Dovepress

Open Access Full Text Article

R E V I E W

Spotlight on daclizumab: its potential in the treatment of multiple sclerosis

Ron Milo[1,2]
Olaf Stüve[3,4]

[1]Department of Neurology, Barzilai University Medical Center, Ashkelon, Israel; [2]Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; [3]Department of Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, TX, [4]Neurology Section, VA North Texas Health Care System, Medical Service, Dallas, TX, USA

Correspondence: Ron Milo
Department of Neurology, Barzilai University Medical Center, 2 Hahistadrut St., Ashkelon 7830604, Israel
Tel +972 8 674 5117
Fax +972 8 674 5463
Email ronm@bmc.gov.il

Abstract: Multiple sclerosis (MS) is a chronic inflammatory-demyelinating disease of the central nervous system of a putative autoimmune etiology. Although the exact pathogenic mechanisms underlying demyelination and axonal damage in MS are not fully understood, T-cells are believed to play a central role in the pathogenesis of the disease. Daclizumab is a humanized binding monoclonal antibody that binds to the Tac epitope on the α-subunit (CD25) of the interleukin-2 (IL-2) receptor, thus effectively blocking the formation of the high-affinity IL-2 receptor, which is expressed mainly on T-cells. A series of clinical trials in patients with relapsing MS demonstrated a profound effect of daclizumab on inflammatory disease activity and improved clinical outcomes compared with placebo or interferon-β, which led to the recent approval of daclizumab (Zinbryta™) for the treatment of relapsing forms of MS. Enhancement of endogenous mechanisms of immune regulation rather than inhibition of effector T-cells might explain the effects of daclizumab in MS. These include expansion and improved function of regulatory CD56^{bright} NK cells, inhibition of the early activation of T-cells through blockade of IL-2 transpresentation by dendritic cells and reduction in the number of intrathecal proinflammatory lymphoid tissue inducer cells. The enhanced efficacy of daclizumab is accompanied by an increased frequency of adverse events and risks of serious adverse events, thus placing it as a second-line therapy and calling for the implementation of a strict risk management program. This review details the mechanisms of action of daclizumab, discusses its efficacy and safety in patients with MS, and provides an insight into the place of this novel therapy in the treatment of MS.

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

Introduction

Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS) and a leading cause of disability in young peoples. It is characterized pathologically by various degrees of perivascular inflammation, demyelination, axonal damage and gliosis scattered in the brain and spinal cord, and clinically by a variety of neurological symptoms and signs disseminated in time and space.[1] The cause of MS is still unknown; however, complex interactions between environmental factors, genes, and appropriate timing are believed to underline an immune dysregulation and breakdown in immune tolerance to myelin antigen(s) that lead to autoimmune attack on myelin and axons in the CNS.[2] Although all arms of the immune systems are involved in the pathogenesis of MS, it is considered to be primarily a T-cell-mediated

95

autoimmune disease due to the observations of T-cell subset alterations in the blood and cerebrospinal fluid (CSF) of MS patients, clonotypic accumulation of activated T-lymphocytes in MS plaques, and the fact that experimental autoimmune encephalomyelitis, an animal model for MS, can be passively transferred by myelin-reactive T-cells.[3] The cytokine interleukin-2 (IL-2), the first interleukin molecule to be identified and characterized as "T-cell growth factor", is the main and critical regulator of growth and differentiation of T-cells.

Daclizumab is a humanized monoclonal blocking antibody of the IgG1 isotype that binds selectively to the Tac epitope (binding site for IL-2) on the α-subunit (CD25) of the high-affinity IL-2 receptor (IL-2R). Early investigations of daclizumab activity in vivo included attempts to block virally transformed T-cell proliferation in adult T-cell leukemia induced by human T lymphotropic virus I (HTLV-I).[4] A previous form of daclizumab for intravenous (IV) administration (Zenapax®, manufactured at Roche's Nutley, NJ, USA, and referred to as DAC Nutley) was initially approved for use in allograft renal transplantation,[5] but its marketing has been discontinued as of 2009 due to insufficient demand. Daclizumab was also shown to reduce autoimmune inflammation in early clinical trial in uveitis.[4,6] Two other distinct forms of daclizumab were later evaluated: IV or subcutaneous (SC) DAC-Penzberg (AbbVie Biotherapeutics, Redwood City, CA, USA), which has been used in the Phase II CHOICE clinical trial but not further developed nor approved for commercial use, and a newly manufactured material, SC Daclizumab High Yield Process (DAC-HYP, Zinbryta™), which was jointly developed by Biogen and AbbVie Biotherapeutics for MS through a program including various complete stand-alone clinical evaluation in patients with MS. DAC-HYP is manufactured using new NSO-derived cell line and process, resulting in different PK parameters optimized for SC dosing, and changes in the glycosylation pattern of the molecule, which affects the binding of daclizumab to Fc receptors, thus decreasing antibody-dependent cellular cytotoxicity.[7] The rationale for using daclizumab in MS stems from the central role played by CD4^{+} and CD8^{+} T-cells in the pathogenesis of the disease. Furthermore, alleles of the IL-2RA gene are associated with the risk of developing MS.[8] Daclizumab was anticipated to suppress activation and proliferation of autoreactive T-cells by blocking IL-2 signaling, thus reducing inflammation in MS. Indeed, in vitro binding of daclizumab to IL-2R results in the inhibition of T-cell proliferation in response to antigenic stimuli.[9,10] However, further research and clinical experience revealed normal in vivo T-cell activation and proliferation,

with additional surprising and complex immunomodulatory effects responsible for the clinical activity and adverse event profile of daclizumab observed in MS patients. These effects are associated mainly with enhancement of endogenous mechanisms of immune regulation and tolerance, probably resulting in the restoration of immune imbalance in MS.[7,11]

IL-2 and the IL-2 receptor

IL-2 is produced predominantly by activated T-cells, and to a lesser degree by activated dendritic cells (DCs), natural killer (NK) cells, and NK T-cells.[12] IL-2 signaling is mediated by a receptor complex consisting of an α (CD25), β (CD122), and γ (CD132) chain. The unshared IL-2 receptor (IL-2R) α-subunit is not known to contain an intracellular signaling domain and mainly increases the affinity of ligand binding. The β- and γ-subunits which are also shared by other cytokine receptors participate in both ligand binding and signal transduction.[12,13] The high-affinity IL-2R consists of all three subunits and is expressed mainly on activated T-cells and constitutively on CD4^{+}CD25^{+} FoxP3^{+} regulatory T-cells (Tregs). Binding of IL-2 to the IL-2Rα is required to form a stable receptor–ligand complex on the plasma membrane, which is then internalized. IL-2Rα is recycled back to the cell surface, but IL-2 and the other receptor subunits are degraded.[14] The intermediate-affinity IL-2R consists of a β and a γ chain only and is constitutively expressed on resting T- and B-lymphocytes, NK cells, and NK T-cells. Upon activation of T- and B-lymphocytes, the CD25 molecule is rapidly upregulated to help increase the receptor affinity for IL-2 by 10–100 fold. NK cells, which are important players in the innate immune system, can also regulate adaptive immunity by formulating antigen-specific immunological memory and killing activated effector T-cells. NK cells, unlike NK T-cells, do not express T-cell receptor or the pan T-cell marker CD3 and can be activated and expanded by IL-2 binding to the intermediate affinity receptor without the engagement of the trimolecular complex and other costimulatory interactions between the T-cell and the antigen-presenting cell.[15] However, due to the absence of the CD25 chain, high levels of IL-2 may be needed for this activation. The two major subtypes of NK-cells are CD56^{bright} CD16[dim(−)] (the majority of NK cells in secondary lymphoid tissues but only 5%–10% of NK cells in the blood) and CD56^{dim} CD16[(+)].[16] CD56^{bright} NK cells can regulate T-cell activation through cytokine production and have the ability to kill immature DC in lymph nodes. These cells also migrate to inflammatory lesions and regulate the local immune response by killing autologous activated T-cells.[17] CD25 by itself has a low affinity to IL-2,

and therefore is called "low-affinity receptor". It is involved mainly in transpresentation of IL-2 by DC for early activation of antigen-specific T-cells.[18]

IL-2 is an important regulator of the immune system rather than simply a T-cell activator: knockout mice lacking genes for IL-2 or IL-2R are not immunodeficient but rather develop lethal autoimmunity characterized by increased proliferation and polyclonal activation of T-lymphocytes and exhaustive differentiation of B cells into plasma cells producing large amounts of immunoglobulins G1 and E as well as autoantibodies.[19,20] This autoimmunity can be prevented by CD4+CD25+ FoxP3+ Tregs, which require IL-2 for their expansion, homeostasis, and function in the periphery as well as in the thymus and play an essential role in the suppression of autoreactive T-cells that escape thymic elimination.[21,22] The situation is even more complex in humans, where both autoimmunity[23] and severe immunodeficiency[24] have been observed in patients carrying mutations in the CD25 gene.

