Review

New treatment strategies in multiple sclerosis

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Abstract

Multiple sclerosis is the most common, non-traumatic, disabling neurological disease of young adults, affecting an estimated two million people worldwide. At onset multiple sclerosis can be categorised clinically into relapsing remitting MS (RRMS — 85–90% of patients) or primary progressive MS (PPMS). Relapses typically present sub-acutely over hours to days with neurological symptoms persisting for days to weeks before they gradually dissipate. At first full recovery is the norm, later patients accumulate deficits and ultimately most convert to a secondary progressive phase (SPMS), characterised by deficits that increase in the absence of further relapses. The clinical picture reflects the complex interplay of focal inflammation, demyelination and axonal degeneration occurring within the central nervous system. Since the introduction of a genuine disease-modifying drug, interferon-beta1b in 1993, there has been a growing interest from academia and pharmaceutical companies alike in multiple sclerosis therapy. In part this effort has focused on investigating the “window of therapeutic opportunity” within the natural history of the disease: it is becoming increasingly clear that immunotherapies are not useful in the secondary phase of the disease but may offer long-term benefit if given early in the relapsing–remitting phase. In part, attention is being paid to the details of dosing and administration of the various licensed therapies, but there is also a significant research effort to explore new ways to treat the disease. In this review, we first sketch the landscape of novel therapies in multiple sclerosis and then discuss in detail approaches which are likely to emerge over the next few years.

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Contents

The landscape of novel therapies in multiple sclerosis ........................................................................................................... 0
New and emerging therapies and the rationale behind their use ........................................................................................................... 0
Lymphocyte migration ........................................................................................................................................................ 0
Natalizumab ........................................................................................................................................................................ 0
Fingolimod (FTY720) ........................................................................................................................................................ 0
Targeting T cells ....................................................................................................................................................................... 0
Daclizumab ........................................................................................................................................................................ 0
Targeting B cells ....................................................................................................................................................................... 0
Rituximab ........................................................................................................................................................................ 0
Mixed targets ....................................................................................................................................................................... 0
Alemtuzumab ....................................................................................................................................................................... 0
Cladribine ........................................................................................................................................................................ 0
In conclusion.......................................................................................................................................................................... 0
References .......................................................................................................................................................................... 0

The landscape of novel therapies in multiple sclerosis

Broadly speaking, novel therapies of multiple sclerosis aim to either disable a component of the immune system or to prevent neurodegeneration, as seen in progressive forms of the disease.

Disabling the immune system can be achieved in a number of ways, including: i) targeting whole populations of immune cells believed to be involved in disease pathogenesis (e.g. daclizumab neutralising the CD25 molecule on all T cells), ii) blocking the migration of peripheral lymphocytes in to the CNS (natalizumab and fingolimod) or iii) “resetting the immune system” by wiping out the existing immune repertoire, including pathogenic myelin reactive clones, then allowing a pool of new and healthy immune cells to...
regenerate, either from residual, non-depleted haematopoietic precursor cells (rituximab for B cells, alemtuzumab for all lymphocytes) or from autologous haematopoietic stem cells transplanted back into the patient following chemotherapy (autologous stem cells transplantation). Whilst a number of these approaches have been shown to be efficacious, a fundamental problem is that they deplete or functionally inhibit “normal immune cells” as well as pathogenic cells, potentially compromising immune protection. Ideally MS therapy would be more directed, selectively targeting disease-specific autoreactive cells, whilst leaving the rest of the immune system competent to respond to infections. To date targeted approaches have included peptide-specific therapies, in which MBP peptides, solubilised MHC–peptide complexes, or altered peptide ligands (APL) of MBP peptides are administered orally, intravenously or transnasally with the intent of re-establishing peripheral tolerance by engaging the T cell receptor complex without co-stimulatory signals, so inducing a) anergy, b) clonal deletion of myelin reactive cells or c) bystander suppression by the induction of regulatory T cells or tolerant antigen presenting cells. A fundamental problem with this approach is that the primary target antigen in multiple sclerosis remains unknown. Several targets are likely to be involved and epitope spreading, that is the emergence of immune reactivity against an expanding array of myelin targets, is likely to occur during the course of the disease. Injecting plasmids, that encode multiple peptides or the entire myelin basic protein, may be a way of circumventing this problem. Other directed therapies include vaccination with attenuated autologous peripheral or CSF myelin specific T cells, or with synthetic TCR peptides similar to those expressed by encephalitogenic T cells, with the aim of specifically depleting myelin reactive cells.

