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Efficacy and Side Effects of Natalizumab Therapy in Patients with Multiple Sclerosis

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ABSTRACT: Natalizumab (Nat) is a humanized monoclonal antibody used for the treatment of relapsing multiple sclerosis (MS). Nat inhibits lymphocyte migration via the blood brain barrier (BBB) by blockage of an integrin adhesion molecule, very late antigen 4. During the phase III clinical trials, it was shown that Nat reduces disease activity and prevents disability progression. In addition, several smaller studies indicate a positive influence of Nat on cognition, depression, fatigue, and quality of life (Qol). Therapeutic efficacy has to be weighed against the risk of developing potentially fatal progressive multifocal leukoencephalopathy (PML), an opportunistic infection by JC-virus (JCV) with an incidence of 3.4/1000 (95% CI 3.08–3.74) in Nat treated MS patients. In this review article, we will review data on the presumed mechanism of Nat action, clinical and paraclinical efficacy parameters, and adverse drug reactions with a special focus on PML.

KEYWORDS: immunomodulatory therapy, progressive multifocal leukoencephalopathy, side effects, risk management strategies

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Introduction

Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS) that affects nearly 2.5 million people worldwide, with preponderance for female gender.[1–4] Data from some geographical regions suggest an increase of MS incidence.[5] MS is associated with significant disability.[3] In addition, approximately 60% of MS patients report cognitive symptoms[6,7] and up to 75% complain about fatigue.[8] Overall, MS leads to a marked reduction in quality of life (Qol) with major socioeconomic burden.[9]

Current disease modifying therapy is focused on modulation and suppression of the immune system with encouraging results in relapsing phenotypes. The monoclonal antibody Natalizumab (Nat) represents a paradigm where identification of a major immunological mechanism in an animal model, experimental autoimmune encephalomyelitis (EAE) rapidly led to the development of a specific immunotherapy. However, the introduction of this first monoclonal antibody for MS therapy targeting a specific antigen involved in leukocyte migration was set off by the occurrence of a previously unanticipated adverse drug reaction. The occurrence of an opportunistic CNS infection, progressive multifocal leukoencephalopathy (PML), led to therapy restriction due to risk-benefit considerations. Like other monoclonal antibodies with presumed highly specific mechanisms of action, initial expectations for Nat on therapeutic efficacy and favorable safety profile were high. However, early reports of PML that had occurred during the phase III trials[10,11] and with an increasing number in the post marketing setting has led to different risk stratification strategies.

According to the European Medicines Agency (EMA), Nat is approved for the treatment of relapsing remitting

multiple sclerosis (RRMS) patients with high disease activity despite treatment with glatiramer acetate or beta interferon. Patients should have at least one relapse in the previous year and at least nine T2-hyperintense lesions on cranial MRI, or at least one gadolinium enhancing lesion on T1 weighted MRI.[12] A non-responder to therapy is defined as a patient with unchanged or increased relapse rate or severe relapses compared to the previous year in which a full and adequate treatment was performed.[12] Nat was also approved by EMA for patients with high disease activity independent of pre-treatment (≥2 disabling relapses within one year and at least one gadolinium enhancing lesion or a significant increase of MRI lesion load on T2 weighted imaging).[12,13] Following the US Food and Drug Administration (FDA) approval, Nat can additionally be prescribed to patients, who are unable to tolerate an alternate MS therapy.[14]

Mode of Action and Pharmacology of Nat

In their 1992 landmark paper, Yednock et al. demonstrated in an animal model of MS that blockade of α4β1 integrins (very late antigen 4, VLA4) inhibited the transmigration of encephalitogenic lymphocytes via the blood brain barrier (BBB) into the CNS-parenchyma.[15] Only 13 years after the identification of VLA4 as a major determinant of leukocyte migration in the animal model, the introduction of the therapeutic monoclonal antibody Nat in 2005 represented a major milestone in the development of MS therapies.

