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Long-term safety evaluation of natalizumab for the treatment of multiple sclerosis.

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Abstract

Introduction

Natalizumab is a humanized monoclonal antibody highly effective in relapsing-remitting multiple sclerosis (MS). Important concerns about its safety have been pointed out because of the risk of progressive multifocal leukoencephalopathy (PML), caused by the opportunistic John-Cunningham virus (JCV).

Areas covered

This review analyzes all the safety aspects related to the use of natalizumab in MS patients. Fatigue and allergic reactions are not-severe adverse events (AEs) occurring more frequently than placebo and no differences in serious AEs (SAE) have been observed comparing to interferon-β1a during clinical trials. No clear teratogenic effects have been observed, but natalizumab is still considered a pregnancy category "C" drug.

The risk of PML depends on 3 factors: concomitant or previous immunosuppression, exposure duration, and anti–JCV antibody level. In natalizumab-related PML the average survival is 77%; prognostic features and information for the earliest identification of PML have been identified to maximally reduce its incidence, mortality and morbidity. In addition, a few other safety issues need to be taken into account.

Expert opinion

Natalizumab is a highly effective drug for MS patients but its safety issues represent a relevant limitation and impose strict clinical surveillance of treated patients. No significantly higher difference in SAEs occurrence was observed during the clinical trials, in spite of PML. Other post-marketing safety red-flags have been pointed out, and if PML and its consequences are considered the most relevant issues for natalizumab patients, a continuous surveillance must be maintained in all patients also regarding other possible SAEs like liver diseases and malignancies.

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1) Introduction

Multiple sclerosis (MS) is a frequent chronic inflammatory disease affecting the central nervous system (CNS) [1]. Recent data report that MS affects over 2.3 million people in the world and 85%–90% of patients experience relapsing and remitting course (RR) of neurologic symptoms [2]. The intermittent formation of inflammatory lesions in brain and spinal cord is the hallmark of RRMS, with the development of demyelinating plaques and axonal loss [3]. The formation of MS inflammatory lesions in CNS is primarily referred to a lymphocyte migration across the blood–brain barrier [BBB][3]. The process of lymphocyte migration in the CNS inflammation areas is mediated by the interaction between the α4-β1 integrin (a lymphocyte surface protein) and the vascular-cell adhesion molecule 1 (VCAM-1), expressed on the surface of endothelial cells of the BBB vessels [4]–[8].

2) Mechanisms of action, pharmacodynamics and pharmacokinetics of natalizumab

Natalizumab (Tysabri®, Biogen, Boston, USA) is a humanized monoclonal antibody (MAb) against the α4 subunit of α4-β1 and α4-β7 integrins; its main mechanism of action is the blockade of the binding of the α4 integrins to their endothelial receptors (VCAM-1 and mucosal addressin-cell adhesion molecule-1), preventing inflammatory cells from crossing the BBB and entering the CNS [9], [10]. Another supposed mechanism of action of natalizumab is the modulation of ongoing inflammatory reactions by inhibiting the binding between α4-leukocytes and both fibronectin and osteopontin, thus modulating the leukocyte activation and proliferation within the CNS [11], [12].

The pharmacokinetics of natalizumab was analyzed in a phase I, randomized, placebo-controlled, dose-escalating trial in MS patients [13]; in this study the maximum serum concentrations and area under the curve (AUC) values for natalizumab resulted proportional to the administered dose [13]. Natalizumab shows a mean half-life of 16 ± 4 days with a clearance of 13.1 ± 5 ml/hour[14]. In the pharmacokinetics study [13] the body weight, in the range of 40-100 kg, did not significantly influenced the clearance of natalizumab; this justified the use of a fixed dose of 300 mg every 28 days. In addition the Authors found a plateau effect for efficacy at doses of 300 mg or more[13].

Natalizumab determines a mean α4-integrin saturation levels greater than 70% at 4 weeks after administration [15], the drug is measurable in circulation for up to 12 weeks and variations in in the distribution of cerebrospinal fluid (CSF) cells were detectable for about 6 months after natalizumab interruption[16].

3) Review of clinical utility

In 2004 natalizumab was introduced in the market after the US Food and Drug Adminsitration (FDA) approval for its use in RRMS, upon the interim analysis of 2 phase-III trials, the AFFIRM and SENTINEL studies [17], [18].