Despite success in animal studies these more directed, and in many ways immunologically more sophisticated approaches have proved disappointing when used in humans. Perhaps a fundamental problem with trying to employ such a targeted approach is that we know surprisingly little about the immunology of MS, for example only recently has it become evident that there are extranodal lymphoid follicles with the meninges of patients with multiple sclerosis (Serafini et al., 2004). Surprisingly, a few years ago, it became apparent that neutralising the cytokine TNFα increased multiple sclerosis disease activity (etanercept and infliximab) (van Oosten et al., 1996). More recently, and again unexpectedly, it has become clear that blocking the B cell cytokines BAFF and APRIL, with the TACI receptor fusion protein atacicept, leads to similar problems (ClinicalTrials.gov website, identifier NCT006424902, accessed 20 October 2009).

If little is known about the immunology of MS, then even less is known about mechanisms driving neurodegeneration. Ken Smith, Steve Waxman and others have proposed a model of neurodegeneration by “energy failure”, as the metabolic load on the demyelinated axon becomes unbearable. In theory, and in the animal laboratory, this is ameliorated by sodium channel blockade, a strategy which so far has been disappointing in humans: a trial of lamotrigine in progressive multiple sclerosis has proved negative (presented at MS Frontiers, London 2009). A number of other agents appear to have neuroprotective effects in the laboratory. Broadly these agents either inhibit toxic pathways (in particular glutamate), and/or activate trophic pathways. Examples include the glutamate antagonistsriluzole and memantadine and low dose cannabinoids, which are believed to exert protective effects by reducing glutamate excitotoxicity, inhibiting cell death pathways and reducing the influx of calcium ions (Croxford et al., 2008). It remains to be seen if any of these therapies will deliver tangible results in the clinical setting. CUPID (cannabinoid use in progressive inflammatory brain disease) a trial comparing oral dronabinol (an FDA-approved cannabinoids with an indication for appetite stimulation) with placebo in progressive forms of the disease is underway. Statins, also known as 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-COA) reductase inhibitors, have also been shown to have some neuroprotective effects in the laboratory (Miron et al., 2007; Painthia et al., 2005, 2006). Statins may also have immunomodulatory functions (Kuipers and van den Elsen, 2007), although results from early trials in RRMS have produced variable results (Birnbaum et al., 2008; Markovic-Plese et al., 2008; Paul et al., 2008; Vollmer et al., 2004).

A major question, yet to be resolved, is the relationship between neurodegeneration and inflammation. It is now clear that strategies aimed at disabling the immune system fail to prevent or reverse disability once patients have entered the progressive phase of the disease. This has led some to suggest that the inflammatory and degenerative phases of the disease are entirely independent. We and others disagree. Our position is that neurodegeneration occurs predominantly through non-inflammatory mechanisms, but that these mechanisms are set up by, and depend upon, prior inflammation. If this is so, neurodegeneration may be best prevented by hitting the inflammatory phase of the disease hard by effective immunotherapies.

New and emerging therapies and the rationale behind their use

Lymphocyte migration

Dysregulation of the blood–brain barrier and migration of encephalitogenic inflammatory cells into the central nervous system are key events in the immunopathogenesis of multiple sclerosis and, as such, are strategic targets for MS therapies.

Natalizumab

The migration of inflammatory cells across the blood brain barrier depends on the interaction of adhesion molecules such as VLA-4 (very late antigen–4) expressed on activated lymphocytes and monocytes, with its ligand VCAM-1 (vascular cell adhesion molecule-1) expressed on cerebrovascular endothelial cells (Elcises et al., 1999). Natalizumab (Tysabri, Biogen Idec) is a humanised IgG4 monoclonal antibody that binds to VLA-4. Like other human IgG4 antibodies, natalizumab does not activate complement and thus does not lyse its target cell. Rather, natalizumab is thought to exert its effect by physically preventing the interaction of VLA-4 with VCAM-1, so impairing the trafficking of inflammatory cells into the CNS. In keeping with this notion, natalizumab has been shown to reduce the number of lymphocytes, particularly CD4+ T cells, within the cerebrospinal fluid; an effect that persists for at least 6 months following cessation of treatment (Stuve et al., 2006a,b).