Nat is a humanized antibody on an IgG4 backbone, which does not activate complement.[16] It is directed against the α4 chain of the α4β1 and α4β7 integrin heterodimers.[17,18]

It is infused every four weeks with a standard dosage of 300 mg, achieving a mean plasma concentration of 110 ± 52 µg/mL.[13,19] While half-life of Nat is 16 ± 4 days,[20] saturation of its target structure VLA4 amounts to >70%, four weeks after the last infusion.[21] Several factors influence Nat pharmacokinetics after application. Co-medication with interferon beta-1a increases the serum concentration of Nat.[22] Higher body weight reduces Nat concentration.[13] Antibodies against Nat cause a three-fold increased Nat clearance with reduced Nat serum concentration.[13] Consequently, patients with persistently positive anti-Nat antibodies have significantly lower Nat serum concentration and reduced therapeutic efficacy.[23] Plasma exchange (PLEX) or immunoadsorption rapidly decreases Nat concentration and VLA4 saturation.[21] After three sessions of PLEX, Nat concentration is reduced by 93% in comparison to baseline Nat concentration.[21] At a Nat concentration below 1 µg/mL, which is achieved after five PLEX performed on alternate days with 1.5 plasma volumes per PLEX, saturation of VLA4 is reduced to less than 50% in at least 95% of the patients.[21]

Efficacy of Nat

A summary of reported therapeutic effects of Nat using different clinical and paraclinical outcome parameters is given in Table 1.

Clinical and MRI Disease Activity

In an early phase II study, Tubridy et al. investigated the effect of Nat on disease activity in 72 MS patients[24] in a randomized, double blind placebo controlled trial (RRMS and secondary progressive MS). Patients were treated with two iv infusions of Nat or placebo. Nat treated patients had fewer new active or enhancing lesions on MRI during the first 12 weeks of treatment.[24] Based on this promising results, the GLANCE study, a phase two randomized (1:1) double blind placebo controlled trial, investigated 110 RRMS patients treated with Nat + placebo or Glatiramer acetate + placebo[25]. Here Nat demonstrated to be more effective than Glatiramer acetate on MRI outcome parameters.[25]

The following two pivotal phase III clinical trials were the basis for approval of Nat (see AFFIRM and SENTINEL).[19,26] In the two year placebo controlled AFFIRM trial, in a total of 942 patients with at least one relapse in the previous year and an Extended Disability Status Scale (EDSS) score between 0 and 5.0 were randomized 2:1 to Nat (300 mg iv every four weeks, n = 627) or placebo (n = 315).[19]

The second phase III clinical trial (SENTINEL) included 1171 RRMS patients in total with at least one relapse during the previous year and an EDSS score between 0 and 5.0.[26] Of these, 589 patients received interferon beta 1a (30 µg once weekly im) in combination with Nat (300 mg every four weeks) and 582 patients received interferon beta 1a plus placebo.[26] Primary endpoints in both studies were the rate of clinical relapse at one year and the cumulative probability of sustained disability progression at two years.[19,26] With regard to these primary endpoints, in the placebo controlled AFFIRM trial, annualized relapse rate was reduced after one year by 68% ($P < 0.001$).[19] This relative reduction at one year was also maintained at the end of study after two years ($P < 0.001$).[19] Additionally, over a study period of two years, the randomized phase III SENTINEL trial demonstrated a strongly reduced annual relapse rate in the patients treated with Nat and interferon beta 1a compared to interferon beta 1a monotherapy (0.34 vs. 0.75, $P < 0.001$).[26]

In AFFIRM, Nat reduced the risk of 12 weeks sustained disability progression as quantified by EDSS by 42% over a period of two years ($P < 0.001$).[19] Likewise, a beneficial effect of Nat on disability progression was confirmed by SENTINEL. Here the combination of Nat and interferon beta 1a led to a 24% reduction in comparison to IFNb1a monotherapy ($P = 0.02$).[26] The accumulation of new or enlarging T2-hyperintense lesions as well as gadolinium uptake on T1 weighted imaging on cranial MRI were studied in AFFIRM and SENTINEL as secondary MRI endpoints.[19,26] Both trials showed a profound reduction in these MRI parameters of disease activity (T2-lesions – 83% in AFFIRM, – 83% in SENTINEL; Gd – 92% AFFIRM, – 89% in SENTINEL).[19,26]

Analysis of Combined Clinical and MRI Endpoints

Havrdova et al. retrospectively analyzed the AFFIRM data and introduced the absence of clinical and radiological disease

Table 1. Therapeutic effects of Nat.

