients as to treatment options, and improved treatment accessibility from dermatology clinics, particularly in rapidly developing countries.

10.2. AA treatment cost reduction is needed

A study investigating medical costs for AA patients was conducted in the U.S.A. between 2014 and 2019. Patients with non-AT/AU AA had high medical ($6303) and pharmacy ($1284) costs per year. AT/AU patients had larger pharmacy costs ($1918) [159]. Treatment costs today are likely much higher with the recent advent of JAK inhibitor approval in several countries. Indeed, some 'alarm bells' are ringing for public health care systems as to the considerable costs that may be involved. With the approval of JAK inhibitors baricitinib and ritlecitinib in the UK, AA treatment costs to the National Health Service (NHS) were nominally calculated at £4 billion annually [157] for 400,000 patients at a point prevalence of 0.58% [56]. This is clearly unrealistic, but even if the penetrance rate into the UK AA market was just 10%, the gross annual cost would still be £400 million. Overall, there is a considerable potential market size for new and improved AA drugs that remains largely unmet, but for widespread adoption of any treatment, the costs involved will have to be addressed.

10.3. Improvements and alternatives to JAK inhibitors are needed

While systemic JAK inhibitors have shown significant efficacy in recent clinical trials, questions remain as to their safety. The long-term use of systemic JAK inhibitors for AA will probably need to be based on the assessments of specific patient risk factors (age, cardiovascular history, smoking status, cancer risk, etc.) to develop risk minimization treatment plans. For high-risk groups, dosage reduction or intermittent dosing strategies might balance efficacy with safety. Developing topical or skin-localized JAK inhibitor application would significantly increase the desirability of this treatment category, as well as reduce costs, and alleviate fears of long-term use side effect risks.

The concentrated investment of time, effort, and resources into JAK inhibitor drugs has inadvertently overshadowed the exploration and development of better therapeutic strategies. As a result, potentially valuable treatments targeting other molecular pathways, efficacy improvements, and more holistic therapeutic approaches remain largely unexplored. Attention to JAK inhibitors could limit the breadth of available treatments and slow the advancement of more comprehensive therapeutic options for AA.

10.4. Alternative therapeutic pathways need exploration

Other molecular pathways that might be targeted with new treatment development are detailed above. Promoting the IP defense of HFs, reactivation of immune system immunoregulatory functions, and interference with HF antigen presentation seem particularly promising avenues for exploration. Such treatments also offer the possibility of not only enabling hair growth in the short term but also achieving long-lasting changes to the immune system and/or HFs that increase resistance to future disease relapse. Refocusing attention on other aspects of AA pathogenesis might also reduce side-effect risks, particularly for drugs that need to be taken long-term.

10.5. Optimization of current and prospective treatments is needed

Current treatments have not been systematically optimized for AA. More comprehensive evaluations of the best dosage for efficacy and side effect minimization are needed. More effective drug vehicles or modes of application could significantly improve treatment efficiency. Most notably, the need for localized AA treatments seems to be largely ignored at the current time. Potentially, reformulation of JAK inhibitors, or other immunosuppressive drugs, could overcome the issues with deep skin penetrance needed for AA topical treatment.

The drug half lives of many immunosuppressive drugs, including JAK inhibitors, last just a few hours. Improved formulations to enable slow release, or a depot effect, could enhance their therapeutic efficacy. These approaches could provide a more consistent drug release minimizing the peaks and troughs associated with daily oral dosing. Maintaining stable plasma concentrations over extended periods could reduce the frequency of medication. In turn, this should improve patient adherence to treatment regimens, which will be particularly relevant for long-term disease management.

10.6. Adjunctive treatments may synergistically increase efficacy

Adjunctive therapeutics to synergistically improve AA treatments need more attention. Small-scale studies suggest that anti-histamines [153], desensitization therapy [160], and/or dupilumab [154] may be effective for patients with atopic AA when combined with immunosuppressive drugs. Other adjunctive therapies could be investigated, such as antidepressants for patients with chronic stress induced AA, or microbiome modifiers for those identified with significant alterations to their gut or scalp microbiota. Minoxidil is used as an adjunctive treatment to directly promote hair growth alongside immunomodulatory drugs [155]. The studies are limited and small scale; proper randomized control trials are needed to really determine the full synergistic effects and optimal dosage.

10.7. Final thoughts

The arena of AA enquiry is small compared to research into other skin autoimmune diseases, but the field is expanding. The number of medical publications on AA has increased almost exponentially in the last few years [103,161]. In no small part, this is due to the discovery of JAK inhibitor drugs for treating AA. Yet, there is a much larger potential for new therapeutics beyond JAK inhibitors that remains untapped. In the next 5 years, we expect to see further increases in research publications and a greater effort to understand the nature of AA, including its molecular disease pathogenesis, clinical prognostics, and patient stratification, as well as new treatments. We look forward to the new discoveries that will be made and the development of new therapeutics we can employ in our clinics for AA patients.

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Declaration of interest

KJ McElwee is a shareholder, consultant, and investigator for Replicel Life Sciences Inc. KJ McElwee is also the Director of McElwee Consulting Ltd. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Immunomodulatory Role and Therapeutic Potential of HLA-DR$^+$ Regulatory T Cells in Systemic Lupus Erythematosus

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease that affects multiple organ systems. A key element in maintaining immune tolerance and preventing autoimmunity is the role of regulatory T cells (Treg cells). Among these, HLA-DR$^+$ Treg cells represent a distinct subset, and their altered expression and functionality in SLE are closely associated with the progression of the disease. This review explores the biological characteristics of HLA-DR$^+$ Treg cells, their mechanisms of action in SLE, as well as their potential and the challenges they pose as therapeutic targets.

Methods and results: This review offers a comprehensive analysis of the mechanisms by which HLA-DR$^+$ Treg cells regulate immune responses. It highlights their direct interactions with autoreactive T cells and antigen-presenting cells, which contribute to the suppression of autoimmunity. Additionally, the review explores the critical role of these cells in maintaining immune tolerance and their promising potential in the context of antigen-specific immunotherapy.

Discussion: The potential of HLA-DR$^+$ Treg cells in the treatment of systemic lupus erythematosus (SLE) is considerable, particularly due to their capacity to generate antigen-specific Tregs. The development of Treg-based therapies, including the expansion of both polyclonal and antigen-specific Tregs, is an area of active investigation. Nonetheless, several challenges persist, such as the need to optimize protocols for Treg generation and expansion, ensure the stability of the Treg phenotype, and address potential safety concerns associated with cellular therapies.Continued research is essential to fully harness the potential of HLA-DR$^+$ Treg cells in the treatment of SLE and other autoimmune diseases.

KEYWORDS

HLA-DR$^+$ regulatory T cells; immune tolerance; systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a complex, chronic autoimmune disease in which the body's immune system erroneously targets its own tissues, resulting in widespread inflammation and damage across various organ systems. The clinical manifestations of SLE differ among individuals and may involve multiple organ systems, including malar rash,

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arthritis, and nephritis. The disease typically follows a course characterized by periods of remission and relapse.

The incidence of SLE exhibits significant geographical variation. It is notably higher in North and South America compared to Asia and Europe, where the incidence is relatively lower. The global incidence of SLE is estimated at 5.14 cases per 100,000 population, with a range from 1.4 to 15.13 cases. Additionally, SLE is more prevalent in women than in men, particularly among those of reproductive age, specifically between 15 and 44 years (Barber et al., 2021).

The etiology of SLE is multifaceted and not attributable to a singular cause. Research indicates that the onset of SLE is significantly influenced by a combination of environmental factors, genetic predispositions, and immune system abnormalities. The interplay among these elements results in immune system dysregulation, subsequently provoking an autoimmune response (Crow, 2023). Tregs play a crucial role in the pathogenesis of autoimmune disorders, particularly in SLE, where Treg dysfunction is closely linked to the progression of the disease (Honing et al., 2024). Recent studies have highlighted the existence of two distinct subsets of Treg based on the expression of human leukocyte antigen-DR (HLA-DR): HLA-DR$^+$ and HLA-DR$^-$ Tregs. These subsets exhibit distinct biological properties and plasticity, which may have significant implications for the pathogenesis and treatment of SLE (Dikiy & Rudensky, 2023). The aberrant expression and function of HLA-DR$^+$ Treg cells, a distinct subpopulation in systemic lupus erythematosus (SLE), are closely associated with disease progression (Raeber et al., 2024). Investigating their functional characteristics not only enhances our understanding of the underlying mechanisms of the disease but also offers new avenues for future therapeutic strategies.

Biological properties of HLA-DR$^+$ Treg cells

Tregs that express HLA-DR represent a distinct subset of CD4$^+$ T cells with unique functional attributes. Constituting approximately one-third of adult peripheral blood CD4$^+$ effector Tregs (Schaier et al., 2013). Studies have shown that HLA-DR$^+$ Tregs are more immunosuppressive compared to HLA-DR$^-$ Tregs (Gootjes et al., 2024). This property allows HLA-DR$^+$ Tregs to play a key role in maintaining immune tolerance and preventing the progression of autoimmune diseases such as SLE (Schaier et al., 2013).

Basic characteristic

HLA-DR$^+$ Treg cells represent a distinct class of Tregs characterized by unique biological properties. These cells exhibit the hallmark features of conventional Tregs, including the expression of CD4 and FOXP3, in addition to HLA-DR, a specific surface molecule (Gootjes et al., 2024). The presence of these markers confers a unique identity to HLA-DR$^+$ Treg cells within the immune system. Notably, HLA-DR$^+$ Tregs typically express elevated levels of FoxP3, a crucial transcription factor that plays a vital role in maintaining the suppressive function of Tregs (Z. Li et al., 2015). Furthermore, HLA-DR$^+$ Tregs may also express additional surface markers, such as CD25 and CD38, which are significantly elevated in CD4+ and CD8+ T cell subsets among patients with SLE. These cells are essential for

maintaining self-tolerance and immune homeostasis, as they inhibit the onset and progression of autoimmune diseases by suppressing hyperactive immune responses (Raeber et al., 2024).

Functional characteristic

HLA-DR$^+$ Tregs constitute a functionally heterogeneous group that inhibits immune responses through a variety of multifunctional and complementary mechanisms. This inhibition is often highly specific to certain types of immune responses, suggesting that customized approaches can address different immune challenges. Although the molecular mechanisms by which HLA-DR$^+$ Tregs exert their suppressive activity have not been clearly defined, we discuss some immunosuppressive mechanisms that have been proposed (Figure 1).

Figure 1 Mechanisms for Maintaining Treg: Teff Balance.This diagram illustrates the various mechanisms by which HLA-DR$^+$ Tregs interact with effector T cells (Teffs) and antigen-presenting cells (APCs) to modulate immune responses and maintain self-tolerance. (a) Induction of Apoptosis:HLA-DR$^+$ Tregs induce apoptosis in Teffs through direct cell contact, thereby reducing their numbers and activity (Chen & Oppenheim, 2011). (b) Release of Immunosuppressive Cytokines: HLA-DR$^+$ Tregs secrete various cytokines, including IL-10 and TGF-β, which inhibit effector T-cell activity and promote immune tolerance (Islam et al., 2021; Palomares et al., 2021). (c) Metabolic Competition: HLA-DR$^+$ Tregs compete with Teffs for glucose, a critical resource for T cell activation and proliferation, thereby limiting Teff expansion (Clever et al., 2016). (d) Modulation of Antigen Presentation: By interacting with dendritic cells (DCs), HLA-DR$^+$ Tregs reduce the capacity of these cells to present antigens effectively, which in turn downregulates Teff activation (Kenison et al., 2023).

Differences and plasticity between HLA-DR$^+$ and HLA-DR$^-$ Tregs

The functional and phenotypic attributes of HLA-DR$^+$ Tregs markedly diverge from those of their HLA-DR$^-$ counterparts. HLA-DR$^+$ Tregs are characterized by their potent immunosuppressive properties, which enable them to significantly suppress the activity and proliferation of effector T cells (Machicote et al., 2018; Zhou et al., 2024).

Furthermore, these cells exhibit enhanced stability and plasticity within inflammatory milieus, attributes that are pivotal for the preservation of immunological homeostasis (Ma et al., 2023). Conversely, HLA-DR$^-$ Tregs have been observed to undergo functional anergy and, under specific conditions, may transdifferentiate into effector T cells, thereby potentially contributing to immune dysregulation (W. Li et al., 2019).

Mechanisms of HLA-DR$^+$ Treg cells in SLE

HLA-DR$^+$ Treg cells show a tendency to change in number and function in patients with SLE. These changes not only affect the patient's ability to immunomodulate, but may also exacerbate disease symptoms. By understanding these cellular changes, researchers and clinicians can better assess the activity of SLE and explore new therapeutic strategies to improve patient prognosis and quality of life.

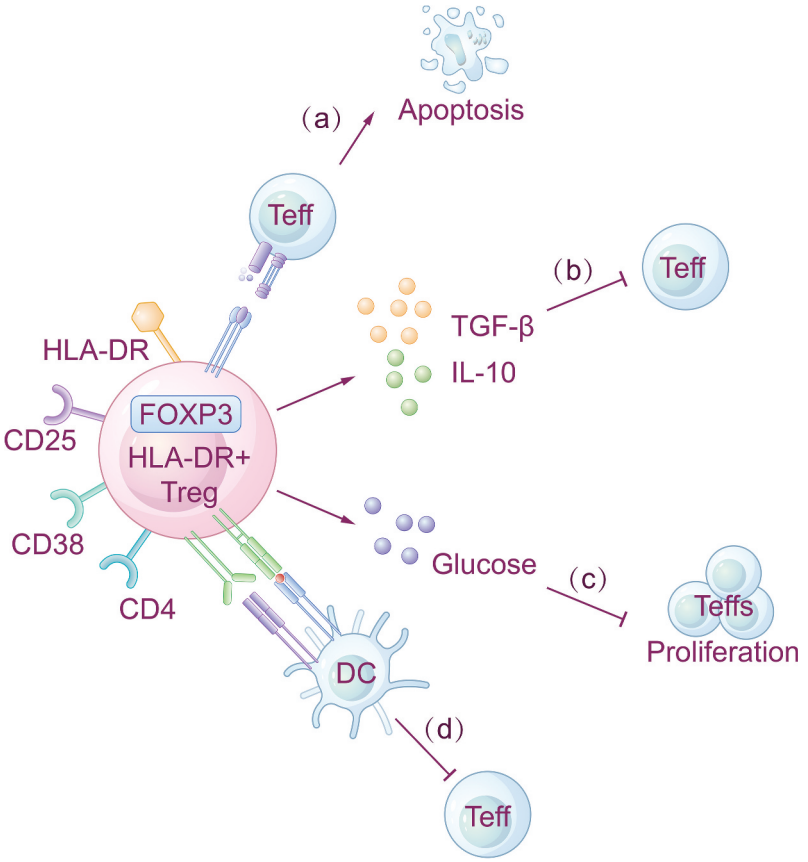

Figure 1. Mechanisms for maintaining Treg: Teff Balance. This diagram illustrates the various mechanisms by which HLA-DR$^+$ Tregs interact with effector T cells (Teffs) and antigen-presenting cells (APCs) to modulate immune responses and maintain self-tolerance. (a) Induction of Apoptosis: HLA-DR$^+$ Tregs induce apoptosis in Teffs through direct cell contact, thereby reducing their numbers and activity. (b) Release of Immunosuppressive Cytokines: HLA-DR$^+$ Tregs secrete various cytokines, including IL-10 and TGF-β, which inhibit effector T-cell activity and promote immune tolerance. (c) Metabolic competition: HLA-DR$^+$ Tregs compete with Teffs for glucose, a critical resource for T cell activation and proliferation, thereby limiting Teff expansion. (d) Modulation of antigen presentation: By interacting with dendritic cells (DCs), HLA-DR$^+$ Tregs reduce the capacity of these cells to present antigens effectively, which in turn downregulates Teff activation.

Changes in the number of HLA-DR$^+$ Treg cells

In SLE, there is a significant alteration in the number of HLA-DR$^+$ Treg, which play a pivotal role in maintaining immune homeostasis. Studies have consistently reported a reduction in the frequency of HLA-DR$^+$ Treg cells in SLE patients, particularly during active phases of the disease. This decrease is associated with a compromised capacity to regulate autoreactive immune responses, suggesting that HLA-DR$^+$ Treg cells are integral to the pathogenesis of SLE. The diminished presence of these cells may be attributed to intrinsic abnormalities within SLE, potentially involving autoantibody-mediated depletion, which targets and reduces the population of HLA-DR$^+$ Treg cells, thereby exacerbating the disease. The work by Shirakawa et al. (1985) and Daca et al. (2011) further underscores the

correlation between the reduced number of HLA-DR$^+$ Treg cells and disease activity, highlighting the importance of these cells in the immune dysregulation observed in SLE.

In SLE patients, different HLA-DR genotypes are associated with changes in the number of Treg cells. For example, certain risk alleles may be associated with reduced numbers of Treg cells and diversity in clinical manifestations of the disease. A previous Taiwanese study included 234 SLE patients and 346 healthy controls (Yen et al., 2023). HLA-DR genotyping was performed on each subject using the HLA FluoGene DRDQ kit. The results showed that HLA-DR2 was significantly more frequent in SLE patients than in controls (ratio [OR] = 2.05, 95% CI, 1.44–2.92, $p < .001$.) HLA-DR6 appeared to be negatively correlated with SLE, whereas HLA-DR8 appeared to be positively correlated with SLE. SLE patients harboring HLA-DR2 had an earlier age of disease onset and a higher incidence of oral ulcers, osteonecrosis, and kidney involvement (lupus nephritis). And HLA-DR2 was associated with susceptibility to SLE in the Taiwanese population and was associated with a lower age of disease onset and more severe clinical manifestations. Although the Treg cells in this study were not directly associated with HLA-DR genotypes, extensive evidence supports their critical role in maintaining immune tolerance and suppressing autoimmune responses (Blinova & Zhdanov, 2024). The observed correlation between specific HLA-DR risk alleles and reduced Treg cell numbers may therefore suggest compromised immunomodulatory capacity in SLE patients carrying these genotypes, potentially contributing to clinical heterogeneity (Lu et al., 2021). In addition, studies have explored the association between HLA-DR genotypes and Treg cells and have proposed the hypothesis that HLA-DR risk alleles may increase autoimmune risk by restricting highly variable regions of the T cell receptor (Ishigaki et al., 2022). However, this association needs to be further validated by studies that specifically address the relationship between Treg cells and HLA-DR genotypes.