Daclizumab: mechanism of action

Daclizumab acts by masking and blocking the Tac epitope on the CD25 molecule, thus preventing IL-2 binding and signaling through the high-affinity IL-2R without depleting T-cells.[4,12,25] This blockade results in inhibition of antigen- and mitogen-induced T-cell proliferation and cytokine secretion by activated T-lymphocytes, which are dependent on IL-2, as well as inhibition of the late CD28-dependent CD40 ligand expression.[26] Daclizumab also decreases CD25 expression on activated CD4+ T-cells through daclizumab-Fc domain interaction with Fc receptors on monocytes and trogocytosis of the CD25 antigen, thus contributing to daclizumab inhibition of IL-2 signaling.[27] On the other hand, there is evidence to suggest that daclizumab may activate T-cell immunity: blocking CD25, which is constitutively expressed in high levels on IL-2-dependent CD4+CD25+ FoxP3+ Tregs, results in reduction in the number of these cells and inhibition of their proliferation and suppression of effector T-cells.[15,28] Moreover, CD25 blockade by daclizumab may results in inhibition of the IL-2-induced apoptosis of effector T-cells.[29]

Early clinical studies with daclizumab in MS (to be discussed later) showed prominent anti-inflammatory effect as reflected by profound reduction in the number of gadolinium-enhancing (Gd+) MRI lesions.[30–33] However, this effect could not be explained entirely by the modest decline only in the number of T-cells. Further mechanistic studies revealed other novel mechanisms of action of daclizumab on several immune cell subsets that may

explain its beneficial effects, as well as its adverse events (AEs), in MS.

Effects on NK cells

A surprising but consistent observation in clinical trials with daclizumab in MS was the marked (up to 7–8 fold) and sustained expansion of CD56^{bright} NK cells in the blood and CSF in daclizumab, but not in placebo-treated patients, that highly correlated with the reduction in the number of Gd+ MRI lesions and the clinical effects at population level.[17,32–34] These IL-2-dependent NK cells are not affected by daclizumab alone in peripheral blood mononuclear cell culture, but can be expanded 7-fold by IL-2 alone and 24-fold by IL-2 combined with daclizumab.[35] Blocking IL-2Rα on recently activated T-cells results in increased IL-2 production and decreased IL-2 consumption by these cells.[12] The in vivo expansion of CD56^{bright} NK cells can be explained by the diversion of excess IL-2 produced by activated T-cells (which cannot consume it due to their high-affinity IL-2R blocked by daclizumab) to NK cells carrying the intermediate affinity receptor (β/γ chains), which are now activated by the excess of IL-2 and subsequently expanded. The higher expression of IL2-Rβ chain on CD56^{bright} NK cells[7] enables them to capture more IL-2 than CD56^{dim} CD16[(+)]NK cells or resting T-cells that also express the intermediate-affinity IL-2R. CD56^{bright} NK cells (also known as regulatory NK cells) produce IL-10 and have an important role in the regulation of inflammation and autoimmunity: their number is increased during pregnancy, which correlates with decreased relapse rate in pregnant MS patients.[36] Elimination of NK cells from the CNS results in disease exacerbation in experimental autoimmune encephalomyelitis, while their expansion in the CNS results in disease amelioration, preferentially mediated by suppression of myelin-reactive TH17 cells.[37,38] In addition, CD56^{bright} NK cells are capable of killing activated T-cells, activated macrophages, DCs, and immature microglia[32,39–42] via perforin-mediated degranulation by granzyme A (GzA) and GzK.[7] Daclizumab therapy was found to enhance expression of GzK and to augment CD56^{bright} NK cells killing of autologous activated CD4+ and CD8+ T-cells whose number was inversely correlated with the expansion of CD56^{bright} NK cells in daclizumab-treated patients, suggesting that CD56^{bright} NK-mediated lysis of activated T-cells might also occur in vivo.[7,17,32,43] Overall, the expansion of CD56^{bright} NK regulatory cells (the major NK cell subset in the CSF) and their enhanced cytotoxicity against autoreactive T-cells and resting microglia in the CNS may partially explain the efficacy of daclizumab in MS. However, other mechanisms

must be involved, as not all patients who respond clinically to daclizumab show an increased number of CD56^{bright} NK regulatory cells.[7]

Effects on DCs

DCs that efficiently activate antigen-specific T-cells in the context of the trimolecular complex and other costimulatory interactions appear to be another target of daclizumab therapy. DCs are able to secrete small amounts of IL-2 and express the IL-2Rα chain (CD25, the low-affinity IL-2R) earlier than activated, nonantigen-experienced T-cells. The immunologic synapse that is formed during T-cell activation includes the engagement of the IL-2Rα chain on DC with the IL-2R β and γ chains from the T-cell to form a high-affinity IL-2R. IL-2 secreted in limited amounts by the DC into this small and confined immunological synapse, while unable to activate the DC that lack the signaling chains of the IL-2R, can now be delivered by CD25 in an antigen-specific manner directly to T-cells that have just received their T-cell receptor and costimulatory signals from DC and thus can efficiently expand and mount an effective immune response. This mechanism by which DC present IL-2 to T-cells is referred to as transpresentation and can be blocked by daclizumab, resulting in limited T-cell proliferation.[7,18] Furthermore, daclizumab was demonstrated to shift the cytokine profile of LPS-activated DC from proinflammatory (M1) to anti-inflammatory (M2), resulting in diminished T-helper priming and polarized immune response toward Th2.[44]

Effects on lymphoid tissue inducer cells

Lymphoid tissue inducer (LTi) cells are proinflammatory lymphocytes that belong to the innate immune system (innate lymphoid cells, ILCs) and originate from CD34^{+} hematopoietic precursor cells. Their function has not been fully elucidated; however, they may be involved with CD4^{+} T-cell memory[45] and related B-cell/plasma cell responses.[46] They can also form ectopic meningeal lymphoid follicles associated with cortical inflammation in MS patients.[47] Untreated MS patients have significantly higher levels of circulating ILCs, including LTi cells, compared with healthy individuals.[7] In contrast, their number is significantly lower in MS patients treated with daclizumab (comparable to those of healthy individuals) and correlates with the expansion of CD56^{bright} NK regulatory cells,[48] although others reported no change in circulating LTi cells.[49] Daclizumab therapy can inhibit the formation and maintenance of meningeal lymphoid follicles and reduce the associated immune memory responses in the CNS of MS patients by enhancing IL-2

signaling through intermediate-affinity IL-2R and skewing the differentiation of CD34^{+} hematopoietic stem cells from LTi lineage toward CD56^{bright} NK cells. This mechanism is suggested by decreased intrathecal production of the CXCL13 (a chemokine highly expressed in tertiary lymphoid aggregates) and of IgG (but not of any immunoglobulin in the blood) during daclizumab therapy.[48]

Effects on other immune cells

Daclizumab indirectly inhibits early activation, proliferation, cytokine production, and CD4^{+} cell memory of effector T-cells by all three mechanisms described earlier, namely, expansion of regulatory CD56^{bright} NK cells, inhibition of IL-2 transpresentation by DC, and suppression of intrathecal LTi cells. In contrast to the prominent inhibition of effector T-cells by daclizumab in vitro,[9] there is only a modest decrease (~5%–10%) in CD4^{+} and CD8^{+} T-cell counts in MS patients treated with daclizumab, and there is no reduction in T-cell proliferation and cytokine production after ex vivo stimulation.[17,32] On the other hand, rapid and sustained 40% reductions in the number of activated T-cells expressing HLA-DR, CD62L, and CD25 were observed in daclizumab-treated patients, and these were reversed after treatment discontinuation.[50] The complex interactions of daclizumab with immune networks in vivo and the microenvironmental alterations in cytokine production and availability seem to limit some of the direct effects of daclizumab on effector T-cells seen in vitro and highlight its indirect effects in MS.

CD4^{+}CD25^{+} FoxP3^{+} regulatory T-cells (Tregs) constitutively express the high-affinity IL-2R. These IL-2-dependent regulatory cells are important in suppressing antigen-specific T-cells, and their function has been reported to be impaired in MS patients.[51] Treatment of MS patients with daclizumab was shown to be associated with a 60% reduction in the number of Tregs that was not predictive of MS outcomes or AEs.[52,53] In addition, other studies demonstrated impaired immunoregulatory function of Tregs by daclizumab.[15,28,39] Nevertheless, this does not seem to significantly counteract the beneficial effects of daclizumab seen in MS, possibly because the increase in CD56^{bright} NK cells may provide sufficient immunoregulation and reduce the dependency on Tregs cells for immune homeostasis. Another possible explanation could be the low level of IL-2 signaling that is sufficient for Treg activation and function despite binding of daclizumab to the IL-2Rα on their cell surface.[54] In addition, daclizumab increases the expression of IL-7Rα (CD127) on Tregs[39] whose signaling may compensate for the lack of IL-2,

although the role of IL-7 signaling in Tregs functioning is controversial.[55,56] On the other hand, daclizumab reprograms activated Tregs (CD45RA−) to downregulate their transcription factor FoxP3, which is important for their suppressive function but has minimal effects on resting Tregs (CD45RA+).[57] In addition, daclizumab treatment was shown to maintain Foxp3 expression and lineage stability in the declined Treg population.[58] Taken together, daclizumab treatment seems to preserve immunoregulation and reduce the MS pathology by differential effects on regulatory cell subsets.

The effects of daclizumab on various immune cells are summarized in Table 1.