EFFECT OF NATALIZUMAB	RESULT
Parameters of clinical progression	
Annual relapse rate	81% ↓,[36] African Americans 60% ↓[97]
Reduction of Annual relapse rate	1,26[98]
Relapse	68% ↓[19]
Risk of sustained disability progression	42% ↓[19]
Probability of progression	17%[19]
Proportion of relapse free patients	64%[27]
Disease free (no new Gd+ lesions and no relapse)	37%,[27] 57%,[99] 62%[100]
Parameters of radiological progression	
Gd+ lesions	No Gd uptake in 95%,[27] 92%↓[19]; African Americans 79%↓[97]
Evoked potentials	
VEP	Improvement in 33%[101]
MEP	Improvement in 32%[101]
SEP	No difference[101]
Visual improvement	
Low contrast acuity	Cumulative probability of visual improvement 57%[102]
EDSS and disability	
EDSS improvement	Decrease from 2.7 to 1.9 (difference 0,8) in a paediatric population,[103] 29% of the patients (EDSS ≥1)[100]
Overall disability progression	64% ↓[36]
Scores of ambulation	
25 foot walk test	Responders walk 24–45% faster[104]
6 minute walk test	Improvement[38]
Cognition	
Risk of cognitive decline	43% ↓[42]
Memory tasks	Improvement[44]
Executive function	Improvement[44]
Overall reduction	Decrease from 29% cognitive impaired patients to 19% (difference 10%)[39]
Fatigue	
Motoric	Improvement[38]
Cognitive	Improvement[38]
Unclassified	Improvement[39,48]
Depression	
Change	Improvement[38]
Measurements of Quality of life	
Visual analogue scale	Improvement[28]
Overall Quality of life	Improvement[38]
Hospitalization	64% ↓[42]
Ability to work	
Sickness benefits	41% ↓[49]

Note: A publication bias could not be excluded.

activity as a compound endpoint ("free of disease activity").[27] 64% of the Nat versus 39% of the placebo treated patients were free of clinical disease activity, both in terms of relapses and disability progression. In comparison to 14% patients under placebo, 58% Nat treated patients were free of radiological disease activity.[27] Combination of clinical and radiological parameters resulted in 37% of the patients in the Nat treatment group who were free of disease activity in contrast to 7% of the placebo treated patients.[27] Yet, the combination of these endpoints may be biased by the inclusion of the MRI endpoint as this was the main source of differences between the treatment groups.[27]

Nat and the Progressive Phase of MS

In the randomized double blind placebo controlled phase II trial, 69 of the 213 patients included were in the secondary progressive phase of the disease, however with superimposed relapses.[28] Placebo (n = 26) or Nat was given every 28 days for six months in a dosage of 3 mg/kg (n = 21) or 6 mg/kg (n = 22). In this secondary progressive MS population, a reduction of gadolinium enhancing lesions on T1 weighted MRI was found in the 3 mg/kg (n = 68) treatment groups.[28]

Currently, the phase IIIb, placebo controlled ASCEND study (NCT01416181) is ongoing with 856 secondary progressive MS patients planned with disability progression as primary endpoint.[29] Final data collection for primary outcome measurements is expected for December 2014.[29]

Withdrawing Nat. A clinical problem is the withdrawal of Nat in patients with high disease activity prior to initiation of Nat. Normally disease activity returns to baseline levels starting as early as approximately three months,[30] however several reports about rebound of disease activity after cessation of Nat have been published.[31,32] Treatment after cessation of Nat needs to take into account (a) a sufficient wash out interval, (b) latency of treatment effects of subsequent therapy, and (c) disease activity. As yet existing data preclude firm recommendations.[33] At least in a proportion of patients, the switch to fingolimod appears to be safe and efficacious.[34,35]

The promising results of the phase III clinical trials led to the approval of Nat. Given its risk profile, the use of Nat is restricted to a selected group of patients with active disease despite immunotherapy or highly active therapy naïve patients[12] (see above). It is noteworthy that these were not the primary target populations investigated in these two trials.[19,26]

