The efficacy and safety of daclizumab and its potential role in the treatment of multiple sclerosis

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Abstract: Daclizumab is a humanized monoclonal antibody of the immunoglobulin G1 (IgG1) isotype that binds to the α-subunit (CD25) of the high-affinity interleukin-2 (IL-2) receptor expressed on activated T cells and CD4+CD25+FoxP3+ regulatory T cells. Based on the assumption that it would block the activation and expansion of autoreactive T cells that are central to the immune pathogenesis of multiple sclerosis (MS), daclizumab was tested in several small open-label clinical trials in MS and demonstrated a profound inhibition of inflammatory disease activity. Surprisingly, accompanying mechanistic studies revealed that the most important biological effect of daclizumab was rather a dramatic expansion and activation of immunoregulatory CD56^{bright} natural-killer (NK) cells that correlated with treatment response, while there was no or only minor effect on peripheral T-cell activation and function. These CD56^{bright} NK cells were able to gain access to the central nervous system in MS and kill autologous activated T cells. Additional and relatively large phase IIb clinical trials showed that daclizumab, as add-on or monotherapy in relapsing–remitting (RR) MS, was highly effective in reducing relapse rate, disability progression, and the number and volume of gadolinium-enhancing, T1 and T2 lesions on brain magnetic resonance imaging (MRI), and reproduced the expansion of CD56^{bright} NK cells as a biomarker for daclizumab activity. Daclizumab is generally very well tolerated and has shown a favorable adverse event (AE) profile in transplant recipients. However, several potentially serious and newly emerging AEs (mainly infections, skin reactions, elevated liver function tests and autoimmune phenomena in several body organs) may require strict safety monitoring programs in future clinical practice and place daclizumab together with other new and highly effective MS drugs as a second-line therapy. Ongoing phase III clinical trials in RRMS are expected to provide definite information on the efficacy and safety of daclizumab and to determine its place in the fast-growing armamentarium of MS therapies.

Keywords: CD25, CD56^{bright} NK cells, clinical trial, daclizumab, IL-2, IL-2 receptor, multiple sclerosis, T cell

Introduction

The past two decades have seen major advances in the treatment of multiple sclerosis (MS). Better understanding of the immune pathogenesis of MS has resulted in a plethora of therapies capable of reducing disease activity. With the advent of monoclonal antibody (mAb) technology, several humanized and fully human mAbs were designed as 'guided missiles' to target specific key molecules and immune cell subpopulations involved in the immune cascade that is believed to lead to inflammation, demyelination and axonal loss in MS. The interaction of a particular mAb with its target antigen may result in binding, blocking or signaling, depending on its F_{ab} activity. Depending on its F_c domain, the mAb can deplete target cells by complement-dependent cytotoxicity, Ab-dependent cellular cytotoxicity phagocytosis or apoptosis [Bielekova and Becker, 2010]. mAbs may also mask the target antigen, thus preventing

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it from interacting with its ligand, or activate signaling pathways [Linker and Kieseier, 2008].

The first mAb to be approved for the treatment of relapsing-remitting (RR) MS in 2006 was natalizumab, a humanized immunoglobulin G4 (IgG4) mAb that targets very late antigen-4 (VLA-4), an α4β1 integrin expressed on circulating immune (mainly T) cells. The interaction of VLA-4 with vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells is crucial for the transmigration of immune cells across the blood–brain barrier into the central nervous system. Its blockade by natalizumab proved to be highly effective in reducing relapse rate, disability progression and magnetic resonance imaging (MRI) activity in patients with RRMS [Chataway and Miller, 2013]. Several other promising mAbs are in late stages of development for MS. The cytolytic alemtuzumab (anti-CD52 which is expressed on the cell surface of lymphocytes, macrophages, monocytes and eosinophils) which causes profound and prolonged depletion of white blood cells has recently shown superiority over and above subcutaneous interferon-β 1a (IFNβ1a) in RRMS [Cohen *et al.* 2012; Coles *et al.* 2012]. The effect of several mAbs directed at the CD20 antigen on B cells on MRI and disease activity in MS (rituximab, ocrelizumab and ofatumumab) highlighted the importance of B cells in the pathogenesis of MS [Hauser *et al.* 2008; Kappos *et al.* 2011], and finally, since MS is speculated to be a T-cell mediated autoimmune disease, the anti-CD25 mAb daclizumab was thought to be an ideal candidate for treatment of the disease by blocking T-cell activation.

Properties and mode of action

Daclizumab is a humanized mAb of the human IgG1 isotype that binds specifically to the Tac epitope on the α-subunit (CD25) of the interleukin-2 receptor (IL-2R). IL-2 was the first interleukine molecule to be identified and characterized as 'T-cell growth factor' due to its mitogenic effect on T cells [Smith *et al.* 1980]. It is produced mainly by activated T cells, and to a lesser degree by activated dendritic cells and monocytes/macrophages. Activation of the T-cell receptor (TCR) by a relevant antigenic peptide presented by the appropriate major histocompatibility complex (MHC) class II molecule (the ' trimolecular complex') results in the upregulation of the heterotrimeric high-affinity IL-2R that is proportional to the strength of the TCR stimulus, the production of large amounts of IL-2 that is responsible for the clonal proliferation and differentiation of the T cells in an autocrine-loop pathway, and the secretion of other pro-inflammatory cytokines [Malek, 2008]. The high-affinity IL-2R consists of three chains: a signaling β-chain (CD122, shared also by IL-15R), a signaling γ-chain (CD132, shared also by receptors for IL-2, IL-4, IL-7, IL-15 and IL-21), and a nonsignaling unshared α-chain (CD25). It should be noted that the property of the immune system to share signaling molecules between receptors and cytokines may contribute to competition for different cytokines and explain the diverse and at times opposing effects of a single cytokine on specific immune responses under various conditions and in various environments.

The intermediate-affinity IL-2R consists of a β and a γ chain only, which mediate all signaling capability to IL-2 and internalization and are constitutively expressed on resting T and B lymphocytes, natural killer (NK) cells and NK T cells [Sakaguchi, 2004]. Cells that carry the intermediate-affinity IL-2R are not affected by daclizumab. NK cells are large granular cytotoxic lymphocytes critical to the innate immune system that are capable of cell lysis and cytokine production without prior antigenic stimulation, and provide rapid responses to virally infected and tumor cells. They may also regulate self-tolerance and play a role in the adaptive immune system by formulating antigen-specific immunological memory and killing activated effector T cells. Unlike NK T cells, NK cells do not express TCR or the pan T-cell marker CD3 or surface immunoglobulin B-cell receptor, but they usually express the surface markers CD16 and CD56. The two major subsets are $CD56^{bright}$ $CD16^{dim(-)}$, which constitute the majority of NK cells in secondary lymphoid tissues but only 5–10% of NK cells in the blood, and $CD56^{dim}$ $CD16^{(+)}$ [Poli *et al.* 2009]. $CD56^{bright}$ NK cells can affect T-cell priming through cytokine production and kill immature dendritic cells in lymph nodes. These cells also migrate to inflammatory lesions and participate in termination of the immune response by killing autologous activated T cells [Bielekova and Becker, 2010]. While T-cell activation requires, in addition to IL-2 stimulation, the engagement of the trimolecular complex and other costimulatory interactions between the antigen-presenting cell and the T cell, NK cells can be activated and expanded by IL-2 recognition alone *via* the intermediate affinity receptor

[Martin, 2012]. However, high levels of IL-2 may be needed for this activation.