Clinical experience in MS
Early Phase IIa clinical trials
Five small open-label single-center baseline-vs-treatment Phase IIa proof-of-concept clinical trials have been conducted using IV daclizumab (DAC-Nutley) in active relapsing–remitting (RR) and secondary progressive (SP)-MS patients who responded insufficiently to other treatments (Table 2).[30–33,59] All 5 studies showed prominent and highly

Table 1 The effect of daclizumab on immune cells in MS

Cell type	Effects
Activated T-lymphocytes	Inhibition of proliferation and cytokine secretion[26,49]
	Inhibition of the CD28-dependent CD40 ligand expression[26]
	Inhibition of the IL-2-induced apoptosis of effector T-cells[29]
Monocytes	Trogocytosis of CD25 from T-cells[27]
CD56^{bright} NK cells	Expansion through availability of excess IL-2 to intermediate-affinity IL-2R[17]
	Enhanced killing of autologous activated T-cells through enhanced expression of GzK[7,17,32,39–40,43]
Dendritic cells	Blockade of transpresentation of IL-2 to T-cells carrying the intermediate-affinity IL-2R and inhibition of early T-cell activation[18]
	M1-M2 shift[44]
LTi cells	Contraction → inhibition of CD4+ memory T-cells and reduced formation of ectopic meningeal lymphoid follicles[48,49]
CD4+CD25+FoxP3 regulatory T-cells	Mixed effects: contraction, impaired immunosuppressive function, increased expression of IL-7Rα, downregulation of FoxP3[11,28,39]

Abbreviations: GzK, Granzyme K; IL, interleukin; IL-2R, interleukin-2 receptor; LTi, lymphoid tissue inducer; NK, natural killer; MS, multiple sclerosis.

Table 2 Early clinical trials with daclizumab

Trial	Phase	Design	Population	Sample Size	Treatment arms	Duration	Outcomes
Bielekova et al,[30] 2004	IIa	Open-label Baseline vs treatment	RRMS/SPMS	11	DAC + IFN-β	30 weeks	↓ 78% in new CEL ↓ 70% in total CEL ↑ Scripps NRS
Rose et al,[31] 2004	IIa	Open-label Baseline vs treatment	RRMS/SPMS	19	1. DAC alone (n=16) 2. DAC + IFN-β, then DAC alone (n = 2) 3. DAC + MP (n = 1)	5–25 months	↓ CEL ↓ ARR ↓ EDSS
Rose et al,[59] 2007	IIa	Open-label Baseline vs treatment	RRMS/SPMS	9	DAC + IFN-β, then DAC alone	Up to 27.5 months	↓ in CEL ↓ ARR ↓ EDSS ↑ Scripps NRS
Bielekova et al,[32] 2009	IIa	Open-label Baseline vs treatment	RRMS/SPMS	15	DAC + IFN-β, then DAC alone	16 months	↓ 72% in new CEL ↓ 77% in total CEL ↓ EDSS ↑ MSFC ↑ Scripps NRS
Rojas et al,[60] 2009	IIa	Retrospective	RRMS	12	DAC	24–60 months	↓ ARR ↓ EDSS
Bielekova et al,[33] 2011	IIa	Open-label Baseline vs treatment crossover	RRMS	16	DAC	54 weeks	↓ 88% in new CEL ↓ EDSS ↑ MSFC ↑ Scripps NRS

Abbreviations: ARR, annualized relapse rate; CEL, contrast enhancing lesions; DAC, daclizumab; EDSS, expanded disability status scale; IFN, interferon; MP, methylprednisolone; MSFC, multiple sclerosis functional composite; NRS, neurologic rating scale; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

significant reductions in the number of new and total contrast-enhancing lesions (CEL) on brain MRI, reflecting the profound anti-inflammatory effect of daclizumab. Despite the small number of patients in each study, clinical outcome measures related to relapse rate, disability, and other MS clinical scales (Scripps Neurological Rating Scale and components of the Multiple Sclerosis Functional Composite [MSFC]) were improved as well (Table 2). Patients with more active disease at baseline and shorter disease duration generally responded better to daclizumab treatment.[31] Although daclizumab monotherapy was effective in most patients with ongoing disease activity while on interferon β (IFNβ), some MS patients with more difficult to treat disease required the additive effect of IFNβ–daclizumab combination or higher doses of daclizumab for better disease control.[32,59] Immunological studies demonstrated expansion of CD56^{bright} NK cells in the blood[32] and CSF,[33] which correlated with concomitant contraction in the number of CD4^{+} and CD8^{+} cells[17,32] that could serve as biomarkers for treatment response and help differentiate between full and partial daclizumab responders. The expanded population of CD56^{bright} NK cells was also shown to kill activated autologous T-cells in a contact-dependent manner.[17] An additional small retrospective study also confirmed the long-term (up to 60 months) safety and clinical efficacy of daclizumab in MS.[60]

Daclizumab treatment was well tolerated by most patients. The side effect profile related to daclizumab was favorable in all trials and included increased number of common infections, elevated liver function tests (LFT), transient increase in bilirubin levels, paresthesias, rashes and other skin reactions (eczema, granuloma annulare, dermatographia, and interface dermatitis), lymphadenopathy, mild leukopenia, systemic immune responses in two patients that developed after withdrawal of IFNβ (characterized by photosensitivity rash, mouth ulcers, and transient formation of autoantibodies, requiring corticosteroid therapy for resolution), and single cases of posttreatment febrile reaction, palindromic rheumatism, and psoriasis.

In a small retrospective case series in pediatric MS, seven patients aged 12.8–17.2 were treated with IV daclizumab 1 mg/kg for 12–40 months. Results showed a reduction in the mean annualized relapse rate (ARR) from 2.6 to 0.62, stabilization in the EDSS scores in five patients, and improvement in another two patients. The median number of CEL on MRI decreased from 3 (range, 0–11) to 0 (range, 0–2) following a median of 10.5 months (range, 6–27 months) of daclizumab treatment. However, relapses and new CEL were observed in four of the seven patients during daclizumab treatment.

Possible reasons for this breakthrough disease activity may include a natural high relapse rate in pediatric-onset MS and the use of a low fixed dose of 1 mg/kg without adjusting it to higher doses in cases of continuous disease activity. The treatment was well tolerated, with mostly mild AE including headaches on the day of infusions (1), anemia (3), leukopenia (2), and elevation of LFT that improved after reducing the concomitant IFNβ dose (1).[61]

Although these were noncontrolled nonrandomized, small observational studies, they provided consistent results indicative of a potential benefit for daclizumab in relapsing MS patients, served as a proof-of-concept for daclizumab in MS, and paved the way for additional larger controlled studies. Later studies would investigate the role of daclizumab in treatment-naïve or treatment-experienced MS patients, as add-on therapy or in comparison with a standard therapy with IFNβ treatment.

Phase II–III clinical trials

Daclizumab was tested in two large Phase IIb clinical trials in RRMS either as add-on therapy to IFNβ (the CHOICE study using SC DAC-Penzberg),[62] or as monotherapy (the SELECT study[63] followed by the SELECTION[64] and SELECTED extension studies using SC DAC-HYP). These were followed by a randomized Phase III trial looking at the efficacy, safety, and tolerability of SC DAC-HYP vs. IM IFNβ-1a (the DECIDE study).[65] Another open-label Phase III study looked at the immunogenicity and pharmacokinetics (PK) of DAC-HYP (the OBSERVE study).[66] Participants of the DECIDE, SELECTED, and OBSERVE studies now have the chance to continue long-term, open label follow-up in the EXTEND study.

The CHOICE[62] (the Study of Subcutaneous Daclizumab in Patients With Active, Relapsing Forms of Multiple Sclerosis; Clinicaltrials.gov identifier: NCT00109161) trial randomized 230 RRMS (92%) and SPMS (8%) patients with active disease despite treatment with IFNβ to receive add-on SC daclizumab 1 mg/kg every 4 weeks (IFNβ/low-dose daclizumab group), SC daclizumab 2 mg/kg every 2 weeks (IFNβ/high-dose daclizumab group), or placebo (IFNβ/ placebo group) for 24 weeks. The study met its primary end point: the adjusted mean number of new or enlarged CEL was reduced by 25% in the low-dose arm ($p=0.51$) and by 72% in the high-dose arm ($p=0.004$). Mean increase in total volume of new or enlarged CEL was nonsignificant ($p=0.21$) and significantly ($p=0.046$) lower in the low and high dose arms, and the mean number of new or enlarged T2 lesions was reduced by 35% ($p=0.60$) and 68% ($p=0.007$),

respectively. No difference in the change in volume of T1 hypointene lesions or in the volume of T2 lesions was observed. The presence of neutralizing antibodies to IFNβ, which are known to reduce the efficacy of 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. Clinical outcome measures of ARR, EDSS, or MSFC scores did not differ between groups in this short-term clinical trial that was not designed to detect any significant clinical benefit. Treatment discontinuation led to the return of MS lesion activity to about the baseline level in all groups after 2–3 months. Common AEs, including infections, were generally equally distributed across treatment groups. However, serious AEs (SAEs) were more common in the daclizumab groups (13%) than in the placebo group (5%), consisting mainly of infections (5% vs 1%) which resolved with standard therapy. There were no opportunistic infections or deaths. A variety of cutaneous events, including injection site irritation and rash, were more frequent in the daclizumab groups. Malignant diseases (breast cancer and recurrent pseudomyxoma peritonei) were observed in two patients treated with daclizumab. Compared with IFNβ, daclizumab treatment was not associated with significant changes in absolute number of T-cells, B cells, or NK cells,

or in the T-cell proliferative response; however, the number of CD56^{bright} NK cells was 7–8 times higher in both daclizumab groups than in IFNβ/placebo group. This correlated with decreased number of new CEL and provided support to the theory that expansion of CD56^{bright} NK cells might mediate some of the beneficial effects of daclizumab and serve as a biomarker for its activity in MS[62] (Table 3).