Functional changes in HLA-DR$^+$ Treg cells

In addition to the observed reduction in numbers, the functionality of HLA-DR$^+$ Treg cells may also be compromised (La Cava, 2018). While some studies suggest that their suppressive function may not be significantly impaired (J. Huang et al., 2024), Treg cell dysfunction is generally regarded as a critical component of the pathological mechanisms underlying SLE. Consequently, it is plausible that both the quantity and functionality of Tregs are affected in SLE; however, the extent of this impact may differ among individuals. This variability underscores the need for more precise phenotypic and functional analyses to further clarify the role of Tregs in the disease.

In patients with SLE, HLA-DR$^+$ Tregs demonstrate significant alterations in surface marker expression. Notably, there is an upregulation of activation markers, such as CD69 and CD71, which suggests that these cells are in an activated state (Bonelli et al., 2008). CD69 serves as an early activation marker that contributes to lymphocyte retention and tissue localization (Cibrián & Sánchez-Madrid, 2017), while CD71, also known as the transferrin receptor, is crucial for iron uptake and cellular proliferation (Xiong et al., 2023). Despite exhibiting an activated phenotype, the immunosuppressive function of these Tregs may be compromised, indicating a potential ineffectiveness in regulating immune responses (Wegrzyn et al., 2022). This phenomenon may stem from the dysregulation of Treg cell function in SLE patients, which is essential for maintaining immune tolerance and controlling autoimmune reactions. The interplay between the activation

state and the dysfunction of Treg cells in SLE represents a complex area of investigation. Research has indicated that the heightened expression of activation markers such as CD69 and CD71 in Treg cells may correlate with their diminished immunosuppressive capabilities (Hu et al., 2021). These observations align with the established role of Tregs in preserving immune homeostasis and suggest that, in conditions like SLE, Tregs may struggle to efficiently execute their regulatory functions.

In SLE patients, there is a significant correlation between HLA-DR$^+$ Treg cells and autoantigen responses (Sumida et al., 2024). The self-antigen recognition and suppressive functions of HLA-DR$^+$ Treg cells may be impaired. Studies have shown that the ability of these cells to respond to self-antigens may be reduced, resulting in insufficient suppression of the autoimmune response and thus exacerbating the disease process (Xiao et al., 2011).

Interactions between HLA-DR$^+$ Treg cells and other immune cells

HLA-DR$^+$ Treg cells engage with various immune cell types through multiple mechanisms, thereby playing a pivotal role in maintaining immune homeostasis and modulating inflammatory responses. For instance, HLA-DR$^+$ Treg cells interact with CD80/CD86 on dendritic cells (DCs) via CTLA-4, which inhibits the costimulatory activity of DCs and consequently diminishes the activation of effector T cells (Walker & Sansom, 2015). Furthermore, cytokines secreted by HLA-DR$^+$ Treg cells, such as IL-10 and TGF-β, not only impede the maturation of dendritic cells but also regulate the functions of other immune cells. During inflammatory responses, HLA-DR$^+$ Treg cells suppress the activity and migration of neutrophils by secreting TGF-β, thereby attenuating the inflammatory response (Josefowicz et al., 2012; J. Wang et al., 2023). Additionally, HLA-DR$^+$ Treg cells can inhibit the cytotoxicity of natural killer (NK) cells in a cell contact-dependent manner, reducing their capacity to kill target cells; this inhibitory effect may involve the Fas/FasL pathway (Cencioni et al., 2015). Regarding monocytes and macrophages, HLA-DR$^+$ Treg cells diminish their activation and decrease the secretion of inflammatory factors through the release of IL-10 and TGF-β (Ou et al., 2023). Moreover, HLA-DR$^+$ Treg cells can induce apoptosis in macrophages via the Fas/FasL pathway, further curtailing the inflammatory response (Caulfield et al., 2014). In autoimmune diseases such as SLE, the interaction between HLA-DR$^+$ Treg cells and endothelial cells is also crucial. HLA-DR$^+$ Treg cells mitigate the inflammatory response of endothelial cells by secreting inhibitory cytokines (e.g., IL-10), thereby regulating the immune response at the site of inflammation (Tsokos, 2024).

HLA-DR$^+$ Treg cells play a significant role in regulating humoral immunity by suppressing B cell activation and antibody production. In SLE, the dysregulation of T cells leads to aberrant B cell activation, which subsequently triggers the production of autoantibodies (Paredes et al., 2021). HLA-DR$^+$ Tregs modulate B cell function through various mechanisms, including both cell-contact-dependent interactions and the secretion of inhibitory cytokines such as IL-10 and TGF-β. These cytokines diminish B cell proliferation and their capacity to produce antibodies (Chien & Chiang, 2018). Consequently, HLA-DR$^+$ Tregs mitigate autoantibody production by inhibiting B cell activation. In SLE patients, excessive antibody production is a major contributor to disease symptoms, highlighting the critical regulatory role of HLA-DR$^+$ Tregs in controlling disease progression (Lyu et al., 2023).

These interactions illustrate that HLA-DR$^+$ Treg cells exert a wide array of immunosuppressive functions across various immune cell types, which is essential for sustaining immune homeostasis and controlling autoimmune responses. These mechanisms also provide a theoretical foundation for the development of immunotherapy strategies targeting HLA-DR$^+$ Treg cells.

Signaling pathways associated with HLA-DR$^+$ Treg cells in SLE

In SLE, the dysregulation of T cells and the subsequent aberrant activation of B cells play a crucial role in disease pathogenesis (Tenbrock & Rauen, 2022). HLA-DR$^+$ Tregs have emerged as a key subset in modulating immune responses and maintaining immune homeostasis in this context. These cells exhibit unique signaling pathways and mechanisms that contribute to their immunoregulatory functions.

HLA-DR$^+$ Tregs, similar to classical CD4+FOXP3+ Tregs, rely on cell-to-cell contact for their suppressive functions, primarily through the interaction of CTLA-4 with its ligands (Walker, 2013). This pathway is critical for inhibiting the activation of effector T cells and maintaining peripheral tolerance. Additionally, the programmed death-1 (PD-1) pathway is implicated in the regulation of HLA-DR$^+$ Tregs, particularly in the context of chronic inflammation. PD-1 signaling can modulate the suppressive capacity of these cells, highlighting its importance in autoimmune diseases like SLE (Ahmed et al., 2018; Machicote et al., 2018; Sagrero-Fabela et al., 2024).

The mammalian target of rapamycin (mTOR) pathway is another key regulator of T cell function and differentiation. In SLE, increased mTORC1 activity has been observed in double-negative T cells, correlating with disease flares. mTORC1 activation promotes the differentiation of pro-inflammatory T cell subsets, such as Th1 and Th17 cells, while inhibiting the development of regulatory T cells (Chi, 2012; Zhao et al., 2022). Conversely, inhibition of mTORC1 has been shown to expand the CD4+FOXP3+ Treg population and suppress Th17 cells, thereby reducing disease activity in SLE patients (Apostolidis et al., 2016; Zhao et al., 2022).

HLA-DR$^+$ Tregs secrete inhibitory cytokines such as IL-10 and TGF-β, which are essential for their suppressive functions. These cytokines inhibit the proliferation of B cells and reduce antibody production, thereby mitigating autoantibody-mediated pathology in SLE. Furthermore, TGF-β signaling is implicated in the differentiation and stability of Tregs, although its role may vary depending on the inflammatory context (Komai et al., 2018).

Recent studies have highlighted the role of CD132 signaling in SLE pathogenesis. Elevated CD132 expression on T cells and B cells is associated with increased disease activity and pro-inflammatory cytokine production. Blocking CD132 signaling with monoclonal antibodies has been shown to reduce the production of autoantibodies and pro-inflammatory cytokines, suggesting a potential therapeutic strategy for SLE (Yin et al., 2024).

HLA-DR$^+$ Tregs can also modulate B cell activation and antibody production through the expression of CD40L (Zhang et al., 2022). CD40L, expressed on the surface of HLA-DR$^+$ Tregs, interacts with CD40 on B cells, providing a co-stimulatory signal that enhances B cell proliferation and antibody production. This interaction is particularly relevant in SLE, where dysregulated T cell function leads to aberrant B cell activation and autoantibody

production. Targeting the CD40/CD40L pathway has been proposed as a potential therapeutic strategy to control B cell-mediated pathology in SLE (Pucino et al., 2020). However, the therapeutic effect of single-targeting CD40L in clinical trials is not ideal. Early clinical trials of monoclonal antibodies targeting CD40L were suspended due to platelet-related thromboembolic complications (Pucino et al., 2020), suggesting the need for a more comprehensive treatment strategy.

Understanding the signaling pathways associated with HLA-DR$^+$ Tregs in SLE provides valuable insights into the mechanisms underlying disease progression and offers potential targets for therapeutic intervention. Modulating these pathways, either through pharmacological agents or targeted biologics, could enhance the suppressive functions of Tregs and improve clinical outcomes in SLE patients.

HLA-DR$^+$ Treg cells as a potential target for SLE therapy

Currently, the primary classes of drugs employed to treat SLE include the following: Antimalarials: Hydroxychloroquine remains the cornerstone of SLE treatment, indicated for nearly all manifestations of the disease. It is effective in alleviating skin symptoms and joint pain while significantly reducing disease activity (Gordon et al., 2018). Glucocorticoids: Agents such as prednisone are highly effective in managing acute symptoms and organ-threatening manifestations. However, prolonged use may increase the risk of organ damage, particularly when dosages exceed 5–7.5 mg/day (Van Staa et al., 2000). Immunosuppressants:Medications such as azathioprine and cyclophosphamide are utilized for severe cases, particularly those involving organ systems, such as lupus nephritis (Basta et al., 2020; Fanouriakis et al., 2024). Their administration necessitates rigorous monitoring to mitigate the risk of adverse effects. Biologics: In recent years, biologics like belimumab have been introduced for the treatment of active SLE, especially in cases where conventional therapies are ineffective. These agents help control the disease by modulating B cell activity (Raja et al., 2020).

New therapeutic strategies are being developed as the mechanisms underlying SLE are intensively studied. Research on HLA-DR$^+$ Treg cells indicates that these cells play a crucial role in immune tolerance. They help maintain immune tolerance and prevent autoimmune reactions through the regulation of cytokine secretion, direct cell contact, metabolic reprogramming, and antigen presentation (Eggenhuizen et al., 2024). However, in SLE patients, functional defects in HLA-DR$^+$ Tregs may lead to an overreaction to autoantigens, thereby exacerbating the disease process (W. Li et al., 2019). Therefore, enhancing the function of HLA-DR$^+$ Tregs may become a new strategy for the treatment of SLE, which deserves further research and exploration (Fanouriakis et al., 2023; Liossis & Staveri, 2021).

Research indicates that strategies aimed at enhancing Treg cell function could be beneficial for patients with SLE. Notably, interleukin (IL)-2, IL-33, and IL-6 have demonstrated potential in improving both the function and quantity of Treg cells (Crayne et al., 2019; Venkatadri et al., 2021). IL-2 plays a crucial role in the maintenance and functionality of Treg cells. Notably, low-dose IL-2 therapy has been demonstrated to increase Treg cell populations, diminish autoimmune responses, and enhance disease outcomes in patients with SLE (Lykhopiy et al., 2023; Raeber et al., 2024). IL-33, an alarmin cytokine, has exhibited protective effects in various inflammatory contexts by modulating the intrinsic immune and inflammatory responses; however, its specific role in lupus nephritis (LN)

requires further investigation (Sarrand & Soyfoo, 2022; Yuan, 2022). While IL-6 is typically associated with disease severity in SLE, recent studies suggest that IL-May 6 also have a protective role in LN by facilitating the expansion of RORγt+Foxp3+ bifunctional Treg cells, which possess enhanced suppressive capabilities (Hagenstein et al., 2019; Korn & Hiltensperger, 2021; Mercader-Salvans et al., 2023; Nepal & Gazeley, 2023; Yi et al., 2020).

SLE is characterized by alternating phases of flare-up and remission, with immune-mediated tissue damage during active disease states linked to lymphoid organ dysregulation (Crow, 2023). Conventional therapies often fail to achieve sustained remission, driving interest in Treg-based strategies. These therapies exploit Tregsdual mechanisms of immune tolerance: cytokine modulation (e.g., IL-10 secretion) and direct suppression via cell-contact molecules like CTLA-4 (A. Wang et al., 2024). In this context, researchers developed Fox19CAR-Tregs (Hagen et al., 2024; M. Li et al., 2024), a CD19-targeted chimeric antigen receptor-engineered Treg product. In vitro, these cells inhibited pathogenic B cell proliferation and IL-6 production in a dose-dependent manner within a co-culture system (Doglio et al., 2024). In a humanized mouse model of SLE, a single infusion of Fox19CAR-Tregs was able to limit autoantibody production, delay lymphopenia (a key feature of SLE), and restore the composition of the human immune system in lymphoid organs without detectable toxicity. Despite the short survival time of these engineered cells, target organs in SLE appear to be protected. In conclusion, the ability of Fox19CAR-Tregs to break the vicious cycle that leads to autoimmunity and sustained tissue damage represents an effective and safe therapeutic strategy that permits the restoration of homeostasis in vivo in SLE. This study provides promising preliminary results for CAR-Treg cell therapy in SLE and lays the foundation for future clinical applications.

Immunosuppressive drugs, such as rapamycin, have been developed to enhance the function of Treg cells (Chen et al., 2021). These agents promote Treg cell proliferation and function by modulating various signaling pathways, particularly the mTOR signaling pathway. As an mTOR inhibitor, rapamycin enhances Treg cell stability and inhibits inflammatory responses, which consequently alleviates symptoms of SLE (Kim et al., 2020).

Novel and repurposed therapeutic agents targeting T cell-associated pathways may offer benefits to specific subpopulations of patients with SLE. These agents have been designed to enhance the function of HLA-DR$^+$ Treg cells while simultaneously suppressing the autoimmune response (Raffin et al., 2020). For instance, Dapirolizumab pegol, an anti-CD40 ligand antibody, inhibits T-cell activation and B-cell differentiation by blocking the interaction between CD40 and its ligand, CD154 (Furie et al., 2021). Although clinical trials of Dapirolizumab pegol in SLE did not meet their primary endpoints, the agent demonstrated an acceptable safety and tolerability profile. Despite the absence of significant efficacy, it exhibited potential in reducing anti-double-stranded DNA antibody levels and improving immunologic markers in SLE patients (Acharya et al., 2023). Another agent, Dalazatide, is an inhibitor of the Kv1.3 potassium channel, which is expressed in a diverse array of immune cells, including effector memory T-cells (Tem) that are implicated in autoimmune diseases (Gubič et al., 2021; Selvakumar et al., 2022). Dalazatide reduces the production of inflammatory cytokines by inhibiting specific channels, which may influence T cell migration and activation. In preclinical and in vitro studies involving SLE, Dalazatide demonstrated the capacity to inhibit the differentiation of Th17 and Th1 cells, while also exhibiting a favorable safety profile in the early phases of clinical trials (Bui & Wilensky, 2010). Tacrolimus,

a calmodulin phosphatase inhibitor, impedes T-cell activation and proliferation by blocking IL-2 expression. Preclinical studies in SLE indicated that Tacrolimus, in conjunction with the STAT3 inhibitor ST21, has the potential to enhance Treg cell numbers and suppress the production of B cells, plasma cells, and TNF-alpha within the germinal center (Kogina et al., 2009; Park et al., 2018). Sirolimus, an mTOR inhibitor, reduces T cell activation, proliferation, and differentiation by targeting the mTOR signaling pathway (Kraaijeveld et al., 2019). Clinical trials in SLE revealed that Sirolimus effectively decreased SLEDAI (SLE Disease Activity Index) and BILAG (British Lupus Assessment Group) scores, with Treg cell expansion observed after 12 months of treatment (Eriksson et al., 2019; Hoi et al., 2024; Lai et al., 2018; Sharabi & Tsokos, 2020). These findings suggest that Sirolimus is both safe and effective for patients with SLE; however, not all therapeutic agents have advanced in clinical trials. For instance, anti-IL-12 and IL-23 monoclonal antibodies (Ustekinumab) and a CTLA-4 agonist (Abatacept) did not meet anticipated outcomes in clinical trials for SLE (Han et al., 2023; van Vollenhoven et al., 2022).