The α-chain (CD25) that forms the high-affinity IL-2R together with the β and a γ chains is expressed at low levels in resting T cells, but in constitutive high levels in CD4+CD25+ FoxP3+ regulatory T cells (Tregs), which are also dependent on IL-2, and is rapidly upregulated in activated T cells. CD25 has no known intracellular signaling function, but increases the receptor affinity for IL-2 by ~10–100-fold. The high level expression of the high-affinity IL-2R in resting regulatory cells enables them to successfully compete for and steal small amounts of IL-2 from other T cells for their immunoregulatory functions [Feinerman *et al.* 2010]. Finally, homodimeric α-chains (IL-2RA) result in low-affinity receptor.

Daclizumab, the first therapeutic humanized mAb, was initially designed to block virally transformed T-cell proliferation in human T lymphotropic virus I (HTLV-I) induced adult T-cell leukemia [Waldmann *et al.* 1985; Lehkey et al, 1998]. It was effective in transplantation and in reducing autoimmune inflammation in early clinical trial in uveitis [Waldmann, 2007; Nussenblatt *et al.* 1999]. It has been approved since 1997 for the prevention of allograft rejection in patients undergoing renal transplantation as intravenous (IV) formulation containing 5 mg/ml (Zenapax®, manufactured at Roche's Nutley, New Jersey, and referred to as DAC Nutley), whose marketing has been discontinued in 2009 due to insufficient demand. Another two formulations of daclizumab have been developed: DAC-Penzberg (Penzberg, Germany) suitable for IV or subcutaneous (SC) administration at 100 mg/ml, which has been used in the phase II CHOICE clinical trial but is not approved for commercial use; and SC Daclizumab High Yield Process (DAC-HYP, PDL BioPharma, Redwood City, CA, USA, manufactured using a new NSO-derived cell-line and process, identical to DAC Penzberg, with the exception of protein concentration and formulated at 100- or 150 mg/ml), currently in use in clinical trials in MS. The rationale for blocking IL-2 dependent activation of T cells in MS by daclizumab seems obvious, as activated CD4+ and CD8+ T cells against central nervous system (CNS) autoantigens which can be found in the blood and CNS lesions of MS patients are thought to play a central role in the pathogenesis of the disease. Furthermore, alleles of the CD25 gene (IL-2RA) were identified as heritable risk factors for MS [International Multiple Sclerosis Genetics Consortium *et al*. 2007].

Daclizumab works by masking the IL-2 binding site on the low- and high-affinity IL-2R without depleting T cells by antibody-dependent cellular cytotoxicity, complement mediated lysis or apoptosis, or activating the receptor and signaling pathways. This blockade results in the inhibition of several IL-2 dependent T-cell functions, including antigen- and mitogen-induced proliferation, cytokine secretion by activated Th1 and Th2 lymphocytes, and interference with CD28-dependent CD40 ligand expression [McDyer *et al.* 2002]. Daclizumab also decreases CD25 expression on activated CD4+ T cells through a FC-receptor mechanism [Sheridan *et al.* 2006].

As mentioned before, CD4+CD25+ FoxP3+ Tregs which play an important immunoregulatory role in MS by suppressing effector autoreactive T cells, constitutively express high levels of CD25 and are dependent on IL-2 for their survival and proliferation. Blocking CD25 on these cells results in reduction in their number and inhibition of their *in vivo* proliferation and *in vitro* suppression of effector T cells [Martin *et al.* 2010; Oh *et al.* 2009]. In addition, high-affinity IL-2 signaling is pro-apoptotic [Lenardo, 1991], and its blocking by daclizumab results in inhibition of apoptosis of effector T cells [Baan *et al.* 2003; Wuest *et al.* 2011]. Taken together, it may be assumed that daclizumab therapy should activate T-cell immunity. In fact, all clinical trials to be discussed below clearly showed that MS disease activity was rather remarkably suppressed. Mechanistic studies revealed a surprising new mode of action (MOA) for daclizumab in MS.

Interestingly, while IL-2 alone could induce a 7-fold expansion of $CD56^{bright}$ IL-10 producing regulatory NK cells (expressing little or no CD25, high levels of CD122, and strong IL-2 mediated CD25 independent STAT-5 phosphorylation) and daclizumab alone had no effect in peripheral blood mononuclear cell culture, the combination of IL-2 and daclizumab resulted in 24-fold expansion of the $CD56^{bright}$ NK cells [Sheridan *et al.* 2008]. In small, phase II, open-label clinical trials, daclizumab therapy was associated with only mild functional blockade of CD4+ T cells and a moderate reduction of CD4+ and CD8+ T-cell numbers *ex vivo*, but a significant expansion of $CD56^{bright}$ NK cells *in vivo* was

observed [Bielekova *et al.* 2006, 2009]. This response is most likely mediated through excess of IL-2 produced by recently activated T cells that is unavailable for interactions with the high-affinity IL-2R blocked by daclizumab, but readily available for binding to the intermediate affinity receptor (β/γ chains) on NK cells, which are activated by this signal and subsequently expanded. The expansion of the $CD56^{bright}$ NK cells highly correlated with a profound treatment response of reduction in brain inflammatory activity as demonstrated by marked reduction in the number of MRI gadolinium (Gd)-enhancing lesions. *In vitro* studies showed that these NK cells killed autologous activated T cells by a contact-dependent mechanism and perforin-mediated degranulation. The expansion of $CD56^{bright}$ NK cells by daclizumab therapy enhanced this immunomodulatory mechanism of T-cell killing and correlated with contraction of CD4+ and CD8+ T cell numbers *in vivo*, supporting NK cell mediated negative immunoregulation of activated T cells during daclizumab therapy. The investigators suggested a mechanism of action of daclizumab *via* a regulatory circuit between innate and adaptive immune responses that involves the action of immunoregulatory $CD56^{bright}$ NK cells on T cells. The expansion of $CD56^{bright}$ NK cells along with decrease in T cells/NK cells and B cells/NK cells ratios and IL-12p40 in the *cerebrospinal fluid* (CSF) of MS patients treated with daclizumab suggest that $CD56^{bright}$ NK cells may gain access to the CNS and suppress activation of pathogenic immune responses [Bielekova *et al.* 2011]. The strong correlation between daclizumab-driven expansion of $CD56^{bright}$ NK cells and inhibition of inflammatory and clinical disease activity in MS has been shown again in additional larger clinical trials conducted with daclizumab, suggesting that this is the principal MOA of daclizumab in MS.