In a subgroup analysis, 209 patients of the AFFIRM (Nat n = 148 vs. placebo n = 61) and 169 RRMS patients of the SENTINEL trial (Nat + interferon beta 1a n = 74 vs. interferon beta 1a + placebo n = 95) fulfilled the criteria for highly active disease (≥2 relapses in the year prior to study inclusion and ≥1 gadolinium enhancing lesion on T1 weighted MRI at study entry).[36] In this subgroup, annualized relapse rate was reduced by 81% in the AFFIRM and by 76% in the SENTINEL trials, respectively (each $P < 0.001$).[36] With respect to radiological outcome parameters, the mean number of new gadolinium enhancing lesions decreased by 84% in AFFIRM and by 96% in the SENTINEL trial ($P < 0.001$, respectively).[36] Thus, current approval is supported by post hoc subgroup analyses of the two phase III clinical trials.

Efficacy of Nat on Neuropsychological Parameters (Fatigue, Cognition, Depression)

Fatigue. Fatigue is a major complaint affecting approximately 75% of MS patients.[8,37] In the phase III clinical trial, AFFIRM fatigue was classified as an adverse event during the treatment period in 27% of the Nat treated and 21% of the placebo treated patients ($P = 0.048$).[19] However there was no additional assessment of fatigue. Following smaller observations, the TYNERGY study focused on fatigue as a potential therapeutic outcome parameter. In this one arm open-label trial on 195 RRMS patients treated with Nat for 12 months, there was a reduction of fatigue measured by the fatigue scale for motor and cognitive function.[38] Other parameters (eg, Qol, sleepiness, depression, cognition) were also improved. Although the results have to be regarded as preliminary due to the open-label uncontrolled design of the study, a positive influence of Nat on fatigue was additionally observed by Iaffaldano et al.[39] Here, a reduction of fatigue measured by the fatigue severity scale after one and two years of Nat therapy was found in an open-label trial including 153 patients.[39] Summarizing, some data point to a possible beneficial influence of Nat on fatigue, which could be another facet of the therapeutic efficacy.

Cognition. Prevalence of cognitive impairment in MS is nearly 60%.[6,7] Cognitive impairment influences occupational and social status and has a negative impact on the Qol.[6,40,41] In a pooled data analysis of the AFFIRM and SENTINEL trials, Weinstock-Guttman et al. focused on prespecified tertiary outcomes, among the progression of cognitive deficits as based on the paced auditory serial addition test-3 score (PASAT-3). Whereas a reduced risk of a neuropsychologically confirmed cognitive deterioration by 43% was found in Nat treated vs. placebo treated patients,[42] no such changes were seen in SENTINEL. In addition, PASAT-3, originally invented for measuring the recovery of head-injured patients, only addresses selected cognitive functions such as auditory information processing speed, flexibility, and calculation ability.[43] Thus PASAT-3 has limitations in the evaluation of cognitive function in MS.

In a small, uncontrolled observational study, Mattiolli et al. demonstrated that memory tasks and executive function improve after 12 months of Nat therapy.[44] Additionally, it was shown in an open-label two year observational study that the percentage of cognitively impaired MS patients was reduced by 10% after 12 months of Nat monotherapy and mean values of the cognitive impairment index significantly improved.[39]

In summary, Nat therapy may affect cognitive performance in MS patients. However, due to study design, data have to be interpreted with caution and possible interrelated confounders (eg, fatigue, depression) have to be taken into account.

Depression. In MS patients, the lifetime prevalence of any depressive episode is approximately 50%.[45] Nearly one third of MS patients in a primary care setting have a moderate to severe depressive episode according to the "clinically significant depressive symptom score".[46] In the open-label, uncontrolled TYNERGY trial, Svenningson et al. also investigated the effect of Nat on depression using the center of epidemiologic studies depression scale.[38] An improvement in comparison to baseline was described[38] as in the open-label observational study by Iaffaldano using the Beck depression index.[39] However, differences were small and the design does not preclude other confounders as well as a regression to the mean.[39] Therefore these data warrant confirmation in independent trials.