Prospects and challenges of HLA-DR$^+$ Treg cells in SLE therapy

HLA-DR$^+$ Tregs demonstrate significant potential in the study and treatment of SLE. Firstly, the levels of HLA-DR$^+$ Tregs may serve as a novel biomarker for monitoring SLE activity and therapeutic response. In patients with active SLE, the overall percentage of Tregs was significantly lower compared to healthy controls, indicating that a reduction in Tregs may play a role in the pathogenesis of SLE (Eggenhuizen et al., 2024). Furthermore, fluctuations in HLA-DR$^+$ Tregs during treatment highlight their potential value in assessing the response to SLE therapy. Notably, the baseline frequency of Ki67$^+$ Tregs (including HLA-DR$^+$ Tregs) was higher and showed an increase throughout the treatment period, which correlated with a lower frequency of Ki67$^+$ Tregs in patients who did not experience a relapse compared with those who did (S.-W. Huang et al., 2024). These findings suggest that levels of HLA-DR$^+$ Tregs may be elevated during the active phase of SLE and diminished in the stable phase of the disease, thereby providing a scientific rationale for their potential as biomarkers of SLE. Consequently, monitoring HLA-DR$^+$ Tregs may be beneficial in evaluating SLE activity and anticipating therapeutic responses, which carries significant implications for the clinical management of patients with SLE. Second, immunotherapeutic strategies targeting HLA-DR$^+$ Tregs may bring new therapeutic options for SLE patients. Conventional treatments for SLE focus on suppressing the overreaction of the immune system, but are often accompanied by significant side effects. By enhancing the function or number of HLA-DR$^+$ Tregs, on the other hand, it can be expected to improve the immune tolerance of patients, thereby reducing disease symptoms. This approach would not only be effective in controlling disease activity, but would also provide a safer and more effective treatment pathway by reducing drug dependence and side effects. Finally, several clinical trials are currently evaluating the use of Treg cell therapy in SLE (Table 1). These studies aim to validate the role of HLA-DR$^+$ Tregs in improving graft survival and inducing graft tolerance, thus providing a new scientific basis for the treatment of SLE. The successful conduct of these clinical trials may revolutionize the treatment of SLE patients, improve their quality of life, and shed light on the treatment of other autoimmune

Table 1. Clinical trials of immunomodulatory therapies in systemic lupus erythematosus.

NCT Number	Study Title	Status	Intervention	Primary Outcomes	Key Findings/Mechanistic Insights
NCT06560775	Effects of Nigella Sativa on T Cells and Cytokine Profile in Pediatric SLE	COMPLETED	Nigella Sativa oil + Standard SLE therapy	SLEDAI score reduction; Treg/Th17 balance modulation	Improved SLEDAI scores and Treg/Th17 ratio in pediatric SLE patients.
NCT02084238	Low-dose IL-2 Treatment in SLE	COMPLETED	Interleukin-2 (IL-2)	Increased Treg cells; Reduced SELENA-SLEDAI scores	IL-2 expanded Treg populations (SRI response: 60%).
NCT03312335	Low-dose Interleukin-2 for SLE (Charact-IL-2)	COMPLETED	Low-dose Aldesleukin (IL-2)	Treg percentage increase (CD4+ T cells)	Enhanced Treg functionality and reduced Th17/Tfh cells.
NCT04447053	Sequential Belimumab and T-cell Based Therapy in SLE	RECRUITING	Belimumab + Standard of Care (SOC)	Treg/Teff ratio; TCR sequencing	Modulated Treg subsets and TCR diversity.
NCT04077684	Efficacy and Safety of Low-dose IL-2 in SLE	COMPLETED	IL-2 (0.2–1 MIU)	SLE Responder Index-4 (SRI-4)	Restored Treg/Th17 balance (SRI-4 response: 65%).
NCT02428309	Autologous Polyclonal Tregs for Lupus	TERMINATED	Autologous Polyclonal Tregs	Safety (Grade ≥3 AEs)	Terminated due to safety concerns, but Treg infusion showed transient immunomodulation.
NCT04835883	CS20AT04 (Allogenic Stem Cells) in SLE	RECRUITING	Allogenic bone marrow-derived MSCs	Corticosteroid reduction; Hematologic/ renal improvement	MSCs promoted Treg activity in lupus nephritis/cytopenia.
NCT06013995	BMS-986326 (Treg Modulator) in Lupus	RECRUITING	BMS-986326 (anti-CD28 antagonist)	Safety; Treg/Tconv ratio	Enhanced Treg stability without significant AEs.
NCT03171194	Mesenchymal Stem Cells for SLE	COMPLETED	Umbilical cord-derived MSCs	Safety profile; Treg expansion	Increased Treg levels and reduced IL-6/TNF-α.
NCT01413230	Vitamin D Supplementation in SLE	COMPLETED	Cholecalciferol (Vitamin D)	Treg/Th17 balance; Gene expression profiling	Vitamin D increased Treg frequency and reduced Th17 cells.
NCT01988506	Induction of Treg by Low-dose IL-2 in Autoimmune Diseases (TRANSREG)	COMPLETED	IL-2	Treg percentage increase across 14 autoimmune diseases	IL-2 universally expanded Tregs, highest efficacy in SLE.

Abbreviations: Treg: Regulatory T cells; Th17: T helper 17 cells; Tfh: Follicular helper T cells; SLEDAI: SLE Disease Activity Index; SRI: SLE Responder Index; MSCs: Mesenchymal Stem Cells.

diseases. Overall, the research and application of HLA-DR$^+$ Tregs in SLE is promising and deserves further in-depth exploration.

In SLE research, HLA-DR$^+$ Tregs have shown remarkable potential, yet their application faces several substantial challenges. Cellular heterogeneity is a primary concern. HLA-DR$^+$ Tregs are not a uniform cell population; they exhibit significant heterogeneity, which may influence their function and therapeutic efficacy. The specific roles of different subpopulations in immunomodulation remain incompletely understood, necessitating in-depth studies to elucidate their contributions to SLE and optimize therapeutic strategies. Moreover, SLE is a complex, multifactorial disease driven by a combination of genetic, environmental, and immunologic factors. The

function of HLA-DR$^+$ Tregs may be modulated by the interplay of these factors, complicating their role in disease pathogenesis. A comprehensive understanding of HLA-DR$^+$ Tregs in SLE requires accounting for these variables, highlighting the need for multidisciplinary collaborative research that integrates insights from immunology, genetics, and clinical medicine. Another challenge is the potential for unintended consequences when enhancing the function of HLA-DR$^+$ Tregs. While this strategy holds promise for improving immune tolerance, it may also lead to immunosuppression and increased infection risk. Ensuring the safety and efficacy of HLA-DR$^+$ Treg-based therapies is thus paramount, requiring a delicate balance that avoids compromising overall immune defense mechanisms. Lastly, the lack of standardized methods for assessing the function and number of HLA-DR$^+$ Tregs poses a significant barrier. Without uniform assays, comparing and validating results across studies becomes difficult. Establishing reliable and consistent assessment methods is crucial for advancing research and facilitating the translation of findings from bench to bedside. In summary, while HLA-DR$^+$ Tregs hold great potential for SLE research and treatment, addressing cellular heterogeneity, disease complexity, therapeutic safety, and the need for standardized methods remains essential. Overcoming these challenges will pave the way for personalized treatment approaches and improved prognoses for patients with SLE.

Directions for future research

The immunomodulatory role and therapeutic potential of HLA-DR$^+$ Tregs in SLE have gradually emerged; however, their mechanisms and clinical applications require further exploration. Future research should focus on several key aspects: First, a comprehensive analysis of the mechanisms of action of HLA-DR$^+$ Tregs is essential, including investigations into their gene expression profiles, proteomic characteristics, and interactions with other immune cells. Second, it is important to optimize the clinical application of HLA-DR$^+$ Tregs by determining the optimal timing and dosage for treatment through clinical trials, as well as exploring methods to enhance cell expansion efficiency in vitro and survival rates in vivo. Additionally, research should be conducted on individualized treatment approaches based on the genetic information and immune characteristics of individual patients, utilizing bioinformatics tools to predict treatment responses and facilitate precision medicine. Finally, strengthening multidisciplinary collaboration, establishing standardized detection methods, and accelerating the translation of basic research into clinical applications are crucial steps. These research directions will provide new insights and strategies for the treatment of SLE and ultimately improve patient prognosis.

Conclusion

HLA-DR$^+$ Tregs play an important role in the pathogenesis and treatment of SLE. By enhancing the function of these cells or modulating the cytokines they secrete, it is expected to provide new therapeutic options for SLE patients, thereby improving their quality of life and prognosis. This not only opens a new direction for the treatment of SLE, but also provides a valuable reference for the management of other autoimmune diseases. Future studies should continue to focus on the mechanism and clinical application of HLA-DR$^+$

Tregs, with the aim of achieving more precise and effective immunomodulatory treatment strategies.

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Autoimmune neuro-ophthalmic disorders: pathophysiologic mechanisms and targeted biologic therapies

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REVIEW

Autoimmune neuro-ophthalmic disorders: pathophysiologic mechanisms and targeted biologic therapies

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ABSTRACT

Introduction: Autoimmune neuro-ophthalmic disorders encompass a diverse array of conditions, including thyroid eye disease (TED), myasthenia gravis (MG), optic neuropathy due to giant cell arteritis (GCA), and optic neuritis related to multiple sclerosis (MS), neuromyelitis optica spectrum disorder (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD). While traditional treatments have shown efficacy in managing symptoms, the rapid emergence of biologic therapies has brought forth new avenues for targeted intervention, revolutionizing treatment approaches for these conditions.

Areas covered: This review highlights the pathophysiologic pathways and FDA-approved biologic therapies utilized in the management of autoimmune neuro-ophthalmic disorders. We explore multiple therapeutic approaches for autoimmune neuro-ophthalmic disorders, including IGF-1 R antagonism, IL-6 inhibition, complement inhibition, FcRn targeting, B-cell depletion and T-cell modulation. Literature from clinical trials, observational studies, and meta-analyses through 2024 was evaluated to assess efficacy, safety, and long-term outcomes.

Expert opinion: Biologic therapies represent a significant advancement in autoimmune neuro-ophthalmic disorders, offering targeted approaches with improved efficacy and safety profiles compared to traditional treatments. Ongoing developments in biomarker identification and delivery systems suggest an increasingly personalized approach to treatment. Future advances will likely focus on optimizing patient selection, reducing costs, improving accessibility, and developing novel therapeutic targets.

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1. Introduction

Autoimmune neuro-ophthalmic disorders encompass a diverse range of conditions that affect both the nervous system and the visual system, often resulting in complex, debilitating symptoms. These disorders include a variety of conditions such as optic neuropathies, ocular motility dysfunctions, and cranial nerve palsies. Neuro-ophthalmic disorders have historically posed significant diagnostic and therapeutic challenges, requiring an intricate understanding of both neuroanatomy and the physiology of vision.

In the past, treatment approaches for these disorders were largely supportive and symptomatic, commonly employing corticosteroids and other immunosuppressive therapies to manage inflammation and prevent progression in conditions such as optic neuritis (ON), myasthenia gravis (MG), and thyroid eye disease (TED). Surgical interventions, such as thymectomy in some patients with MG or strabismus surgeries for ocular motility dysfunctions, have also played a crucial role in symptom management. However, many of these treatments are limited by long-term inefficacy, risk of side effects, and potential for disease recurrence.

In recent years, the landscape of neuro-ophthalmology has been transformed by the advent of biologic therapies. These treatments, which target specific molecular pathways involved in disease pathogenesis, offer a more precise and targeted approach to managing autoimmune neuro-ophthalmic disorders. Monoclonal antibodies (mAb) and other biologics that modulate immune responses, block inflammatory cytokines, or inhibit abnormal cell signaling have shown promise in treating conditions such as MG, inflammatory optic neuropathies, and certain types of autoimmune-related ocular diseases. The potential for biologic therapies to provide more durable, effective, and safer options for patients represents a new frontier in neuro-ophthalmology.

This article explores the evolution of treatment strategies in autoimmune neuro-ophthalmic disorders, highlighting the historical challenges in managing these complex conditions. It also examines the emerging role of biologic therapies, focusing on mechanisms of action, clinical applications, and their transformative potential in patient care. To maintain a clear scope, only therapies approved by the United States Food and Drug Administration (FDA) will be cited as examples, despite the breadth of novel treatments currently in clinical development.

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Article highlights

- Biologic therapies have transformed the treatment landscape of autoimmune neuro-ophthalmic disorders, including thyroid eye disease (TED), myasthenia gravis (MG), and optic neuritis related to multiple sclerosis (MS), giant cell arteritis (GCA), and neuromyelitis optica spectrum disorder (NMOSD).
- The targeted nature of biologic therapies addresses specific disease mechanisms while dramatically improving the risk-benefit profile compared to traditional broad immunosuppression with corticosteroids, delivering more effective and sustained disease control and avoiding serious complications such as hypertension, diabetes, osteoporosis, and psychiatric effects.
- IGF-1 R antagonism with teprotumumab has demonstrated robust efficacy in TED, achieving significant reductions in proptosis and offering the first targeted therapy for this condition.
- IL-6 inhibition with tocilizumab has demonstrated significant efficacy in GCA, allowing for reduction in corticosteroid use while maintaining disease control.
- Complement inhibition through agents like eculizumab and ravulizumab has revolutionized the treatment of MG and NMOSD, providing effective disease control with favorable long-term safety profiles.
- The emergence of FcRn inhibitors, such as rozanolixizumab and efgartigimod, represents a novel therapeutic approach for MG, offering more convenient administration options and potentially better tolerability compared to traditional treatments.
- B-cell depletion strategies such as ocrelizumab, ofatumumab and ublituximab for MS, and inebilizumab for NMOSD, have shown remarkable efficacy, reducing relapse rates and disease progression.
- T-cell modulating agents like natalizumab and fingolimod work by targeting specific T-cell pathways to reduce inflammation in neurological conditions like MS, showing promise in improving visual outcomes.
- Despite the transformative potential of biologics, challenges remain regarding patient selection, treatment optimization, and healthcare access, highlighting the need for continued research and development of biomarkers.
- The future of neuro-ophthalmic care lies in the development of these more selective targeting mechanisms, improved delivery systems, and personalized treatment approaches guided by biomarker-driven decision making.

2. Pathophysiology of common autoimmune neuro-ophthalmic disorders

The pathophysiology of autoimmune neuro-ophthalmic disorders is diverse and complex, ultimately involving a wide array of mechanisms including inflammation, ischemia, and neurodegeneration (Table 1). These conditions can affect the optic nerve, orbital tissues, extraocular muscles, and visual processing pathways in the brain. A deeper understanding of these mechanisms is crucial for developing targeted therapies to treat vision symptoms, prevent damage, and improve the quality of life for affected individuals.

2.1. Optic neuritis, multiple sclerosis, neuromyelitis optica spectrum disorder, and myelin oligodendrocyte glycoprotein antibody-associated disease

Optic neuropathies refer to a group of conditions that affect the optic nerve, leading to visual impairment. When an optic neuropathy is caused by acute inflammation, the disorder is termed ON and can lead to demyelination and axonal damage with resulting disruption of visual signal transmission from the retina to the brain. The causes of ON are extensive. However, multiple sclerosis (MS)-associated ON is the most common presentation, accounting for roughly 50–80% of ON cases and, therefore, is conventionally termed 'typical' ON [1]. It is important to note that there is significant geographic variability in the underlying causes of ON; while MS-associated ON predominates in Western countries like the United States and Europe, other causes of ON such as neuromyelitis optica spectrum disorder (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) are thought to be more prevalent causes than MS in Asian populations, particularly in countries like Japan, China, and India.

Typical (acute demyelinating) ON most commonly affects Caucasian women between 18 and 50 years and presents with the typical features of subacute monocular visual loss with associated pain during eye movements [2]. Vision declines over the first 7 to 10 days and reaches a nadir by 2 weeks. Other signs and symptoms include dyschromatopsia, visual field loss, reduced contrast sensitivity, and the presence of an ipsilateral relative afferent pupillary defect. Optic disc edema is present in approximately one-third of cases, and the remaining two-thirds do not exhibit optic disc edema due to retrobulbar involvement [3]. These typical features can also be noted in a demyelinating clinically isolated syndrome without a known underlying systemic cause but may carry a risk of conversion to MS. The Optic Neuritis Treatment Trial (ONTT) found that 72% of patients with one or more brain lesions on a baseline magnetic resonance imaging (MRI) scan developed MS within 15 years compared to only 25% of patients with no brain lesions at baseline [4]. Idiopathic acute clinically isolated demyelinating ON is also a common cause of ON, though not as common as MS-ON (1).

The pathogenesis of MS-ON appears to be multifactorial and a definitive target antigen has yet to be identified. The optic nerve lesions appear pathologically similar to MS brain lesions. In the active phase, inflammatory demyelination occurs, resulting in vision loss from conduction block. The acute phase is predominantly due to systemic activation of peripheral myelin-reactive CD4+ T cells [5]. The T lymphocytes then infiltrate the central nervous system (CNS) through interaction with endothelial cellular adhesion molecules such as integrin α4β1 and endothelial vascular cell adhesion molecule 1 (VCAM-1), allowing migration across the blood-brain barrier [6,7]. The aberrant T-cells recognize CNS self-antigen, resulting in myelin destruction and axonal damage. This triggers Th1 and Th17 pro-inflammatory cell activation and the release of pro-inflammatory cytokines such as interferon-γ and IL-17 [8]. The role of B cells in MS is mainly through the production of antibodies to self-antigens such as anti-myelin basic protein (MBP). Autoantibodies cloned from MS patients against myelin antigen have been shown to be capable of inducing complement-mediated robust oligodendrocyte loss and microglial activation, indicating the potential role of myelin-binding autoantibodies in MS pathogenesis [8]. Improvement in inflammation and vision occurs over weeks to months while remyelination occurs, although it may be incomplete [9].

In contrast, non-MS ON is less frequent and can be related to infectious and immune-mediated diseases such as NMOSD and MOGAD. These cases are more likely to have atypical features, including male gender, age less than 18 or greater than 50, absence of pain, and bilateral presentation [2].

NMOSD, originally known as Devic's disease, is a chronic inflammatory autoimmune disease of the CNS classically

Table 1. Autoimmune neuro-ophthalmic disorders: key features.