Natalizumab, given as a monthly intravenous infusion, has been evaluated as a treatment of relapsing remitting multiple sclerosis in two phase III clinical trials [the AFFIRM monotherapy trial, and the SENTINEL add-on trial with interferon beta-1a (Avonex)]. AFFIRM demonstrated that, compared to placebo, natalizumab reduced the risk of relapse by 68%, and the risk of sustained accumulation of disability by 42% at 2 years (Polman et al., 2006). On the SENTINEL trial, the addition of natalizumab to interferon beta-1a (Avonex), led to a 56% reduction in relapse rate, and to a 24% reduction in the risk of sustained accumulation of disability when compared to Avonex monotherapy, again over two years (Rudick et al., 2006). Both trials demonstrated comparable reductions in MRI lesion activity (Polman et al., 2006; Rudick et al., 2006). Based on these encouraging findings, natalizumab was approved for the treatment of RRMS in November 2004. Surprisingly, only three months later, Biogen Idec announced its voluntary withdrawal after two patients with RRMS and one patient with Crohn’s disease developed progressive multifocal leukoencephalopathy (PML) following treatment. Two of the cases were fatal; all occurred in the context of concomitant immunotherapies. Following a review of safety and efficacy by the FDA and EMEA, natalizumab was re-introduced into the market in 2006 as a monotherapy for patients with particularly aggressive or treatment-resistant RRMS. Since this time, up until August 2009, a further 13 cases of PML have occurred in approximately 43,000
patients receiving natalizumab. There is some suggestion that rapid removal of natalizumab by plasma exchange can reduce the mortality associated with PML, although useful neurological recovery remains very rare (source: Hans Peter Hartung, ECTRIMS). Additional adverse effects have included allergic reactions (type I, but also type III) in up to 4% of patients, in many cases linked to the formation of neutralising antibodies (Krumholz et al., 2007; Leussink et al., 2008), and deranged liver function tests with clinically significant liver damage in a small number of cases (US Food and Drug Administration, 2009).

The cause of PML post-natalizumab is debated; for example, it has been suggested that natalizumab leads to PML by mobilising JCV-infected cells, particularly B cell precursors, from bone marrow stores (Bonig et al., 2008; Krumholz et al., 2008; Ransohoff, 2005) Alternatively, or additionally, PML may occur due to reduced CNS immunosurveillance by lymphocytes. If reduced immunosurveillance alone is sufficient to cause PML, the strategy of targeting lymphocyte trafficking must be called into question.

**Fingolimod (FTY720)**

Fingolimod is an oral immunosuppressant under evaluation as a treatment of MS. It is thought to exert its effect by sequestering lymphocytes in secondary lymphoid tissue, so preventing the migration of lymphocytes into the CNS. Fingolimod achieves its effect by acting as a super-agonist to the sphingosine-1-phosphate receptor, which is predominantly expressed on lymphocytes and regulates their migration from lymphoid tissue. Excessive stimulation of the receptor leads to its internalisation, so depriving these cells of crucial migration signals. Interestingly, experimental evidence suggests that fingolimod differentially affects the sequestration of T regulatory cells and enhances their suppressive function (Daniel et al., 2007; Sawicka et al., 2005).

In a proof-of-concept trial, daily oral fingolimod was shown to reduce the number of new gadolinium-enhanced lesions on MRI and the annualised relapse rate, compared with placebo (from 0.77 to: 0.35 on 1.25 mg fingolimod, and to 0.36 on 5 mg fingolimod) (Kappos et al., 2006). This benefit was sustained during the 24 month extension study (O'Connor et al., 2009). Based on these promising efficacy results Novartis initiated a phase III study programme to evaluate fingolimod vs. placebo (FREEDOMS study) or interferon beta-1a (TRANSFORMS study) in patients with RRMS. In addition, the role of this drug in PPMS will also be assessed in a Phase III trial comparing high dose fingolimod with placebo (INFORMS study).