Not in line with this MOA, the observation of a patient who responded well to daclizumab treatment by more than 80% suppression of Gd-enhancing lesions on brain MRI and stabilization of clinical disability, but still did not expand $CD56^{bright}$ NK cells at all, has led to the elucidation of another possible MOA for daclizumab – a direct effect on dendritic cells. It has been shown that dendritic cells that efficiently activate antigen-specific T cells in the context of MHC class II-antigen-TCR interactions, combined with leukocyte function-associated antigen-1 (LFA1)/intracellular adhesion molecule-1 (ICAM1) and CD28–CD80/86 costimulation, express also CD25 molecules which serve to present IL-2 to the T cell undergoing activation *via* the immunological synapse in an antigen-specific manner. In this way, IL-2 secreted by the dendritic cells in limited amounts into the small and confined immunological synapse can reach concentrations high enough to be captured by CD25 expressed on dendritic cells and colocalize to the immunologic synapse with the antigen-specific T cell. This IL-2 is unable to activate dendritic cells which are lacking the signaling chains CD122 and CD132 of the IL-2R, but is rather delivered by CD25 specifically and directly to T cells that have just received full TCR and costimulatory signals and thus can mediate an effective immune response. This additional IL-2 signal is necessary for efficient expansion of antigen-specific T cells, and when it is abrogated (by blocking CD25 on dendritic cells with daclizumab), T cells proliferate poorly [Bielekova, 2013].

Daclizumab can also normalize the abnormally high number of circulating lymphoid tissue inducer (LTi) cells found in MS patients. LTi cells are a subtype of pro-inflammatory lymphocytes that belong to the innate immune system and originate from CD34+ hematopoietic precursor cells. The function of LTi cells is not entirely clear, although they may be involved with the evolution and maintenance of CD4+ memory T cell and related B cell/antibody responses and with tertiary ectopic lymphoid follicles formation associated with chronic inflammation, as can be seen in the meninges of MS patients. Daclizumab therapy can skew the differentiation of CD34+ hematopoietic stem cells from LTi lineage toward $CD56^{bright}$ NK cells by enhancing IL-2 signaling through intermediate-affinity IL-2R. As a result, formation and maintenance of meningeal lymphoid aggregates and associated immune memory responses in the brains of MS patients may be inhibited, as suggested by decreased intrathecal production of the chemokine CXCL13 (which is highly expressed in tertiary lymphoid aggregates) and of IgG (but not of any Ig in the blood) during daclizumab therapy [Perry *et al.* 2012; Bielekova, 2013].

Clinical experience in MS

Four small open-label baseline *versus* treatment single-center phase IIa proof-of-concept clinical trials have been conducted using IV daclizumab in active RRMS and secondary-progressive (SP)

MS patients who failed other treatments; two of them were performed at the US National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), and two at the University of Utah, Salt Lake City. These were followed by two large phase IIb, randomized, controlled multi-center trials in RRMS (the CHOICE study using SC DAC-Penzberg and the SELECT study using SC DAC-HYP followed by the SELECTION extension study). Phase III trials are ongoing, looking at the efficacy, safety and tolerability of SC DAC-HYP *versus* IM IFNβ1a (the DECIDE study) and at the immunogenicity, pharmacokinetics and pharmacodynamics of DAC-HYP (the OBSERVE study) (Table 1).

Initial open-label trials

An initial crossover clinical trial conducted at the NINDS, NIH, enrolled 11 RRMS and SPMS patients who failed treatment with IFNβ and showed contrast-enhancing lesions (CEL) on baseline MRI. Patients were treated with IV daclizumab at 1mg/kg/dose 2 weeks apart for the first two doses and every 4 weeks thereafter for a total of seven infusions. Compared with baseline, daclizumab therapy resulted in a 78% decrease in new CEL and 70% decrease in total CEL (primary outcome measures) which developed gradually over 1.5–2 months. All secondary outcome measures improved, some nonsignificantly (the changes in T2 lesion volume, black hole volume, Expanded Disability Status Scale (EDSS) and timed 25-foot walk) and others significantly [73% reduction in the volume of CEL, 81% reduction in exacerbation rate, and improvement in Scripps Neurological Rating Scale (NRS) and 9-hole peg test]. Treatment was generally well-tolerated and main adverse effects (AEs) included increase in the number of mild infections and transient elevation of liver function tests (LFTs) [Bielekova *et al.* 2004].

In a second trial at the University of Utah, 19 active RRMS and SPMS patients on other therapies were treated with IV daclizumab for 5–25 (average 13.6) months using the same NIH protocol. With continuous treatment, dose was adjusted to 0.8–1.9 mg/kg based on clinical response. A total of 10 patients had clinical improvement and nine patients stabilized, with an average reduction of 1.5 points on the EDSS during the treatment period ($p < 0.0004$). Annualized relapse rate (ARR) dropped by 74% from 1.23 to 0.32, and the number of Gd-enhancing MRI scans was significantly reduced ($p < 0.05$) during treatment. Patients with shorter disease duration and more active disease at baseline responded better to daclizumab treatment. AEs included paresthesias in six patients, rash in four, and mild leukopenia without lymphopenia, transient elevation in liver enzymes and upper respiratory tract infection (one each) [Rese *et al.* 2004]. Another small trial with nine clinically and radiologically active RRMS patients on IFNβ from the same center also showed reduction in total and new CEL as well as significant clinical improvement over a treatment period of 27.5 months [Rose *et al.* 2007].

