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Monoclonal antibody therapy in multiple sclerosis

Paradigm shifts and emerging challenges

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Therapeutic approaches to multiple sclerosis (MS) are based on altering the functions of the immune system, either by using broad immunosuppressive drugs used for transplantation rejection and rheumatology, or by modulating them more discreetly with beta interferon and synthetic amino-acid copolymers. These strategies are only partially successful, have important safety and tolerability limitations, and have shown to be mostly effective in earlier stages of the disease, in which acute relapses dominate the clinical picture. For progressive phenotypes of MS there are currently no effective therapeutic options. As very specific and potent immunosuppressive agents, monoclonal antibodies (mAbs) may offer considerable advantages over other therapies for MS. During the last decade, anti-a4 integrin natalizumab became the first approved mAb for treatment of relapsing MS, after convincingly demonstrating clinically significant effects on two large Phase 3 trials. Moreover, the concept of disease remission was introduced for the first time to describe patients who show no signs of clinical or imaging markers of disease activity during therapy with natalizumab. Of the mAbs under development for MS, alemtuzumab and rituximab have also shown promising evidence of effectiveness and potentially expanded the therapeutic horizon to reversal of disease progression in early relapsing patients and progressive patients who previously had not been studied. However, the appearance of progressive multifocal leukoencephalopathy (PML) in natalizumab-treated MS patients, as well as in patients with lymphoma, lupus and rheumatoid arthritis, treated with rituximab and autoimmune-type complications in alemtuzumab-treated MS patients underlines the fact that extended efficacy comes with significant clinical risks. The challenge is then how best to utilize therapies that have evidently superior efficacy in a chronic disease of young adults to obtain the best benefit-risk ratio and how to monitor and prevent emergent safety concerns.

Key words: monoclonal, antibody, multiple sclerosis, therapy, natalizumab, rituximab, alemtuzumab

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Introduction
Current Perspectives on Multiple Sclerosis Therapy

Until the 1990s, multiple sclerosis (MS) was seen as mainly an intractable disease for which clinicians and patients alike had little else to do but manage the inexorable progress of neurological deficit. MS is a clinically heterogeneous disease in which initially acute and reversible periods of neurological worsening affecting virtually any area of the central nervous system (CNS, brain and spinal cord) predominate; this is the relapsing-remitting form of the disease. In most patients, this is followed by a so-called progressive period, in which the clinical picture becomes dominated by insidious neurological worsening, manifesting itself as a spinal cord-dementia syndrome.[1]

Initially, the mainstays of therapy were steroids for the treatment of acute relapses and sporadic use of immunosuppressive drugs in an attempt to curb progression; although these therapies could have

beneficial effects on reducing the length and severity of relapses and occasionally providing periods of relapse suppression in selected patients, overall their impact on disease progression was seen as negligible.[2] This situation changed with the approval of interferon beta (IFNb) and glatiramer acetate (GA) for the treatment of relapsing-remitting MS and later mitoxantrone for relapsing forms of MS, including transitional progressive patients. At the same time, there was a burgeoning in the knowledge-base regarding the immunopathology of this disease[3] and development of magnetic resonance imaging (MRI) as the main biomarker of disease activity, including its inclusion as part of the current diagnostic criteria, and as a major endpoint for clinical trials.[4]

The efficacy of these drugs has been repeatedly confirmed in several Phase 3 trials, including trials in relapsing-remitting forms of MS and clinically isolated syndrome (CIS) patients at high-risk of developing MS;[5] also, apart from a single positive trial that included a significant percentage of progressive patients who still had relapses, IFNb and GA have failed to have an impact in secondary or primary progressive MS.[6,7] In summary, clinical efficacy with these drugs (sometimes collectively called the ABCR drugs, an acronym derived from the commercial names Avonex, Betaseron, Copaxone, Rebif) has been shown to be grossly similar—all effect marked reductions in MRI disease activity, decrease by about 30–35% the relapse rate, have marginal but significant impact on sustained short-term disease progression and have been shown to delay the transition from CIS to MS.

Probably too many clinical trials have been conducted in recent years in an attempt to prove the existence of a dose-response and frequency effect between different IFNb formulations, and in head-to-head trials between IFNb and GA, with the final results apparently being that, apart from tolerability (all agents have injectable formulations, but differ in frequency and route of administration, which are subcutaneous or intramuscular), there appears to be no real difference between these therapies.[7] It has also been argued that recent changes in the demographics and clinical characteristics of patients entering MS trials is making it progressively harder to attempt comparisons between drugs, including between the ABCR generation and newer agents in development.[8]

Even though the current scenario is clearly a substantial improvement from the situation only two decades ago, there is still a large unmet need in MS therapeutics, both for therapies with increased efficacy, as well as for progressive phenotypes of the disease. Naturally, this is besides the need for better symptomatic therapy to address complaints such as fatigue, sexual dysfunction and cognitive impairment and the whole field of regenerative medicine, which is not covered in this paper. Fortunately, there are currently several new therapies in late-stage development, in large measure due to our increased understanding of disease pathophysiology. Growth in this area has been based on a combination of data derived from experimental animal models and cutting-edge pathological data (both microscopical and molecular, including genomics and proteomics) that have contributed to the identification of new drug targets.[9,10]

Therapies currently in development have attempted to push the ceiling for clinical efficacy, such as having larger effects on relapse rate reduction and disease progression and providing better tolerability and convenience, e.g., by adopting the oral route of administration (**Table 1**). Current lead candidates as "oral MS therapies" include cladribine and fingolimod (FTY720), which have reported Phase 3 data (CLARITY, FREEDOMS and TRANSFORMS trials), followed by drugs such as teriflunomide, laquinimod and dimethyl-fumarate (BG-12) which are still in the early stages of Phase 3.[11,12] Briefly, data from the cladribine and fingolimod programs is clearly encouraging from the perspective of increased efficacy, but also raised significant safety concerns such as carcinogenicity (cladribine) or fatal herpes virus infections (fingolimod). This is perhaps not unexpected from drugs that have widespread effects on human biology and whose target lacks specificity for a particular disease process.