In May 2009 data from TRANSFORMS were presented at the American Academy of Neurology (AAN), showing a 52% reduction in relapse rate on high dose fingolimod and a 38% reduction on low dose fingolimod. However, whilst its efficacy appears promising, there are increasing concerns about its safety profile. Although usually well tolerated (nasopharyngitis, headache and flu-like symptoms are the most common side effects), more serious adverse events have included: 3 cases of herpes viral infections (2 of which were fatal - TRANSFORMS, also see Leyboldt et al., 2009) 1 case of posterior leukencephalopathy (Kappos et al., 2006), and an increase risk of local skin malignancy (TRANSFORMS) (Kappos et al., 2006; O'Connor et al., 2009).

An effective, orally available, well tolerated drug would be a landmark in MS therapy, however, these side effects call into question the validity of interfering with lymphocyte homing and migration. Further analyses from all three phase III studies will be important in clarifying the long-term safety of this drug.

**Targeting T cells**

T cells are believed to be pivotal in the pathogenesis of multiple sclerosis, and as such are obvious therapeutic targets.

**Daclizumab**

IL-2 is a critical growth factor for the expansion and maturation of activated T cells. Daclizumab is a humanised IgG1 monoclonal that binds to the IL-2 receptor-α chain (CD25), expressed on activated but not resting T cells, blocking IL-2 binding without triggering cytolysis. Daclizumab is licensed for the treatment of renal-transplant rejection, and has been used “off label” with some success as a treatment of haematological malignancies and in a number of T cell mediated autoimmune diseases (Waldmann, 2007).

With the intent of specifically targeting activated, autoreactive T cells, daclizumab has been trialled as a treatment in multiple sclerosis. In a phase II, open-label, baseline-versus-treatment study of 10 MS patients with an incomplete therapeutic response to IFN-β, the addition of daclizumab led to a 78% reduction in new gadolinium-enhancing lesions (Bielekova et al., 2004). Preliminary results from CHOICE, a phase II randomised, double-blind, placebo-controlled, add-on trial of 230 patients with active relapsing remitting multiple sclerosis are also encouraging; showing a 72% reduction in new contrast-enhancing lesions at 24 weeks for those receiving high dose daclizumab in addition to concurrent IFN-β therapy (Montalban X, 2007).

To date, daclizumab appears to be well tolerated. Side effects seen in the CHOICE study include: a higher rate of urinary tract infections (17% vs. 13%), transient photosensitivity-type rashes, mouth ulcers, transient headaches, and transient elevations of bilirubin, liver transaminases and autoantibodies. Two cases of generalised lymphadenopathy have been reported. In one case lymph node biopsy revealed non-specific reactive changes. Both cases resolved after stopping the drug.

In contrast to predictions from in vitro studies (Goebel et al., 2000; Tkaczuk et al., 2001) daclizumab appears to have little effect on T cell function in vivo: peripheral CD4+ and CD8+ T cell counts are only slightly reduced following treatment, CD4+ T cell proliferation is reduced by just 20%, CD8+ T cell proliferation is unaffected, and T cell mediated cytokine production is unchanged (Bielekova et al., 2006). However, daclizumab expands a subset of NK cells expressing high levels of the NK cell marker CD56 (CD56bright NK cells). The size of this population correlates with reduced MRI disease activity, supporting a role for these cells in the therapeutic effect of daclizumab (Bielekova et al., 2006). Besides their well known antiviral and anti-tumour properties, CD56bright NK cells have been shown to have immunoregulatory properties, including inhibiting the survival of activated T cells in a contact-dependent manner (Bielekova et al., 2006; Smeltz et al., 1999).

Long-term efficacy and safety data does not yet exist for daclizumab treatment of multiple sclerosis and further trials are needed. However, given the expansion of NK cells seen after daclizumab, it seems unlikely that this treatment will be complicated by the reactivation of latent viruses such as herpes viruses and the JC virus.

**Targeting B cells**

MS is characterised by the intrathecal production of oligoclonal IgG. In addition, clonally expanded activated B cells and plasma cells accumulate in MS lesions (Baranzini et al., 2000; Owens et al., 1998) and in the spinal fluid of patients with MS (Colombo et al., 2000; Qin et al., 1998; Monson et al., 2005), suggesting local antigen-driven B cell activation rather than a bystander response. B cells may contribute to MS pathology in a number of ways; either directly by the production of anti-myelin autoantibodies (Berger et al., 2003; Genain et al., 1996), or indirectly by regulating T cell responses via antigen presentation, cytokine release and via the induction of regulatory T cells (Sifakis et al., 2007). Depleting B cells is, therefore, a reasonable therapeutic strategy in MS.