In the SELECT[63] (Safety and Efficacy Study of Daclizumab HYP to Treat Relapsing-Remitting Multiple Sclerosis; Clinicaltrials.gov identifier: NCT00390221) trial, 621 subjects with RRMS (76% treatment-naïve) were randomized in a 1:1:1 ratio to receive SC placebo, 150 mg, or 300 mg DAC-HYP every 4 weeks for 1 year. Daclizumab treatment (150 mg, 300 mg) resulted in a significant reduction in ARR (primary endpoint) vs placebo (54%, $p<0.0001$ and 50%, $p=0.00015$, respectively). There was a higher proportion of relapse-free patients vs placebo (81%, $p<0.0001$ and 80%, $p=0.00032$ vs 64%), the mean number of new CEL between weeks 8 and 24 was significantly reduced (1.5, 1.0 vs 4.8; a reduction of 69% and 78%, respectively, $p<0.0001$) as was the mean number of new/newly enlarging T2 lesions (2.4, 1.7 vs 8.1; a reduction of 70% and 79%, respectively, $p<0.0001$). There was a 27.3% increase in the volume of T2 hyperintense lesions in the placebo group and a 11.1% and 12.5% decrease

Table 3 Large clinical trials with daclizumab

Trial	Phase	Design	Population	Sample size	Treatment arms	Duration	Outcomes
CHOICE Wynn et al,[62] 2010	IIb	Randomized, double-blind, add-on	RRMS/SPMS	230	IFN-β + placebo IFN-β + high-dose DAC IFN-β + low-dose DAC	6 months	High-dose/low-dose: ↓ 72%/25%[a] in CEL ↓ 68%/35%[a] in new T2 lesions No effect on clinical measures
SELECT Gold et al,[63] 2013	IIb	Randomized, double-blind, placebo-controlled	RRMS	621	Placebo DAC 150 mg DAC 300 mg	12 months	150 mg/300 mg: ↓ ARR by 54%/50% ↓ CDP by 57%/43%[a] ↓New CEL by 69%/78%
SELECTION Giovannoni et al,[64] 2014	IIb	Double-blind controlled extension	RRMS	517	Placebo→DAC 150/300 mg DAC→DAC original dose DAC→24 weeks washout then DAC original dose	12 months	Sustained efficacy for treatment continuation High efficacy similar to SELECT for treatment initiation Loss of effect but no rebound after washout
DECIDE Kappos et al,[65,75] 2015	III	Randomized, double-blind, double-dummy, active comparator	RRMS	1841	DAC IFN-β-1a (Avonex)	24–36 months	↓ ARR by 45% ↓ 54% in new/enlarge T2 lesions ↓ 60% in CEL ↓ CDP by 16% (12 week)[a] ↓ CDP by 27% (24 week) ↑ Mean change in MSFC score ↑ Mean change in SDMT score
OBSERVE Tran et al,[66] 2016	III	Open-label, single arm	RRMS	150	DAC	11 months (+36 months)	

Notes: [a]Nonsignificant.
Abbreviations: ARR, annualized relapse rate; CDP, confirmed disability progression; CEL, contrast enhancing lesions; DAC, daclizumab; EDSS, expanded disability status scale; IFN, interferon; MP, methylprednisolone; MSFC, multiple sclerosis functional composite; RRMS, relapsing-remitting multiple sclerosis; SDMT, symbol-digit modalities test; SPMS, secondary progressive multiple sclerosis.

in the 150 mg and 300 mg groups, respectively (*p*<0.0001), and the volume of T1 hypointense lesions increased by 18% in the placebo group but decreased by 10.5% and 12.9% in the 150 mg and 300 mg groups, respectively (*p*<0.0001). There were no differences in brain volume between groups. A trend toward improvement in the Multiple Sclerosis Impact Scale (MSIS)-29 physical score was observed (*p*=0.00082 and *p*=0.13 vs placebo, respectively). The disability progression sustained for 3 months was reduced by 57% (*p*=0.021) and 43% (*p*=0.091), respectively. The effect on disability progression was mediated by both reductions in proportions of patients with disabling relapses and in disability progression independent of relapses. Prevention of sustained disability progression in MS typically requires 2-year trial duration and larger number of patients as seen in Phase III trials because statistical power to detect treatment effects on disability progression is generally lower than that for other MS end points. Nevertheless, this end point has been achieved in this Phase II trial, despite its shorter (1 year) duration and the low rate of events (13%) observed in the placebo group, underscoring the beneficial effect of daclizumab on this key goal of MS treatment. Subsequent subanalyses from the select trial showed that the impact of daclizumab on disability progression was evident even in patients with highly active disease[67] and that daclizumab treatment resulted in a reduction in the proportion of new MRI-CEL evolving to permanent black holes[68] (suggesting less destructive lesions) and a higher proportion of patients free of disease activity compared with placebo (39% vs 11%, *p*<0.0001).[69] There was an improvement in health-related quality of life with daclizumab compared to placebo, partially driven by reduction in disease activity and attenuation of the adverse impact of relapse on health-related quality of life.[70] AEs related to daclizumab in the 150 mg treatment group included an increase in serious infections in 2% (compared to none in the placebo group), serious cutaneous events in 1% (rash, atopic, allergic or exfoliative dermatitis, and erythema nodosum) and elevations in liver enzymes (ALT/AST) >5× the upper limit of normal (ULN) range in 4%. Immune-mediated events (autoimmune thyroiditis, Crohn's disease, lymphadenopathy and hypersensitivity reaction) were observed in 1%, all in the daclizumab 300 mg group. One patient who was recovering from a serious rash died due to a complication of a psoas abscess. There were two cases of cervical carcinoma (one in the placebo group and one in the daclizumab 150 mg group), and two cases of melanoma in the daclizumab 300 mg group. There was no association between the reduced number of Tregs observed during treatment with daclizumab and the clinical/MRI outcomes or AEs.[71] On the other hand, the marked expansion in CD56^{bright} NK cells was associated with fewer clinical relapses and new MRI T2 lesions.[34] T-cell counts decreased by about 7%–10% at week 52 on daclizumab treatment, but 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 2 (<1%) at week 52, indicating their transient nature.[63]

Five hundred and seventeen patients who completed the SELECT enrolled into the 1-year double-blind controlled extension SELECTION trial (ClinicalTrials.gov identifier: NCT00870740).[64] Patients who received placebo in the SELECT trial were randomized 1:1 to receive daclizumab 150 mg or 300 mg; those originally treated with daclizumab were randomized either to continue prior treatment, or to undergo a washout period of 24 weeks followed by reinitiation of their original dose. The study aimed to assess the safety and efficacy of daclizumab in subjects initiating treatment after 1 year on placebo or in those treated continuously for 2 years, and to assess whether a washout period can evoke rebound disease activity, and the safety and efficacy after treatment reinitiation (Table 3).

After 1 year of treatment, patients who switched from placebo to either dose of daclizumab had a 59% reduction in ARR (*p*<0.001) and a 54% reduction in the 3-months confirmed disability progression (*p*=0.033). These patients had 74% reduction in the number of new or enlarging MRI T2 lesions and 86% reduction in the number of CEL. The ARR achieved in year 1 was sustained in year 2 among patients who remained on daclizumab, and 88% were free of confirmed disability progression at 2 years. The reduction in new/newly enlarging T2 lesions in this group was more pronounced in the second year than in the first year of treatment for both doses (1.2 vs 1.85; *p*=0.032). Clinical and MRI activity returned to pretreatment levels after 24 weeks of washout from daclizumab, without evidence of rebound of disease activity above the pretreatment period.[64,72] Resuming daclizumab treatment for 6 months resulted in a fallback of the mean lesion counts to the end of SELECT trial levels. There was a similar incidence of serious infections (2% vs 2%) and serious cutaneous events (1.1% vs 1.0%) in SELECTION and SELECT trials, whereas liver enzyme elevations >5x ULN were less common (2% vs 4%). Three cases of immune-mediated SAEs occurred during the SELECT trial, including one patient who died because of autoimmune hepatitis and liver failure. The number of CD56^{bright} NK cells that increased during the SELECT trial plateaued in the continuous treatment arms of the SELECTION trial, but gradually decreased to the

baseline level during the 6-month washout period.[70] There was a reciprocal effect on FoxP3+-Tregs.[73]

Overall, the SELECTION trial showed that the effects of daclizumab were maintained through the second year of treatment and that AEs and immunogenicity were not increased in the second year of continuous treatment or during treatment washout and reinitiation.

The Phase III, multicenter, randomized, double-blind, double-dummy, parallel-group, active-control DECIDE trial (Efficacy and Safety of Daclizumab High Yield Process vs Interferon β 1a in Patients With Relapsing-Remitting Multiple Sclerosis; ClinicalTrials.gov identifier: NCT01064401) randomized (1:1) 1,841 patients with active RRMS to either SC DAC-HYP monotherapy administered at a dose of 150 mg every 4 weeks or to IFNβ -1a (Avonex) administered intramuscularly at a dose of 30 μg once a week for 96–144 weeks.[65] The primary end point was the ARR over a period of up to 144 weeks. Secondary efficacy end points were the number of new or newly enlarged hyperintense lesions on T2-weighted MRI scans of the brain over a period of 96 weeks, the proportion of patients with confirmed progression of disability at 12 weeks over a period of up to 144 weeks, the proportion of patients who were relapse free during the study, and the proportion of patients with a ≥7.5-point worsening from baseline on the MSIS-29 physical subscale at 96 weeks. Several clinical (including visual and cognitive) and MRI outcome measures served as tertiary end points.