These trials reported the great efficacy of natalizumab in RRMS, demonstrating a relevant reduction of the annualized relapse rate (ARR) as compared with placebo (0.26 for the natalizumab group vs. 0.81 for the placebo group) [17] and leading to a new goal in MS therapy: the “freedom from disease activity” [19]. However, natalizumab was temporarily withdrawn from the market after the occurrence of 3 cases of progressive multifocal leukoencephalopathy (PML) in 2 MS patients and in one patient treated for Crohn's disease [20]–[22].

Given to its efficacy, natalizumab was reintroduced into the market and released in the European

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Union in 2006, together with a Global Risk Management Plan. In particular, the "TOUCH®" program (TYSABRI Outreach: Unified Commitment to Health Prescribing Program) [23] has been developed in conjunction with the FDA to facilitate the appropriate use of natalizumab and to assess the natalizumab-associated incidence and risk factors for PML and other opportunistic infections. As of September 2016, approximately 161.300 patients received natalizumab in post-marketing setting worldwide, and as of December 2016, 698 treated patients reported a confirmed PML [24]. Some other adverse events are associated to the every 28 day i.v. infusion of natalizumab, encompassing liver diseases, infections, reported to occur in 1.9–4% of patients, opportunistic infections other than PML, with an incidence of 0.2%-1%, hypersensitivity reactions and anaphylaxis, occurring in 0.2%-1.3% of patients [17], [18], [25], [26]. Overall, these adverse events have a marginal negative impact in the management of patients treated with natalizumab, in consideration of their low incidence, morbidity and mortality rate, and given the high efficacy of the treatment; on the contrary, the risk of PML represents a critical limiting factor in natalizumab start or continuation. Additionally, there are a few authors reporting the onset of lymphoproliferative disorders in natalizumab treated patients; these represent serious and potentially fatal AEs although their low incidence and the lack of biological evidences raised doubts on the cause-effect correlation with the use of natalizumab.

This paper reviews all the available data on natalizumab safety, analyzing and discussing all the issues related to the natalizumab therapy. In the context of an increasing armamentarium of approved MS treatments and a more complex management of MS patients, this review aims to provide updated information and to discuss the potential risks correlated to the use of one of the most efficacious disease-modifying therapy for MS.

For this review, a PubMed search was performed using the terms “natalizumab", "natalizumab AND multiple sclerosis”, “natalizumab AND PML”, “natalizumab AND safety”, "natalizumab AND side effects" without time restriction.

4) Adverse events other than PML

Including MS relapses, the global incidence of serious adverse events (SAE) reported in the phase-III clinical trials AFFIRM and SENTINEL [17], [18] is 19% and 18% respectively. In the AFFIRM trial [13] 6% of patients had a MS relapse, 3.2% a serious infection (4 cases of pneumonia, 5 cases of urosepsis and various other infection encompassing pilonidal cyst infection, cellulitis, febrile infection, gastroenteritis, cryptosporidial diarrhea, mononucleosis, osteomyelitis, sinusitis, tonsillitis, viral infection, appendicitis, and an infection of unclear cause), <1% malignancies (3 breast cancers, 1 cervical cancer and 1 malignant melanoma), 27% fatigue. Infusion reactions are defined as any event that occurred within two hours after the start of the one-hour infusion and occurred in 24% of patients; the most common infusion reaction was headache (5%), while 4% of patients had a hypersensitivity reaction: 12 cases of urticaria or generalized urticaria, 1 of allergic dermatitis, 8 of a reaction called hypersensitivity and 5 of anaphylactoid reactions (urticaria plus other signs) [17]. In the AFFIRM study, the only adverse events significantly more frequent in the natalizumab than in the placebo group were fatigue (27% versus 21%) and allergic reaction (9% versus 4%) [17].