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Rituximab

Rituximab is a chimeric murine/human IgG1k mAb directed against CD20, a protein present on the surface of pre-B cells and mature B cells (but not plasma cells, or bone marrow progenitor cells), triggering cytolysis. In a recent Phase II, randomised, double-blind, placebo-controlled trial (Hauser et al., 2008), patients with RRMS were given two rituximab infusions (or placebo infusions), two weeks apart. Compared to placebo-treated patients, those receiving rituximab had significantly (91%) fewer gadolinium-enhancing T1 brain lesions on MRI at weeks 12, 16, 20 and 24. A 58% relative reduction in the proportion of patients who experienced a relapse was noted after 24 weeks of therapy and patients in the rituximab group, compared with those in the placebo group, had a lower annualised relapse rate at 24 weeks (0.37 vs. 0.84, p = 0.04), unfortunately this was not sustained to 48 weeks. On the whole, rituximab was well tolerated. Mild to moderate infusion reactions due to cytokine release during B cell lysis were common and consistent with those reported in patients with receiving rituximab for rheumatoid arthritis (Cohen et al., 2006). Despite the rapid and complete depletion of peripheral B cells, there was no higher risk of infection (Hauser et al., 2008). However, as the authors point out, this trial was not designed to assess long-term safety, or to detect uncommon adverse events. Opportunistic infections, such as JC virus resulting in progressive multifocal leukoencephalopathy, have been reported in other patient populations treated with rituximab (Carson et al., 2009a; Molloy and Calabrese, 2008), with an estimated risk of 1:4000, associated with 90% mortality (Carson et al., 2009b).

Critically, the results from this study support the idea that B cells play an important role in the pathogenesis of MS. It also lends weight to the therapeutic strategy of targeting B cells. As plasma cells are not depleted by rituximab, and antibody body levels are not significantly affected, it is unlikely that rituximab exerts its effect through the reduction of pathogenic autoantibodies, but rather by modulating antigen presentation and pro-inflammatory B cell cytokine production (Bar-Or et al., 2008). Further trials with rituximab are unlikely, but its humanised successor, ocrelizumab, has been taken to a phase 3 trial.

Mixed targets

Alemtuzumab

Alemtuzumab (formally known as Campath-1H) is a humanised monoclonal antibody directed against CD52, a protein on the surface of lymphocytes and monocytes with unknown function. Treatment rapidly produces a profound lymphopenia. Lymphocytes regenerate, but the speed and degree of recovery varies between cell types: CD4+ T cells are particularly slow to recover, taking five years to reach pre-treatment levels (Coles et al., 2006). Alemtuzumab is licensed for the treatment of chronic lymphocytic leukaemia (Robak, 2005), and has been used successfully in a variety of autoimmune diseases (Hale and Waldmann, 1996; Isaacs et al., 1992, 1995, 1996; Killick et al., 1997; Lim et al., 1993; Lockwood et al., 1996), as well as in renal transplantation (Watson et al., 2005) and non-myeloablative conditioning prior to stem cell therapy (Maloney et al., 2002).

In 1991, studies of alemtuzumab in the treatment of SPMS revealed efficacy in the suppression of relapses, but not in preventing the continued progression of disability. In contrast, open-label studies in RRMS showed that alemtuzumab stabilised and even improved existing deficits. These findings led to the hypothesis that the progressive phase of the disease is due to post-inflammatory neurodegeneration and that immunosuppression would influence long-term disability only if given early in the disease course. These concepts informed the design of CAMMS-223; a phase II randomised trial comparing alemtuzumab with subcutaneous interferon beta-1a (Rebif) in the treatment of patients with early active RRMS. CAMMS-223 demonstrated alemtuzumab to be highly effective in the treatment of RRMS, reducing the risk of relapse and risk of sustained accumulation of disability by >70% when compared to Rebif. In addition, in line with early studies, mean disability scores improved among those receiving alemtuzumab, but worsened among those on Rebif. This was paralleled by an increase in brain volume on T1 MRI between months 12 and 36, whereas brain atrophy continued among patients on Rebif. This positive effect on disability is unprecedented in trials of MS.