The main alternative to oral MS therapies are monoclonal antibodies (mAbs), which combine high biological potency and selectivity of action. This paper provides an overview of the development of the main mAb candidates for MS therapy, outlines what advantages they may provide over current standard of care and what they have taught us about MS as a disease and highlights the emerging safety concerns regarding their use and how that impacts their clinical use.

Development of mAb Therapy for Multiple Sclerosis: Failures and Lessons Learned

The therapeutic effects of mAbs are determined by their target molecule and its function; in the case of MS, target molecules have been either key players in the immune response, or markers for cells that play central roles in them. Furthermore, to truly understand the end-result of mAb therapy in MS, we need to consider the characteristics of the interaction between mAb and target, e.g., binding, blocking or signaling, mAb effector functions, such as complement and cell-mediated cytotoxicity, and finally access to the CNS compartment.[13] As to the last point, CNS access for large antibody molecules is predictably poor, with only an estimated 0.1% of systemically administered mAb reaching the cerebrospinal fluid (CSF) compartment;[14] theoretically, this percentage may be higher in case of blood-brain barrier (BBB) disruption, as occurs in acute inflammatory CNS lesions.

Nonetheless, it is plausible that most of the therapeutic benefit of mAbs in MS comes from altering the functions of the immune system in the peripheral, i.e., non-CNS, compartment. In principle, this limits the potential for development of antibodies targeting CNS-specific molecules, such as the axonal regrowth and remyelination inhibitors Nogo-A or LINGO, which might offer hope of recovery of established neurological deficits.[15,16] Nevertheless, clinical experience with anti-beta amyloid mAbs such as bapineuzumab have shown that parenchymal penetration may not be such a rate-limiting step for mAb therapy in particular CNS diseases.[17]

Almost since their inception as viable therapies, monoclonal antibodies have been tried in MS. Early pilot attempts using murine antibodies targeting

Table 1. Main industry-sponsored studies for oral NME currently in development for multiple sclerosis

Name	Development Phase	Clinicaltrials.gov identifier	Company	Route	Mechanism	Comparator	MS type
Cladribine	Phase 3 completed (CLARITY)	NCT00213135	Merck-Serono	p.o.	Purine analogue	Placebo IFNb1a s.c. (rescue)	RMS
	Phase 3 ongoing (ORACLE)	NCT00725985	Merck-Serono	p.o.	Purine analogue	Placebo	CIS
Fingolimod	Phase 3 completed (FREEDOMS I & II, TRANSFORMS)	NCT00340834 NCT00289978 NCT00355134	Novartis	p.o.	Sphingosine 1 phosphate agonist	Placebo Placebo IFNb1a i.m.	RMS
	Phase 3 ongoing (INFORM)	NCT00731692	Novartis	p.o.	Sphingosine 1 phosphate agonist	Placebo	PPMS
Teriflunomide	Phase 3 ongoing (TOWER, TEMSO, TENERE)	NCT00751881 NCT00134563 NCT00883337	Sanofi-Aventis	p.o.	Dihydroorotate dehydrogenase inhibitor	Placebo Placebo IFNb1a s.c.	RMS
	Phase 3 ongoing (TOPIC)	NCT00622700	Sanofi-Aventis	p.o.	Dihydroorotate dehydrogenase inhibitor	Placebo	CIS
Laquinimod	Phase 3 ongoing (ALLEGRO & BRAVO)	NCT00509145 NCT00605215	Teva/Active Biotech	p.o.	Dihydro-quinoline derivative	Placebo Placebo & IFNb1a i.m.	RMS
Dimethyl-fumarate (BG-12)	Phase 2 POC completed, ongoing (EXPLORE) Phase 3 starting (DEFINE, CONFIRM)	NCT00168701 NCT01156311 NCT00420212 NCT00451451	Biogen Idec	p.o.	Second generation fumaric acid derivative Nrf2 pathway activator	Placebo Add-on to IFN and GA Placebo Placebo & GA	RMS
BAF312	Phase 2 ongoing	NCT00879658	Novartis	p.o.	Sphingosine 1 phosphate agonist	Placebo	RMS
ACT-128800	Phase 2 ongoing	NCT01006265	Actelion	p.o.	Sphingosine 1 phosphate agonist	Placebo	RMS
CDP 323	Phase 2 terminated	NCT00484536	Biogen Idec UCB	p.o.	Integrin alpha4 antagonist	Placebo	RMS
Firategrast	Phase 2 completed (no POC study)	NCT00469378	GSK	p.o.	Integrin alpha4 antagonist	N/A	RMS

CIS, clinical isolated syndrome; GA, glatiramer acetate; IFN, interferon; i.m., intramuscular; MS, multiple sclerosis; p.o., per os; POC, proof-of-concept; PPMS, primary progressive MS; RMS, relapsing MS; s.c., subcutaneous.

differentiation antigens present on T lymphocytes, such as CD2, CD4 and CD6, were followed by larger trials with OKT3, an anti-CD3 (pan-T lymphocyte) antibody in progressive MS patients. Besides proving a functional effect on the immune system, including in the treatment of animal models of autoimmune disease,[18] these initial experiments had no impact on disease course and highlighted some of the main caveats for mAb therapy in a chronic disease, such as immunogenicity derived from animal protein sequences present in the antibody structure, acute toxicity caused by cell depletion and cytokine release, and safety related to reactivation of latent viruses.[19]

Beyond that, purely as pharmacological tools, mAbs proved their benefit early on; in another trial, use of an anti-tumor necrosis factor (TNF) mAb (cA2) resulted in a paradoxical increase in MRI disease activity and other markers of immune activation,[20] a finding which went against all predictions derived from animal models of MS and was later confirmed in a trial using lenercept, a decoy TNF receptor-Ig fusion protein.[21] These observations prompted a reevaluation of the role this cytokine plays in the immune response in MS patients, and illustrated the often paradoxical ("Janus-like") ways in which immune regulation works. TNF is undoubtedly a key pro-inflammatory cytokine, but also plays a role in remyelination by promoting the growth of myelin-producing cells.[22]