After 144 weeks, the ARR was reduced from 1.6 to 0.39 (–76%) in the IFNβ-1a group and from 1.5 to 0.22 (–85%) in the daclizumab group, representing a 45% relative reduction for daclizumab compared with IFNβ-1a ($p<0.001$). The number of new or newly enlarged hyperintense lesions on T2-weighted images at week 96 was 54% lower in the daclizumab group than in the IFNβ-1a group ($p<0.001$). The estimated incidence of disability progression confirmed at 12 weeks was 16% with daclizumab and 20% with IFNβ-1a ($p=0.16$). The lack of superiority of daclizumab over IFNβ-1a in reducing disability progression can be attributed to low event rates for this end point seen also in other recent clinical trials and the significant impact of IM IFNβ-1a by itself on disability progression (37.2% relative reduction compared with placebo in the MSCRG trial[74]). This highlights the reduced statistical power of active comparative studies and the shortcoming of studies 2–3 years long in capturing the long-term treatment effect on disability progression. On the other hand, additional analyses of 24-weeks confirmed disability progression and outcomes on the MSFC, which were

tertiary end points and are published as supplemental material,[75] suggested a better effect of daclizumab. Analyses of additional two secondary endpoints – the on-study proportion of relapse-free patients and a clinically meaningful worsening in the physical subscale of the MSIS-29 did not show a significant difference between daclizumab and IFNβ-1a.

The overall incidence of AE was similar (91%) in both treatment arms. SAEs were reported in 15% of daclizumab patients vs 10% of those in the IFNβ-1a group, and more patients discontinued daclizumab because of AEs (14% vs 9%). Infections, the most frequent AE, were more common with daclizumab (65% vs 57%), as were serious infection (4% vs 2%). Cutaneous events (rash, eczema, erythema, seborrheic dermatitis, acne, and pruritus) were more common with daclizumab (37% vs 19%), classed as serious in 2% vs less than 1% and led to treatment discontinuation in 5% and 1% of patients, respectively. Elevations of hepatic transaminase levels >5 ULN occurred in 6% of the patients in the daclizumab group and in 3% of those in the IFNβ-1a group. Malignancies were reported in seven patients in the daclizumab group and in eight in the IFNβ-1a group.

Further analyses presented at recent scientific meetings demonstrated the superiority of daclizumab over IFNβ-1a also in subgroups of patients with baseline characteristics predictive of less or more active diseases.[76,77] Daclizumab was also more effective than IFNβ-1a in reducing the rate of brain volume loss,[78] improving cognitive outcomes,[79] and achieving no evidence of disease activity status over 2 years (24.3% vs 13.9%, $p<0.001$).[80]

The results of these large studies led to the approval of daclizumab-HYP 150 mg administered by SC injections monthly for the treatment of relapsing forms of MS by the US Food and Drug Administration in May 2016[81] and by the European Medical Agency in July 2016.[82]

Safety and tolerability

Daclizumab was generally well tolerated in all clinical trials reported so far. The rates of treatment discontinuation were similar between daclizumab and placebo groups in the large Phase II trials, but more patients discontinued daclizumab because of AEs in the Phase III DECIDE trial (14% vs 9%).[65] AEs reported with IV DAC-Nutley in the early open-label trials included skin reactions, lymphadenopathy, upper respiratory and urinary tract infections, transient elevation of liver enzymes, transient thrombocytopenia, mild lymphopenia and leukopenia, granuloma annulare, headache, paresthesias, autoantibodies, breast tenderness, and exacerbation of depression. No death or other SAEs were reported.[30–33,58–59] The

safety and tolerability of monthly IV DAC-Nutley 1 mg/kg have also been evaluated in 55 MS patients who received the drug off-label in a single center.[83] Most common AEs included fatigue (8), gastrointestinal upset (4), generalized weakness (3), and rash (3). Two cases with skin rashes were diagnosed as pityriasis rosacea and folliculitis. Interestingly, another patient with a family history of psoriasis developed this autoimmune condition for which daclizumab has been proposed as a treatment option. Lymphadenopathy, common infections including viral meningitis, allergic reactions, headache, paresthesias, and cardiac toxicity were reported in two patients each. One case of cardiac toxicity was accompanied by eosinophilia and pericardial and pleural effusions without evidence for infectious or malignant origin, which may represent an immune-mediated reaction. The other patient developed acute necrotizing eosinophilic myocarditis associated with a systemic necrotizing inflammatory process involving also the skin, liver, muscles, and spinal cord 5 days after starting treatment with modafinil, consistent with a severe drug-induced (probably modafinil) hypersensitivity reaction. The patient quickly deteriorated and died 20 days later. Another case consistent with an immune-mediated reaction was reported in a patient who continued IV daclizumab after completing one of the NIH open-label trials[33] and developed small-vessel CNS vasculitis associated with decrease in $CD4^+CD25^+$ FoxP3^{+} Tregs but without the expected expansion of CD56^{bright} NK cells. The authors speculated that the daclizumab-induced inhibition of Tregs without concomitant expansion of immunoregulatory CD56^{bright} NK cells (probably genetically determined) left the patient vulnerable to the effect of a short period of daclizumab interruption that led to decreased blood levels of the drug, which deprived her also from the other mechanism of action of daclizumab – inhibition of activation of antigen-specific T-cells by blocking IL-2 transpresentation by DCs.[84] The authors also suggested that genetic studies may identify susceptibility alleles that predispose patients to this immunologic complication and that the safety of daclizumab treatment may be enhanced by screening susceptible individuals or by implementing preventive therapy (eg, corticosteroids) during the vulnerable period after conclusion of daclizumab dosing.[84]

Five patients died during the DECIDE trial, including one in the daclizumab HYP group and four in the IFNβ-1a group. None of the deaths were considered by the investigators to be related to the study treatment.[65] On the other hand, for two deaths reported in the SELECT and the SELECTION trials the contributory role of daclizumab treatment was not excluded: one patient developed severe dermatitis then

psoas abscess that led to thrombosis of an adjacent mesenteric artery and fatal ischemic colitis.[63] Another patient in the washout and reinitiation group of the SELECTION trial[64] died because of autoimmune hepatitis (confirmed by increased serum IgG and liver histology findings at autopsy) after reinitiation of 300 mg daclizumab.

Generally, AEs attributed to daclizumab can be categorized into four main groups: infections, LFT abnormalities, skin reactions, and immune-mediated phenomena.

Infections (mainly of the upper respiratory and urinary tracts) were slightly increased in the initial open-label studies as well as in the DECIDE[65] and SELECTED[85] trials. Serious infections were more common in the daclizumab groups than in the placebo or IFNβ-1a groups in the CHOICE (5% vs 1%), SELECT (2% vs 0%), or DECIDE (4% vs 2%) trials. These infections were generally typical for the MS population without any pattern of specific organ or system involvement and usually resolved with standard therapy, often without treatment discontinuation. It is hypothesized that the expanded CD56^{bright} NK cell population may contribute to effective immunity against opportunistic infections and potentially also against cancer. In addition, unlike natalizumab and fingolimod, daclizumab does not prevent immune cells from accessing the CNS and protecting against opportunistic infections.[7] Indeed, no cases of progressive multifocal leukoencephalopathy (PML) have been reported with daclizumab, and the risk for herpes virus infections, which are associated with impaired T-cell-mediated immunity, was not increased.

Elevations of liver enzymes were frequent, typically occurred late during daclizumab treatment, resolved, and tended not to recur with continued treatment. The increased rate of elevation of LFT >5x the ULN in the daclizumab-treated patients and the case of fatal liver failure in the SELECTION trial led to the implementation of onsite testing of LFT immediately prior to each dosing of daclizumab in current clinical trials and monthly monitoring of LFT in clinical practice.

A variety of cutaneous AEs have been reported in the open-label studies and in 20%, 19%, and 37% of subjects treated with daclizumab in the SELECT,[63] SELECTION,[64] and DECIDE[65,86] trials, respectively. Most skin reactions were allergic in nature or rashes of various types and intensities, but eczema, contact dermatitis, erythema, urticaria, pruritus, folliculitis, exfoliative dermatitis, pityriasis rosea, leukocytoclastic vasculitis, alopecia, acne, lichenoid keratosis, seborrheic dermatitis, papulosquamous conditions, dry skin, and psoriasis have also been reported.[75,86–89] These cutaneous events were mostly mild to moderate in intensity

and resolved either spontaneously or after standard interventions such as topical steroid treatment; however, some severe and prolonged (albeit not life threatening) skin reactions required systemic steroids and/or discontinuation of therapy.[86] Serious cutaneous events (eg, exfoliative rash or dermatitis, toxic skin eruption) developed in about 1%–2% of the daclizumab-treated subjects. The development of diffuse or highly inflammatory rash may require referral to a dermatologist and discontinuation of daclizumab.[88] Sun-exposed areas and dry, scaling skin may predispose daclizumab-treated patients to skin rashes, and the routine use of sunscreen and skin moisturizers has been advised by some.[7] Inhibition of CD4+CD25+ Foxp3+ Tregs leading to unmasking or activation of resident T-cell populations that mediate skin reactions [28] and the expansion and local tissue infiltration of CD56^{bright} NK cells[90] have been hypothesized to underlie daclizumab-associated skin reactions, but this relationship is still inconclusive.[86,91]