The principal adverse effect of alemtuzumab is autoimmunity, arising months to years after treatment in the setting of lymphopenia. Typically, this is directed against the thyroid gland with 20–30% of patients developing some form of thyroid autoimmunity (most commonly hyperthyroidism) following treatment. In addition, in CAMMS-223 6 patients (2.8%) receiving alemtuzumab, and one patient (0.9%) receiving Rebif developed idiopathic thrombocytopenia purpura (ITP), an immune mediated condition directed against blood platelets. The index patient suffered a fatal brain haemorrhage before diagnosis. All subsequent cases were identified by the risk management programme. Of those treated with alemtuzumab, remission of ITP occurred without treatment in one patient, after corticosteroid therapy in two patients, and after rituximab therapy in two patients (Coles et al., 2008). All are currently off treatment and have platelet counts within the normal range. In addition to ITP and thyroid autoimmunity, we have also observed two cases of Goodpasture’s disease (one in the Cambridge cohort, and one on the phase 3 programme) and single cases of autoimmune neutropenia (Coles et al., 2006) and autoimmune haemolytic anaemia (unpublished observation). We have shown that autoimmunity arises in those patients with greater T cell apoptosis and cell cycling in response to lymphopenia, driven by genetically determined higher levels of IL-21 (Jones et al., 2009), i.e., autoimmunity arises, not due to lymphocyte depletion per se, but rather as a result of the homeostatic response it induces. Importantly, serum IL-21 levels measured before alemtuzumab can predict, with 70% accuracy, whether or not someone will develop an autoimmune complication of treatment. If confirmed, this will allow for improved counselling of patients considering alemtuzumab treatment, as an individualised risk of autoimmunity can be given, it will also permit focused surveillance of high-risk individuals.

In the CAMMS-223 trial, three cancers (non-EBV-associated Burkitt's lymphoma, breast cancer and cervical carcinoma in situ) were reported in patients in the alemtuzumab group, with onset ranging from 22 to 64 months after the first annual cycle; one patient treated with Rebif developed colon cancer at 36 months (Coles et al., 2008). Whilst cancers were not statistically more frequent after alemtuzumab (1.4% of patients vs. 0.9%) we speculate that the case of Burkitt's lymphoma, breast cancer and cervical carcinoma in situ (0.9%) receiving Rebif developed idiopathic thrombocytopenia purpura (ITP), an immune mediated condition directed against blood platelets. Of those treated with alemtuzumab, remission of ITP, an immune mediated condition directed against blood platelets. In addition, in line with early studies, mean disability scores improved among those receiving alemtuzumab, but worsened among those on Rebif. This was paralleled by an increase in brain volume on T1 MRI between months 12 and 36, whereas brain atrophy continued among patients on Rebif. This positive effect on disability is unprecedented in trials of MS.

The rationale behind using alemtuzumab as a treatment of MS was to disable the aberrant immune response by simply removing lymphocytes. This is now known to be an oversimplified view of its mechanism of action; indeed the composition of lymphocyte pool is radically altered following alemtuzumab. B cell numbers recover rapidly (Thompson et al., 2010) whereas T cells take up to 5 years to reach pre-treatment levels. Memory T cells dominate the depleted T cell pool, and for the first 6 months there is a predominance of cells with a regulatory phenotype (CD4+ CD25hi FoxP3+), with very reduced constitutive cytokine expression (Cox et al., 2005), providing a tolerogenic environment for newly generated lymphocytes to emerge.

Two phase III trials; Care-MS I trial comparing low dose alemtuzumab with Rebif in the treatment of RRMS, and Care-MS II trial comparing two doses of alemtuzumab with Rebif in patients with RRMS who have continued to relapse whilst on one of the licensed disease-modifying therapies are underway.
Cyclophosphamide (1952–1980) was introduced as a chemotherapy drug to treat a variety of cancers. It was later found to be effective in treating autoimmune diseases such as rheumatoid arthritis and lupus. However, its use was limited due to severe side effects such as hemorrhagic cystitis and myelosuppression.

In conclusion, while cyclophosphamide demonstrated some efficacy in treating autoimmune diseases, it was often accompanied by significant side effects. Thus, while it provided a stepping stone for the development of safer and more targeted immunosuppressive therapies, it paved the way for the modern pharmacological approach to treating these conditions.