Disorder	Key clinical features	Key diagnostic tests	Pathophysiology
Multiple Sclerosis Associated Optic Neuritis (MS-ON)	• Most common in Caucasian women 18–50 years • Subacute monocular visual loss with pain during eye movements • Vision decline over 7–10 days • Dyschromatopsia • Visual field loss • Reduced contrast sensitivity • Ipsilateral RAPD • Mild optic disc edema (⅓ of cases)	• Visual function testing (visual acuity, color vision, pupillary examination, visual field testing) • OCT • MRI brain/orbits (demyelinating lesions, focal enhancement of the optic nerve)	• T-cell mediated demyelination • CD4+ T cells infiltrate CNS via interaction with integrin α4β1 and VCAM-1 • B cells produce antibodies to self-antigens • Release of pro-inflammatory cytokines (IFN-γ, IL-17)
Neuromyelitis Optica Spectrum Disorder (NMOSD)	• Profound and persistent visual loss • Atypical optic neuritis, can be bilateral • Longitudinally-extensive Ttransverse myelitis	• Visual function testing • OCT • MRI brain/orbits and spine (enhancement of the optic nerve, bilateral optic nerve or chiasmal or hypothalamic involvement, longitudinally-extensive transverse myelitis) • AQP4 antibody testing (positive in 70–80%)	• AQP4 antibody-mediated astrocyte destruction • Complement activation • Blood-brain barrier disruption • Infiltration of inflammatory cells • Secondary oligodendrocyte injury
MOG Antibody-Associated Disease (MOGAD)	• Atypical optic neuritis, can be bilateral • Frequent anterior optic nerve involvement • Optic disc edema, may be moderate or severe • Longitudinally-extensive transverse myelitis	• Visual function testing • OCT • MRI brain/orbits and spine (optic nerve enhancement, optic nerve sheath enhancement, longitudinally-extensive transverse myelitis) • MOG antibody testing	• Antibodies against MOG • T-cell mediated inflammation • Complement activation • Increased pro-inflammatory cytokines • Myelin-laden macrophage infiltration
Giant Cell Arteritis (GCA)	• Age >50 years • More common in white women • Temporal headache • Scalp tenderness • Jaw claudication • Profound vision loss • Pallid optic disc edema	• Visual function testing • OCT • Fluorescein angiography • ESR/CRP/platelets • Temporal artery ultrasound • Temporal artery biopsy	• Granulomatous inflammation of medium/large vessels • Inflammatory cytokine production • Vessel wall inflammation and luminal occlusion • Tissue ischemia
Myasthenia Gravis (MG)	• Fatigue worsens throughout day • Fluctuating muscle weakness • Ocular involvement (ptosis, diplopia) in 50% • Ocular MG can progress to generalized MG	• Ice pack test • Ocular alignment and movements • AChR, MuSK, LRP4 antibody testing • EMG repetitive nerve stimulation • Single fiber EMG	• Autoantibodies against AChR, MuSK, LRP4, others • Complement activation • Neuromuscular junction dysfunction • Impaired neuromuscular transmission
Thyroid Eye Disease (TED)	• Double vision • Dry eye • Proptosis • Eyelid retraction • Periorbital swelling • Extraocular muscle enlargement and restriction • Exposure keratopathy • Optic nerve compression	• Clinical activity score • Visual function testing • Ocular alignment and movements • Forced duction testing • Exophthalmometry • Thyroid function tests • Anti-TSHR antibodies • Orbital imaging (CT/MRI)	• IGF-1 R/TSHR complex activation • Orbital fibroblast activation • Increased glycosaminoglycan production • Orbital tissue inflammation • Orbital fat and muscle expansion • Late fibrosis

Abbreviations: RAPD = Relative Afferent Pupillary Defect, OCT = Optical Coherence Tomography, AQP4 = Aquaporin-4, MOG = Myelin Oligodendrocyte Glycoprotein, ESR = Erythrocyte Sedimentation Rate, CRP = C-Reactive Protein, AChR = Acetylcholine Receptor, MuSK = Muscle-Specific Kinase, LRP4 = Low-density lipoprotein receptor-related protein 4, EMG = Electromyography, TSHR = Thyroid Stimulating Hormone Receptor, IGF-1 R = Insulin-like Growth Factor 1 Receptor.

characterized by acute ON and transverse myelitis. A hallmark feature of NMO-ON is profound and persistent visual loss, unlike that of MS-ON. NMO-ON may also be differentiated from MS-ON based on imaging. MRI enhancement of the optic nerve in NMO-ON generally involves longer contiguous segments of the optic nerve ('longitudinally extensive lesions') and these lesions are more likely to involve the optic chiasm or adjacent hypothalamus than in MS-ON [10]. In 2004, a pathogenic NMO-associated IgG antibody was discovered that targeted the water channel membrane protein aquaporin-4 (AQP4) [11]. The AQP4 antibody (AQP4-Ab) is found exclusively in NMOSD patients; however, 20–30% of NMOSD patients are seronegative for AQP4-Ab. The International Panel for NMO Diagnosis revised the diagnostic criteria for NMOSD in 2015 (Table 2) [12]. AQP4 is expressed in astrocytes throughout the CNS including the optic nerve. In NMOSD,

Table 2. The 2015 international panel diagnostic criteria for adult patients with NMOSD. Reproduced with permission from [12], © 2015 American Academy of Neurology, licensed under the CC BY-NC-ND 4.0 license.

Core clinical characteristics
- Optic neuritis
- Acute myelitis
- Area postrema syndrome: episode of otherwise unexplained hiccups or nausea and vomiting
- Acute brainstem syndrome
- Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions
- Symptomatic cerebral syndrome with NMOSD-typical brain lesions

Diagnostic criteria for NMOSD with AQP4-IgG
- At least 1 core clinical characteristic
- Positive test for AQP4-IgG using best available detection method (cell-based assay strongly recommended)
- Exclusion of alternative diagnoses

Diagnostic criteria for NMOSD without AQP4-IgG or NMOSD with unknown AQP4-IgG status
- At least 2 core clinical characteristics occurring as a result of one or more clinical attacks and meeting all of the following requirements:
  - At least 1 core clinical characteristic must be optic neuritis, acute myelitis with LETM, or area postrema syndrome
  - Dissemination in space (2 or more different core clinical characteristics)
  - Fulfillment of additional MRI requirements, as applicable
- Negative tests for AQP4-IgG using best available detection method, or testing unavailable
- Exclusion of alternative diagnoses

Additional MRI requirements for NMOSD without AQP4-IgG and NMOSD with unknown AQP4-IgG status
- Acute optic neuritis
  - Requires brain MRI showing normal findings or only nonspecific white matter lesions, or optic nerve MRI with T2-hyperintense lesion or T1-weighted gadolinium-enhancing lesion extending over > 1/2 optic nerve length or involving optic chiasm
- Acute myelitis
  - Requires associated intramedullary MRI lesion extending over ≥ 3 contiguous segments (LETM) or ≥ 3 contiguous segments of focal spinal cord atrophy in patients with history compatible with acute myelitis
- Area postrema syndrome
  - Requires associated dorsal medulla/area postrema lesions
- Acute brainstem syndrome
  - Requires associated periependymal brainstem lesions

Abbreviations: AQP4 = aquaporin-4; IgG = immunoglobulin G; LETM = longitudinally extensive transverse myelitis lesions; NMOSD = neuromyelitis optica spectrum disorders.

CNS lesions show perivascular deposition of immunoglobulin and complement, corresponding to areas of high AQP4 expression. The binding of AQP4-Ab on astrocytes activates antibody-dependent cell-mediated cytotoxicity, resulting in astrocyte destruction. The release of inflammatory factors, including cytokines and chemokines, disrupts the blood-brain barrier and allows for infiltration of neutrophils, eosinophils, and macrophages. This results in demyelination through secondary injury of oligodendrocytes [13–16].

MOGAD is an antibody-mediated demyelinating disease that affects the brain, optic nerves, and spinal cord. Myelin oligodendrocyte glycoprotein (MOG) is a transmembrane protein on the outer surface of CNS myelin. High titers of autoantibodies to MOG are associated with a spectrum of demyelinating diseases, including ON, transverse myelitis, acute disseminated encephalomyelitis, and cerebral cortical encephalitis. MOGAD is similar to NMO in that it may cause an atypical ON and transverse myelitis. Like NMO-ON, MOG-ON may differ radiologically from MS-ON in that optic nerve enhancement may be bilateral and include (longitudinally-extensive lesions'). MOGAD also more commonly involves the anterior optic nerves and can have more severe optic disc edema than MS-ON and NMOSD, while NMO more commonly involves the posterior optic nerves, optic chiasm, and optic tracts. Additionally, perineural sheath and orbital fat enhancement are more typical of MOGAD than MS and NMOSD [17]. The pathophysiology of MOGAD is still not clearly understood. A study of biopsy samples showed an abundance of myelin-laden macrophages/microglial cells in areas of active demyelination, with most infiltrating lymphocytes being CD4+ T cells and a few B cells and CD8+ T cells [18] (Figure 1). This study also revealed complement activation in active lesions, resembling pattern II demyelinating lesions as seen in MS. Additionally, the CSF cytokine/chemokine profile of MOGAD shows increased proinflammatory cytokines, which include TNF-α, IFNγ, IL-13, IL-6, IL-8, CXCL12, APRIL, BAFF, CXCL13, CCL19, and others [20].

Diagnosis of acute ON begins with history and clinical exam, followed by orbital MRI and NMO/MOG antibody testing when there is clinical suspicion (very young or older age, lack of pain, bilateral or severe vision loss, bilateral or severe optic disc edema). The current standard of treatment for typical ON has been profoundly influenced by the ONTT. In cases of typical acute ON, intravenous (IV) methylprednisolone treatment at 1000 mg/day for three days followed by oral prednisolone (1 mg/kg) for 11 days led to more rapid recovery of vision compared to only oral prednisone at 1 mg/kg body weight for 14 days or placebo treatment. However, final measured outcomes for visual acuity, visual fields, and contrast/color vision were similar among the three groups [21,22]. Patients with MS-ON who were treated with IV methylprednisolone had fewer MS relapses in the following two years compared to the oral prednisolone and placebo groups; however, the IV group caught up with the 1 mg/kg/day oral prednisone and placebo groups after two years, with no definite long-term protective benefit. Subsequent studies revealed that oral-equivalent dosing of oral prednisone at 1,250 mg per day produced similar outcomes to 1,000 mg IV methylprednisolone per day for the treatment of acute demyelinating ON [23,24] Furthermore, ON caused by MOGAD or NMOSD

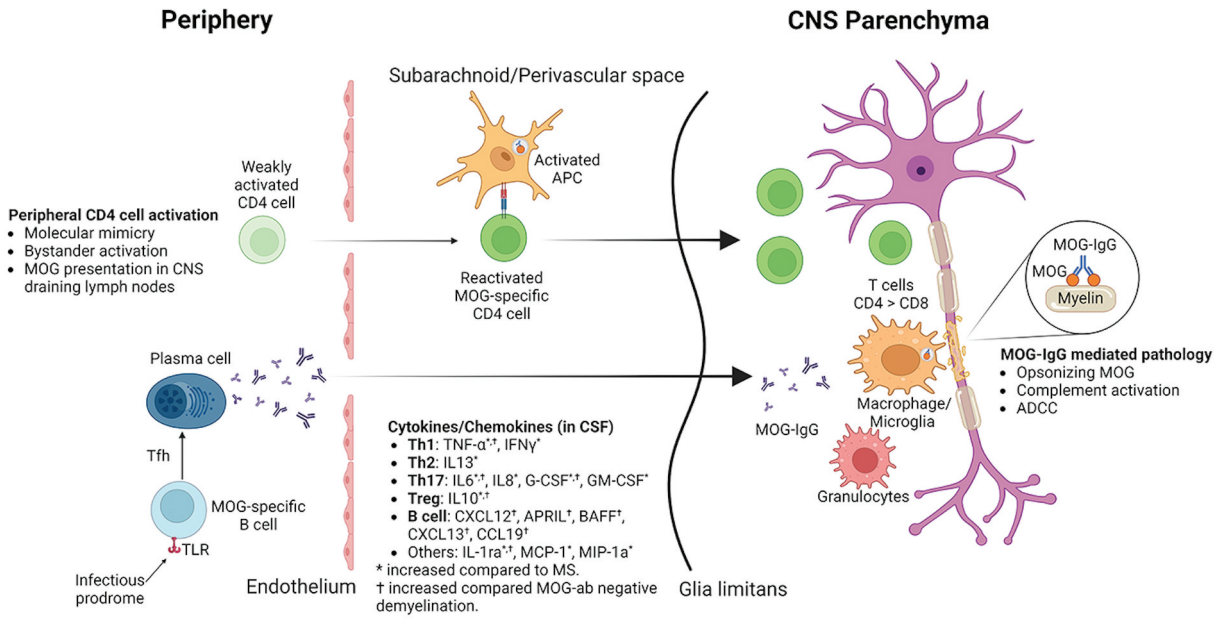

Figure 1. Illustration of the cells and cytokines involved in the pathophysiology of MOGAD. Reproduced from [19], © 2023 Corbali and Chitnis, licensed under the CC by 4.0 license.

was not studied by the ONTT and therefore the results are not directly translatable; other studies suggest that early intervention with corticosteroids and other immunosuppression is critical for the management of these diseases [1]. In cases where there is a lack of improvement with high-dose IV corticosteroids or when NMO is confirmed or highly suspected, plasmapheresis is often indicated [25,26].

2.2. Ischemic optic neuropathy and giant cell arteritis

Ischemic optic neuropathy (ION) results in optic nerve damage due to vascular insufficiency. ION is classified based on the location of the ischemic damage and the etiology. Anterior ION (AION) accounts for 90% of cases and involves ischemia of the optic nerve head, resulting in optic disc edema in the acute phase. Posterior ION (PION) involves parts of the optic nerve posterior to the optic nerve head and, therefore, lacks disc edema.

Based on the presence or absence of vasculitis, ION is further classified as arteritic or non-arteritic, respectively [27]. Non-arteritic AION (NAION or NA-AION) presents as isolated, sudden, painless, monocular vision loss with diffuse or segmental optic disc edema. The disc edema resolves over six to 11 weeks with resulting disc pallor. The severity of vision loss varies from normal visual acuity with visual field defects to profound vision loss. The Ischemic Optic Neuropathy Decompression Trial (IONDT) showed that by six months after onset of symptoms, 43% of patients with visual acuity worse than 20/64 had at least a three-line improvement on the Snellen eye chart without therapeutic intervention [28]. Patients with NAION typically have a 'disc at risk' with a crowded optic nerve head with a small physiological cup. NAION patients often have systemic risk factors such as hypertension, diabetes mellitus, hypercholesterolemia, ischemic heart disease, tobacco use, obstructive sleep apnea, and/or stroke, which predispose them to blood supply disruption to the small vessels supplying the anterior portion of the optic nerve [27,29,30]. There is no established treatment for NAION, and management is geared toward detecting and controlling vascular risk factors. The IONDT showed no benefit from surgical intervention with optic nerve sheath fenestration [28].

Arteritic AION (AAION) is most commonly associated with giant cell arteritis (GCA), a chronic granulomatous inflammatory disease primarily affecting large and medium-sized arteries. GCA exists on a spectrum that includes polymyalgia rheumatica. The disease almost exclusively affects patients older than 50 years. Incidence increases with age and is more common in white women. Interestingly, immune checkpoint inhibitors, which have been increasingly used for cancer therapy, may increase the risk of GCA by disturbing normal immune tolerance mechanisms in the body, although there may be milder symptoms and better treatment responses compared with traditional GCA [31,32]. Symptoms of GCA may include temporal headache, scalp tenderness, jaw claudication, malaise, weight loss, fever, diplopia, and transient or permanent vision loss. Vision loss in GCA is most commonly due to AAION, which typically presents with 'pallid' optic disc edema, in contrast to the optic nerve edema in NAION, which may be more hyperemic. However, vision loss from GCA can also be due to cilioretinal or central retinal artery occlusion, PION, choroidal infarction, ocular ischemic syndrome, or occipital lobe stroke [33]. Laboratory testing commonly shows elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and may include thrombocytosis. When there is sufficient clinical suspicion for GCA, confirmatory testing with a temporal artery biopsy is helpful in definitively establishing the diagnosis. The biopsy result is ideally obtained within 14 days of starting steroid therapy. Temporal artery color Doppler ultrasound can detect mural edema of the temporal artery, seen as the 'halo sign,' in patients with active GCA [34]. When

clinical suspicion is sufficiently high, treatment is initiated with high-dose corticosteroids (often IV), even before the diagnosis is confirmed. There is no consensus on the tapering protocol for corticosteroids, though the duration of treatment is typically on the scale of months. Long-term corticosteroid therapy is associated with adverse effects and can cause complications for patients with osteoporosis, diabetes mellitus, hypertension, and infections.

2.3. Myasthenia gravis

MG is an autoimmune disorder that affects the neuromuscular junction (NMJ), causing weakness in voluntary muscles, including those responsible for ocular movements. It is characterized by autoantibodies directed against acetylcholine receptors (AChR) or, less commonly, against muscle-specific kinase (MuSK) at the NMJ. AChR facilitates nerve-to-muscle signaling by binding the neurotransmitter acetylcholine, while MuSK organizes and stabilizes AChR clusters at the NMJ to ensure efficient signal transmission. In MG, autoantibodies impair neuromuscular transmission either by blocking the AChR, promoting receptor degradation, or activating the complement system, which leads to damage of the postsynaptic membrane, causing muscle fatigue and weakness [35]. Approximately 50% of MG patients initially present with only ocular symptoms such as diplopia, ptosis, and orbicularis weakness that is variable. Ocular MG (OMG) is characterized by ocular symptoms exclusively, but can evolve into generalized MG (gMG) in 20–60% of cases [36].