The fourth study was designed to address three questions. (1) Is the therapeutic effect of daclizumab dependent on combination with IFNβ? (2) Is a higher dose of daclizumab more efficacious in patients with persistent disease activity? (3) Can biomarkers predict full *versus* partial therapeutic response to daclizumab? A total of 15 RRMS or SPMS patients, who experienced one or more relapses or sustained increase in disability in preceding 12 months on standard IFNβ treatments, and had at least two CEL during 3 monthly baseline brain MRIs were treated with IV daclizumab 1 mg/kg every 2 weeks for the first two doses and every 4 weeks thereafter in combination with IFNβ (months 0–5.5) and as monotherapy (months 6.5–15.5). Daclizumab monotherapy was efficacious in nine of 13 MS patients who completed the trial and combination therapy with IFNβ was needed according to the protocol in additional four patients to control disease activity. Daclizumab dose was increased to 2 mg/kg in one patient who did not fulfill the criteria for IFNβ withdrawal. Overall, 72% inhibition of new ($p = 0.002$) and 77% inhibition of total CEL ($p < 0.001$) by daclizumab were observed. All clinical measures of disability improved [for EDSS $p < 0.001$, for Scripps NRS $p < 0.001$ and for *Multiple Sclerosis Functional Composite* (MSFC) $p = 0.002$]. Two patients developed systemic immune responses 1–2 months after withdrawal of IFNβ, characterized by mouth ulcers, photosensitivity rash and transient formation of autoantibodies that required corticosteroid therapy for resolution, and did not complete the trial. Other AEs included lymphopenia (1), generalized lymphadenopathy (1), and transient elevation of bilirubin (1) and liver enzymes (1). Immunological studies showed that further expansion of $CD56^{bright}$ NK cells and contraction of CD4+ and CD8+ T cells occurred with daclizumab monotherapy after cessation of

IFNβ, which could differentiate between full and partial daclizumab responders [Bielekova *et al.* 2009].

In a small retrospective study in pediatric-onset MS, seven patients aged 12.8–17.2 were treated with IV daclizumab 1 mg/kg for 12–40 months. Mean ARR was reduced from 2.6 to 0.62, EDSS stabilized in five patients and improved in another two patients, and the median number of CEL decreased from 3 (range 0–11) to 0 (range 0–2) on the last available MRI following a median of 10.5 months (range 6–27 months) of daclizumab treatment. However, four of the seven patients had relapses and new CEL during daclizumab treatment. The treatment was well-tolerated, and AEs were usually mild and included headaches on the day of infusions (1), leukopenia (2), anemia (3) and elevation of LFT (1) that improved after halving the concomitant IFNβ dose [Gorman *et al.* 2012].

A recent retrospective analysis of frequent MRI scans performed on 70 patients at the NIH showed that daclizumab therapy was associated with a significant reduction in the meaningful measure of the rate of brain atrophy compared with other therapies (3.72 ml/year *versus* 5.17 ml/year, $p < 0.001$) [Borges *et al.* 2013].

Although these were noncontrolled, nonrandomized, small observational studies, the consistent results indicated a potential benefit for daclizumab as a monotherapy, especially in RRMS and early SPMS patients for up to 27.5 months. One study also suggested synergistic effects of IFNβ and daclizumab, the need for combination treatment with IFNβ or a higher dose of daclizumab for optimal response in some patients, and a possible biomarker for response to daclizumab [Bielekova *et al.* 2009]. These studies served also as a proof-of-concept for daclizumab in MS and paved the way for additional larger controlled studies that would investigate the possible role of daclizumab in treatment-naïve MS patients as well as add-on therapy or in comparison with a standard therapy with IFNβ treatment.

CHOICE, SELECT and SELECTION clinical trials

Two relatively large randomized, multi-center, placebo-controlled phase IIb clinical trials have been conducted with daclizumab either as add-on therapy to IFNβ (CHOICE) or as monotherapy (SELECT) in RRMS.

The CHOICE study (Study of Subcutaneous Daclizumab in Patients With Active, Relapsing Forms of Multiple Sclerosis) [Clinicaltrials.gov identifier: NCT00109161] included 230 RRMS (92%) and SPMS (8%) patients with active disease defined as having at least one relapse, or CEL on MRI in the previous year despite receiving IFNβ treatment. Patients were randomized to receive add-on SC daclizumab (DAC-Penzberg) 2 mg/kg every 2 weeks (IFNβ/high-dose daclizumab group), daclizumab 1 mg/kg every 4 weeks (IFNβ/low-dose daclizumab group), or placebo (IFNβ/placebo group) for 24 weeks. The adjusted mean number of new or enlarged CEL (primary endpoint) was reduced by 72% in the high-dose arm ($p = 0.004$) and by 25% in the low-dose arm ($p = 0.51$). Mean number of new or enlarged T2 lesions was reduced by 68% ($p = 0.007$) and 35% ($p = 0.60$) and the mean increase in total volume of new or enlarged CEL was significantly ($p = 0.046$) and nonsignificantly ($p = 0{\cdot}21$) lower in the high and low dose arms, respectively. There was no difference in the change in volume of T1 hypointense lesions or in the volume of T2 lesions. Interestingly, the presence of neutralizing antibodies to IFNβ was associated with an increase in the number of new or enlarged gadolinium CEL in the IFNβ/placebo group but not in the daclizumab groups. There were no significant differences in clinical outcome measures (ARR, EDSS or MSFC scores) between groups. MS lesion activity returned to about the baseline level in all groups 2–3 months after treatment discontinuation. Overall, daclizumab was well tolerated and common AEs, including infections, were generally equally distributed across treatment groups and there were no opportunistic infections or deaths. However, serious AEs (SAEs) were more common in the daclizumab groups (13%) than in the placebo group (5%), most frequently consisting of infections (5% *versus* 1%) which resolved with standard therapy. More patients in the daclizumab groups had a variety of cutaneous events, including injection site irritation and rash. There were no opportunistic infections or deaths. Two patients, both of whom were treated with daclizumab, developed malignant diseases (breast cancer and recurrent pseudomyxoma peritonei). Daclizumab treatment was not associated with significant changes in absolute numbers of T cells, B cells or NK cells, or T-cell proliferative response compared with IFNβ. The number of $CD56^{bright}$ NK cells was 7–8 times higher in both daclizumab groups than in IFNβ/placebo group. This was associated with fewer new CEL and provided

support to the theory that expansion of CD56^{bright} natural killer cells might mediate some of the effects of daclizumab on reducing MS lesion activity [Wynn *et al.* 2010]. The IFNβ/low-dose daclizumab group in the CHOICE study had a higher disease activity at baseline and during the washout period. However, the smaller nonsignificant treatment effect in this group may have been related to the low-dose daclizumab rather than to the baseline imbalance between groups, as it was still present when patients with high numbers of baseline lesions were excluded from the analyses. This seems somewhat puzzling, as CD25 saturation data suggest that there is a dose-dependent exposure efficacy relationship for daclizumab in MS, the IFNβ/low-dose daclizumab regimen used in this trial was similar to the initial IV dose used in earlier trials of daclizumab in MS which led to 100% saturation of CD25 Tac epitope on peripheral blood mononuclear cells, and that efficacy of daclizumab decreases 4–6 weeks after the last IV dose, despite the fact that the CD25 epitope remains almost completely saturated (>95%) in the blood [Bielekova *et al.* 2004; Rose *et al.* 2004, 2007]. In fact, the effects of daclizumab on T cells and dendritic cells, and their ability to present IL-2 to recently activated T cells as discussed above, take place mainly in sites in which the antigen presentation happens, such as lymphoid organs and inflamed tissues where concentrations of mAbs are lower than in the blood and thus may not saturate all CD25 molecules in these compartments [Bielekova, 2013]. This may explain the insufficient response in the low-dose daclizumab group in the CHOICE study or why some patients in the earlier clinical trials responded only after doubling the dose of daclizumab, and suggest that daclizumab doses above the minimum required to saturate peripheral CD25 receptors might be needed for greater efficacy in patients with MS.