More recently, the results of the anti-IL12/23 mAb ustekinumab Phase 2 trial in MS, if disappointing from the efficacy standpoint, again underlined the difficulties mAb therapy face in MS. The interleukin 12p40 family of cytokines, including IL-12 and IL-23, are integral components of the T lymphocyte differentiation pathway that leads to the generation of Th1 and Th17 cells, respectively.[23] These T-helper phenotypes are thought to be key players in the pathogenesis of MS, and strategies targeting them have been shown to be effective in the treatment of the animal model experimental autoimmune encephalomyelitis (EAE).[24] In a randomized, double-blind, placebo-controlled, multicenter, dose-ranging Phase 2 trial, 249 relapsing-remitting MS patients were treated with four dose levels of ustekinumab and observed for change in

the number of new gadolinium-enhancing T1 lesions, a standard imaging endpoint. At the end of the 23-week treatment period, there was no significant change in the cumulative number of new lesions and no sign of a dose-response effect between the 4-dose levels of ustekinumab (from 27–180 mg, subcutaneous, q4w).[25] This is particularly striking since other proof-of-concept trials in psoriasis and psoriatic arthritis gave strong positive results and, in Crohn disease, promising results have led to further development.

The likeliest explanation for these findings, given our acceptance of the current autoimmune model of MS pathogenesis, is that the lack of penetration of ustekinumab into the disease target-organ (CNS) compromises its ability to modify the disease course; the lack of any pharmacodynamic dose-response effect in this trial does not support an under-dosing explanation.[26] This is an important lesson for the development of mAb in MS, since it makes a strong argument for the concept that clinical efficacy is related to effects on the immune system outside the CNS, even if repercussions can extend to brain and spinal cord parenchyma.

There is one mAb currently approved for the treatment of MS and three others in late-stage development. These four are natalizumab (targeting the alpha-4 integrin molecule), daclizumab (targeting CD25), alemtuzumab (targeting CD52) and rituximab (targeting CD20) (**Table 2**). Several papers provide excellent reviews on their modes of action, pharmacological characteristics and development paths.[19,27-29] The purpose of this review is not so much to add further to this list, but to comment and put into perspective the impact that these novel therapies can have in MS clinical management and the main challenges facing their development and usefulness. While there is accumulating evidence from clinical efficacy that mAb therapy has resulted in a clear paradigm shift in MS therapeutics, it is still unclear how these powerful biological weapons can be safely used in the management of MS patients.

A First Success: Targeting Lymphocyte Migration with Natalizumab

Natalizumab is currently the only approved mAb therapy for MS being used for the treatment of relapsing-remitting patients that have failed previous immunomodulatory therapy or are judged to have aggressive clinical courses.[30,31] The product is a humanized IgG4κ mAb raised against human alpha4 integrin, a target expressed on lymphocytes, monocytes and hematopoietic cells that forms heterodimers with β1 and β7 integrin. The heterodimer α4β1-integrin (VLA-4) is the counter-receptor for vascular adhesion molecule-1 (VCAM-1) and the key-player in the transmigration of lymphocytes across the blood-brain barrier,[32] as was elegantly shown almost two decades ago in the EAE model.[33]

Phase 1 studies of natalizumab started in the late 1990s, the results of a proof-of-concept Phase 2 trial were published in 2002 and in 2004 the FDA approved this therapy based on an interim analysis of 1-year data coming from the Phase 3 program.[34] The Phase 3 program consisted of two trials, AFFIRM (monotherapy, enrolling 942 relapsing-remitting MS patients) and SENTINEL (add-on to IFNb, enrolling 1,171 patients), in which the study drug was given as a monthly intravenous 300 mg infusion for 116 weeks. At the end of these trials, there was clear evidence that natalizumab therapy resulted in a very significant reduction in all measures of disease activity both as monotherapy and in addition to IFNb: relapse rate, proportion of relapse-free patients, proportion of patients with clinically confirmed disease progression, mean and cumulative number of new T1-gadolinium enhancing lesions and T2 lesions.[35,36]

It is probably fair to say that these results were groundbreaking and, as of then, clearly the most robust efficacy data reported. In fact, in a substantial proportion of treated patients (circa 28% of the total population), for the duration of treatment there was a complete absence of any new clinical or imaging activity of disease, leading some to propose that something like "disease remission" (an oncological concept with interesting connotations) had become achievable.

Natalizumab works primarily by blocking the interaction of alpha4 integrin with the VCAM-1 receptor on endothelial cells, thereby preventing the transmigration of lymphocytes into the CNS. This is a key step in the genesis of new acute MS lesions, as was originally shown in animal models, and which then translated into a remarkable reduction in the number of new contrast enhancing MRI lesions in natalizumab-treated patients.[33,37] Initially, it was thought that the α4β1 heterodimer might constitute a CNS-specific signal, i.e., the "zip code hypothesis," but this was rapidly dismissed as too simplistic. Also, several other potential biological impacts of natalizumab therapy have been identified. These include a specific decrease in the CD4/CD8 lymphocyte ratio in the CSF, an impairment in the transmigration of $CD3^+CD25^+FoxP3^+$ regulatory T cells and reduction in CSF B lymphocytes and plasma cells, modulation of the activation threshold of immune cells, reduction in the number of antigen-presenting cells such as dendritic cells present in the brain perivascular spaces and potentially induction of apoptosis in activated T cells.[38-41] All these mechanisms might potentially contribute to the clinical efficacy of the compound, but biological effects extending beyond and outlasting simple blockage of brain penetration might also be related to emerging side effects, such as opportunistic infections.