MG is diagnosed by history and examination findings, but the diagnosis is supported by pharmacologic, electrophysiologic, and laboratory testing. Edrophonium chloride was used in the past as a confirmatory diagnostic test, as the medication inhibits the degradation of ACh at the NMJ and results in an observable improvement in muscle weakness when the test is positive. Electromyography (EMG) can elicit evidence of MG by measuring the response to repetitive nerve stimulation (RNS), which shows a decremental response in cases of MG. However, in cases of OMG, RNS may only be abnormal in 30–50% of cases [37]. Single-fiber EMG of the frontalis or orbicularis muscles is more sensitive for detecting MG in purely ocular cases. Several serological tests are used in MG diagnosis as well. AChR antibodies are the most common serological test and have a sensitivity of 80–90% in gMG. However, the sensitivity is thought to be lower in OMG, although more recent availability of newer cell-based assays has increased sensitivity in 'seronegative OMG' [38]. Anti-MuSK antibodies have been reported in 38–54% of patients who are seronegative for AChR antibodies (5–8% overall), but they are rarely present in OMG [39]. Other previously seronegative cases of MG can be identified by the presence of antibodies to low-density lipoprotein receptor-related protein 4 (LRP4) or agrin, or less commonly titin or ryanodine receptor antibodies. LRP4 normally facilitates the activation of MuSK, and LRP4 antibodies are found in approximately 1–5% of MG cases. LRP4 antibody-associated MG is less well-studied than AchR or MuSK antibody-associated MG. Immune checkpoint inhibitors have also been reported to precipitate or exacerbate MG [40,41].

Medical treatment of MG has historically focused on restoring muscle strength through symptom management (e.g. limiting acetylcholine breakdown via acetylcholinesterase inhibitors such as pyridostigmine), immunosuppressive therapies (e.g. corticosteroids or disease modifying therapies), thymectomy, and immunomodulation through IV immunoglobulin (IVIG). Plasmapheresis can be utilized in acute exacerbations that are unresponsive to corticosteroids and acetylcholinesterase inhibitors [42].

Several nonmedical treatments are available for the ophthalmic manifestations of MG if there is incomplete to no response to medical therapy [43]. For ptosis, mechanical elevation of the weak eyelid by a 3D printed crutch or applying cosmetic eyelid tape may be beneficial. For diplopia, monocular occlusion may be the most effective treatment due to the variable incomitance of the strabismus, but at the expense of binocularity and stereopsis. In stable cases of diplopia, prism spectacles and strabismus surgery may also be viable treatment options.

2.4. Thyroid eye disease

TED is an autoimmune condition affecting the orbital tissues that may result in an increase in orbital fat and proptosis (forward displacement of the eye), extraocular muscle enlargement and restrictive strabismus, orbital fibrosis, conjunctival injection, chemosis, and eyelid retraction and edema. It is most commonly associated with Graves' disease, though it can also occur in patients with other thyroid conditions such as Hashimoto's thyroiditis or precede subsequent hyperthyroidism. Although the precise triggers of TED are not fully understood, a key early step in its development involves the production of autoantibodies targeting the thyroid-stimulating hormone receptor (TSHR), found in both the thyroid gland and in the orbital tissues of TED patients [44,45]. Central to TED pathophysiology is an intricate interaction between the TSHR and insulin-like growth factor-1 receptor (IGF-1 R) on orbital fibroblasts and preadipocytes [46]. IGF-1 R is a transmembrane protein structurally similar to the insulin receptor; it mediates the actions of insulin-like growth factor 1 (IGF-1), a hormone critical for cell growth and division [47]. Importantly, TSHR does not act alone in promoting disease progression; instead, it forms a complex with IGF-1 R, which involves cross-talk (transactivation) between TSHR and IGF-1 R, an established signaling mechanism between G protein-coupled receptors and receptor tyrosine kinases [46,48]. This process sets off a cascade of inflammatory events driving the characteristic symptoms of TED. In TED, IGF-1 R is overexpressed in orbital fibroblasts, leading to increased sensitivity and response to IGF-1, which exacerbates inflammation, extraocular muscle and fat expansion and subsequent complications, with eventual fibrosis of the affected tissues [48,49]. Hyaluronic acid and other glycosaminoglycans are released, leading to fluid accumulation in orbital tissues. The ensuing expansion of orbital fat and muscle, results in proptosis and impaired venous drainage, which exacerbates fluid retention in the orbit. Impaired extraocular muscle function from globe displacement and muscle body engorgement produces a mechanical strabismus, often presenting as diplopia. Globe exposure may cause dry eye, conjunctivitis, and blurred vision. The orbital inflammatory reaction often produces periorbital swelling and orbital discomfort, with severe cases resulting in optic nerve compromise from direct compression

by enlarged extraocular muscles and surrounding orbital tissues near the orbital apex and/or significant traction from axial proptosis.

TED is a clinical diagnosis based on characteristic ocular symptoms like proptosis, eyelid retraction, restrictive strabismus, and periorbital swelling. Hyperthyroidism and Graves' disease can be confirmed by measuring TSH, free T4, total T3 levels, and testing for anti-TSHR antibodies. TED is then confirmed through an eye exam, noting signs like periorbital swelling, chemosis, lid retraction, limited ductions, strabismus, and measuring proptosis with an exophthalmometer.

Traditional TED treatments have aimed to reduce inflammation and alleviate symptoms. For decades, corticosteroids were the cornerstone of TED management due to their potent anti-inflammatory effects [50]. In many parts of the world, IV corticosteroids remain the recommended first-line treatment for moderate to severe TED [51]. Corticosteroids reduce the inflammatory milieu in the orbit, providing symptomatic relief during the active phase of the disease. While corticosteroids effectively reduce acute inflammation, they show limited impact on long-term outcomes, such as proptosis and diplopia. Furthermore, they do not address the underlying autoimmune mechanisms or halt disease progression. Relapse rates are high once corticosteroids are tapered. Supportive therapies include artificial tears and selenium supplements. However, these approaches offer limited relief and have minimal impact on the disease's underlying mechanisms. Orbital decompression surgery, strabismus surgery, and eyelid retraction surgery offer mechanical solutions to moderate and severe TED. Indications for these procedures may include severe proptosis, cornea-threatening exposure, or compressive optic neuropathy for orbital decompression surgery, stable large-angle and/or incomitant strabismus for strabismus surgery, and exposure keratopathy for eyelid retraction surgery.

3. Biologic therapies in autoimmune neuro-ophthalmic disorders

mAbs have emerged as promising therapies in the management of several autoimmune neuro-ophthalmic disorders (Table 3). Their precision has led to significant enhancements in clinical outcomes, including reductions in relapse rates and disease progression, preservation of visual function, and overall improvements in patient quality of life [52]. These therapies have also helped minimize treatment adverse effects and have helped improve the response to treatment. FDA-approved mAbs used in the treatment of autoimmune neuro-ophthalmic disorders will be reviewed from those that target end organ specific receptors involved in disease pathophysiology to those that affect entire subsets of immune system effector cells.

3.1. IGF1-R antagonism

IGF-IR is a receptor tyrosine kinase that plays a pivotal role in cellular growth, differentiation, and survival. As previously noted, in TED, IGF-IR is overexpressed on orbital fibroblasts. This overexpression is functionally significant, as IGF-IR forms a signaling complex via crosstalk or transactivation with TSHR, enhancing the pathological effects of thyroid stimulating immunoglobulins [46,48]. The activation of this IGF-IR /TSHR complex drives fibroblast proliferation, adipogenesis, and the secretion of pro-inflammatory cytokines and extracellular matrix components such as hyaluronan. These processes contribute to the characteristic tissue expansion, fibrosis, and chronic inflammation seen in TED. Interestingly, IGF-1 has been shown to amplify the effect of TSH on thyroid follicular epithelial cells [53]. Furthermore, disrupting IGF-1 signaling in vitro blocks downstream effects of TSHR activation, such as Erk activation [48], supporting the role of IGF-IR as an important therapeutic target in TED, providing a dual hit approach to interrupt signaling from both TSHR and IGF-IR.

Teprotumumab-trbw (Tepezza, Amgen, Thousand Oaks, CA, U.S.A.) is a fully humanized mAb that was approved in 2020 by the FDA for the treatment of TED. Teprotumumab binds IGF-1 R and prevents the downstream signal transduction pathway of IGF-1 R/TSHR complexes. In two registration studies, a Phase 2 study and a Phase 3 study (OPTIC), teprotumumab demonstrated efficacy in reducing inflammation, proptosis, diplopia, and improving quality of life in TED patients [54,55]. These clinical trials investigated teprotumumab (administered IV at 10 mg/kg of body weight for the first dose followed by 20 mg/kg thereafter at three-week intervals over a period of 21 weeks) in active, moderate-to-severe TED of acute (≤ nine months) onset [55]. Compared to placebo, teprotumumab demonstrated significantly better outcomes in achieving at least 2 mm of proptosis improvement at 24 weeks (69% vs 20% and 83% vs 10%, respectively, in each trial), Clinical Activity Score of 0 or 1, diplopia response, and mean change in Graves' ophthalmopathy-specific quality-of-life overall score [54,55].

The Phase 3 OPTIC-X trial investigated the use of IV teprotumumab in the patients who had initially received placebo in the OPTIC study [56]. Notably, this treatment group had a longer duration of TED compared to the treatment group in OPTIC (median 12.9 months in OPTIC-X vs 6.3 months in OPTIC). This study demonstrated that TED patients with longer disease duration responded similarly to those treated earlier in the disease course. This study also evaluated patients with an insufficient initial response or flare after receiving teprotumumab, and suggested benefit from additional teprotumumab therapy [56].

In 2023, the FDA expanded the label of teprotumumab's approval for TED to include any disease activity or duration [57]. This update followed the results of a Phase 4 study evaluating the efficacy of teprotumumab in patients with chronic TED and low disease activity [58]. At week 24, proptosis improvement was greater with teprotumumab than with placebo ($p < 0.01$), while the proportion of adverse events were similar between the groups. These results established the efficacy of teprotumumab independent of disease duration or activity.

During clinical trials for teprotumumab, reported side effects included muscle spasm, nausea, alopecia, diarrhea, fatigue, hyperglycemia, hearing impairment, dysgeusia, headache, dry skin, weight decreased, nail disorders and menstrual disorders [55,56,59]. Less common but more severe reactions included

Table 3. Autoimmune neuro-ophthalmic biologic therapeutic classes and fda-approved therapies.

Drug Class and Mechanism of Action	FDA-approved Agents and Route	Route	FDA-Approved Neuro-Ophthalmic Indications	Key Features
1. IGF-1R Antagonists • Bind IGF-1R and prevent downstream signaling • Block IGF-1R/TSHR transactivation	**Teprotumumab** (Tepezza, Amgen)	IV	• TED (regardless of disease activity or duration)	• Administered IV every 3 weeks x 8 infusions • First targeted therapy for TED • Significant proptosis reduction • Effective in both active and chronic TED
2. IL-6 Inhibitors • Bind IL-6 receptor • Block IL-6 signaling pathway • Reduce inflammatory cascade	**Tocilizumab** (Actemra, Genentech)	SC	• GCA	• Blackbox warning regarding serious infections • Requires TB testing prior to use • Administered SC once every week, in combination with a tapering course of corticosteroids
	Satralizumab (Enspryng, Genentech)	SC	• AQP4 Ab-positive NMOSD	• Administered SC at weeks 0, 2, and 4, followed by maintenance every 4 weeks • First subcutaneous NMOSD treatment
3. Complement Inhibitors • C5 Inhibitors • Prevent terminal complement activation	**Eculizumab** (Soliris, Alexion)	IV	• AQP4 Ab-positive NMOSD • Anti-AChR Ab-positive gMG	• Blackbox warning regarding infections caused by Neisseria meningitidis • Requires meningococcal vaccination prior to use • Administered IV weekly at weeks 0-5, followed by maintenance every 2 weeks • First complement inhibitor approved for MG
	Ravulizumab (Ultomiris, Alexion)	IV	• AQP4 Ab-positive NMOSD • Anti-AChR Ab-positive gMG	• Blackbox warning regarding infections caused by Neisseria meningitidis • Requires meningococcal vaccination prior to use • Administered IV every 8 weeks, starting 2 weeks after loading dose • Similar efficacy to eculizumab with fewer infusions
	Zilucoplan (Zilbrysq, UCB)	SC	• Anti-AChR Ab-positive gMG	• Not a mAb, but a small, synthetic peptide • Blackbox warning regarding infections caused by Neisseria meningitidis • Requires meningococcal vaccination prior to use • Administered SC daily • Only FDA-approved self-administered complement C5 inhibitor for the treatment of gMG
4. FcRn Inhibitors • Bind to FcRn • Prevent IgG recycling • Decrease IgG levels	**Rozanolixizumab** (Rystiggo, UCB)	SC	• Anti-AChR and MuSK Ab-positive gMG	• Administered in cycles SC once weekly for 6 weeks • The safety of initiating subsequent cycles sooner than 63 days from the start of the previous treatment cycle has not been established • First approved for both AChR and MuSK MG
	Efgartigimod (Vyvgart, Argenx)	IV	• Anti-AChR Ab-positive gMG	• Not a full length mAb, but a Fc fragment of IgG1 • Administered in cycles IV once weekly for 4 weeks • The safety of initiating subsequent cycles sooner than 50 days from the start of the previous treatment cycle has not been established
	Efgartigimod + Hyaluronidase (Vyvgart Hytrulo, Argenx)	SC	• Anti-AChR Ab-positive gMG	• Not a full length mAb, but a Fc fragment of IgG1 • Administered in cycles SC once weekly for 4 weeks • The safety of initiating subsequent cycles sooner than 50 days from the start of the previous treatment cycle has not been established
5a. B-Cell Depleting Agents • Target CD20+ B cells • Lead to B cell depletion via ADCC and CDC	**Ocrelizumab** (Ocrevus, Genentech)	IV	• RMS • PPMS	• Administered IV at weeks 0 and 2 then every 6 months • Twice-yearly maintenance dosing • First approved for PPMS • Reduces relapse rates and disability progression • Contraindicated in active hepatitis B infection
	Ocrelizumab + Hyaluronidase (Ocrevus Zunovo, Genentech)	SC	• RMS • PPMS	• Administered SC every 6 months • No loading dose • Premedicate with oral corticosteroid and antihistamine at least 30 minutes prior to each injection • Contraindicated in active hepatitis B infection
	Ofatumumab (Kesimpta, Novartis)	SC	• RMS • Active Secondary Progressive MS	• Administered SC at Weeks 0, 1, and 2, then monthly starting at Week 4 • First self-administered anti-CD20 therapy
	Ublituximab (Briumvi, TG Therapeutics)	IV	• RMS • Active Secondary Progressive MS	• Administered IV at weeks 0 and 2, then 24 weeks after the first infusion and then every 24 weeks • Twice-yearly maintenance dosing

(*Continued*)

Table 3. (Continued).

Drug Class and Mechanism of Action	FDA-approved Agents and Route	Route	FDA-Approved Neuro-Ophthalmic Indications	Key Features
5b. B-Cell Depleting Agents • Target CD19+ B cells • Broader B cell depletion	**Inebilizumab** (Uplizna, Amgen)	IV	• AQP4 Ab-positive NMOSD	• Administered IV at weeks 0 and 2, then 6 months from the first infusion and then every 6 months • Twice-yearly maintenance dosing
6a. T-Cell Modulators • Integrin receptor antagonists • Block α4β1 integrin • Prevent T-cell CNS migration	**Natalizumab** (Tysabri, Biogen)	IV	• RMS	• Blackbox warning regarding increased risk of progressive multifocal leukoencephalopathy (PML), an opportunistic viral infection of the brain • Administered IV every 4 weeks
6b. T-Cell Modulators • S1P receptor modulators • Prevent lymphocyte egress	**Fingolimod** (Gilenya, Novartis)	Oral	• RMS • Active Secondary Progressive MS • Pediatric MS (ages 10 and older)	• Not a full length mAb, but a sphingosine 1-phosphate receptor modulator • Administered orally once daily • First oral MS therapy

Abbreviations: IGF-1R = Insulin-like Growth Factor 1 Receptor, TSHR = Thyroid Stimulating Hormone Receptor, TED = Thyroid Eye Disease, IL-6 = Interleukin 6, NMOSD = Neuromyelitis Optica Spectrum Disorder, AQP4 = Aquaporin-4, gMG = Generalized Myasthenia Gravis, AChR = Acetylcholine Receptor, MAC = Membrane Attack Complex, MS = Multiple Sclerosis, PPMS = Primary Progressive Multiple Sclerosis, ADCC = Antibody-Dependent Cell-mediated Cytotoxicity, CDC = Complement-Dependent Cytotoxicity, RMS = Relapsing forms of Multiple Sclerosis, PPMS = Primary Progressive Multiple Sclerosis, PML = Progressive Multifocal Leukoencephalopathy, S1P = Sphingosine-1-Phosphate, FcRn = Neonatal Fc Receptor, MuSK = Muscle-Specific Kinase

hearing loss, Hashimoto's encephalopathy, and exacerbation of preexisting inflammatory bowel disease (IBD). The majority of adverse events experienced with teprotumumab treatment were graded as mild to moderate and were manageable in the trials, with few discontinuations or therapy interruptions.

Teprotumumab represents a major advancement in TED treatment. Unlike corticosteroids, which suppress inflammation broadly, IGF1-R antagonism specifically inhibits the pathological processes driving TED. The clinical trials described above demonstrate that teprotumumab achieves significant and sustained reductions in proptosis, a key feature of TED that is resistant to other therapies [60]. Moreover, its effects on orbital tissue remodeling, as demonstrated on orbital imaging in chronic TED, suggest that it may also reverse some of the structural changes in extraocular muscles and orbital fat which characterize the chronic phase of the disease [58]. As teprotumumab becomes more widely used among patients with TED, continued monitoring by healthcare providers will enhance our understanding of its risk-benefit profile in broader patient populations.