In the SENTINEL trial [18] 5% of patients treated with natalizumab plus interferon-β1a had a MS relapse, 2.7% had a serious infection, encompassing viral infections and urosepsis, 1% malignancies, 24% infusion reactions (1.9% hypersensitivity reaction without cardiopulmonary compromise). No SAEs had a statistically significant higher incidence between combination therapy and interferon-β1a alone group; the mild adverse events associated with combination therapy were

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anxiety, pharyngitis, sinus congestion, and peripheral edema. In the open label, prospective, multi-national, single-arm post-marketing safety STRATA study [25] on 1094 MS patients receiving a median of 56 infusions, 16% reported at least one SAE (excluding MS relapse): 4% infections and infestations, 2% gastrointestinal disorders, 2% neoplasms (benign, malignant and unspecified). Infusion reactions were experienced by 5% of patients (more frequently during the second or third infusion).

The TOP study [26], another large post-marketing open-label 10-year prospective study, observed 4821 natalizumab treated subjects, reporting 2,6% of patients with a SAE related or possibly related to natalizumab. The most common SAE was infection (1.9%); the serious hypersensivity reaction incidence was 0.5%, while malignancies occurred in 0,5% of patients (12 different types of malignancies reported).

Regarding the presence of natalizumab antibodies, 9% had detectable antibodies at some time during the AFFIRM study and 6% reported persistent antibodies with an increase in infusion-related adverse events and a loss of efficacy of therapy [17]. In the SENTINEL study, 12% of the combination-therapy group had antibodies to natalizumab, but only 6% reported persistent anti-natalizumab antibodies, resulting in a loss of efficacy and an increase in infusion-related adverse events [18]. In the post-marketing STRATA and TOP studies [25], [26], about 3% of patients were found persistently positive for anti-natalizumab antibodies, even though in the latter study the antibody status data were not collected routinely. In the TOP study [26] the presence of anti-natalizumab antibodies was listed as a reason for discontinuing the therapy.

As a known pharmacodynamic effect, increases in the number of lymphocytes, monocytes, eosinophils, basophils and also nucleated red cells were seen in patients treated with natalizumab, in absence of elevation in the number of neutrophils. All changes reported were not correlated with clinical effects [17], [18].

While the clinical trials AFFIRM and SENTINEL [17], [18] reported a similar incidence of hepatobiliary disorders between the patients treated with natalizumab and the control groups, the Post-Marketing FDA Adverse Reaction Reporting System (FAERS) contains 628 cases of different degrees of liver injury associated with natalizumab from 2009 to 2014, comprising 22 cases of liver failure and 12 cases of autoimmune hepatitis [27]. To date, 12 case reports of significant liver injury associated with natalizumab are described [27], 50% of which with autoimmune hepatitis antibodies and compatible biopsy. Monitoring of liver function is recommended for patients treated with natalizumab. The adverse events other than PML are summarized in Table 1.

5) Pregnancy

Experience concerning the influence of natalizumab on fertility and pregnancy is limited. In animal studies, natalizumab proved alteration of fertility in females, but not in males; however, no clear teratogen effects have been observed nowadays, and normal outcomes of pregnancy have been reported in some published cases of patients treated for the whole gestational period [28], [29]. A study with a small case series, on the contrary, found mild hematological alterations in 10 of 13 children of mothers receiving natalizumab during the 3rd pregnancy trimester [30].

Nowadays there are no controlled data in human pregnancy and the effects of natalizumab on fertility, pregnancy and breastfeeding still need to be ascertained [31], [32]. Thus, at the time of prescription, patients must be correctly informed of the possible consequences of the drug on fertility and of the need for contraception. At the same time, pregnancy should be planned in advance and the treatment suspension evaluated in a pre-conception time period adapted to the half-life of the drug [33]. To date, natalizumab is a pregnancy category "C" drug [34].

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Breastfeeding is contraindicated in natalizumab treated patients since it is excreted into human milk. Although the drug is not orally bioavailable, the effects of exposure on infants are unknown and a decision to discontinue breastfeeding or discontinue the drug should be made [35].