In the clinical trial setting, natalizumab was generally well-tolerated, with infusion/hypersensitivity reactions being the main concern in a minority of patients; however, the drug was withdrawn from the market only 3 months after its approval, due to the appearance of three cases, two of which were fatal, of progressive multifocal leukoencephalopathy (PML), a rare viral demyelinating disease that up until then had been unknown in MS.[42] The appearance of fatal PML cases in natalizumab-treated patients confronted the MS clinical community for the first time with the challenge of making a potentially life-threatening risk-benefit decision and led to the enforcement of a strict pharmacovigilance program (TOUCH in the USA, TYGRIS in the rest of the world) and the

Table 2. Current mAb therapies under development for multiple sclerosis

Name	mAb type	Target	Mechanism	Company	Development phase
Natalizumab	IgG4κ	Alpha4 integrin	Blocking lymphocyte migration CNS	Biogen Idec Elan	FDA/EMA approved

Studies	Clinicaltrials.gov identifier	Patient numbers	MS type	Comparator	Duration	Benefit/Risk
AFFIRM	NCT00027300	942	RMS	Placebo	2 years	Clear reduction ARR, disease-free status and MRI activity
SENTINEL	NCT00030966	1171	RMS	NAT + IFNb1a i.m. NAT + placebo	2 years	Hypersensitivity reactions 2 PML cases detected

Name	mAb type	Target	Mechanism	Company	Development phase
Daclizumab	IgG1	Alpha-chain CD25	Blocking IL-2 binding to T cells Expansion NK CD56^{bright} regulatory cells	Biogen Idec Facet Biotech	Phase 2 ongoing Phase 3 started

Studies	Clinicaltrials.gov identifier	Patient numbers	MS type	Comparator	Duration	Benefit/Risk
CHOICE	NCT00109161	230	RMS	DAC + IFNb1a Placebo + IFNb1a	6 months	Reduction in MRI activity
SELECT	NCT00390221	600 pln	RMS	Placebo	12 months	Slight increase in infection rates
DECIDE	NCT01064401	1500 pln	RMS	IFNb1a i.m.	2–3 years	

Name	mAb type	Target	Mechanism	Company	Development phase
Alemtuzumab	IgG1κ	CD52	Immune cell depletion Post-reconstitution increase in regulatory T cell population	Genzyme Bayer-Schering	Phase 2 completed Phase 3 planning

Studies	Clinicaltrials.gov identifier	Patient numbers	MS type	Comparator	Duration	Benefit/Risk
CAMMS223	NCT00050778	344	RMS*	IFNb1a s.c.	2 years	Very significant reduction in ARR and MRI activity. Reversal in EDSS progression
CARE MS I	NCT00530348	581 pln	RMS*	IFNb1a s.c.	2 years	Trial prematurely stopped: 6 cases of ITP (1 fatal), thyroid disease
CARE MS II	NCT00548405	840 pln	RMS	IFNb1a s.c.	2 years	

Name	mAb type	Target	Mechanism	Company	Development phase
Rituximab	IgG1κ	CD20	B cell depletion Downstream effects on immune system	Genentech	Phase 2 completed

Studies	Clinicaltrials.gov identifier	Patient numbers	MS type	Comparator	Duration	Benefit/Risk
HERMES	NCT00097188	104	RMS	Placebo	48 weeks	Very significant reduction in MRI activity, reduction in ARR
OLYMPUS	NCT00087529	439	PPMS	Placebo	96 weeks	Trend for reduction in EDSS; subgroup analysis positive in younger patients with active MRI
						No significant safety issues in MS trials; PML cases have been reported in cancer and rheumatological diseases with RTX

Name	mAb type	Target	Mechanism	Company	Development phase
Ocrelizumab	IgG1κ	CD20	B cell depletion Downstream effects on immune system	Roche Genentech	Phase 2 ongoing

Studies	Clinicaltrials.gov identifier	Patient numbers	MS type	Comparator	Duration	Benefit/Risk
Phase 2	NCT00676715	250 pln	RMS	Placebo IFNb1a i.m.	96 weeks	Results pending

*Early active RMS: Disease onset <5 years, >2 relapses last 2 years, EDSS 0–3. ARR, annualized relapse rate; DAC, daclizumab; EDSS, Expanded Disability Status Scale; EMA, European Medicines Agency; FDA, United States Food and Drug Administration; IFN, interferon; i.m., intramuscular; MRI, magnetic resonance imaging; MS, multiple sclerosis; NAT, natalizumab; PPMS, primary progressive MS; Pln, planned; RMS, relapsing MS; RTX, rituximab; s.c., subcutaneous; PML, progressive multifocal leukoencephalopathy; ITP, immune thrombocytopenic purpura.

generation of patient selection and monitoring algorithms.[43] Natalizumab was restricted to use as monotherapy and only in patients failing other therapies or with clearly aggressive courses, thereby negating its potential as a first-line therapy for MS patients. In retrospect, this was probably the best decision, although initially it was felt that the reported PML cases could be due to the combined effects of natalizumab and other immune-altering therapies (IFNb in the MS cases or several

immunosuppressant drugs in the Crohn disease case), with increased experience in many other cases (up to 28 in a recent count, for a total cumulative experience of almost 85,000 patient-years) have been reported, including a few in treatment-naïve patients.[44] The incidence of PML increases proportionally with the duration of exposure up until the 3 year mark,[44] and a recent evidence-based assessment by the American Academy of Neurology put the risk of PML at 1:1,000 patients, treated for an average of 17.9 months.[45] This could mean that after approximately 2 years of natalizumab exposure, the patients' benefit-risk ratio should be re-evaluated and continued therapy justified.

Treatment of natalizumab-related PML has generally consisted of plasmapheresis or immunoabsorption to remove the drug and restore immunosurveillance, which has led, in a few cases, to the appearance of immune reconstitution inflammatory syndrome (IRIS) when the re-awakened immune system rushes to the CNS to fight off JC virus infection. This typically causes a worsening in the patients' clinical status that may last several weeks and needs to be managed by the use of high-dose steroids.[44] In addition to mAb removal, several drugs with potential antiviral properties have been used, although no clear evidence for efficacy has emerged; recently, these have included the antimalarial mefloquine (based on an in vitro screening process) and the antidepressant mirtazepine, which would supposedly block viral entry into oligodendrocytes via the serotonin 5HT2A receptor.[46,47]

As experience builds with the use of natalizumab in the clinical setting, other rare adverse events have become apparent, including single cases of primary CNS lymphoma[48] and melanoma,[49] and the full spectrum of potentially life-threatening complications remains unknown, especially in the setting of chronic administration of this mAb. As for any low-risk event, only with the expansion of the safety database and continued use can the approximate incidence of these complications be truly ascertained. This should not detract from the really remarkable clinical benefit that patients can obtain from natalizumab therapy, but clearly means that dealing with PML (and safety monitoring in general) is going to be the key determinant of future success for this drug.