These mechanisms may potentially underlie the development of other immune-mediated disorders including lymphadenopathy, noninfectious colitis and others that occur with daclizumab. Serious immune-mediated AEs developed in four patients in the SELECT trial (autoimmune thyroiditis, Crohn's disease, hypersensitivity, and lymphadenopathy)[63] and in five patients in the SELECTION trial (autoimmune hepatitis with fatal liver failure, Graves' disease, glomerulonephritis, and two cases of ulcerative colitis).[64] Surprisingly, no immune-mediated conditions secondary to daclizumab treatment were reported in the Phase III DECIDE trial.[65] Nevertheless, the Zinbryta US Prescribing Information does refer to these immune-mediated phenomena and notes that "Overall, serious immune-mediated conditions were observed in 5% of patients treated with Zinbryta", and that "in the active-control study (DECIDE), immune-mediated disorders were observed in 32% of Zinbryta-treated patients compared with 12% for Avonex-treated patients".[87] Across all clinical studies, immune-mediated disorders occurred in 28% of patients on daclizumab, the most common of which were skin reactions and lymphadenopathy or lymphadenitis. Mild generalized lymphadenopathy has been frequently observed in daclizumab-treated MS patients, since the early clinical trials, throughout the treatment period and resulted in discontinuation in 0.6% of the patients.[87] In several cases evaluated by fine needle biopsy, no pathology profile or changes in flow cytometry have been found.[7] Other immune-mediated conditions such as celiac disease, type I diabetes, immune hemolytic anemia, autoimmune thyroiditis, rheumatoid arthritis, pancreatitis, thrombocytopenia,

glomerulonephritis, sarcoidosis, and sialadenitis have also been observed in 2 or more daclizumab-treated patients. In some cases, patients had concurrent or sequentially occurring disorders while taking daclizumab. Some of the immune-mediated disorders may be life threatening or fatal, as in the case of autoimmune hepatitis in the SELECTION trial[64] and the case of severe dermatitis, psoas abscess, ischemic colitis and death in the SELECT trial.[63] If a patient develops a serious immune-mediated disorder, discontinuation of daclizumab should be considered and the patient should be referred to a specialist for further evaluation and treatment.

Because of the risks of severe liver injury and immune-mediated disorders, daclizumab has a boxed warning and is available in the United States only through a restricted distribution program under a Risk Evaluation and Mitigation Strategy called Zinbryta REMS Program that includes the need to obtain transaminase and bilirubin levels before starting daclizumab, monthly before the next dose during treatment, and up to 6 months after the last dose, and a contraindication for using daclizumab in patients with preexisting hepatic disease or impairment.

Additional AEs that have been observed with daclizumab include depression, seizures, oropharyngeal pain not associated with upper respiratory tract infection, and pancreatitis. Of interest is an unrelated case of fatal pancreatitis reported in a series of patients who underwent intestinal transplantation and were treated with daclizumab (Zenapax) for induction of immunosuppression.[92] Lymphopenia may be observed with daclizumab, which is generally mild to moderate ($\geq$500/mm^3) and similar to that observed in the control groups in clinical trials. Although sustained severe lymphopenia (<500/mm^3) was not observed in clinical studies with daclizumab, monitoring of complete blood count is recommended every 3 months. Three malignancies (cervical carcinoma and two cases of melanoma) considered not to be related to daclizumab treatment were reported in the SELECT trial, and one patient in the SELECTION trial developed breast carcinoma. The rate of malignancies did not differ between daclizumab and IFNβ-1a in the DECIDE trial (7 and 8, respectively).

Despite the mild immunosuppressive effects of daclizumab and the increased incidence of infectious complications in clinical trials, patients with MS under daclizumab therapy mount normal antibody responses to influenza vaccinations.[93] However, vaccination with live vaccines is not recommended during treatment and up to 4 months after discontinuation of daclizumab.[87]

Studies in monkeys showed no adverse effects related to daclizumab on fertility, maternal well-being, embryo–fetal development, and postnatal development and growth. Thirty eight pregnancies were reported across the clinical development program in MS, with no increased risk of spontaneous abortion or adverse fetal or maternal outcomes.[94]

Conclusion

Daclizumab, the first therapeutic humanized monoclonal antibody (mAb), is also the first-in-class IL-2R modulator to join the rapidly growing armamentarium of immune modulators for the treatment of relapsing MS. The original assumption that blocking IL-2 signaling in T-cells with daclizumab will lead to inhibition of effector T-cells and suppression of detrimental pathways in MS has been replaced by novel and unique mechanisms of expansion, differentiation, and enhanced cytotoxicity of regulatory CD56^{bright} NK cells, attenuation of early T-cell activation via blocking IL-2 transpresentation by DCs, and possible reduction in the number of proinflammatory LTi cells, resulting in enhancement of endogenous mechanisms of immune regulation. A series of in vivo observations and mechanistic in vitro studies conducted along with clinical trials in active MS patients that demonstrated robust effect of daclizumab on inflammatory disease activity which correlates with the expansion of CD56^{bright} NK cells underly these discoveries. Monoclonal antibodies that target a single molecular epitope by definition are expected to be highly selective in their mode of action, thereby providing improved efficacy and lesser off-target toxicity. Yet, mAbs exert multiple biological effects as has been demonstrated with daclizumab, attributed to the pleiotropy of functions and situational diversity characteristic of the complex human immune system. Therefore, it is not unlikely that additional mechanisms of action and newly emerging AEs of daclizumab will be unveiled in the future.

The clinical program that evaluated daclizumab in MS demonstrated significant and consistent beneficial effects on clinical and MRI disease measures that led to the recent approval of the drug for patients with the relapsing forms of MS. However, the enhanced efficacy of daclizumab is accompanied by an increased frequency of AEs and risks of serious AEs, which , according to the US Prescribing Information but not the European one, restrict its use to patients who have had an inadequate response to two or more disease-modifying agents and necessitates the implementation of a strict risk management program.

In addition to efficacy, safety, and tolerability issues, other factors should be considered when selecting a treatment option in a given patient, such as compliance, adherence to treatment and monitoring programs, convenience of administration, comorbidities, possible interactions with other drugs, suitability for the patient's lifestyle and preferences, pregnancy issues, immunization status, previous immunosuppressive therapies, risks of specific AE, treatment access and logistics, regulatory status, social and family support systems, and cost. Unlike other therapeutic mAbs for MS that need to be administered IV, the SC administration of daclizumab is not associated with infusion-related reactions and enables self-injection at home rather than in specialized infusion centers. The once-monthly administration of daclizumab also provides an advantage over other injectable MS drugs that are administered more frequently. This may improve compliance and adherence to treatment. To date, daclizumab is not known to interact with immunomodulatory drugs or other drugs commonly used by MS patients. However, caution should be used when using concomitant hepatotoxic drugs.

Serious safety limitations (eg, increased risk of PML after 2 years of natalizumab treatment, or prolonged immunosuppression with alemtuzumab) may restrict the duration of treatment with other highly effective mAbs. 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 clinical trials.

MS is a highly variable disease and may be highly aggressive and disabling in certain patients, taking a high toll on patients, caregivers, and health systems. Such patients may need highly effective treatment early in the course of their disease in order to halt disease progression and prevent accumulation of further disability. Daclizumab seems to have a favorable risk–benefit ratio in such patients and may even be considered as a first-line treatment option in highly active patients, especially those with poor prognostic factors that may predict rapid disease progression.[95] Using daclizumab as an additional therapeutic option for MS is also in line with current concepts in MS therapy of "No Evidence of Disease Activity", "treat to target", and "zero tolerance for disease activity", adopted by many neurologists in order to meet higher treatment goals and reach better disease outcomes.[96]

Disclosure
The authors report no conflicts of interest in this work.

References
1. Compston A, Coles A. Multiple sclerosis. *Lancet.* 2008;372(9648):1502–1517.
2. Milo R, Kahana E. Multiple sclerosis: geoepidemiology, genetics and the environment. *Autoimmun Rev.* 2010;9(5):A387–A394.

3. Hemmer B, Nessler S, Zhou D, Kieseier B, Hartung HP. Immunopathogenesis and immunotherapy of multiple sclerosis. *Nat Clin Pract Neurol.* 2006;2(4):201–211.

4. Waldmann TA. Anti-Tac (daclizumab, Zenapax) in the treatment of leukemia, autoimmune diseases, and in the prevention of allograft rejection: a 25-year personal odyssey. *J Clin Immunol.* 2007;27(1):1–18.

5. Adu D, Cockwell P, Ives NJ, Shaw J, Wheatley K. Interleukin-2 receptor monoclonal antibodies in renal transplantation: meta-analysis of randomised trials. *BMJ.* 2003;326(7393):789.

6. Nussenblatt RB, Fortin E, Schiffman R, et al. Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: a phase I/II clinical trial. *Proc Natl Acad Sci U S A.* 1999;96(13): 7462–7466.

7. Bielekova B. Daclizumab therapy for multiple sclerosis. *Neurotherapeutics.* 2013;10(1):55–67.

8. Hafler DA, Compston A, Sawcer S, et al. Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med.* 2007;357(9): 851–862.

9. Depper JM, Leonard WJ, Robb RJ, Waldmann TA, Greene WC. Blockade of the interleukin-2 receptor by anti-Tac antibody: inhibition of human lymphocyte activation. *J Immunol.* 1983;131(2):690–696.

10. Cavazzana-Calvo M, Fromont C, Le Deist F, et al. Specific elimination of alloreactive T cells by an anti-interleukin-2 receptor B chain-specific immunotoxin. *Transplantation.* 1990;50(1):1–7.

11. Wiendl H, Gross CC. Modulation of IL-2R alpha with daclizumab for treatment of multiple sclerosis. *Nat Rev Neurol.* 2013;9(7):394–404.