The main limitation of the CHOICE trial was its short duration (6 months). It was also not powered to detect any significant clinical benefit. However, this study was consistent with the four previous smaller trials and showed again the significant reduction of inflammatory activity in active MS patients as measured by Gd-enhanced MRI scans. The overall good tolerability and safety profile of daclizumab were also encouraging. In addition, the study confirmed the serendipitous observation of the expansion of CD56^{bright} NK cells by daclizumab treatment and its negative correlation with inflammatory activity, suggesting that this pharmocodynamic marker may serve as a biomarker for daclizumab activity in MS.

Continuous treatment with daclizumab over more than 1 year revealed an increasing effect on clinical and MRI measures in previous studies [Rose *et al.* 2004, 2007; Bielekova *et al.* 2009] and prompted a larger and longer duration study with daclizumab as monotherapy. In February 2008, enrolment began to the SELECT (Safety and Efficacy Study of Daclizumab HYP to Treat Relapsing-Remitting Multiple Sclerosis) [Clinicaltrials.gov identifier: NCT00390221] trial [Giovannoni *et al.* 2011; Gold *et al.* 2013]. This was a large-scale phase IIb double-blind, placebo-controlled study to evaluate the safety and efficacy of DAC-HYP in subjects with RRMS that randomized 621 subjects in a 1:1:1 ratio to receive SC placebo, 150 mg, or 300 mg DAC-HYP every 4 weeks for 1 year. A total of 21 patients at one of the study sites were excluded from the efficacy analysis due to misdosing. Primary endpoint was the ARR; secondary endpoints included the number of new or enlarged CEL at weeks 8–24 in a MRI sub-study (n = 309, MRI scans performed at weeks 8, 12, 16, 20, 24), new T2 lesions on MRI at week 52, the proportion of patients relapsing within 1 year, and change in Multiple Sclerosis Impact Scale (MSIS) 29 Physical Score (a quality of life measure) over 1 year. EDSS progression at week 52 was a tertiary endpoint. To be eligible, patients had to have EDSS score ≤5.0, at least one clinical relapse in the preceding year plus MRI evidence of MS, and/or a MRI scan with CEL within 6 weeks prior to randomization. Baseline characteristics were comparable, apart for median time since diagnosis of 2.0 years in placebo group and 3.0 years in both daclizumab groups, and a moderately lower number of CEL (1.4 *versus* 2.1 and 2.0) and T2 lesions (36 *versus* 45 and 40) in the high-dose group compared with the low-dose and placebo groups, respectively. Most patients (93%) completed study treatment, representing a high adherence rate. At 1 year, daclizumab treatment (150 mg, 300 mg) resulted in a significantly lower ARR *versus* placebo (0.21, 0.23 *versus* 0.46; a reduction of 54%, $p < 0.0001$ and 50%, $p = 0.00015$, respectively,); a higher proportion of relapse-free patients *versus* placebo (81%, $p < 0.0001$ and 80%, $p = 0.00032$ *versus* 64%); reductions in the mean number of new CEL between weeks 8–24 (n = 309; 1.5, 1.0 *versus* 4.8; a reduction of 69% and 78%, respectively,

$p < 0.0001$ for both doses) and in the mean number of new/newly enlarging T2 lesions (2.4, 1.7 *versus* 8.1; a reduction of 70% and 79%, respectively $p < 0.0001$ for both doses). The volume of T2 hyperintense lesions increased by 27.3% in the placebo group and decreased by 11.1% and 12.5% in the 150 mg and 300 mg groups, respectively ($p < 0.0001$), and the volume of T1 hypointense lesions increased by 18% but decreased by 0.5% and 12.9% in the 150 mg and 300 mg groups, respectively ($p < 0.0001$). No differences in brain volume between groups were evident after 24 and 52 weeks. There was a trend towards improvement in MSIS-29 physical score ($p = 0.00082$ and $p = 0.13$ *versus* placebo, respectively). After 1 year, 13% of the placebo patients had a 3-month sustained disability progression, which was reduced to 6% (–57%, $p = 0.021$) in the daclizumab 150 mg group and to 8% (–43%, $p = 0.091$) in the daclizumab 300 mg group. These positive results, including early impact on disability progression that was evident also in highly active patients [Giovannoni *et al.* 2012], were seen in a cohort of mildly to moderately disabled patients (mean EDSS of 2.6–2.8 at baseline) with early RRMS (median disease duration 2–3 years) and medium disease activity in a short duration trial. The effect on disability progression was mediated not only by reductions in proportions of patients who had disabling relapses (3% *versus* 6% in the placebo group), but also by a reduction in disability progression independent of relapses (4% *versus* 7%). Additional analyses from the SELECT trial showed that daclizumab treatment resulted in a reduction in the proportion of new MRI-CEL evolving to permanent black holes [Radue *et al.* 2013] and a higher proportion of disease-activity free patients compared with placebo (39% *versus* 11%, $p < 0.0001$) [Havrdova *et al.* 2013]. AEs related to daclizumab included an increase in serious infections (2%, including one case each of appendicitis, gastroenteritis, hepatitis B, peritonsillar abscess, psoas abscess sinusitis, urinary tract infection, CMV, *Yersinia* and ' viral' infections), serious cutaneous events (1%), elevations in liver enzymes [alanine aminotransferase/aspartate transaminase (ALT/AST) >5× upper limit of normal (ULN)] (4%) and immune mediated events (1%, autoimmune thyroiditis, Crohn's disease, hypersensitivity and lymphadenopathy, all in the daclizumab 300 mg group). Four malignancies were detected during the trial, including two cases of melanoma in the daclizumab 300 mg group. One patient who was recovering from a serious rash died due to a complication of a psoas abscess. The number of Tregs counts was reduced during treatment with daclizumab, but this was not associated with clinical/MRI outcomes or AEs [Selmaj *et al.* 2013], and the marked expansion in $CD56^{bright}$ cells was associated with lower numbers of new MRI T2 lesions and fewer clinical relapses, suggesting $CD56^{bright}$ NK cell counts as a daclizumab efficacy biomarker [Elkins *et al.* 2012]. CD4+ and CD8+ T-cell counts decreased by about 7–10% at week 52 in patients given daclizumab, and the CD4/CD8 ratio remained constant. Neutralizing antibodies to daclizumab were detected in six (2%) patients in the daclizumab groups at week 24, and in only two (<1%) at week 52.