To date, natalizumab remains the most effective disease-modifying therapy for relapsing MS patients and should be used in situations where the clinical benefit clearly outweighs the risks. For populations outside the scope of the current label, for example in progressive populations, there is no clinical trial evidence to support its use. It is possible that with off-label use and by "downgrading" secondary progressive patients to relapsing-remitting MS (a boundary that is, admittedly, not easy to demarcate), clinical experience with the use of natalizumab in progressive populations will start to accrue and that could lead to a less formal evaluation of its efficacy in this phenotype.

Alternative mAb Targets for MS: Expanding the Treatment Horizon

Besides natalizumab, three other mAbs are transitioning to late stage development for MS: daclizumab, alemtuzumab and rituximab. To briefly discuss the first of these, daclizumab is a humanized IgG1 mAb raised against the alpha chain of the human IL-2 high-affinity receptor, CD25 and currently approved for the treatment of renal allograft rejection. It has also been tested in several autoimmune disorders, including MS. The target, CD25, is specifically expressed on activated T and B lymphocytes, NK cells, monocytes, as well as regulatory T and NK cells. By blocking the interaction between IL-2 and CD25, daclizumab selectively inhibits the immune response in conditions of overactivation of the immune response, as is presumed to happen in MS. Besides this, daclizumab treatment has been shown to cause an expansion of a regulatory subset of immune cells, $CD56^{bright}$ NK cells,[50] although more recently evidence for reduced numbers and function of "canonical" regulatory T cells ($CD4^{+}CD25^{+}$) has also been reported.[51] It is unclear what the net effect of these alterations in regulatory function on the immunopathogenesis of MS may be.

Clinical development of daclizumab has consisted of pilot uncontrolled clinical trials, and more recently two Phase 2 trials, CHOICE and SELECT. Initial trials such as the NIH-sponsored open-label Phase 2 add-on trial of daclizumab to IFNb in relapsing and progressive MS patients showed a robust (78%) reduction in the number of new contrast-enhancing MRI lesions in comparison to baseline.[52] A second, recently reported, small scale NIH trial consisting of an initial adjunctive IFNb plus daclizumab period of 5.5 mo followed by daclizumab monotherapy for the following 10 mo, showed that in a significant minority of patients the combination appeared to have synergistic effects when compared to monotherapy, and that higher doses of daclizumab might be more efficacious.[53]

To address both these questions, the CHOICE Phase 2 trial looked at the MRI effects of high dose daclizumab (2 mg/Kg s.c. q4w) plus IFNb, low dose (1 mg/kg s.c., q4w) plus IFNb, and IFNb alone in 230 relapsing-remitting MS patients during 6 mo. The results confirmed that high-dose daclizumab add-on therapy results in a statistically significant reduction in the accumulation of new inflammatory MRI lesions, whereas low-dose merely showed a positive trend, and that daclizumab has an immunomodulatory effect by increasing $CD56^{bright}$ NK cells.[54] The therapy appeared to be overall well tolerated, and although there was an increase in the number of serious adverse events, mainly infections, there were no significant safety concerns.[54] In conclusion, daclizumab looks promising as an augmentation therapy to IFNb, but it is unclear how this would fit into the treatment algorithm in the future landscape of MS therapies.

From the clinical management viewpoint, the prospect of having an additional parenterically delivered therapy on top of another already cumbersome drug, needs to be compared with the emergence of clinically effective oral compounds and the existence of high-potency biologicals. Both of these latter options might become more appealing alternatives to clinicians and patients as second (or even first)-line therapies, and a daclizumab add-on option may have a difficult time in establishing itself in the treatment algorithm.

Alemtuzumab is humanized IgG1k mAb raised against human CD52, a glycoprotein present on most normal

peripheral blood mononuclear cells except for plasma cells and on thymocytes. As a cell-depleting antibody, it is an approved therapy for fludarabine-refractory B-cell chronic lymphocytic leukemia. The use of alemtuzumab is expected to cause massive immunosuppression, akin to what would be found in immune ablation before bone marrow transplantation, and for autoimmune diseases the concept would be that a "reboot" of the immune system might lead to a normalization of the immune response pattern. Other potentially relevant mechanisms of action include an increase in regulatory populations in the immune reconstitution phase, induction of regulatory T-cell differentiation and inhibition of T-cell transmigration. There is scant evidence for all of these, however.

In MS, alemtuzumab has been studied now for almost 20 years (pilot trials first came out in 1991). Initially, there were safety and tolerability concerns with the use of this therapy, probably related to excessive dosing and regimen, which resulted in transient increase in symptoms caused by massive release of pro-inflammatory cytokines.[55] In subsequent uncontrolled trials, it was shown that alemtuzumab had a significant effect on clinical measures of disease activity and especially in relapsing MS patients very early in their disease course; also, about a third of treated patients developed clinically significant autoimmune thyroid disease.[56] It was postulated that in the immune reconstitution post-alemtuzumab phase, MS patients might have an intrinsic bias toward the generation of self-reacting lymphocytic repertoires, leading to the appearance of other autoimmune phenomena.