12. Malek T. The biology of interleukin-2. *Annu Rev Immunol.* 2008;26: 453–479.

13. Smith KA. Interleukin-2: inception, impact, and implications. *Science.* 1988;240(4856):1169–1176.

14. Hémar A, Subtil A, Lieb M, Morelon E, Hellio R, Dautry-Varsat A. Endocytosis of interleukin 2 receptors in human T lymphocytes: distinct intracellular localization and fate of the receptor α, β, and γ chains. *J Cell Biol.* 1995;129(1):55–64.

15. Martin R. Anti-CD25 (daclizumab) monoclonal antibody therapy in relapsing–remitting multiple sclerosis. *Clin Immunol.* 2012;142(1):9–14.

16. Poli A, Michel T, Thérésine M, Andrès E, Hentges F, Zimmer, J. CD56bright natural killer (NK) cells: an important NK cell subset. *Immunology.* 2009;126(4):458–465.

17. Bielekova B, Catalfamo M, Reichert-Scrivner S, et al. Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2R alpha-targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci U S A.* 2006;103(15):5941–5946.

18. Wuest SC, Edwan JH, Martin JF, et al. A role for interleukin-2 transpresentation in dendritic cell-mediated T cell activation in humans, as revealed by daclizumab therapy. *Nat Med.* 2011;17(5):604–609.

19. Sadlack B, Löhler J, Schorle H, et al. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+ T cells. *Eur J Immunol.* 1995;25(11):3053–3059.

20. Suzuki H, Kündig TM, Furlonger C, et al. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor β. *Science.* 1995;268(5216):1472–1476.

21. Malek TR, Yu A, Vincek V, Scibelli P, Kong L. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rβ-deficient mice. Implications for the nonredundant function of IL-2. *Immunity.* 2002;17(2):167–178.

22. Bayer AL, Yu A, Malek TR. Function of the IL-2R for thymic and peripheral CD4+CD25+Foxp3+ T regulatory cells. *J Immunol.* 2007;178(7):4062–4071.

23. Caudy A, Reddy ST, Chatila T, Atkinson JP, Verbsky JW. CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. *J Allergy Clin Immunol.* 2007;119(2):482–487.

24. Sharfe N, Dadi HK, Shahar M, Roifman CM. Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. *Proc Natl Acad Sci U S A.* 1997;94(7):3168–3171.

25. Bielekova B, Becker B. Monoclonal antibodies in MS: mechanisms of action. *Neurology.* 2010;74(Suppl 1):S31–S40.

26. McDyer JF, Li Z, John S, Yu X, Wu CY, Ragheb JA. IL-2 receptor blockade inhibits late, but not early, IFN-gamma and CD40 ligand expression in human T cells: disruption of both IL-12-dependent and -independent pathways of IFN-gamma production. *J Immunol.* 2002;169(5): 2736–2746.

27. Zhang Y, McClellan M, Efros L, et al. Daclizumab reduces CD25 levels on T cells through monocyte-mediated trogocytosis. *Mult Scler.* 2014; 20(2):156–164.

28. Oh U, Blevins G, Griffith G, et al. Regulatory T cells are reduced during anti-CD25 antibody treatment of multiple sclerosis. *Arch Neurol.* 2009;66(4):471–479.

29. Baan CC, Balk AH, van Riemsdijk IC, et al. Anti-CD25 monoclonal antibody therapy affects the death signals of graft-infiltrating cells after clinical heart transplantation. *Transplantation.* 2003;75(10):1704–1710.

30. Bielekova B, Richert N, Howard T, et al. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. *Proc Natl Acad Sci U S A.* 2004;101(23): 8705–8708.

31. Rose JW, Watt HE, White AT, Carlson NG. Treatment of multiple sclerosis with an anti-interleukin-2 receptor monoclonal antibody. *Ann Neurol.* 2004;56(6):864–867.

32. Bielekova B, Howard T, Packer AN, et al. Effect of anti-CD25 antibody daclizumab in the inhibition of inflammation and stabilization of disease progression in multiple sclerosis. *Arch Neurol.* 2009;66(4):483–489.

33. Bielekova B, Richert N, Herman ML, et al. Intrathecal effects of daclizumab treatment of multiple sclerosis. *Neurology.* 2011;77(21): 1877–1886.

34. Elkins J, Sheridan J, Amaravadi L, et al. CD56(bright) natural killer cells and response to daclizumab HYP in relapsing-remitting MS. *Neurol Neuroimmunol Neuroinflamm.* 2015;2(2):e65.

35. Sheridan JP, Zhang Y, Efros L, Shi JD, Vexler V. Anti-CD25 antibody daclizumab *in vitro* greatly enhances IL-2-induced expansion of CD56bright NK cells [abstract]. *Neurology.* 2008;70(Suppl 1):A376.

36. Airas L, Saraste M, Rinta S, et al. Immunoregulatory factors in multiple sclerosis patients during and after pregnancy: relevance of natural killer cells. *Clin Exp Immunol.* 2008;151(2):235–243.

37. Hao J, Liu R, Piao W, et al. Central nervous system (CNS)-resident natural killer cells suppress Th17 responses and CNS autoimmune pathology. *J Exp Med.* 2010;207(9):1907–1921.

38. Hao J, Campagnolo D, Liu R, et al. Interleukin-2/interleukin-2 antibody therapy induces target organ natural killer cells that inhibit central nervous system inflammation. *Ann Neurol.* 2011;69(4):721–734.

39. Martin JF, Perry JS, Jakhete NR, Wang X, Bielekova B. An IL-2 paradox: blocking CD25 on T cells induces IL-2-driven activation of CD56(bright) NK cells. *J Immunol.* 2010;185(2)1311–1320.

40. Cooper MA, Fehniger TA, Turner SC, et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood.* 2001;97(10):3146–3151.

41. Della Chiesa M, Vitale M, Carlomagno S, Ferlazzo G, Moretta L, Moretta A. The natural killer cell mediated killing of autologous dendritic cells is confined to a cell subset expressing CD94/NKG2A, but lacking inhibitory killer Ig-like receptors. *Eur J Immunol.* 2003;33(10):1657–1666.

42. Lünemann A, Lünemann JD, Roberts S, et al. Human NK cells kill resting but not activated microglia via NKG2D- and NKp46-mediated recognition. *J Immunol.* 2008;181(9):6170–6177.

43. Jiang W, Chai NR, Maric D, Bielekova B. Unexpected Role for Granzyme K in CD56bright NK Cell-Mediated immunoregulation of multiple sclerosis. *J Immunol.* 2011;187(2):781–790.

44. Mnasria, K. Lagaraine C, Velge-Roussel F, Oueslati R, Lebranchu Y, Baron C. Anti-CD25 antibodies affect cytokine synthesis pattern of human dendritic cells and decrease their ability to prime allogeneic CD4+ T cells. *J Leukoc Biol.* 2008;84(2):460–467.

45. Withers DR, Gaspal FM, Mackley EC, et al. Cutting edge: lymphoid tissue inducer cells maintain memory CD4 T cells within secondary lymphoid tissue. *J Immunol.* 2012;189(5):2094–2098.

46. Lane PJ, Gaspal FM, McConnell FM, Kim MY, Anderson G, Withers DR. Lymphoid tissue inducer cells: innate cells critical for CD4 (+) T cell memory responses? *Ann N Y Acad Sci U S A.* 2012;1247:1–15.

47. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain.* 2007;130(pt 4):1089–1104.

48. Perry JS, Han S, Xu Q, et al. Inhibition of LTi cell development by CD25 blockade is associated with decreased intrathecal inflammation in multiple sclerosis. *Sci Transl Med.* 2012;4:145ra06.

49. Gillard GO, Saenz SA, Huss DJ, Fontenot JD. Circulating innate lymphoid cells are unchanged in response to DAC HYP therapy. *J Neuroimmunol.* 2016;294:41–45.

50. Sheridan JP, Zhang Y, Vexler V, et al. Daclizumab treatment reduces activated T cells: results from the CHOICE MS study. *Neurology.* 2009;72(11 Suppl 3):A35.

51. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J Exp Med.* 2004;199(7):971–979.

52. Gold R, Selmaj K, You X, Sheridan J, Fontenot J, Elkins J. Regulatory T-cell numbers in patients with RRMS were not associated with clinical or MRI outcomes in the SELECT study. *Mult Scler.* 2012; 18(Suppl 4):195.

53. Diao L, Hang Y, Othman AA, et al. Population PK-PD analyses of CD25 occupancy, CD56^bright NK cell expansion, and regulatory T cell reduction by daclizumab HYP in subjects with multiple sclerosis. *Br J Clin Pharmacol.* 2016;82(5):1333–1342.

54. Yu A, Zhu L, Altman NH, Malek TR. A low interleukin-2 receptor signaling threshold supports the development and homeostasis of T regulatory cells. *Immunity.* 2009;30(2):204–217.

55. Heninger AK, Theil A, Wilhelm C, et al. IL-7 abrogates suppressive activity of human CD4 + CD25 +FOXP3+ regulatory T cells and allows expansion of alloreactive and autoreactive T cells. *J Immunol.* 2012;189(12):5649–5658.

56. Bayer AL, Lee JY, de la Barrera A, Surh CD, Malek TR. A function for IL-7R for CD4+CD25+Foxp3+ T regulatory cells. *J Immunol.* 2008; 181(1):225–234.

57. Rech AJ, Mick R, Martin S, et al. CD25 blockade depletes and selectively reprograms regulatory T cells in concert with immunotherapy in cancer patients. *Sci Transl Med.* 2012;4(134):134ra62.

58. Huss DJ, Mehta DS, Sharma A, et al. In vivo maintenance of human regulatory T cells during CD25 blockade. *J Immunol.* 2015;194(1):84–92.