Of note is the large number of subjects (600) who were evaluated for 1 year in this phase II trial, unlike other phase II clinical trials in MS that usually recruit less than half the number of patients, last for 6 months and use MRI measures as primary outcome. This phase II trial was even larger than previous pivotal phase III trials that compared two doses of IFNβ with placebo and led to the approval of SC IFNβ1b (Betaseron/Betaferon) 20 years ago [IFNB Multiple Sclerosis Study Group, 1993] and SC IFNβ1a (Rebif) 15 years ago [PRISMS Study Group, 1998]. The SELECT study shares some similarities with recent large clinical trials in MS, such as a large placebo effect (65%) for the primary endpoint, and low relapse and disability progression rates in the placebo group (13%). These are quite different from trials conducted in the 1990s, reflecting a substantial change in patient populations which are available for today's clinical trials in MS.

By December 2011, 517 patients who completed the SELECT enrolled into the 1-year double-blind placebo-controlled extension SELECTION trial [ClinicalTrials.govidentifier:NCT00870740]. Patients who received placebo in the SELECT trial were randomized 1:1 to receive daclizumab 150 mg ($n = 86$) or 300 mg ($n = 84$); those originally treated with daclizumab were randomized either to a washout period of 24 weeks followed by re-initiation of their original dose ($n = 86$ for 150 mg, $n = 88$ for 300 mg), or to continue prior treatment ($n = 86$ for 150 mg, $n = 87$ for 300 mg). The three main objectives of this study were to assess: the safety and efficacy of daclizumab in subjects initiating treatment (especially how does MS activity in year 2 after starting treatment compare to year 1 on placebo); the safety and efficacy of daclizumab in subjects treated continuously for 2 years; and the impact of washout period on

rebound disease activity and on the safety and efficacy after treatment re-initiation.

In the former placebo group, switching to either dose of daclizumab resulted in a 59% reduction in ARR (from 0.434 to 0.179, $p < 0.001$) and in a 54% reduction in the proportion of patients with 3-month confirmed disability progression ($p = 0.033$) after 1 year of treatment. The number of new or enlarging MRI T2 lesions was reduced by 74% and the number of CEL was reduced by 86%. Among patients who remained on daclizumab over 2 years, the ARR achieved in year 1 was sustained in year 2 (0.148 *versus* 0.165), and 88% were free of confirmed disability progression at 2 years. The reduction in new/newly enlarging T2 lesions was more pronounced in the second year than in the first year of treatment for both doses (1.2 *versus* 1.85; $p = 0.032$). After 24 weeks of washout from daclizumab, the number of MRI-CEL increased for both doses (from 0.4 to 1.2 in the 150 mg group, and from 0.2 to 0.9 in the 300 mg group), although they remained lower than at pretreatment baseline and was interpreted as 'no evidence for disease rebound (mean Gd+ lesions at pretreatment baseline *versus* end of washout: 1.6 *versus* 1.1)' [Giovannoni et al. 2013]. When these patients had resumed daclizumab treatment for 6 months, mean lesion counts fell back to levels similar to those at the end of SELECT trial. No new safety signals were observed in the SELECTION trial, with the exception of three cases of immune-mediated SAEs, including one patient who died because of autoimmune hepatitis and liver failure. Otherwise, the incidence of serious infections (2% *versus* 2%) and serious cutaneous events (1.1% *versus* 1.0%) was similar in SELECTION *versus* SELECT whereas AST/ALT elevations >5× ULN were less common (1.5% *versus* 4%). The number of $CD56^{bright}$ NK cells that increased during the SELECT trial plateaued during the second year in the continuous treatment arms of the SELECTION trial, but gradually decreased to the baseline level during the 24-week washout period [Giovannoni *et al.* 2013]. A reciprocal effect on FoxP3+-Tregs was observed [Mehta *et al.* 2013].

Ongoing clinical trials

Four additional clinical trials are currently ongoing with DAC-HYP in RRMS:

The SELECTED study [ClinicalTrials.gov identifier: NCT01051349] is a phase II, multicenter, open label, extension study to evaluate the long-term safety and efficacy of SC daclizumab 150 mg (DAC-HYP) monotherapy once a month in subjects with MS who have completed 52 weeks' treatment in the SELECTION study. The estimate study completion date is September 2015.

The OBSERVE study [ClinicalTrials.gov identifier: NCT01462318] is a phase III single arm, open-label study to investigate the immunogenicity, pharmacokinetics and pharmacodynamics of DAC-HYP administered by prefilled syringes. Approximately 150 subjects will be enrolled and treated with DAC-HYP 150 mg SC for up to a 24-week treatment period and then enter a 20-week washout period for monthly assessment of immunogenicity, pharmacokinetics, pharmacodynamics, safety and tolerability.

The DECIDE study (Efficacy and Safety of Daclizumab High Yield Process Versus Interferon β 1a in Patients With Relapsing-Remitting Multiple Sclerosis) [ClinicalTrials.gov identifier: NCT01064401] is a phase III, multicenter, randomized, double blind, double dummy, parallel group, active-control study to determine the superiority of monthly DAC-HYP 150 mg monotherapy compared with Avonex (IFNβ1a) in reducing the ARR in approximately 1800 subjects with RRMS over a 96–144 week treatment period. The primary endpoint is ARR; secondary endpoints include the number of new or newly enlarging T2 hyperintense lesions on brain MRI, Change in the MSFC score, 3-months' sustained disability progression, change in the MSIS-29 physical score and the proportion of relapse-free subjects. Additional safety, clinical, MRI, cognitive, pharmacodynamic and economic outcomes comprise tertiary endpoints. Subjects completing the DECIDE study will be given the opportunity to enter an open-label extension trial with daclizumab (EXTEND study) [ClinicalTrials.gov identifier: NCT01797965]. This study will primarily assess the long-term safety and tolerability of daclizumab monotherapy, and secondarily the immunogenicity of DAC-HYP, clinical and MRI outcomes, safety, tolerability, and efficacy of switching from long-term treatment with IFNβ1a to daclizumab, as well as pharmacodynamics parameters that may be associated with treatment response.