These results prompted the conduction of a Phase 2 open-label and evaluator-blinded trial, CAMS223, in 344 relapsing-remitting, treatment-naïve MS patients early on their disease courses and with evidence of aggressive disease (inclusion criteria required an Expanded Disability Status Scale score of under 3.0, the existence of two relapses in the previous year and at least one enhancing MRI lesion). The trial compared two doses of alemtuzumab (12 or 24 mg/day for 5 days, re-dosed at 1 year for 3 days) with subcutaneous IFNb for 2 years. Both doses of alemtuzumab resulted in a very significant reduction in relapse rate (average 74%) and imaging parameters and, for the first time in MS trials, a reversal in EDSS progression—at the highest dose level, alemtuzumab reduced the EDSS score by 0.45 points on average (0.39 points for both doses combined) at the end of the 2-year treatment period versus an increase with IFNb by 0.38 points.[57] Unfortunately, the trial was prematurely stopped for safety reasons, namely the appearance of three cases of idiopathic thrombocytopenic purpura (ITP), one of which was fatal. At the end of the trial, a total of six cases of ITP were recorded. Also, clinically significant thyroid disease occurred in 11% of alemtuzumab-treated patients (Graves disease in 6.5%).[57]

In conclusion, alemtuzumab is another clear illustration of the challenges of mAb development for MS: undoubted clinical efficacy clouded by significant safety risks. Although ITP can be a potentially life-threatening disease, with early detection and proper management it may not be an overwhelming problem, but clearly something to which neurologists are unaccustomed. Nevertheless, the incidence of autoimmune disease is an added concern in this population; recently, blood level of IL-21 has been proposed as a biomarker for the appearance of autoimmune disease in the reconstitution phase.[58] Overproduction of IL-21 appears to be genetically determined and to cause excessive lymphocytic cycling between apoptosis and expansion, increasing the chances for the emergence of self-reactive populations. If confirmed, a combination of clinical criteria and baseline IL-21 levels might be a way to select the best patients for therapy, maximizing the benefit-risk equation. A Phase 3 program is currently under development for alemtuzumab, consisting of two proposed trials, CARE-MS1 and 2, in treatment-naïve and breakthrough patients.

The final, and currently some of the most promising, mAbs used for the treatment of MS are the anti-CD20 chimeric antibody rituximab and the next generation humanized or human anti-CD20 antibodies such as ocrelizumab[59] and ofatumumab.[60] For all of these mAbs, the target is the CD20 molecule, which is a marker in nearly all B lymphocyte lineage cells, with the exception of stem cells and plasma cells. Rituximab is a chimeric IgG1κ mAb currently approved for the treatment of non-Hodgkin lymphoma and rheumatoid arthritis, but which has been used (and studied) off-label for several other autoimmune conditions;[28] its mechanism of action is through antibody and complement-dependent cell depletion, leading to profound reduction in B cell counts and widespread effects on immune function.

In retrospect, it is curious how long it took to use B-cell depletion strategies in MS, given the weight of evidence for their role in MS pathogenesis. Conceptually, B lymphocytes have several potential roles in MS pathogenesis, the most evident of which is the production of anti-myelin antibodies: B lymphocytes are present in CNS demyelinating lesions and there is evidence for their clonal expansion in the CSF; antibodies and complement have been causally implicated in the appearance of demyelination and the most common pathological pattern (type II) is characterized by antibody and C9neo deposition; oligoclonal bands are almost universally present and anti-myelin protein and lipid antibodies are very frequent in MS patients; and finally, therapies which impact humoral immunity, such as plasmapheresis and intravenous immunoglobulin, have shown clinical benefit in MS.[61,62]

Besides these, however, the central role of B cells in chronic autoimmune diseases has recently been emphasized. Ectopic lymphoid follicular tissue, composed mainly of activated B cells, in the leptomeninges and submeningeal spaces of progressive MS patients has been confirmed and its presence correlated with a worse prognosis.[63] These ectopic follicles could act as a chronic reservoir for immune activation, driving a local inflammatory reaction in more cortical regions of the brain, potentially contributing to the appearance of widespread cortical pathology which is characteristic of later stages of the disease.[64] Importantly, this type of localized cortical and meningeal inflammation is not readily observable by MRI imaging and would in essence constitute a different physiological compartment distinct from the more common perivascular

plaques that have so far been considered the hallmark of MS. Also, B cells can act as important antigen-presenting cells in the adaptive immune response and are capable of producing several key cytokines with widespread effects on immune function, such as IL-6, TNF and IL-10.[65]

In the last few years, clinical trials in MS using rituximab have helped to establish the importance of B cells as therapeutic targets in this disease and, again, have potentially stretched the therapeutic horizon for these patients. The HERMES trial, published in 2008, was a Phase 2 double-blind, multicenter trial lasting 48 weeks, in which 104 patients were randomized to receive either rituximab (1,000 mg on days 1 and 15) or placebo. This was mainly a proof-of-concept study with an imaging primary endpoint consisting of the total number of contrast-enhanced MRI lesions during follow-up, and secondary clinical endpoints such as relapse rate and safety. Overall, the trial was judged a clear success in that there was a very significant reduction in the number of new MRI lesions up to week 48, as well as a reduction in the annualized relapse rate, with no significant safety findings emerging that might impair the usefulness of this drug in MS, although clearly the study was not designed to look at long-term safety or low-frequency events.[66]

The remarkable speed of onset for the imaging findings (with almost complete suppression of total and new lesions achieved at week 12) also supported the significance of the non-antibody related B cell roles in MS such as antigen presentation or cytokine production. Interestingly, in an autopsy report for a patient treated with rituximab for gastrointestinal mantle-cell lymphoma who died from PML, it was noted that B-cell depletion was detectable even in the CNS perivascular spaces.[67] Assuming this could be the case also in MS patients, which is credible especially under conditions of BBB breakdown, the immunomodulatory effects of rituximab might extend from the peripheral blood into the CNS perivascular inflammatory compartment.