59. Rose J, Burns J, Bjorklund J, Klein J, Watt HE, Carlson NG. Daclizumab phase II trial in relapsing and remitting multiple sclerosis: MRI and clinical results. *Neurology.* 2007;69(8):785–789.

60. Rojas MA, Carlson NG, Miller TL, Rose JW. Long-term daclizumab therapy in relapsing-remitting multiple sclerosis. *Ther Adv Neurol Disord.* 2009;2(5):291–297.

61. Gorman MP, Tillema JM, Ciliax AM, Guttmann CR, Chitnis T. Daclizumab use in patients with pediatric multiple sclerosis. *Arch Neurol.* 2012;69(1):78–81.

62. Wynn D, Kaufman M, Montalban X, et al. Daclizumab in active relapsing multiple sclerosis (CHOICE study): a phase 2, randomised, double-blind, placebo-controlled, add-on trial with interferon beta. *Lancet Neurol.* 2010;9(4):381–390.

63. Gold R, Giovannoni G, Selmaj K, et al. Daclizumab high-yield process in relapsing-remitting multiple sclerosis (SELECT): a randomised, double-blind, placebo-controlled trial. *Lancet.* 2013;381(9884):2167–2175.

64. Giovannoni G, Gold R, Selmaj K, et al. Daclizumab high-yield process in relapsing-remitting multiple sclerosis (SELECTION): a multicentre, randomised, double-blind extension trial. *Lancet Neurol.* 2014;13(5):472–481.

65. Kappos L, Wiendl H, Selmaj K, et al. Daclizumab HYP versus interferon beta-1a in relapsing multiple sclerosis. *N Engl J Med.* 2015; 373(15):1418–1428.

66. Tran JQ, Othman AA, Mikulskis A, Wolstencroft P, Elkins J. Pharmacokinetics of daclizumab high-yield process with repeated administration of the clinical subcutaneous regimen in patients with relapsing-remitting multiple sclerosis. *Clin Pharmacol.* 2016;8:9–13.

67. Giovannoni G, Radue EW, Havrdova E, et al. Effect of daclizumab high-yield process in patients with highly active relapsing-remitting multiple sclerosis. *J Neurol.* 2014;261(2):316–323.

68. Radue EW, Sprenger T, Vollmer T, et al. Daclizumab high-yield process reduced the evolution of new gadolinium-enhancing lesions to T1 black holes in patients with relapsing-remitting multiple sclerosis. *Eur J Neurol.* 2016;23(2):412–415.

69. Havrdova E, Giovannoni G, Stefoski D, et al. Disease-activity-free status in patients with relapsing-remitting multiple sclerosis treated with daclizumab high-yield process in the SELECT study. *Mult Scler.* 2014;20(4):464–470.

70. Phillips G, Guo S, Bender R, Havrdová E, Proskorovsky I, Vollmer T. Assessing the impact of multiple sclerosis disease activity and daclizumab HYP treatment on patient-reported outcomes: results from the SELECT trial. *Mult Scler Relat Disord.* 2016;6:66–72.

71. Selmaj K, Gold R, You X, et al. Regulatory T-Cell numbers in patients with relapsing-Remitting MS were not associated with clinical or MRI outcomes in the SELECT study [abstract]. Presented at: 65th Annual Meeting of the American Academy of Neurology; March 16–23; 2013; San Diego, CA, P05.192.

72. Giovannoni G, Gold R, Selmaj K, et al. The safety and efficacy of daclizumab HYP in relapsing-remitting multiple sclerosis in the SELECTION extension study: primary results [abstract]. Presented at: 65th Annual Meeting of the American Academy of Neurology; March 16–23; 2013; San Diego, CA, S01.001.

73. Mehta M, Amaravadi L, Gold R. Reversal of the pharmacodynamic effects of daclizumab HYP following treatment washout: results from the SELECTION study [abstract]. Presented at: 65th Annual Meeting of the American Academy of Neurology; March 16–23; 2013; San-Diego, CA, P05:188.

74. Jacobs LD, Cookfair DL, Rudick RA, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. *Ann Neurol.* 1996;39(3):285–294.

75. Supplementary Appendix to: Kappos L, Wiendl H, Selmaj K, et al. Daclizumab HYP versus interferon beta-1a in relapsing multiple sclerosis. *N Engl J Med.* 2015;373:1418–1428. Available from: http://www.nejm.org/doi/suppl/10.1056/NEJMoa1501481/suppl_file/nejmoa1501481_appendix.pdf. Accessed July 29, 2016.

76. Wiendl H, Kappos L, Selmaj K, et al. Daclizumab high-yield process (DAC HYP) vs intramuscular interferon beta-1a in subgroups predictive of active disease: results from the DECIDE Study. Presented at: Annual Meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 7–10, 2015; Barcelona, Spain. *Mult Scler.* 2015;21(11 Suppl):233–234, P523.

77. Cohan S, Kappos L, Wiendl H, et al. Efficacy of daclizumab HYP vs intramuscular interferon beta-1a on disability progression across patient demographic and disease activity subgroups in DECIDE. Presented at: Annual Meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 7–10; 2015; Barcelona, Spain. *Mult Scler.* 2015;21(11 Suppl):260–261, P561.

78. Arnold DL, Kappos L, Khan O, et al. Reduction in brain volume loss in patients receiving daclizumab HYP versus intramuscular Interferon beta-1a: results of the DECIDE study. Presented at: Annual Meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 7–10; 2015; Barcelona, Spain. *Mult Scler.* 2015;21(11 Suppl):257–258, P558.

79. Benedict RHB, Cohan S, Lynch S, et al. Improved cognitive outcomes in relapsing-remitting multiple sclerosis with daclizumab HYP over 96 weeks in the DECIDE study. Presented at: 68th Annual Meeting of the American Academy of Neurology; April 15–21, 2016; Vancouver, Canada. *Neurology.* 2016;86(16 Suppl):P3.090.

80. Kappos L, Havrdova E, Giovannoni G, et al. Effect of daclizumab HYP versus intramuscular interferon beta-1a on no evidence of disease activity in patients with relapsing-remitting multiple sclerosis: analysis of the DECIDE study. Presented at: Annual Meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 7–10, 2015; Barcelona, Spain. *Mult Scler.* 2015;21(11 Suppl):22. Abstract 89.

81. FDA approves Zinbryta to treat multiple sclerosis. Available from: www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm504000.htm. Accessed July 30, 2016.

82. European Medicinal Agency. Zinbryta. Authorization Details. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003862/human_med_001987.jsp&mid=WC0b01ac058001d124. Accessed July 30, 2016.

83. Ali E, Healy B, Stazzone L, Brown BA, Weiner HL, Khoury SJ. Daclizumab in treatment of multiple sclerosis patients. *Mult Scler*. 2009;15(2):272–274.

84. Ohayon J, Oh U, Richert N, et al. CNS vasculitis in a patient with MS on daclizumab monotherapy. *Neurology*. 2013;80(5):453–457.

85. Gold R, Radue EW, Giovannoni G, et al. Safety and efficacy of daclizumab in relapsing-remitting multiple sclerosis: 3-year results from the SELECTED open-label extension study. *BMC Neurol*. 2016;16:117.

86. Krueger JG, Kircik L, Hougeir F, et al. Cutaneous adverse events in the randomized, double-blind, active-comparator DECIDE study of daclizumab high-yield process versus intramuscular interferon beta-1a in relapsing-remitting multiple sclerosis. *Adv Ther*. 2016;33(7):1231–1245.

87. Zinbryta Prescribing Information. Available from: https://www.zinbryta.com/content/dam/commercial/multiple-sclerosis/zinbryta/na/en_us/pdfs/zinbryta-prescribing-information.pdf. Accessed July 30, 2016.

88. Zinbryta: Summary of Product Information. Available from: http://ec.europa.eu/transparency/regdoc/rep/3/2016/EN/3-2016-4260-EN-F1-1-ANNEX-1.PDF. Accessed July 30, 2016.

89. Milo R. The efficacy and safety of daclizumab and its potential role in the treatment of multiple sclerosis. *Ther Adv Neurol Disord*. 2014;7(1):7–21.

90. Oh J, Saidha S, Cortese I, et al. Daclizumab-induced adverse events in multiple organ systems in multiple sclerosis. *Neurology*. 2014;82(11):984–988.

91. Huss DJ, Mehta DS, Sharma A, et al. In vivo maintenance of human regulatory T cells during CD25 blockade. *J Immunol*. 2015;194(1):84–92.

92. Nishida S, Levi D, Kato T, et al. Ninety-five cases of intestinal transplantation at the University of Miami. *J Gastrointest Surg*. 2002;6(2):233–239.

93. Lin YC, Winokur P, Blake A, et al. Patients with MS under daclizumab therapy mount normal immune responses to influenza vaccination. *Neurol Neuroimmunol Neuroinflamm*. 2016;3(1):e196.

94. Gold R, Stefoski D, Selmaj K, et al. Pregnancy experience: nonclinical studies and pregnancy outcomes in the daclizumab clinical study program. *Neurol Ther*. Epub July 13, 2016.

95. Milo R. Effectiveness of multiple sclerosis treatment with current immunomodulatory drugs. *Expert Opin Pharmacother*. 2015;16(5):659–673.

96. Giovannoni G, Turner B, Gnanapavan S, Offiah C, Schmierer K, Marta M. Is it time to target no evident disease activity (NEDA) in multiple sclerosis? *Mult Scler Relat Disord*. 2015;4(4):329–333.