3.2. IL-6 inhibitors

Interleukin-6 (IL-6) is a pro-inflammatory cytokine involved in the immune response, inflammation, and disease processes. IL-6 is produced by T cells, B cells, monocytes, and fibroblasts in response to infections, tissue injury, or immune activation. Once IL-6 binds to its receptor, IL-6 R, it associates with a signal-transducing protein called gp130, which is ubiquitously expressed on cells [61]. This initiates downstream signaling pathways, particularly the JAK/STAT pathway, which activates gene transcription for pro-inflammatory cytokines and acute-phase proteins, as well as the MAPK and PI3K/Akt pathways, which promote inflammation, cell proliferation, and survival (Figure 2) [63]. Dysregulated IL-6 signaling can generate significant inflammatory cytokine production and be implicated in the pathophysiology of chronic inflammatory diseases, autoimmune disorders, and cancer [64,65].

IL-6 inhibitors can block IL-6 signaling by binding IL-6 itself to prevent receptor interaction, by binding the IL-6 receptor, or by disrupting gp130 signaling. IL-6 inhibitors are used to treat inflammatory and autoimmune diseases, such as rheumatoid arthritis, juvenile idiopathic arthritis, and GCA [66]. By inhibiting IL-6 signaling, inflammation decreases, reducing symptoms such as swelling, pain and joint damage. In addition, acute-phase proteins such as CRP are reduced, and disease progression is mitigated [67,68].

Tocilizumab (Actemra, Genentech, South San Francisco, CA, U.S.A.) is a humanized IL-6 R-inhibiting mAb used in the treatment of rheumatoid arthritis, GCA, systemic sclerosis-associated interstitial lung disease, polyarticular juvenile idiopathic arthritis, systemic juvenile idiopathic arthritis, cytokine release syndrome, and COVID-19. Tocilizumab is administered as a subcutaneous injection and became the first FDA-approved therapy for the treatment of adult patients with GCA. The approval followed the positive results from the Phase 3 Giant Cell Arteritis Actemra (GiACTA) study demonstrating that tocilizumab combined with a six-month steroid regimen achieved remission through 52 weeks in 56% and 53% of patients when administered weekly and biweekly, respectively [67]. This compared to 14% and 18% in the placebo groups combined with a 26-week steroid taper and 52-week steroid taper, respectively. The extension phase of the GiACTA trial included patients who were in clinical remission at one year and were treated at the investigators' discretion including no treatment, tocilizumab, glucocorticoids, methotrexate, or combinations of these, for two years. 42% of patients treated with tocilizumab for one year maintained drug-free remission during the two years following tocilizumab cessation [69]. Both weekly and biweekly tocilizumab groups received significantly less

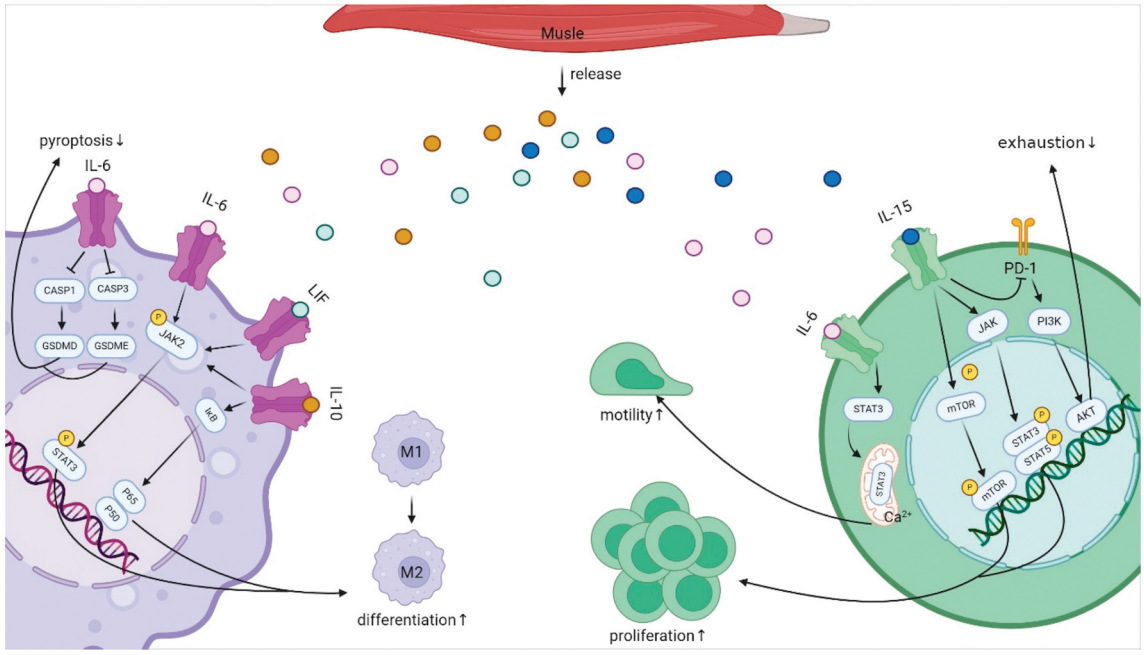

Figure 2. Illustrative representation of interleukin signaling pathways in macrophages and T cells, including IL-6 interaction with the caspase-3-GSDME pathway, caspase-1-GSDMD pathway, JAK/STAT3 pathway, and STAT3/mitochondrial pathway. Reproduced from [62], © 2024 Lu et al., licensed under the CC by 4.0 license.

median cumulative glucocorticoid doses over three years compared to the placebo groups. Tocilizumab also restored clinical remission among patients who relapsed during the two years following tocilizumab cessation [69]. Adverse events associated with tocilizumab include neutropenia, opportunistic infections, temporarily elevated liver enzymes, and higher serum cholesterol levels. Neutropenia occurred at a similar rate in the GiACTA trial (4%) to rates observed in previous trials of tocilizumab. No new or unexpected safety findings were reported over the full three years of the extension phase of the GiACTA trial [69]. Nevertheless, the US prescribing information contains a black box warning regarding serious infection and advises tuberculosis testing prior to use.

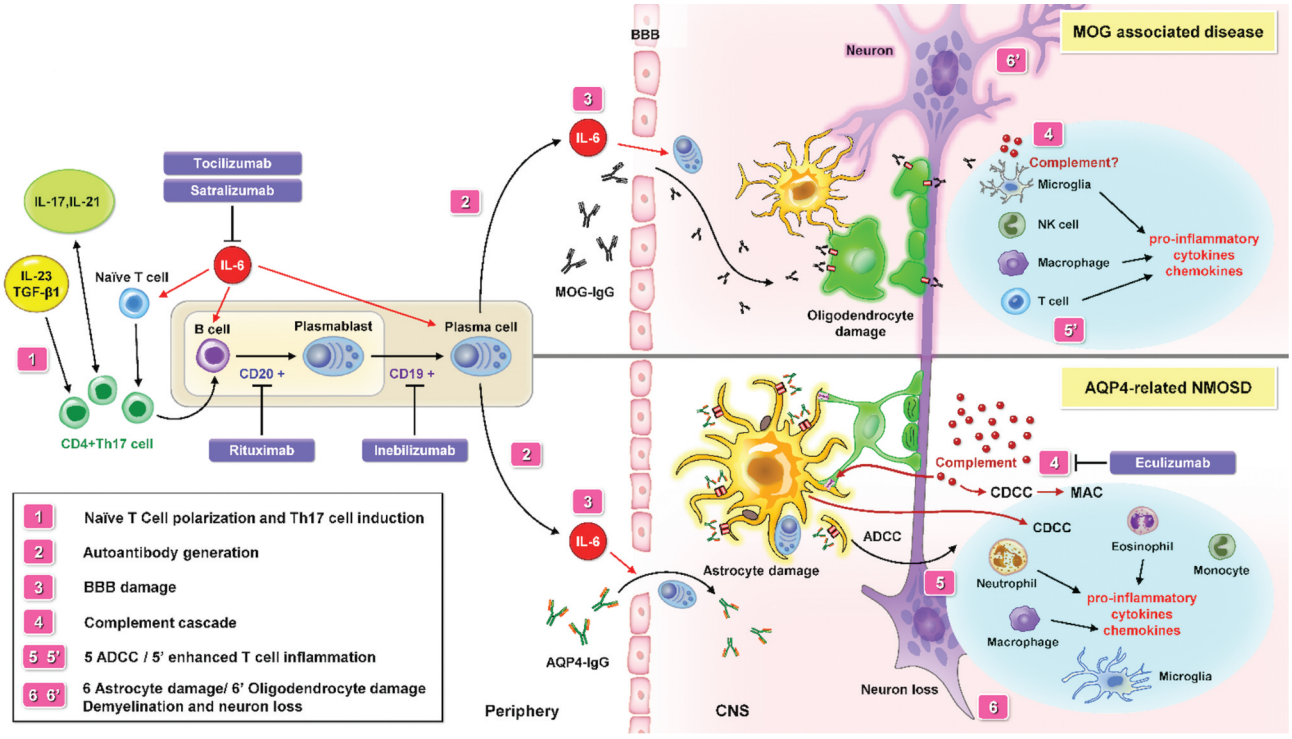

Figure 3. Schematic depiction of the pathologic mechanisms involved in NMOSD and MOGAD, and therapies targeting complement activation (eculizumab), IL-6 R signaling (tocilizumab, satralizumab), and plasma cells producing AQP-4 and MOG IgG abs (rituximab, inebilizumab). Reproduced from [70], © 2022 Huang et al., licensed under the CC by 4.0 license.

Satralizumab-mwge (Enspryng, Genentech, South San Francisco, CA, USA) is an anti-IL-6 R mAb approved for the treatment of NMOSD in adult patients who are AQP4-Ab positive (Figure 3). In 2020, satralizumab became the first subcutaneous NMOSD treatment [71]; it is administered subcutaneously at weeks 0, 2, and 4, followed by maintenance dosing every four weeks. The approval was supported by the results from the Phase 3 SAkuraStar and SAkuraSky studies. SAkuraStar investigated satralizumab monotherapy against placebo while SAkuraSky evaluated satralizumab added to baseline immunosuppressant therapy. The primary endpoint for both studies was the time to first protocol-defined relapse (PDR). Satralizumab monotherapy achieved a 30% PDR rate compared to 50% in the placebo group ($p = 0.02$) [72], while satralizumab added to stable immunosuppressant treatment revealed a PDR of 20% compared to 43% in the placebo group [73]. Satralizumab was well tolerated in these studies, with common side effects including nasopharyngitis, headache, upper respiratory infections, gastritis, rash, joint pain, and fatigue. Rates of serious adverse events did not differ between groups. These studies established satralizumab as a potential valuable treatment option for patients with NMOSD.

3.3. Complement inhibitors

The complement system, an intricate network of more than 50 proteins, plays a vital role in innate immunity, helping to maintain tissue homeostasis and conduct immune surveillance (Figure 4). The system acts as the frontline defense against pathogens through direct cell lysis and enhancing antibody-mediated adaptive immunity. Activation of the complement cascade generates effector molecules that achieve three main goals: (1) opsonization of pathogens for phagocytosis, (2) production of anaphylatoxins that increase vascular permeability and attract immune cells, and (3) cell lysis through targeted membrane damage. These effects are tightly controlled by endogenous inhibitory enzymes to avoid damage to host tissues [75].

Three distinct complement system pathways exist based on their method of activation: the classical, lectin, and alternative pathways [76]. The classical pathway is activated when complement component 1q (C1q) binds the Fc region of immunoglobulins within antibody-antigen complexes on the surfaces of pathogens [77,78], while the lectin pathway is activated by specific carbohydrate patterns on microbial surfaces [79]. Both pathways lead to the downstream formation of C3-convertase, the first major enzyme in the cascade and a key regulatory target involved in all three pathways, which cleaves C3 into C3a and C3b in plasma. C3a acts as an anaphylatoxin to recruit and activate effector cells of the innate immune system [80]. Meanwhile, C3b is released to deposit on local cellular surfaces and acts as an opsonin to coat cell surfaces and mark them for phagocytosis [81]. Host cells feature regulatory proteins which prevent binding of C3b, whereas pathogens lack such regulatory proteins.

Membrane-bound C3b advances the complement cascade by interacting with additional C3-convertase to form C5-convertase on the cell surface, which cleaves C5 into C5a and C5b fragments. Similar to C3a, C5a functions as an anaphylatoxin [80], while C5b binds to C6, C7, C8, and C9 to assemble the pore-forming membrane attack complex (MAC) on the cell surface. MAC causes lysis by disrupting the target cell membrane, and can additionally elicit further localized inflammatory responses, amplifying the immune reaction and contributing to pathogen clearance [82].

In the alternative pathway, activation occurs continuously at low levels in plasma, necessitating constant inhibition by endogenous regulatory proteins on host cell surfaces to

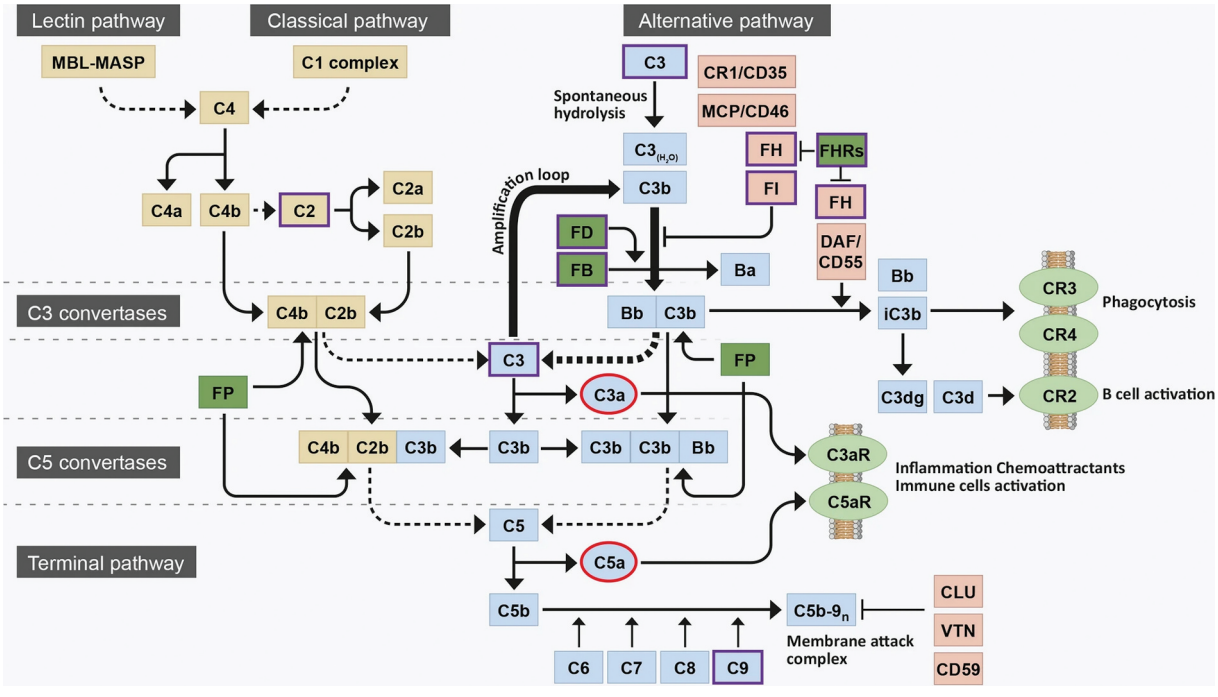

Figure 4. Overview of the complement system and its activation pathway. Reproduced from [74], © 2021 Armento et al., licensed under the CC by 4.0 license.

prevent local tissue damage [83]. The alternative pathway is initiated by spontaneous, slow hydrolysis of C3 into its active C3a and C3b fragments. Soluble C3b binds with soluble complement factor B (CFB) to form C3bB which is subsequently cleaved by CFD to produce the soluble alternative-pathway C3-convertase. This enzyme cleaves additional C3, creating a self-amplifying loop that generates more C3b fragments. On cell surfaces, C3b fragments similarly interact with CFB and CFD to form membrane-bound C3-convertase. This enzyme binds additional C3b to produce membrane-bound C5-convertase, paralleling the activation mechanisms observed in the classical and lectin pathways and propelling the cascade downstream to form MAC and other immune responses in a similar manner as described previously.

The amplification loop of the alternative pathway, if not properly regulated, would rapidly upregulate complement cascade activity [84,85]. To mitigate the risk of excessive immune response and potential local tissue damage, this amplification loop must be tightly regulated by the endogenous inhibitory enzymes CFH and CFI [86]. CFH is expressed exclusively on host cell surfaces and works together with CFI to degrade C3-convertase and inactivate C3b on host cell surfaces. This regulatory mechanism suppresses the amplification loop of the alternative pathway, effectively limiting complement activation and protecting host tissues from collateral damage.

Eculizumab (Soliris, Alexion Pharmaceuticals, Boston, MA, U.S.A.) is a complement inhibitor approved for use in adult patients with refractory MG who are positive for anti-AChR Ab and adult patients with AQP4-Ab-positive NMOSD. Eculizumab is a recombinant humanized monoclonal IgG2/4$_K$ antibody that binds to human C5 complement protein, inhibiting its cleavage to C5a and C5b and preventing the generation MAC, thereby protecting AChR from immune-mediated damage [87]. The FDA approved eculizumab for adult patients with refractory MG who are positive for anti-AChR antibodies in 2017 [88]. A Phase 2 study demonstrated that eculizumab produced clinically meaningful improvements in muscle strength relative to placebo in patients with anti-AChR-Ab positive refractory MG [89]. The Phase 3 REGAIN study evaluated the safety and efficacy of eculizumab in patients with refractory gMG. All patients were required to have previously failed treatment with at least two immunosuppressive agents or failed treatment with at least one immunosuppressive agent and required chronic plasma exchange or IVIG. The primary efficacy endpoint of change from baseline in MG-Activities of Daily Living Profile (MG-ADL) total score at week 26 and the first secondary endpoint of change from baseline in Quantitative MG (QMG) total score, a physician-administered assessment of MG clinical severity, at week 26 were assessed using a worst-rank score analysis. Eculizumab failed to reach statistical significance ($p = 0.07$) for the primary efficacy endpoint; however, fewer patients in the eculizumab arm had MG exacerbation and required rescue therapy [90]. Furthermore, a post hoc worst-rank ANCOVA of the primary endpoint showed a significant benefit with eculizumab over placebo ($p = 0.02$). In the extension phase of the REGAIN trial, improvements with eculizumab in activities of daily living, muscle strength, functional ability, and quality of life were maintained through three years [91]. Additionally, more eculizumab-treated patients achieved 'minimal symptom expression' versus placebo at week 26 ($p < 0.01$), which was sustained through three years [92]. IV eculizumab was generally well tolerated in patients with refractory gMG, with a tolerability profile generally similar to that seen previously in other indications [90]. These results supported eculizumab's FDA approval for this indication, which represented the first complement inhibitor to be approved for use in these patients [87]. It is administered intravenously weekly at weeks 0–5, followed by maintenance every two weeks.