Parameters of Qol

In the placebo controlled phase II trial, the self-reported well-being on a visual analog scale was assessed as additional clinical endpoint.[28] In contrast to placebo, in both Nat groups (3 vs. 6 mg/kg) patients reported an improvement after six months of treatment, whereas the placebo patients reported a decline.[28] Rudick et al. retrospectively analyzed pooled data from the AFFIRM and SENTINEL trial focusing on Qol.[47] Qol was assessed with the short form 36 (SF-36) questionnaire and a visual analog scale at baseline and weeks 24, 52, and 104.[47] Qol, as measured by SF-36, significantly improved in both components (physical and mental) in Nat treated AFFIRM patients at week 104. In the SENTINEL data analysis, a significant difference was observed only in the physical component of the SF-36 in the Nat treated patients.[47] Additionally independent data of two single arm observational studies indicated a beneficial effect of Nat on Qol.[38,48] Wickström et al. investigated the effect of Nat on the ability to work in 288 patients with sickness benefit prior to initiation of Nat therapy.[49] In this special patient population, the ratio of "the ability to work" to "the total employment rate" was approximately doubled and the sickness benefits were reduced by 41%.[49] Therefore, Nat therapy was consistently associated with an improvement of health related Qol as individual parameter of well-being and potentially with socioeconomic factors.

Experimental Outcome Parameters

Neuronal axons contain a high concentration of neurofilaments (Nf)[50] and cerebrospinal fluid (CSF) Nf levels have been

proposed as potential surrogate markers of axonal dysfunction in MS.[51]

Recently, it was demonstrated that Nat modifies CSF Nf concentrations.[52] In CSF samples from 92 Nat treated patients, Gunnarson et al. described a three-fold reduction of the CSF Nf light chain (NfL) concentration in CSF during Nat treatment (6 and 12 months after baseline).[52] Similar results were reported in a cohort of 30 RRMS patients.[51] Further studies are needed to clarify clinical relevance of these findings.

Adverse Drug Reactions to Nat

During the two-year phase III placebo controlled AFFIRM trial, only 6% of the Nat and 4% of the placebo treated patients discontinued the study because of adverse events.[19] According to the summary of product characteristics (SPC), Nat side effects occurring with a probability of more than 1/100 include pharyngitis, urinary tract infection, urticaria, cephalgia, dizziness, nausea, vomiting, arthralgia, fever, and rigidity.[13] Whereas infusion-associated adverse reactions (allergic or non-allergic) are the main immediate side effects, a rare but potentially serious adverse reaction, PML is currently in the focus of scientific interest.

Nat Antibodies and Hypersensitivity Reaction

Despite humanization, Nat remains immunogenic, which results in an at least transient production of antibodies in 9–12% of Nat treated patients.[19,26] Of these patients, 6% have a persistently positive antibody status,[19,26] defined as an antibody detection at least at two different time points with an interval ≥42 days.[19] In 98% of the anti-Nat antibody positive patients, antibodies occurred within the first 24 weeks of therapy (within the Nat infusion 4–6), up to the ninth infusion.[23] If antibodies were detected after six months of Nat therapy, a persistence of the anti-Nat antibody could be assumed.[23] Biological effects of persisting antibodies were demonstrated by Calabresi et al. who reported mean serum Nat concentration of 1.3 µg/mL in the presence of antibodies compared to 14.9 µg/mL in the absence of antibodies at therapy week 12.[23] Most importantly, the occurrence of persisting high titer antibodies is correlated with a reduced efficacy of Nat with more frequent relapses, as well as disability and MRI progression.[23] Additionally, patients with antibodies against Nat more often develop allergic infusion reactions than patients without anti-Nat antibodies.[19,26] As a consequence of this association, the probability of allergic infusion-related side effects parallels the course of anti-Nat antibodies being highest at the second infusion and remains relevant up to the 6th–9th Nat administration.[19] Still, anaphylactic reactions are rare (0.8%).[19] Given reduced clinical efficacy and the higher incidence of allergic reactions, we recommend termination of Nat therapy in the presence of persisting high titer anti-Nat antibodies.