In the OLYMPUS trial, rituximab was used to treat primary progressive MS (PPMS) patients, in what was the first, and so far only, trial of mAbs in this population. PPMS patients make up about 15–20% of the total disease population, have a more serious prognosis and, significantly, have no current approved (or for that matter, effective) therapy, therefore constitute a clear medical unmet need. This multicentral, randomized, double-blind trial studied 439 PPMS patients under treatment with rituximab for 96 weeks (1,000 mg every 24 weeks or 4 courses) or placebo. The primary endpoint was timed to confirmed disease progression as measured by the EDSS scale and confirmed after 12 weeks; other endpoints included imaging metrics such as lesion volume. Although the primary endpoint was not met (96-week progression rates of 38.5% for placebo vs. 30.2% for rituximab), a subgroup analysis showed that there was a statistically significant treatment effect in younger patients (age under 51 years) and patients having gadolinium-enhancing lesions.[68] Even if only in a subset of patients, this positive result brings hope that anti-CD20 mAbs may have a beneficial impact on disease progression in a carefully selected progressive MS population.

Finally, rituximab has also been studied in pilot trials for the treatment of neuromyelitis optica (Devic disease), a rare clinical variant of MS carrying a severe prognosis, for which anti-aquaporin 4 antibodies are currently considered both the main agent responsible for demyelination, as well as diagnostic biomarkers.[69] In a recent report combining the results of several open-label trials conducted in centers in North America and the UK, rituximab was found to reduce the annualized relapse rate and led to a stabilization or even improvement in disability in a subset of patients.[70] In summary, anti-CD20 therapies have proven to be effective in a wide spectrum of CNS demyelinating disorders, including classical relapsing MS, as well as in a subset of primary progressive MS patients and patients with aggressive variants of this disease. Naturally, should this be confirmed in larger trials, mAb therapy would once more lead to a paradigmatic change in current MS therapy. Currently, rituximab has been replaced with ocrelizumab (a humanized version of the antibody) for MS development; a large Phase 2 multicenter, double-blind, active-comparator trial has finished recently and results are expected to be reported this year.

As would be expected, however, there are a few open questions. The first relates to the net effects of B-cell depletion on immune function. While it is known from the large clinical experience with rituximab that rituximab therapy does not lead to significant reduction in total antibody titers (only in IgM isotype) and does not seem to impair the vaccination status of patients, indiscriminate B-cell depletion also leads to the destruction of a regulatory B-cell population (Breg) that has been shown to be relevant in several models of autoimmune disease, including EAE.[71] In fact, it has been proposed that during the reconstitution phase after B-cell depletion, there might be an increase in numbers of naïve IL-10 producing Bregs, as opposed to the inflammatory memory B-cell population.[72] Were this to be the case in humans, a rational argument might be made for the use of intermittent B-cell depletion, in which during the "holiday" periods the immune system might be allowed to reset to a less dysfunctional operating mode.[73]

The second point relates to the long-term safety profile when using anti-CD20 therapies. Realistically, in a non-life threatening chronic disease such as MS, the benefit-risk equation is naturally biased towards safety and recent experience with natalizumab has only stressed that further. Besides infusion related adverse events and hypersensitivity reactions, rates of infection have so far not been superior to placebo, but PML has emerged once more as a main safety concern for this mAb (and presumably for all anti-CD20 molecules) therapy. Although case reports of PML cases in patients with cancer and autoimmune diseases treated with rituximab had been reported previously (leading to a labeling update for rituximab in February 2006), the magnitude of the problem was recently put into perspective with the publication of a report from the Research on Adverse Events and Reports (RADAR) project.[74] In this paper, 57 cases of PML in patients treated with rituximab and, admittedly, together with other immunosuppressive drugs, were collected from cancer centers and academic hospitals,

FDA reports, publications and the manufacturer's database. Of these 57 cases, 52 had lymphoproliferative disorders (mainly chronic lymphocytic leukemia and non-Hodgkin lymphoma), two had lupus erythematosus and one each had rheumatoid arthritis, autoimmune pancytopenia and thrombocytopenia; the case-fatality rate was 90%.[74] Although not frequent, PML is undoubtedly a risk when using anti-CD20 therapies and, even though no cases so far have been reported in the context of MS or other neurological autoimmune disorders, one can presume that once those therapies are widely used, especially for long periods of time, such events will start to occur.

In essence, this report has brought emphasis once again to the challenges and pitfalls of using mAbs in MS therapy. Although mAbs are clearly very potent and efficacious therapeutic weapons, some sort of therapy management plan will need to be implemented in order to be able to fully explore their benefits. This should include pharmacovigilance and risk management plans (as are in place today), but also monitoring for JC virus reactivation and emergence of PML, together with better specific therapies for this viral infection. Also, it will probably not be tenable to maintain MS patients without B cells for extended periods of time. Perhaps such therapies will need to be employed in the same manner as they are in oncology, with periods of induction of clinical remission interspersed with periods of immune reconstitution, during which other complementary therapies may be useful.

Emerging Safety Concerns: Progressive Multifocal Leukoencephalopathy from HIV to MS

PML is a viral infection of the CNS in which the JC polyomavirus causes demyelinating lesions by destruction of myelin-producing oligodendrocytes. Apparently a common asymptomatic infection during childhood and adolescence, the virus becomes dormant in the kidney and lymphoid tissues, such as the bone marrow (probably not in the CNS itself), and is reactivated under conditions of immune suppression or dysfunction.[75-78] After reactivation and rearrangement of the viral non-coding regulatory sequence for neurotropism, the virus then travels to the CNS, presumably carried by lymphoid cells such as B lymphocytes, where it establishes an active infectious foci.[79] The most common clinical situation in which this occurs is AIDS; depletion in cellular immunity leads to frequent opportunistic infections and, in fact, cellular immunity (mediated by $CD8^+$ cytotoxic T lymphocytes specific for viral proteins) has been identified as probably the main protective immune response against JC virus.