In 2019, eculizumab was approved for use in AQP4-Ab-positive NMOSD [93]. Approximately three quarters (73%) of all patients with NMOSD have AQP4 auto-antibodies which activate the complement cascade, leading to demyelination and to the death of neurons, predominantly in the spinal cord and optic nerve [94–96]. The efficacy of eculizumab in the treatment of AQP4-IgG-seropositive NMOSD was established in the Phase 3 PREVENT study (Prevention of Relapses in Neuromyelitis Optica) [97]. Subjects enrolled in the trial were required to have a history of at least two relapses during the previous 12 months or three relapses during the previous 24 months (with at least one relapse in the previous 12 months) and an Expanded Disability Status Scale (EDSS) score of seven or less. Eculizumab significantly reduced the risk of the primary endpoint of adjudicated relapse relative to placebo, with 3% of patients in the eculizumab group experiencing relapses compared to 43% of patients in the placebo group ($p < 0.01$) [97]. Eculizumab has demonstrated safety in the short- and longer-term in patients with AQP4-IgG-seropositive NMOSD [98]. The most common treatment emergent adverse events (TEAE) were upper respiratory tract infection, headache, nasopharyngitis, nausea, diarrhea, urinary tract infection, limb pain, cough, and vomiting [97]. An increased risk of Neisseria meningiditis infections may accompany eculizumab use and vaccination is advised prior to use of the medication. Most TEAEs were mild to moderate in severity and considered to be unrelated to the study medication. Real-world evidence has demonstrated safety and effectiveness results consistent with those from the PREVENT trial in patients with AQP4-Ab-positive NMOSD [99].

Ravulizumab-cwvz (Ultomiris, Alexion Pharmaceuticals, Boston, MA, U.S.A.) is a long-acting C5 inhibitor mAb approved for use in anti-AChR Ab-positive gMG and AQP4-Ab-positive NMOSD. Like eculizumab, ravulizumab is administered intravenously but offers the advantage of less frequent dosing. Two weeks after an initial loading dose, maintenance doses are given every eight weeks, significantly reducing the frequency of infusions. This extended dosing interval not only improves patient convenience and adherence but also reduces the healthcare burden associated with frequent clinic visits. Ravulizumab's extended half-life allows for sustained complement inhibition, providing effective disease control with fewer treatments. Ravulizumab was approved by the FDA in 2022 for gMG following the results of the Phase 3 CHAMPION-MG trial [100]. In the randomized, double-blind, placebo-controlled study, ravulizumab demonstrated superiority over placebo in the primary endpoint of change from

baseline in the MG-ADL and clinician-reported QMG total scores at week 26 [100]. There were also no notable differences in adverse events observed between ravulizumab and placebo. Final results from the open-label extension phase of the study revealed maintenance of improvements in MG-ADL and QMG scores through week 164, supporting the sustained efficacy and long-term safety of ravulizumab in anti-AChR Ab-positive gMG [101].

More recently in 2024, ravulizumab was approved for use in AQP4-Ab-positive NMOSD following the results of the Phase 3 CHAMPION-NMOSD trial [102]. This study compared ravulizumab every eight weeks to an external comparator in the placebo group of the eculizumab Phase 3 PREVENT trial due to the potential long-term functional impact of NMOSD relapses and available effective treatment options. Ravulizumab met the primary endpoint, with no patients sustaining an adjudicated relapse compared to 20 patients with adjudicated relapses in the placebo group of the PREVENT trial [102]. Most TEAEs were mild to moderate, and although two patients taking ravulizumab experienced meningococcal infections, both recovered without sequelae. A long-term extension period of the CHAMPION-NMOSD study is ongoing similar to the CHAMPION-MG trial.

Zilucoplan (Zilbrysq, UCB, Brussels, Belgium) is a subcutaneous C5 inhibitor administered daily and approved for use in anti-AChR Ab-positive gMG. Zilucoplan is a small, synthetic peptide that binds to C5, inhibiting its cleavage into C5a and C5b, and thereby preventing the formation of MAC. The typical dosing regimen is once daily, which allows for steady-state inhibition of the complement system. The ability to self-administer zilucoplan at home enhances its convenience and may improve patient adherence compared to therapies requiring IV infusions. Zilucoplan's subcutaneous administration also offers flexibility in dosing schedules, making it a versatile option in the long-term management of MG. The Phase 3 RAISE trial was a multicenter, randomized, double-blind, placebo-controlled trial comparing the efficacy of zilucoplan to placebo in patients with anti-AChR Ab-positive gMG [103]. Zilucoplan achieved significant improvements in both MG-ADL and QMG total scores with a favorable adverse event profile, which were instrumental in its FDA approval [103]. Interim results from the RAISE-XT open-label extension study revealed a favorable long-term safety profile, good tolerability, and sustained efficacy of zilucoplan through week 60 with consistent benefits in a broad anti-AChR Ab-positive gMG population [104]. Due to the mechanism of action, these complement inhibitors increase the risk of meningococcal and encapsulated bacterial infection, and consequently, the prescribing information contains a black box warning advising meningococcal vaccination prior to use.

3.4. Anti-FcRn therapies

The neonatal Fc receptor (FcRn) plays a critical role in protecting immunoglobulin G (IgG) from degradation, thereby extending its half-life in circulation. FcRn is widely expressed in endothelial, epithelial, and hematopoietic cells and binds to IgG in acidic environments (e.g. intracellular endosomes), shielding IgG from subsequent lysosomal degradation [105]. The endosome is then shuttled back to the cell surface where, at physiological pH, IgG dissociates from FcRn and is recycled back into the bloodstream. In healthy individuals, this process maintains adequate levels of circulating IgG to ensure long-term immunity.

IgG autoantibodies constitute the underlying pathophysiology of several autoimmune disorders, including MG. Anti-FcRn therapies work by inhibiting the interaction between IgG and FcRn, leading to accelerated degradation of IgG antibodies, including pathogenic IgG autoantibodies. This approach has shown promise in reducing disease severity in patients with MG [105]. FcRn blockade with selective targeting of IgG has proven successful and resulted in the FDA approval of FcRn inhibitors for the treatment of adults with gMG who are anti-AChR Ab-positive (efgartigimod) and who are anti-AChR or anti-MuSK Ab-positive (rozanolixizumab). There are additional FcRn inhibitors in clinical development for gMG and other IgG autoantibody-mediated autoimmune disorders.

Rozanolixizumab-noli (Rystiggo, UCB, Brussels, Belgium) is a humanized mAb that targets FcRn and is approved for use in gMG patients who are anti-AChR or anti-MuSK Ab-positive. By binding to FcRn, rozanolixizumab prevents the receptor from recycling IgG, resulting in a reduction of circulating pathogenic IgG autoantibodies, including those against AChR and MuSK. Rozanolixizumab is administered as a subcutaneous injection in cycles once weekly for six weeks, with the duration of treatment tailored to the patient's clinical response and disease severity [106]. The subcutaneous route offers convenience and may be associated with fewer side effects compared to IV administration, enhancing patient adherence to therapy. The FDA approval of rozanolixizumab for the treatment of gMG in June 2023 followed the results of the Phase 3 MycarinG trial [107]. This randomized, double-blind, placebo-controlled trial compared once weekly subcutaneous rozanolixizumab 7 mg/kg and rozanolixizumab 10 mg/kg to placebo. The study found significantly greater reductions in MG-ADL and QMG total scores from baseline to day 43 in the rozanolixizumab groups relative to placebo. Both doses were also generally well tolerated, with the most frequent TEAE being headache, diarrhea, and pyrexia [107]. The open-label extension MG0004 trial including patients from the MycarinG study demonstrated maintenance of MG-ADL and QMG improvements with chronic, weekly rozanolixizumab [108]. Rozanolixizumab represents the only FDA-approved treatment in adults for both anti-AChR and anti-MuSK Ab-positive gMG, the two most common subtypes of gMG.

Efgartigimod alfa (Vyvgart, Argenx, Rotterdam, the Netherlands) was the first FDA-approved anti-FcRn therapy for the treatment of anti-AChR Ab-positive gMG. It prevents recycling of IgG, but unlike rozanolixizumab, efgartigimod is a Fc fragment of IgG_1 that binds to FcRn, instead of a full mAb. Efgartigimod is administered intravenously as an infusion over one hour, with a typical dosing regimen of once weekly for four weeks, followed by a treatment-free period. The cycle may be repeated based on the patient's response and clinical need. The IV administration allows for a higher bioavailability of the drug, which may contribute to its efficacy in rapidly reducing antibody levels. Efgartigimod was approved by the FDA in December 2021 following the results of the Phase 3

ADAPT trial [109]. This study was a randomized, double-blind, placebo-controlled study, in which efgartigimod was shown to provide rapid and sustained improvements in MG-ADL scores, with a favorable safety profile, leading to its approval [109]. A recent prospective, real-world study compared the clinical efficacy, safety, and biological effects of efgartigimod and ravulizumab in anti-AChR Ab-positive gMG [110]. Both biologics demonstrated similar moderate short-term improvement in MG-ADL scores, while only ravulizumab achieved a slight decrease in QMG scores from baseline levels. However, after adjusting for age and sex, both therapies performed similarly [110]. Both ravulizumab and efgartigimod were also well tolerated with no serious adverse events reported.

Efgartigimod alfa and hyaluronidase-qvfc (Vyvgart Hytrulo, Argenx, Rotterdam, the Netherlands), a combination anti-FcRn inhibitor and endoglycosidase combination, is a subcutaneous formulation that has been approved for the treatment of adult patients with anti-AChR Ab-positive gMG and chronic inflammatory demyelinating polyneuropathy. It is administered in cycles of once weekly injections for four weeks. The addition of recombinant human hyaluronidase PH20 (rHuPH20) facilitates the subcutaneous delivery of efgartigimod as a single injection by a healthcare professional over 30 to 90 seconds. The approval of efgartigimod and hyaluronidase was based on the results of the Phase 3 ADAPT-SC trial which revealed noninferiority in total IgG reduction between subcutaneous efgartigimod PH20 1000 mg and IV efgartigimod 10 mg/kg during the study period [111]. Subcutaneous efgartigimod PH20 also performed similarly to IV efgartigimod in improvements in MG-ADL and QMG total scores. Furthermore, continued treatment cycles of subcutaneous efgartigimod PH20 demonstrated long-term safety and consistent improvements in MG-ADL total score [111], offering patients potential improved treatment burden and flexibility.

3.5. B-cell depletion therapies

B cells play a critical role in the pathophysiology of inflammatory conditions such as MS and NMOSD due to their involvement in the immune response, including antigen presentation, cytokine production, antibody secretion, and activation of T cells [112]. mAbs targeting CD20, a B cell surface marker, have demonstrated efficacy in reducing disease activity by depleting B cells responsible for the production of pathogenic autoantibodies [113].

Rituximab (Rituxan, Genentech, South San Francisco, CA, U.S.A.) is a mAb targeting CD20 that became the first anti-CD20 therapy approved by the FDA in 1997 for the treatment of non-Hodgkin's lymphoma. Rituximab has been increasingly used in autoimmune diseases and has gained further approval for use in rheumatoid arthritis, granulomatosis with polyangiitis, microscopic polyangiitis, and pemphigus vulgaris. Although not FDA approved, rituximab has demonstrated efficacy and promise when used off-label for the management of NMOSD [114,115], MOGAD [116–118], and MS [119–121].

Ocrelizumab (Ocrevus, Genentech, South San Francisco, CA, U.S.A.) is another mAb that selectively targets CD20-positive B cells and is approved for use in relapsing MS and primary progressive MS (PPMS). It is administered intravenously at weeks 0 and 2 then every six months. Although the precise mechanism by which ocrelizumab exerts its therapeutic effects in MS is unknown, it leads to destruction of B cells, modulating immune response. The Phase 3 OPERA I and OPERA II studies evaluated the safety and efficacy of IV ocrelizumab 600 mg every six months compared with subcutaneous interferon beta-1a 44 mcg three times per week for relapsing MS. The primary endpoint of annualized relapse rate was 46% and 47% lower with ocrelizumab than with interferon beta-1a in trials I and II, respectively ($p < 0.01$ for both) [122]. Furthermore, ocrelizumab achieved a 40% relative risk reduction in confirmed disability progression (CDP), as measured by the EDSS, sustained for 12 weeks compared with interferon beta-1a in a pooled analysis of OPERA I and OPERA II. Meanwhile, the Phase 3 ORATORIO study evaluated the efficacy and safety of ocrelizumab 600 mg every six months compared with placebo for PPMS. The primary end point of CDP at 12 weeks in a time-to-event analysis showed a 24% relative risk reduction with ocrelizumab compared to placebo [123]. Throughout the three Phase 3 trials, common adverse events included infusion reactions and upper respiratory tract infections, which were mostly mild to moderate in severity [122,123]. The results of these studies are supported by numerous real-world studies which demonstrated similar reductions in relapse rate and disease progression rates, including in studies with more diverse patient populations not well represented in the pivotal trials [124]. A subcutaneous combination of ocrelizumab with hyaluronidase-ocsq is also approved for use in relapsing MS and PPMS, dosed every six months (Ocrevus Zunovo, Genentech, South San Francisco, CA, U.S.A.). Pretreatment with oral corticosteroid and antihistamine at least 30 minutes prior to each injection is indicated.

Ofatumumab (Kesimpta, Novartis Pharmaceuticals, Basel, Switzerland) is a fully human anti-CD20 mAb that can be self-administered by patients and is approved in several countries worldwide for the treatment of relapsing forms of MS. It is administered at weeks 0, 1, and 2, then monthly starting at week 4. Subcutaneous ofatumumab became the first B-cell-targeting therapy intended for self-administration at home. Three Phase 2 trials, including APLIOS, APOLITOS, and MIRROR, revealed subcutaneous ofatumumab achieved significant reductions in new brain lesion activity (from baseline or versus placebo), as measured by MRI, in patients with relapsing forms of MS [125–127]. In two identical Phase 3 ASCLEPIOS I and II trials in adults with relapsing forms of MS, subcutaneous ofatumumab 20 mg once monthly was compared to 14 mg once daily oral teriflunomide (selectively and reversibly inhibits dihydro-orotate dehydrogenase, a key enzyme in the de novo pyrimidine synthesis pathway, leading to a reduction in proliferation of activated T and B lymphocytes). Ofatumumab was more effective in reducing the annualized relapse rate, as well as reducing MRI-detected lesion activity and limiting worsening of disability for up to 30 months [128]. Ofatumumab was well tolerated in patients with relapsing forms of MS with a similar rate of adverse events to oral teriflunomide [128]. The most common adverse events reported were injection-related reactions, nasopharyngitis, headache, injection-site reactions, upper respiratory tract

infections and urinary tract infections [129]. The Phase 3 ALITHIOS trial was a long-term safety study including patients from the ASCLEPIOS I and II, APLIOS, or APOLITOS trials. This study found ofatumumab was well tolerated, with no new safety risks identified through 3.5 years of exposure [130].

Ublituximab-xiiy (Briumvi, TG Therapeutics, Morrisville, NC, U.S.A.) is a CD20-directed mAb that is administered intravenously at weeks 0 and 2, and then every six months. Ublituximab targets a unique epitope on CD20-expressing B cells and is uniquely designed to lack certain sugar molecules normally expressed on the antibody to allow for efficient B-cell depletion at low doses. Ublituximab became FDA approved for use in relapsing forms of MS following the results of the Phase 3 ULTIMATE I & II trials [131]. These studies revealed ublituximab reached the primary endpoint of superiority over oral teriflunomide in significantly reducing the annualized relapse rate, in addition to the number of T1 Gd-enhancing lesions and the number of new or enlarging T2 lesions. Infusion-related reactions occurred in 47.7% of the participants in the ublituximab group, while serious infections occurred in 5.0% in the ublituximab group compared to 2.9% in the teriflunomide group [131]. Following its approval, ublituximab became the first and only anti-CD20 mAb approved for patients with relapsing MS that can be administered in a one-hour infusion following the starting dose.

Inebilizumab-cdon (Uplizna, Amgen, Thousand Oaks, CA, U.S.A.) is a recombinant humanized kappa IgG_1 mAb that targets and depletes CD19-expressing B cells. In 2020, the FDA approved inebilizumab for the treatment of adult patients with AQP4-Ab-positive NMOSD as a twice-a-year maintenance regimen following initial doses [132]. This approval followed the results from the pivotal N-MOmentum trial, the largest study ever conducted in a real-world spectrum of adults with NMOSD [133]. This Phase 2/3 study compared 300 mg IV inebilizumab to placebo with the primary endpoint of time to onset of an NMOSD attack. The randomized controlled period was ultimately stopped before complete enrollment due to clear demonstration of efficacy. 12% of participants receiving inebilizumab had an attack compared to 39% of participants receiving placebo (hazard ratio 0.272, 95% CI 0.150–0.496, $p < 0.01$). Furthermore, inebilizumab demonstrated statistically significant benefits in key secondary endpoints, including reductions in NMOSD-related hospitalizations [133]. Inebilizumab also demonstrated a favorable safety and tolerability profile. A post hoc analysis performed in participants receiving inebilizumab for over four years in the randomized controlled period and open-label extension of the N-MOmentum study found the efficacy of inebilizumab may be enhanced after the first year of treatment [134].