PML

PML is caused by an infection of glial cells in the CNS white matter by JC-virus (JCV).[53] It was first described in patients with Hodgkin´s lymphoma and chronic lymphocytic leukemia in 1958 by Astrom et al.[54] JCV is a neurotropic double-stranded DNA polyomavirus.[55] Via binding the 5-HT2a receptor it infects mainly astrocytes and oligodendrocytes in the CNS as well as B-lymphocytes and kidney epithelial cells.[55,56] After the initial infection, the virus resides in the bone marrow or in the kidneys.[57] As an opportunistic CNS infection, PML exclusively affects immunocompromised patients.[53] Therefore, PML primarily affected human immunodeficiency virus (HIV) infected patients or patients with hematologic malignancies prior to introduction of Nat.[58] Meanwhile PML is increasingly diagnosed in the context of immunotherapies such as Nat, and has led to warnings (eg, anti-CD20 monoclonal antibody (mAb) rituximab) or even withdrawal of potent monoclonal antibodies (anti-LFA1 mAB efalizumab).[59] Up to now (November 2013) 418 Nat associated PML cases have been reported with a total of 120,500 Nat treated MS patients worldwide resulting in an incidence of 3.4 per 1000 patients (95% CI 3.08–3.74).[60] Current Nat-PML figures can be accessed in Ref. 60.

Disease Course and Diagnosis of Nat-PML

The initial complaints of PML patients may be misinterpreted as MS relapse.[61,62] However, optic nerve and spinal cord manifestations are exceedingly rare. In comparison to HIV-PML, Nat-PML patients more often report neuropsychological and cognitive deterioration as one of the first symptoms occurring in roughly one third of patients.[61] During the disease course, neuropsychological symptoms increase up to 54%.[63] Seizures are another typical PML manifestation. As demonstrated by Clifford et al. seizures occurred in 36% of Nat-PML cases,[61] in contrast to non-Nat-PML patients where seizures develop less frequently (18%).[64] Seizure semiology was analyzed by our group.[65] In our monocentric cohort of 15 patients, focal initiated grand maux were mostly followed by simple partial motor and psychomotor seizures.[65] Series of seizures or status epilepticus occurred in seven of eight PML patients.[65] Interictal EEG recording demonstrated focal slowing in seven and epileptic discharges in two of the eight patients.[65] EEG changes in terms of focal slowing were described in one patient prior to MRI and CSF alterations.[66] We also demonstrated the temporal association of seizures with immune reconstitution inflammatory syndrome (IRIS, which will be discussed subsequently).[65] This led to the conclusion that more frequent occurrence of seizures in Nat-PML than in non-Nat-PML patients might be due to the more frequent and severe IRIS in Nat-PML patients.[65] Given the high occurrence of epileptic seizures in our PML cohort, we currently treat PML patients prophylactically, eg, using Levetiracetam.[65]

Other frequent PML symptoms are visual complaints, which were reported as presenting symptom in 8 of 28 Nat-PML patients, mostly homonymous hemianopia because of an occipital lesion.[61] Frank optic neuritis has not been reported so far.

PML MRI lesions are hyperintense on T2/diffusion weighted images and hypointense on T1-sequences. Further radiological characteristics include size (>3 cm in diameter), location (subcortical U-fibers, sharp defined to cortex, and diffuse to white matter), and the absence of mass effect.[67] In contrast to HIV-PML patients, 30–40% of the PML patients have gadolinium (Gd) uptake at the time point of diagnosis in the MRI.[63]

Disease course of Nat associated PML is usually severe with significant aggravation of disability. Vermersch et al. published 35 Nat associated PML cases of whom, 29% died.[68] The disease course of PML was analyzed by our group[62]: these 15 monocentrically treated Nat-PML patients survived, but they presented a physical deterioration after 21.5 months of follow-up with an EDSS increase by three points and 13 out of 15 patients had a Karnofsky index lower than 70% after PML.[62]

Establishing PML diagnosis early is relevant, because diagnostic delay appears to have a negative impact on Nat-PML prognosis.[68] Vermersch et al. demonstrated that PML was diagnosed with a delay of 44.2 days in the non-fatal and 62.8 days in the fatal PML cases.[68] In our cohort with 15 non-fatal Nat-PML patients, the interval until diagnosis was 30 days (median, range 1–112 days).[62]

Given the prognostic relevance of early PML diagnosis, clinical vigilance is of high importance in early recognition of PML. Additionally, cranial MRI can assist in early diagnosis.[67]