6) PML and immune reconstitution inflammatory syndrome" (IRIS)

The most important concern regarding the use of natalizumab is related to the possible occurrence of PML, an infective CNS disease caused by the John Cunningham virus (JCV) reactivation [36], [37]. JCV infection often occurs in early life and it is typically asymptomatic or mildly symptomatic, in absence of CNS involvement; then, JCV, a member of the polyoma family, presumably has a whole-life persistence in human body, remaining latent in various tissues, encompassing the kidneys, the bone marrow and the lymphoid tissues [38], [39]. In the general population a 60-70% prevalence of detectable anti-JCV antibodies has been estimated, with the prevalence of seropositive subjects increasing with the age [38]. PML may result from the reactivation of latent JCV infection in the brain [40] or spread from peripheral reservoirs in the kidneys [41] and bone marrow [42] to the brain. The disease affects severely immunosuppressed patients in terms of T-lymphocyte response [39], with a JCV infection primarily involving the myelin-producing oligodendrocytes and causing severe demyelination [40]–[43]. PML infection seems to be more widespread in the brain, since histological observations of JCV are reported also in cerebellum, astrocytes and neuronal cells [44]–[47].

PML has never been reported in MS prior to introduction of natalizumab. The diagnosis of PML in a clinical setting is based on the clinical presentation, imaging findings and the detection of the virus in the CSF using quantitative Polymerase Chain Reaction (PCR); however, the gold standard for the diagnosis of PML is represented by the presence of JCV in histopathology examination of biopsy material [48], [49].

PML is a well known disease in the field of human immunodeficiency virus epidemiology, where it represents a complication of the pathological immunosuppression in about 5% of patients [50]; moreover, the wide use of immunosuppressive treatments in the last decades, including alkylating agents and monoclonal antibodies, has given the contribute to the onset of further cases. The clinical presentation of PML is variable with behavioral, motor, language, and visual symptoms as possible first manifestation of the disease; cognitive changes in particular seem to be more frequent in MS natalizumab-treated patients developing PML, possibly because of the multifocal demyelinating lesions already present in these patients [51]. Visual symptoms are reported in one-quarter to one-half of all PML patients, typically presenting as a field deficit [52] related to the involvement of the visual pathways and not as a direct damage of the optic nerve, never described in PML patients. Seizures can occur in up to one-third of PML cases and are more frequent in cases of juxta-cortical lesions [53].

Distinguishing PML from an acute MS attack can be difficult, as the general symptoms can be similar to the symptoms of an MS relapse. However, the early diagnosis of PML is pivotal since patients whose immune functions can be restored could have an improvement in terms of survival and sequelae [54], [55]. Following natalizumab approval, experience from a higher number of patients treated for a long time period is providing greater accuracy in PML risk estimation. The current known risk factors for JCV reactivation in natalizumab patients are: concomitant or previous immunosuppression and natalizumab exposure duration, particularly after the 24th administration [36]. More recently, the anti–JCV antibody level in serum or plasma has been identified as a further risk of natalizumab-associated PML [56]. According to these data, the European Medicines Agency (EMA) has recently updated the estimate risk for PML in seropositive

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JCV antibody patients treated with natalizumab [57]; the risk is small at antibody index values of 0.9 or less (0.1 – 0.6/1000), and increases substantially in patients with index values above 1.5 who have been treated for more than 24 administrations (0.9 – 10/1000) [54]. Patients with previous immunosuppressant therapy after 24 doses have a PML risk ranging from 0.4 – 10/1000 [55]. Following EMA recommendations, after the 24th natalizumab dose, patients should be informed again about the risk of PML with natalizumab and they are asked to provide a standardized written consent form to continue this therapy [58]. Indeed, after the 24th natalizumab dose, patients should reevaluate with the neurologist the opportunity to continue natalizumab, to switch to any other first or second line MS treatment or, alternatively to quit all therapies. Moreover, patients should be informed to be vigilant about the risk of PML for up to 6 months after discontinuation of natalizumab [58].

PML usually presents in magnetic resonance imaging (MRI) as one or more areas of hyperintensitity on T2/FLAIR sequences in the white matter with a peripheral, bilateral and asymmetric distribution. Little irregular signal intensity within the lesions can have a punctate microcystic appearance [59], [60]. This finding has been suggested to represent small areas of demyelination in the immediate vicinity of infected oligodendrocytes or early immune response within perivascular spaces [61]. Small punctate T2 lesions may be seen in proximity to the lesions. These vary in shape and size, growing and becoming confluent as the disease progresses [57], [58]. PML lesions have subcortical rather than periventricular location and affect U-fibers; FLAIR is the preferred sequence for PML diagnosis, because of its subcortical location. The border is ill-defined toward the white matter and sharp toward the cortical grey matter (GM). Cortical GM involvment is seen in 50% of cases. On T1 weighted imaging lesions become increasingly hypointense, as white matter destruction occurs. No mass effect is present even in large lesions apart from when inflammatory response occurs [60]. Posterior fossa involvement encompasses the cerebellum and middle cerebellar peduncles and, rarely, the brainstem with a “crescent” shape. Optic nerves and spinal cord are spared [59].