Safety

Treatment with mAbs may raise some safety concerns about associated infections, including

opportunistic infections such as progressive multifocal leukoencephalopathy (PML) or herpes simplex (natalizumab, alemtuzumab, rituximab, ocrelizumab), cancer (natalizumab, alemtuzumab) and secondary autoimmunity (alemtuzumab). Daclizumab has been generally very well tolerated in all clinical trials reported so far. AEs reported in the early open-label trials over treatment periods from 6 to 25 months included rashes, lymphadenopathy, upper respiratory and urinary tract infections, paresthesias, transient elevation of liver enzymes, mild lymphopenia and leukopenia, granuloma annulare, transient thrombocytopenia, autoantibodies, breast tenderness, headache and exacerbation of depression. No deaths or SAEs were reported [Bielekova *et al.* 2004, 2009; Rose *et al.* 2004, 2007]. The safety and tolerability of monthly IV daclizumab 1 mg/kg have also been evaluated in 55 MS patients who received the drug offlabel in a single center [Ali *et al.* 2009]. Among 36 patients who reported any AEs, the most common were fatigue (8), gastrointestinal upset (4), rash (3) and generalized weakness (3). Common infections including viral meningitis, lymphadenopathy, allergic reactions, paresthesias, headache and cardiac toxicity were reported in two patients each. Of interest is the previously unreported case of psoriasis in one patient with a family history of this autoimmune disease for which daclizumab has been proposed as a treatment option.

In the CHOICE and SELECT trials, AEs were equally distributed across treatment groups, and the dropout rate was low. However, specific or serious AEs attributed to daclizumab treatment have emerged, that can be categorized into four main groups: infections; skin reactions; LFT abnormalities; and autoimmune phenomena.

Infections, mainly of the upper respiratory and urinary tract systems, were noted to be slightly increased in the initial open-label studies. Moreover, serious infections were more common in the daclizumab groups than in the placebo groups both in the CHOICE (5% *versus* 1%) and the SELECT (2% *versus* 0%) trials. Most patients with serious infections resumed treatment after the infection resolved. This nondramatic increase in the incidence of infections may be explained by the inhibitory effect of daclizumab on T-cell activation, proliferation and cytokine secretion. On the other hand, no opportunistic infections were observed during daclizumab treatment in MS. Unlike natalizumab and fingolimod, daclizumab does not prevent immune cells from accessing the CNS, and the expanded $CD56^{bright}$ NK cell population may contribute to effective immunity against opportunistic infections and potentially also against cancer [Bielekova, 2013].

A variety of skin reactions have been reported in 13% and 20% of subjects treated with daclizumab in the CHOICE and SELECT trials, respectively. These included mainly allergic skin reactions and rashes of various types and intensities, but also eczema, urticaria, contact dermatitis, folliculitis, erythema nodosum, pityriasis rosea, exfoliative dermatitis, seborrheic dermatitis, alopecia, pruritus and psoriasis. Most of these cutaneous events were mild to moderate in intensity and resolved either spontaneously or after topical steroid treatment, however, some prolonged and severe rashes required systemic steroids and/or discontinuation of therapy. About 1% of the daclizumab-treated subjects developed serious cutaneous events. Some of these cases have been characterized as progressive, severe skin eruptions consistent with a hypersensitivity reaction. An increased frequency of skin rashes on sun-exposed areas and with dry, scaling skin have been observed, and the routine use of sunscreen and skin moisturizers has been advised by some [Bielekova *et al.* 2013]. Hypothetical mechanisms that have been proposed to explain daclizumab-associated skin reactions are the inhibition of CD4+CD25+ Foxp3+ Treg cells in the skin [Oh *et al.* 2009], and the expansion and local tissue infiltration of $CD56^{bright}$ NK cells [Oh *et al.* 2012].

Elevation of LFT has been frequently observed in subjects treated with daclizumab, as well as in patients treated with placebo (with or without IFNβ). Abnormalities in LFT that typically took place late in the treatment period with daclizumab resolved and tended not to recur with continued treatment. The occurrence of elevation of LFT ≥5 times greater than the upper limit of normal in 4% of the daclizumab-treated patients compared with <1% of subjects who received placebo in the SELECT trial and the death of one patient due to liver failure in the SELECTION trial prompted the implementation of a strict safety monitoring program in the currently conducted clinical trials, including onsite testing of LFT immediately prior to each dosing.

Serious immune-mediated AEs affecting vital organs developed in four patients in the SELECT trial and three patients in the SELECTION trial,

Table 1. Clinical trials with daclizumab.

Trial	Phase	Design	Population	Sample size	Duration	Results
Bielekova *et al.* [2004]	IIa	Open label Baseline *versus* treatment	RRMS/SPMS	10	6 months	78%↓ in new CEL; 70%↓ in total CEL
Rose *et al.* [2004]	IIa	Open label Baseline *versus* treatment	RRMS/SPMS	19	5–25 months	Significant ↓ in CEL; ARR, EDSS
Rose *et al.* [2007]	IIa	Open label Baseline *versus* treatment	RRMS/SPMS	9	28 months	Significant ↓ in CEL, ARR, EDSS, NRS
Bielekova *et al.* [2009]	IIa	Open-label Baseline *versus* treatment	RRMS/SPMS	15	16 months	72%↓ in new CEL; 77%↓ in total CEL; ↓ In disability measures
CHOICE Wynn *et al.* [2010]	IIb	Randomized, double blind, 'add-on' study	RRMS/SPMS	230	6 months	72%↓ in CEL; 68%↓ in new T2WI
SELECT Gold *et al.* [2012]	IIb	Randomized, double blind, PBO-controlled	RRMS	600	12 months	ARR↓ by 54%, 50%; New CEL↓ by 69%, 78%; Disability↓ by 57%, 43%
SELECTION Giovannoni *et al.* [2013]	IIb	Double blind PBO –controlled extension	RRMS	517	12 months	Sustained efficacy for treatment continuation; high efficacy similar to SELECT for treatment initiation
DECIDE Ongoing	III	Randomized, double blind, double dummy, active comparator	RRMS	1800	24–36 months	
OBSERVE Ongoing	III	Open label, single arm	RRMS	150	11 months (+36 months)	

ARR, annualized relapse rate; CEL, contrast enhancing lesions; EDSS, Expanded Disability Status Scale; NRS, Neurological Rating Scale; PBO, placebo; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis, T2WI, T2 Weighted Images.

including inflammatory bowel disease, glomerulonephritis, thyroiditis and autoimmune hepatitis with fatal liver failure. Several cases of psoriasis in previous trials and the attribution of some daclizumab-associated skin reactions to immune dysregulation raise some concerns about autoimmunity secondary to daclizumab treatment that could be associated with a reduction of CD4+CD25+ Foxp3+ Tregs [Oh *et al.* 2009].

Mild generalized lymphadenopathy without any pathological changes has been associated with daclizumab therapy in early [Bielekova *et al.* 2004, 2009] but not in the later CHOICE and SELECT trials.