If Nat-PML is suspected, a lumbar puncture with consecutive polymerase chain reaction (PCR) of JCV DNA in CSF should be performed. Sensitivity is reported with a range between 74–92% with specificity between 92–100%.[69] We recommend analyzing JCV PCR in reference laboratories that employ sensitive PCR-protocols. In case of clinical/radiological suspicion but negative CSF findings, repeated lumbar punctures are recommended; likewise repeated cMRI can assist in diagnosis. On occasion, brain biopsy with detection of JCV in CNS tissue may be required. Intrathecal production of JCV specific antibodies has been reported to assist in diagnosis of a CSF-JCV DNA and biopsy negative patient.[70] In any case, suspicion of PML should lead to immediate interruption of Nat treatment.

Treatment of Nat-PML

We currently follow a standardized treatment protocol[62,71] that includes the initiation of five PLEX sessions after diagnosis of PML in order to hasten Nat clearance.[21] We further administer mefloquine (250 mg once a week) and mirtazapine (30–60 mg per day) based on *in vitro* data.[72] However, there are no clinical data of higher evidence class supporting this approach. As already discussed, PML patients are at a high risk to develop symptomatic seizures, especially during IRIS.[65] Therefore, PML patients are treated preventively with an antiepileptic drug (eg, levetiracetam 1000–1500 mg).[65] To detect IRIS, we repetitively perform MRI scans. IRIS is often associated with deterioration of neurological status leading to serious complications. Histopathologically, IRIS is characterized by an excessive immune response against the JCV infection with disruption of BBB, massive CD-8 T-cell and macrophage invasion into the lesion and cerebral oedema.[73,74] For treatment of IRIS, steroids iv can be administered. However, preventive treatment without clinical and/or radiological signs of IRIS is discouraged due to decrease of JCV specific T-cell activity.[75]

Until now, using the depicted therapeutic approach, 22 Nat associated PML patients have been treated in our center. Of these, one patient with predominant brainstem involvement died. Further studies are warranted to optimize treatment strategies of PML and IRIS.

PML Risk Stratification

JCV antibody status, immunosuppressive pre-treatment, and duration of Nat therapy are widely accepted as factors in the stratification of the risk to develop PML; other potential biomarkers are currently under validation.[76,77]

Immunosuppressive pre-treatment. Immunosuppressive pre-treatment is described as PML risk factor.[60] It was estimated that PML risk is three to four times higher in patients with a positive history of an immunosuppressive pre-treatment.[78] This increase in risk appears to be independent of the immunosuppressant used and duration of immunosuppressive pre-treatment.

Duration of Nat therapy. There is a strong relationship regarding duration of treatment, with a very low incidence during the first treatment year (0.06; 95% CI 0.02–0.12), a gradual increase during the second year (0.67; 95% CI 0.51–0.86), and a strongly increased risk beginning in the third year (1.84; 95% CI 1.53–2.21, 4th year: 2.36; 95% CI 1.82–2.92), and 5th year (2.33; 95% CI 1.82–2.92).[60] As indicated before, the incidence estimates for later time points should be interpreted with caution because of the relatively low number of cases included so far.[76]

Anti-JCV antibody serostatus and reactivity. It is assumed that after the primary, usually asymptomatic, infection, JCV persists in renal or lymphatic tissue.[79] Thus far, mechanisms of JCV-reactivation and PML development are incompletely understood but presumably include different viral and host factors.[80]

Asymptomatic virus reactivation is frequently seen in Nat treated patients without PML, hence, direct detection of JCV DNA, eg, in blood or urine did not prove to predict the risk to develop PML under Nat.[81] As indicator for previous contact to JCV, antibody responses to JCV are currently recommended as biomarkers that aid in risk stratification.[13,60] For this purpose, a two-step ELISA is available and approved.[82,83] Yet, analytical difficulties have to be kept in mind. Using the first generation ELISA, "seroreverters" were observed in 4.7% of patients in an independent cohort.[83] This group of patients exhibited

low antibody levels reflecting variability because of natural fluctuation of antibody reactivity around the assay cut points. A second generation ELISA is established with reported sensitivity of 98% and an improved validity in patients with low JCV antibody titres.[82] To our best knowledge, a direct independent comparison of the first and second generation ELISA has not been carried out. A recent longitudinal analysis of a patient cohort reported higher than expected rates of seroconversion and index values of anti-JCV antibodies when testing the same patient cohort at a later time point with 2nd generation ELISA.[84] The authors argue that this is rather an effect of prolonged Nat therapy than an effect of improved test procedures as increased antibody reactivity was especially observed in patients still on Nat at the 2nd time point. A selection bias has to be considered in this cohort as patients previously tested positive have mainly stopped Nat treatment.