At the early asymptomatic stage MRI shows focal hyperintense T2 signal in the juxta-cortical white matter, most commonly unilobar in the frontal lobe, involving U-fibers and also quite frequently the adiacent grey matter [59], [62]. Compared with other PML populations, contrast-enhancement can be observed quite frequently in natalizumab-associated PML, occurring in about 30-40% of the cases at the time of diagnosis [59], [62].

The pattern of enhancement is variable and may be patchy, linear, nodular, peripheral or perivascular. It suggests that an inflammatory response is involved. Enhancement at time of diagnosis seems to be correlated with decreased survival and greater disability [59].

Diffusion weighted MR imaging (DWI) varies depending on the stage of the disease and can be negative in asymptomatic patients, probably because the degree of oligodendrocytic and astrocytic damage is milder. Spectroscopy findings in PML are non specific and not useful in differentiate PML lesions from MS relapses [59].

Data are emerging about the evidence of PML six months before the onset of symptoms [63]. Recommendations from an expert group [60], based on the opinion of authors, in the absence of peer-reviewed evidence of the predictive values of MRI at different time points prior to PML diagnosis, indicate that all new patients should be scanned prior to initiating therapy and at least annually on treatment. After 18 months of therapy the frequency of MRI depends on JCV status and – for anti-JCV positive patients – their index. After this time brain MRI is recommended every 6 months for index ≤ 1.5 and every 3-4 months for index > 1.5 [60].

One of the most concerns after the diagnosis of PML and the prompt withdrawal of natalizumab is

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the almost inevitable occurrence of the "immune reconstitution inflammatory syndrome" (IRIS). IRIS typically presents as a sub-acute progression and exacerbation of earlier symptoms of PML within days to weeks after natalizumab withdrawal and/or plasma exchange therapy (PLEX) for the faster removal of the drug from the blood [51].

IRIS is an inflammatory response to clinically apparent or subclinical pathogens, associated with recovery of the immune system after a period of immunosuppression [39]. This immune reconstitution is inferred by an increase in T-lymphocyte counts, which usually follows the commencement of antiretroviral therapy in HIV (Human Immunodeficiency Virus) patients, or with cessation of immunosuppressive therapy in other patients [39]. IRIS has been described in context of infections with most pathogens seen in AIDS [64]–[66].

MRI evidence of enlarging and/or gadolinium enhancement of previous CNS lesions supports the diagnosis of IRIS in MS patients; however, since the inflammatory brain response occurs before MRI changes MRI activity represents a supportive diagnostic finding, not useful in the attempt to prevent IRIS onset [67], [68].

The IRIS reaction is often clinically important because the removal of natalizumab results in renewed immune surveillance, causing a robust inflammatory syndrome. It is possible that the use of PLEX, causing a faster removal of the drug from plasma, could lead to a sudden immune reconstitution and consequently to a more severe IRIS. Nevertheless, this data still needs confirmation by controlled studies.

Currently, there are no evidences of specific and effective treatment for PML; the infection outcome depends completely on the individual's immune reconstitution ability to respond to JCV [69]. The main and mandatory intervention in the suspicion of PML is the immediate withdrawal of natalizumab that had enabled the disease development. Once the PML had confirmed by radiological and CSF findings, one of the most diffuse approach is the fast removal of the residual plasmatic quote of drug by the use of PLEX [70], [71].