There were two malignancies reported in the CHOICE trial (breast cancer and recurrent pseudomyxoma peritonei), both in the daclizumab group, and four malignancies in the SELECT trial (three in the daclizumab-treated subjects – two melanomas and cervix carcinoma, and one – cervix carcinoma, in the placebo group). The association of daclizumab treatment with cancer is not clear yet. However, the daclizumab-induced expansion of $CD56^{bright}$ NK cells may be protective against cancer, as supported by a meta-analysis that found a lower incidence of secondary cancer and opportunistic infections in transplant patients treated with daclizumab in addition to standard immunosuppressive therapy compared with patients treated with the same immunosuppressive therapy without daclizumab [Webster *et al.* 2004]. These data suggest that, in contrast to other mAbs that compromise immune function by eliminating a large number of immune cells (e.g. rituximab, alemtuzumab) or preventing the migration of immune cell to the CNS (natalizumab), anti-CD25 therapy may lead to better control of latent viral infections and malignant cells.

The potential role of daclizumab in the treatment of MS

All clinical trials conducted so far with daclizumab in MS have demonstrated consistent and significant beneficial effects on MRI and clinical disease measures. Confirmation of these results in the ongoing phase III DECIDE clinical trial is

required before daclizumab can be approved as another treatment option for RRMS, which will probably place this mAb together with other potent MS therapies such as natalizumab, alemtuzumab and fingolimod.

By definition, mAbs target only one specific molecule with defined function(s) and thus they are expected to be highly selective in their MOA and to provide improved efficacy along with lesser off-target toxicity. Yet, mAbs exert multiple biological effects, as a result of the pleiotropy of functions and situational diversity characteristic of the complex human immune system. Therefore, it is not unexpected that additional mechanisms of action as well as newly emerging AEs of daclizumab will be unveiled in the future.

In addition to efficacy issues, other factors such as safety, tolerability, compliance, convenience, interactions with other drugs or with specific health conditions and cost should be considered when evaluating the role of a new treatment in a given disease. Daclizumab is administered subcutaneously once a month, an advantage over other mAbs that need to be administered intravenously and are associated with infusion-related reactions, or other injectable MS drugs that are administered more frequently. This may improve compliance with treatment and make daclizumab an attractive therapeutic option in MS. To date, there are no known interactions between daclizumab and immunomodulatory drugs or other drugs commonly used by MS patients.

Overall, treatment with daclizumab is well-tolerated. Since its approval for the prevention of graft rejection more than 15 years ago it has been used in more than 50,000 transplant recipients and has shown a favorable AE profile [Sandrini, 2005]. However, the potentially serious infections, skin reactions, liver abnormalities or autoimmune phenomena in several body organs that were reported in recent clinical trials may restrict its future use to patients with active RRMS as a second line therapy if approved by the regulatory authorities. A strict safety monitoring program, especially for skin and LFT abnormalities, as employed in current clinical trials, may be required in future clinical practice. More research is warranted to elucidate the mechanisms of these SAEs in daclizumab-treated subjects, identify markers for increased risk and screen for patients who may be at risk for these complications.

Duration of treatment with other highly effective mAbs may be restricted by serious safety limitations (e.g. increased risk of PML after 2 years of natalizumab treatment, or prolonged immunosuppression with alemtuzumab). There are no signals yet of any specific risks that may increase over time with daclizumab treatment; however, longer follow up is still needed beyond the limited duration of current extension clinical trials.

MS can be aggressive in certain patients, may cause considerable disability and take a high toll on patients, caregivers and health systems. Highly effective agents are needed in the armamentarium of MS therapies and their value in the treatment of MS should be weighted by evaluating their risk–benefit ratio in the individual patient and by comparing them with currently available therapies. Taking into account the overall low incidence of SAEs reported so far, the recovery of most infections, skin reactions and LFT abnormalities with standard therapies or drug discontinuation and the fact that no opportunistic infections have occurred with daclizumab, a favorable risk–benefit ratio for daclizumab is reasonable in most RRMS patients with disease activity or those who fail other treatments. However, the issue of secondary autoimmune diseases is worrisome and will hopefully be clarified in the ongoing DECIDE study that provides a prolonged follow-up in a larger cohort of patients.

Two new oral therapies have recently been approved for the treatment of relapsing forms of MS: Dimethyl fumarate (BG-12, Tecfidera™) [Fox *et al.* 2012; Gold *et al.* 2012] and teriflunomide (Aubagio®) [O'Connor *et al.* 2011], both having good safety profiles. Another oral agent, laquinimod, demonstrated only marginal efficacy in reducing relapse rate but was effective in reducing disability progression and the rate of brain atrophy and is very safe in RRMS [Vollmer *et al.* 2012]. Alemtuzumab is soon expected to be approved after demonstrating superiority over SC IFNβ1a in both treatment-naïve and treatment experienced RRMS patients [Cohen *et al.* 2012; Coles *et al.* 2012]. Its toxicity (prolonged immunosuppression, high rate of secondary autoimmunity and increased rate of infections and malignancy) may limit its future use for highly active patients who failed one or two lines of MS therapies only, and necessitate close monitoring. The anti-CD20 mAb ocrelizumab has shown pronounced effect on MRI and relapse-related outcomes and an acceptable safety profile in a phase II clinical trial [Kappos

et al. 2011], and is currently in phase III trials for RRMS as well as primary-progressive MS. It would not be appropriate to make any comments regarding the place of daclizumab in comparison with these emerging MS therapies as long as there are no direct head-to-head studies or definite clinical data for daclizumab. Yet, the confirmation of its beneficial effects on clinical and MRI outcomes in ongoing phase III clinical trials combined with acceptable long-term safety profile may make daclizumab an attractive candidate and another therapeutic option for the treatment of active RRMS patients. Moreover, the increased tendency among neurologists to adopt personalized and more aggressive approach to achieve higher treatment goals and optimal outcomes in MS may grant daclizumab a role also as a first-line therapy, especially in patients with prognostic factors for more aggressive disease.

Conclusion

Daclizumab constitutes a new avenue in the research and treatment of MS. The original assumption that blocking IL-2 signaling in T cells with daclizumab will lead to inhibition of effector T cells has been replaced by the elucidation of serendipitous and unexpected effects on both the innate and adaptive immune systems in MS, involving expansion of $CD56^{bright}$ NK cells, inhibition of T-cell activation by dendritic cells and inhibition of LTi cells. These discoveries have been made through a series of *in vivo* observations and mechanistic *in vitro* studies conducted along with initial clinical trials in active MS patients that demonstrated robust effect of daclizumab on inflammatory disease activity and suggested the expansion of $CD56^{bright}$ NK cells as a biomarker for daclizumab activity. Large phase IIb clinical trials added support to the beneficial effect of daclizumab treatment on clinical and MRI outcomes both as add-on and as monotherapy in RRMS that is sustained and may even increase over 2 years. The emergence of new and potentially serious AEs associated with daclizumab treatment may require strict monitoring programs. In any case, more definite results from ongoing phase III clinical trials are needed in order to verify the actual efficacy and safety of daclizumab in MS, and are eagerly awaited.

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