Seroprevalence of anti-JCV antibodies in a multinational cohort of 10.280 MS patients was 57.6%, with certain geographical variation.[85] It is generally higher in males than in females, increases with age,[85,86] and is independent of immunosuppressive pre-treatment or Nat therapy.[85,86] The overall PML risk in anti-JCV antibody positive patients is higher than in seronegative patients.[60] The absence of anti-JCV antibodies is thus associated with a relatively low risk to develop PML. Yet, being seronegative does not exclude PML in Nat treated patients as seroconversion to anti-JCV positive is to be considered. The range of seronconversion rates as described by Trampe et al.[83] and Outteryck et al.[84] is between 9.8 and 14.5%.

In addition to mere serostatus, antibody reactivity has recently been described to potentially identify a subgroup of patients with a higher risk to develop PML.[83,87,88] These data are currently being followed up. Interestingly, an increase in antibody reactivity before or at PML onset has been described in small patient numbers.[83] Hypothetically, increasing antibody reactivity as a marker of an increased immune response to JCV during PML manifestation may be biologically plausible, but will have to be further investigated.

Other potential risk parameters. Recently, patients with a lower body weight during Nat therapy have been described to have higher serum levels of Nat and lower body weight was suggested to occur more often in PML patients than in other Nat treated patients.[77] This may be biologically plausible in terms of an increased bioavailability and thus accumulation of the drug. However, potential confounders of this study include high variability of body weight in different geographical regions because groups of different origin were compared. Still, if further confirmed, this approach may serve as an additional parameter very feasible to be acquired.

The investigation of T-cell subsets and functional characteristics has been focused by different groups.

In this context, intracellular ATP levels of CD4 + T-cells were shown to be decreased in patients with PML of different etiology and after long-term Nat treatment.[89] However, in samples obtained prior to occurrence of PML (STRATA-study), this parameter could not predict higher risk to develop PML in some patients samples.[90] Recently, Schwab et al. investigated the influence of Nat on several molecules, which are relevant for leukocyte migration via BBB (Nat treated MS patients n = 224, other therapy n = 21, untreated n = 28), including 16 PML cases.[91] A significantly lower percentage of L-selectin expression in Nat treated patients compared to the other groups was found. Interestingly, in the pre-PML blood samples (n = 8), the L-selectin expression was nine-fold lower compared to the non-PML Nat treated patients.[91] The analysis of L-selectin expression appears to be a promising approach to improve PML risk stratification strategies. However, future studies with the inclusion of more PML cases need to be performed.

Conclusion

Nat is a highly active therapy for patients with severe relapsing MS course. In addition to conventional parameters of clinical and radiological disease activity, patient-related outcomes as Qol-related parameters, fatigue and cognitive function appear to be positively influenced though mainly evaluated in smaller observational studies. Profound clinical efficacy needs to be weighed against the risk to develop potentially severe or lethal PML.

Given the growing therapeutic armamentarium in MS, the role of Nat needs to be carefully re-evaluated taking into account several alternative strategies. Here, especially, Tecfidera (DEFINE and CONFIRM: relative reduction of annualized relapse rate, 44–53%[92,93]) and Alemtuzumab (CARE-MS 1 and 2: relapse free after two years, 65–78%,[94,95]) could be possible therapeutic options in patients with initial high disease activity or after treatment failure of interferon and glatiramer acetate.

In addition to approaches to (individualized) risk—benefit considerations, future research should also focus on the sequence of therapies, since re-emerging disease activity after cessation of Nat treatment remains a clinical challenge.[96]

Author Contributions

RH, SF, AS, RG and AC analyzed articles, databases, and data included in the manuscript. RH, SF, AS, RG, and AC developed the structure and arguments for the manuscript. All authors reviewed and approved the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review.

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