Several immunosuppressive drugs that impact cellular immunity have been associated with the appearance of PML, as might be expected.[76] For mAb therapies which have PML cases reported (natalizumab, rituximab, efalizumab), there are several potential mechanisms implicated: loss of immune surveillance by reduction of the numbers of dendritic and T cells in the perivascular spaces and CSF (natalizumab and possibly efalizumab), mobilization of hematopoietic progenitor cells potentially vulnerable to JC virus infection into the peripheral blood (natalizumab), upregulation of transcription factor genes that might increase viral synthesis (natalizumab), suppression of the cellular immune system (rituximab), and expansion of the pre-B lymphocyte population carrying JC virus during immune reconstitution (rituximab).[80-83]

The final risk of PML for a patient under mAb therapy will likely be a combination of disease-associated and therapy-associated risks. AIDS is the prototypical high-risk disease, in which reduced cellular immunity may combine with some sort of crosstalk between the HIV and JC viruses that potentiates the appearance of PML. For lupus or rheumatoid arthritis (RA), frequent past and concomitant use of other drugs with effects on the immune system, e.g., steroids, methotrexate, probably compounds the PML risk of mAb therapy. Interestingly, there is also an increase in the spontaneous rate of PML in rheumatological diseases, especially in lupus erythematosus,[84,85] presumably due to the underlying widespread dysfunction of the immune system. Unlike these situations, in MS the background risk of PML is not elevated above the general population, since the dysfunction in the immune system appears to be confined to a breakdown in tolerance to myelin antigens. As for therapy-related risks in MS, natalizumab is so far the only culprit. In this case, the combination of decrease immune surveillance and an increase in the numbers of virus-carrying cells and viral expression within them may result in a higher overall risk compared with other mAb therapies used for MS.

Historically, PML was first identified in patients with hematological cancer, and oncologists have since become adept at managing the risks associated with high potency biological therapies. In other clinical specialties, the reality has been different. It is true that rheumatologists treating lupus and RA patients have been exposed to the dangers of biological therapies, e.g., TNF blockers, for several years now, but PML has only become a concern relatively recently, with the rituximab PML cases. Dermatologists treating psoriasis patients have been shocked by the recent experience with efalizumab (anti-CD11a mAb), in which 3 out of 4 patients with PML died in a relatively short time, leading to withdrawal of the drug.[81] Although the manifestations of PML are not by themselves characteristic (the disease typically behaves as a mass lesion, with the subacute presentation of localized cortical dysfunction), in these clinical settings, the appearance of neurological signs and symptoms naturally leads to suspicion and a work-up to detect the presence of CNS lesions, resulting in a relatively straightforward diagnosis of PML.

The opposite situation occurs in MS. There, the presence of fluctuating multifocal neurological findings is a characteristic of the disease itself and therefore the diagnosis of PML poses further challenges. This is also true given that the imaging findings are not pathognomonic, serological testing will confirm the presence of antiviral antibodies in the majority of healthy adults, and more specific testing for the presence of viral DNA in the CSF is not a standardized procedure.[76] Up until recently, for the specialist in MS care the likeliest scenario in which PML might be encountered was as a differential diagnosis for a patient with unclassified

white matter lesions. This changed dramatically after the appearance of PML cases with natalizumab therapy and the implementation of the pharmacovigilance programs TOUCH and TYGRIS. Awareness of PML, both in MS patients as well as health professionals, needed to be increased, and algorithms were generated to help the differential diagnosis of PML versus a MS relapse.[43]

What has been proposed in this setting is that the appearance of clinical findings that are not characteristic of MS should prompt immediate investigation to rule out the presence of PML and in doubt, should lead to stopping mAb therapy.[43] In the case of natalizumab, this has been followed by removal of the drug by plasmapheresis to reinstate immune status. Naturally, for cell-depleting mAbs, such as rituximab or alemtuzumab, this is not a viable strategy, since immune reconstitution will take much longer in these patients. It has become imperative, therefore, to develop biomarkers that allow a prediction of the PML-risk for a particular patient and once on therapy, monitoring tools that track the emergence of JC virus infection. Recently, asymptomatic reactivation of JC virus in urine samples was detected in patients after 12 months of therapy with natalizumab, in the absence of clinical findings, together with a drop in cellular immunity against the virus;[86] unfortunately, this finding was negated by another study in which viuria or immune responses were not found to change over time under the same mAb therapy.[87] Clearly, much more work needs to be done in the development of biomarkers of viral replication that can be used with confidence in clinical practice.

Conclusions and Future Directions

The field of MS therapy underwent a revolution in the past two decades; from a disease in which therapeutic nihilism predominated and diagnosis was pursued with some degree of leisure to one in which the availability of disease-modifying drugs led to a sense of pressure to make an early diagnosis and institute therapy. The availability of effective medicines led to the widespread appearance of MS clinics and specialists and spawned a series of clinical trials in which MR imaging was validated as one of the mainstays of diagnosis and monitoring. It took some time to understand how best to use these initial medicines, and we have probably reached the efficacy ceiling with them: partial reduction in relapse rate and marginal reduction in disease progression.

mAb therapy has shown the promise of once again revolutionizing the treatment landscape for these patients: offering higher clinical potency, including, for the first time, a robust impact on progression and possibly, efficacy in progressive clinical phenotypes currently not treatable. With this higher promise, however, come, for the first time, significant safety risks, of which PML is certainly the most visible. In this upcoming MS therapeutic era, decisions will become harder. There will be a range of alternative therapies from which to choose (classic immunomodulators, oral compounds, mAbs), with different clinical efficacy, safety and tolerability. Choice of therapy will need to be made on the basis of a rational allocation of the benefit-risk ratio for each individual patient and drivers for initial choice, therapy switch or escalation will need to be identified.

In this context, mAbs may be used either as initial induction therapies for the induction of remission or, provided the safety risks can be managed, for chronic maintenance therapy. In either situation, to make the best use of these therapies neurologists and MS care specialists will need to adapt. Probably, they will need to assume some of the behaviors that oncologists and rheumatologists have used, such as an increased focus on clinical management instead of diagnosis, expertise in complicated therapeutic protocol and comfort in using high-potency drugs and managing the adverse effects and safety risks that accompany them. For MS patients, this will mean that in upcoming years, there will be, for the first time, a wider choice of differentiated therapies, but also that their experience of care will become both more intense and more risky. Hopefully, the development of personalized therapeutics in the near future, based on biomarkers of disease activity and progression, will both facilitate the process of therapy choice and lead to the best outcome for each individual patient.

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