3.6. T-cell modulation agents

T-cell modulating agents target the T cells involved in inflammatory and autoimmune processes and have shown potential in the treatment of numerous autoimmune neuro-ophthalmic diseases.

Natalizumab (Tysabri, Biogen, Cambridge, MA, U.S.A.) is a recombinant humanized anti–α4-integrin antibody for the treatment of MS (Figure 5). Integrin inhibition inhibits T-cell migration into the CNS to prevent optic nerve inflammation through the limitation of T cell and vascular endothelium interaction. Natalizumab has demonstrated an ability to reduce disability progression and clinical relapse rates in MS [136–138]. The AFFIRM (natalizumab monotherapy) and SENTINEL (natalizumab and interferon beta-1a) trials demonstrated a significant reduction in the risk of clinically significant visual loss, defined as two-line worsening of low-contrast visual acuity over 12 weeks, by 35% ($p < 0.01$) and 28% ($p = 0.04$), respectively [139]. Furthermore, the cumulative probabilities of sustained visual improvement were greater in the natalizumab group by 57% for 2.5% contrast ($p = 0.01$) and 39% for 1.25% contrast ($p = 0.01$) [140]. These benefits of natalizumab therapy, however, must be weighed against the potential adverse events. The risk of an uncommon but serious adverse event, progressive multifocal leukoencephalopathy, led to the drug's withdrawal from the market in 2006, although it was subsequently reintroduced later in 2006 after considering its clinical benefits over such risks [141,142]. The US prescribing information contains a black box warning regarding PML. The most common adverse events include infusion-related symptoms, infections, arthralgias, gastroenteritis, depression, and rash [52,137].

Fingolimod (FTY720, Gilenya, Novartis Pharmaceuticals, Basel, Switzerland) is a sphingosine 1-phosphate receptor modulator approved for the treatment of relapsing forms of MS. T-cell-mediated demyelination of the CNS is involved in the pathophysiology of MS. Fingolimod sequesters T cells in lymph nodes by preventing their egress, leading to a reduced infiltration of lymphocytes into the CNS. Preclinical studies also revealed fingolimod may have neuroprotective capabilities due to modulatory effects on neural sphingosine 1-phosphate receptors [143]. The Phase 3 TRANSFORMS (Trial Assessing Injectable Interferon vs. FTY720 Oral in RRMS) trial revealed that fingolimod decreased the relapse rate and disease activity on MRI, as compared to once-weekly, intramuscular injection of interferon beta-1a 30 µg [144]. Furthermore, the Phase 3 FREEDOMS (FTY720 Research Evaluating Effects of Daily Oral therapy in Multiple Sclerosis) trial revealed that oral fingolimod improved the relapse rate, the risk of disability progression, and end points on MRI compared to placebo [145]. These encouraging results, however, need to be weighed against the possible long-term adverse events, including bradycardia and atrioventricular conduction, elevated liver-enzyme levels, and mild hypertension. Macular edema is a recognized ocular adverse event with fingolimod that may cause blurred vision and can be differentiated from MS-ON by clinical exam, optical coherence tomography, and absence of pain.

T-cell modulation agents represent a promising therapeutic strategy in the management of neuro-ophthalmic disorders with an autoimmune or neuroinflammatory etiology. By targeting specific T-cell pathways, these agents help reduce inflammation, prevent tissue damage, and improve long-term visual outcomes. However, their use is often part of a broader immunomodulatory approach, and ongoing

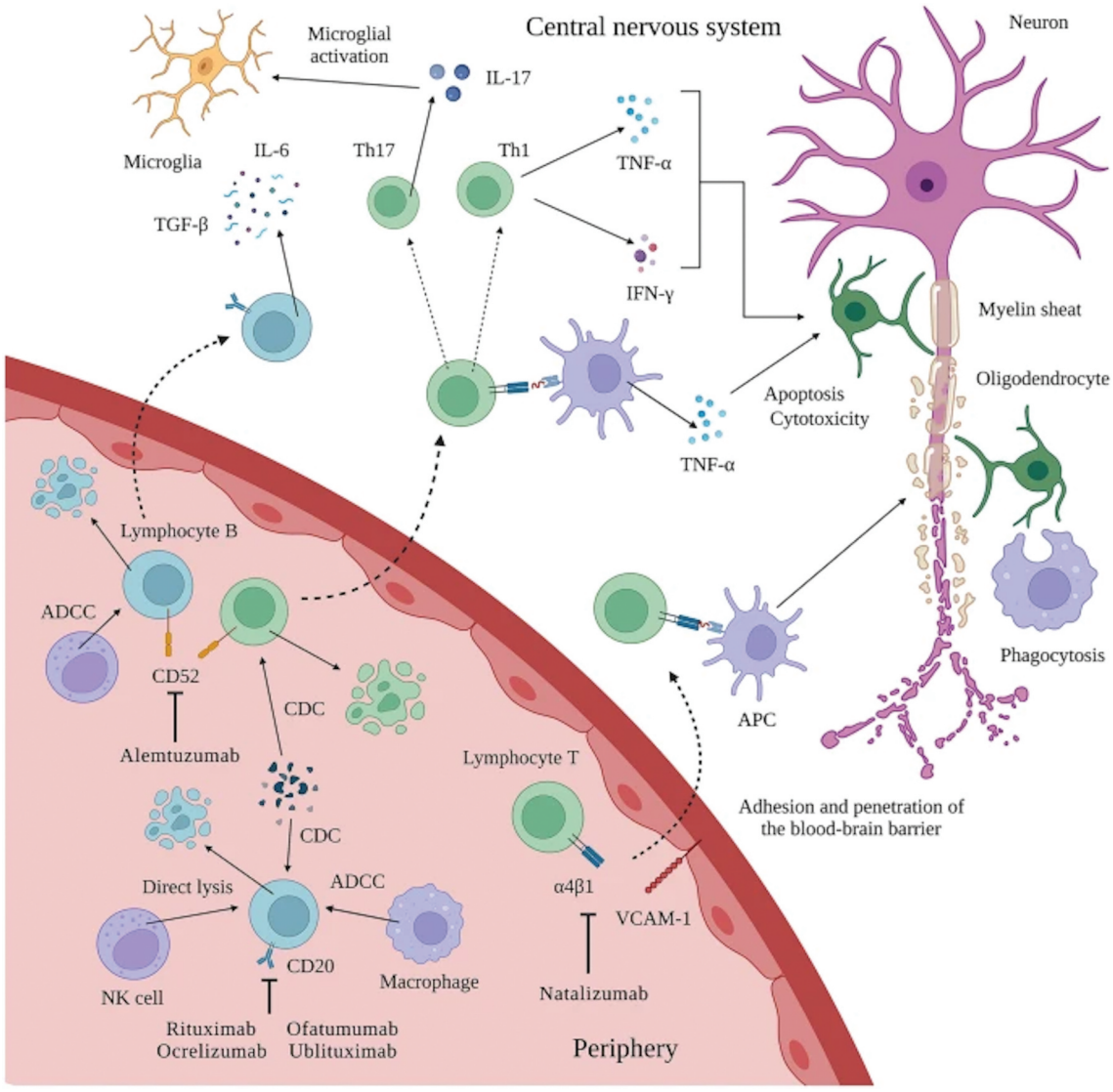

Figure 5. Mechanism of action of mAbs in the treatment of MS, including rituximab, ocrelizumab, ofatumumab, ublituximab, alemtuzumab, and natalizumab. Reproduced from [135], © 2022 Krajnc et al., licensed under the CC by 4.0 license.

research is essential to determine optimal treatment protocols and safety profiles for these agents.

4. Safety and adverse effects

The advent of biologic therapies has dramatically improved the treatment options for patients with autoimmune neuro-ophthalmic disorders by offering targeted effective treatment approaches while avoiding many of the adverse effects associated with nontargeted therapies such as corticosteroids. While biologics can trigger immune responses and infusion-related reactions, these effects are generally manageable and predictable. Anti-drug antibodies may develop, potentially affecting therapeutic efficacy, but infusion reactions can be effectively managed through premedication protocols and optimized infusion rates. This safety profile contrasts favorably with traditional corticosteroid therapy, which is associated with numerous systemic complications including hypertension, hyperglycemia, pancreatitis, and various hematologic, immunologic, and neuropsychologic effects, as well as more serious sequelae such as osteoporosis, aseptic joint necrosis, and adrenal insufficiency.

Long-term safety data from extensive clinical experience and pharmacovigilance programs have reinforced the favorable risk-benefit profile of biologic therapies. While these agents require monitoring for opportunistic infections, particularly with complement inhibitors like eculizumab, the risk can be effectively managed through preventive measures such as vaccination protocols. Newer agents will require ongoing monitoring to fully characterize their long-term safety profiles.

5. Challenges and future directions

The advancement of biologic therapies for autoimmune neuro-ophthalmic disorders faces several significant challenges, particularly in patient stratification and treatment personalization. Current approaches often lack precise biomarkers

to predict treatment response, leading to potential inefficiencies in therapy selection. The heterogeneity of autoimmune neuro-ophthalmic disorders, combined with variable patient responses to biologics, underscores the need for more sophisticated predictive models incorporating genetic, immunological, and clinical parameters.

Novel biologic targets and innovative delivery methods represent promising avenues for advancement in the field. Emerging research focuses on identifying new molecular pathways and developing more selective therapeutic approaches, including dual-targeting antibodies and tissue-specific delivery systems. Integration of biologics into existing treatment algorithms requires careful consideration of timing, sequence, and combination strategies with conventional therapies. Healthcare policy and accessibility remain significant challenges, with issues of cost, insurance coverage, and healthcare infrastructure affecting treatment availability. The development of biosimilars may help address some accessibility concerns, though regulatory frameworks and clinical validation processes need careful consideration. Furthermore, cost reduction strategies in the use of biologic therapies may include therapeutic drug monitoring, ensuring patients receive the minimum effective dose, and establishing evidence-based dose reduction and tapering protocols for patients in remission or with stable disease. These challenges highlight the importance of continued research, collaboration between healthcare stakeholders, and policy development to ensure equitable access to these transformative therapies.

6. Conclusion

Biologics represent an evolving and promising class of treatments for a variety of autoimmune neuro-ophthalmic disorders. Clinical trials and observational studies have demonstrated their potential to target specific immune pathways, reduce inflammation, and protect or restore vision in patients with conditions like ON, NMOSD, MG, GCA, and TED. These therapies have not only improved clinical outcomes but have also enhanced our understanding of disease pathophysiology . The ability to selectively target specific molecular pathways has led to more precise interventions with improved safety profiles compared to traditional broad-spectrum immunosuppression. Ongoing research into novel therapeutic targets, improved delivery systems, and biomarker development suggests potential for even more effective and personalized treatment approaches. The evolution of biosimilars and continued refinement of existing therapies may help address current challenges of cost and accessibility.

7. Expert opinion

The impact of biologic therapies in autoimmune neuro-ophthalmic disorders represents one of the most significant therapeutic advances in recent decades, fundamentally changing treatment paradigms and patient outcomes. The ability to specifically target disease pathways has not only improved treatment efficacy but has also enhanced our understanding of disease mechanisms, creating a virtuous cycle of discovery and therapeutic advancement.

The translation of current advances into clinical practice still faces several practical challenges. While the efficacy of biologics is well-documented, their high cost and complex administration requirements often create barriers to widespread adoption. Healthcare systems must develop infrastructure for proper patient selection, monitoring, and management of potential complications. The development of validated biomarkers for patient stratification and treatment response prediction remains a critical unmet need. Current limitations in predicting individual patient responses lead to potentially inefficient use of these expensive therapies. Additionally, the long-term implications of biological immune modulation require continued surveillance and data collection.

Technical improvements in several key areas could significantly advance the field. Novel delivery systems, particularly those allowing for self-administration and/or extended dosing intervals, could improve treatment adherence and reduce healthcare resource utilization. Improved understanding of the relationship between specific molecular targets and clinical outcomes could lead to more personalized treatment approaches.

Research in autoimmune neuro-ophthalmic disorders shows tremendous promise for continued advancement. Rather than approaching a definitive endpoint, the field appears to be entering an era of accelerated discovery. The identification of new therapeutic targets, development of multi-specific antibodies, and potential for combination therapies suggest numerous avenues for future investigation. The integration of artificial intelligence and machine learning tools for patient stratification and outcome prediction represents another promising frontier.

Looking ahead five years, several key developments are likely to reshape standard clinical practice. Formulations and delivery systems will continue to improve with further progress in the development of subcutaneous auto-injectors, that facilitate home-based self-administration, reducing the treatment burden associated with frequent trips to the IV infusion center. We anticipate the approval of several new biologics currently in late-stage development, particularly for conditions like MG and NMOSD. The availability of biosimilars for current biologics may improve treatment accessibility, though careful validation of therapeutic equivalence will be essential. Treatment algorithms will likely become more sophisticated, incorporating biomarker-driven decision-making and potentially utilizing combination approaches with both biological and conventional therapies.

The field will likely evolve toward more personalized treatment approaches, supported by improved understanding of disease mechanisms and better predictive tools. We may see the emergence of 'precision neuro-ophthalmology,' where treatment selection is guided by individual patient characteristics including genetic, immunological, and clinical parameters. This evolution will require significant investment in research infrastructure and data collection systems but could substantially improve treatment outcomes while optimizing resource utilization. The greatest challenge will be managing the growing complexity of treatment options while ensuring equitable access to these transformative therapies. Success will require continued collaboration between clinicians, researchers, industry partners, and healthcare systems to optimize the development and implementation of these important therapeutic advances.

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Author contributions

All authors have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. All authors drafted and/or reviewed the work critically for important intellectual content. All author gave final approval of the version to be published; All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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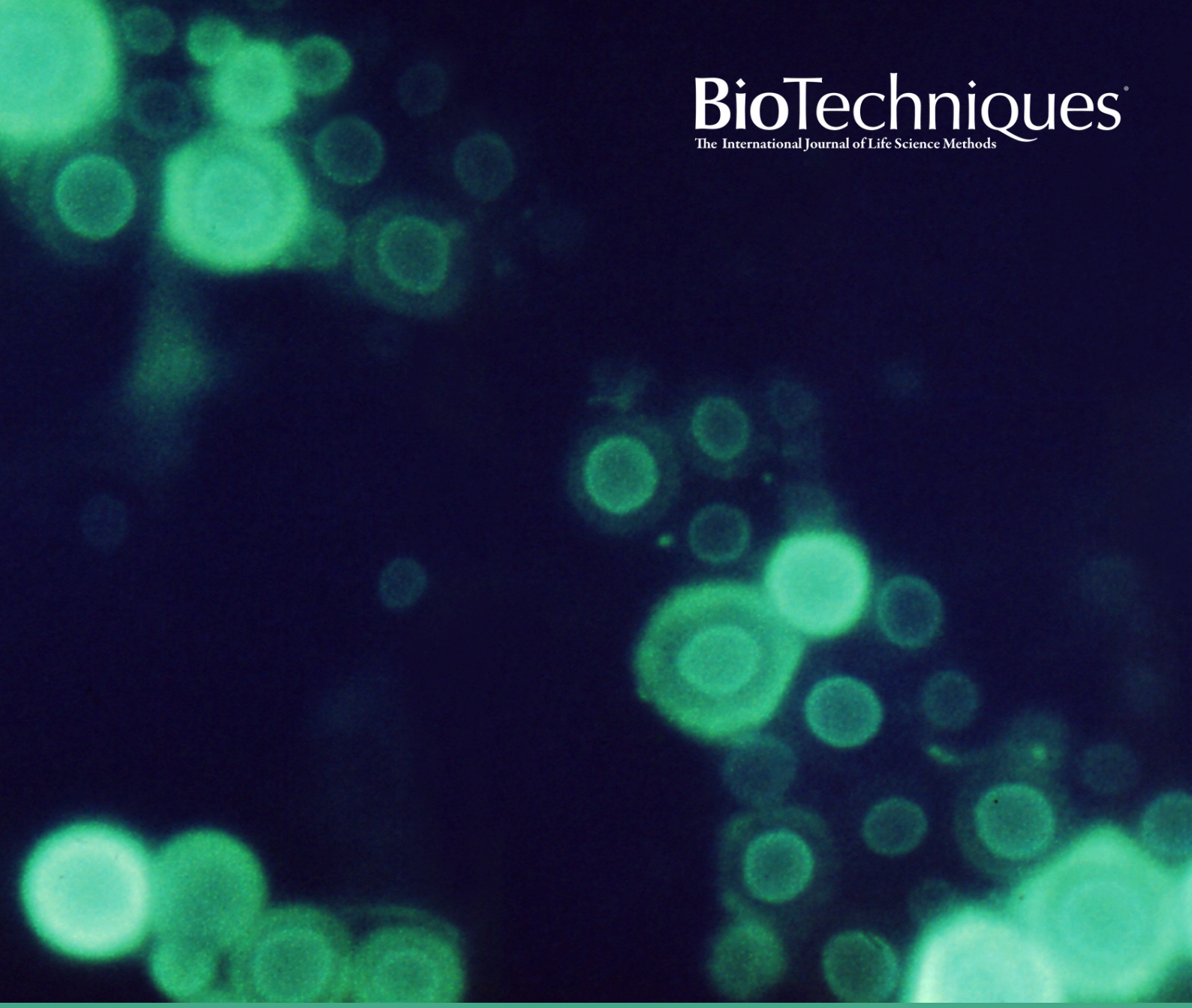

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