The 5-HT2A serotonin receptor has been identified as a receptor for JCV in glial cells [72] and the use of medications selective for these receptors, such as mirtazapine and risperidone, have been shown to inhibit viral entry into unaffected glial cells. These drugs have been used in clinical practice in PML patients with conflicting results [73]–[78]. Also the anti-malarial drug mefloquine, able to pass the BBB, recently proved to have anti-JCV activity in vitro, blocking the virus replication within the infected cells [79]. As for mirtazapine and risperidone, experience with mefloquine comes from case reports and case series, in absence of outcome improvement from large prospective studies [80]. The use of other drug classes, such as inhibitors of DNA replication (Brincidofovir, Ganciclovir, Leflunomide), immune-response modulators (IL-2 and IL-7) and passive or active immunization has been reported in case reports or small case series [69].

Many patients with PML experience rapid worsening of neurologic symptoms after PLEX due to the occurrence of IRIS. PML-IRIS is also observed in the absence of PLEX, even though in these cases it tends to occur later, approximately 90 days after last dose [51], [81], [82].

Corticosteroids are used in the majority of the PML-IRIS patients to reduce the inflammation and to improve the associated clinical symptoms.

There are 2 different approaches in the use of corticosteroids for the management of the PML-IRIS: pulse corticosteroids alone or prolonged corticosteroid course with slowly tapering regimens alternating with pulse corticosteroids. Tan and coll. [83] reported data on 42 patients with natalizumab related PML-IRIS observing that early corticosteroids had no effect on the subsequent development of IRIS while the administration of corticosteroids after the IRIS onset seems to be associated with favorable EDSS outcomes. Scarpazza and coll. [84] analyzed data on 40 Italian

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PML patients observing that PLEX did not improve the clinical outcome and that corticosteroids administered out of the IRIS onset are associated to a negative disability progression. Landi and coll. [85] also compared the clinical outcome and survival of 227 MS cases on the basis of the use of PLEX after PML diagnosis: their results did not show improvement of clinical outcome and survival with PLEX (36.5% of good outcome in the PLEX group versus 44% in the non-PLEX group; 84% of survival in the PLEX group versus 88% in the non-PLEX group).
Average survival is estimated 77% in natalizumab-related PML patients [86], with a low mortality rate in comparison with HIV-PML cases in HIV treated patients [39], [86].Positive prognostic features for survival in MS PML subjects are: younger age, lower pre-PML EDSS, lower JCV load in CSF, unifocal MRI lesion and earlier diagnosis [87], [88]. PML and IRIS diagnostic, clinical features and therapeutic options are summarized in Table 2.

7) Malignancies

Clinical trials on natalizumab didn't show significant differences in malignancies incidence between natalizumab treated patients and control groups [13-15]. However, there are a few authors reporting the onset of lymhpoma in MS natalizumab treated patients during the post-marketing phase. In particular, there are 6 case reports in literature describing the onset of primary central nervous system lymphoma (PCNSL) during natalizumab treatment (5 MS patients and 1 patient affected by Crohn disease) [89]. All authors reported an Epstein-Barr virus (EBV)-negative B-cell lymphoma, raising doubts on the immunosuppressant effect of natalizumab as a causative agent for the PCNSLs development, given the pivotal role of EBV in the PCNSL onset in immunocompromised patients. In addition, natalizumab pre-clinical toxicology studies didn't found evidence of immunosuppression-related lymphoproliferative diseases in nonhuman primates, even when using doses 10 times higher than dose used in human patients [90].

Schowinsky and coll. [91] reported one case of peripheral T cell lymphoma occurring in a MS patient after 17 infusions of natalizumab, without evidence of EBV-driven etiology, while a few cases of melanoma occurring during natalizumab therapy have been also observed [92]. A prospective study on 775 melanocytic skin lesions in 74 MS patients treated with natalizumab found changes in only 1.54% of cases and histologic analysis revealed all the excised lesions to be benign [93].

Finally, Rolfes and coll. in 2013 reported 4 cases of cervical dysplasia in patients treated with natalizumab [94], questioning whether immune suppression related to natalizumab could be responsible for the persistence of an HPV infection and subsequently cervical dysplasia. However, to our knowledge, no further associations between HPV infection and natalizumab patients have been reported since then.

8) Withdrawal issues

Several studies show that after natalizumab discontinuation a disease activity worse than pre-natalizumab status, may occur [95]–[97], indicating a rebound effect, similar to an immune reconstitution inflammatory syndrome [98]. A recent study [99] who had reached clinical and radiological stability after 24 natalizumab courses demonstrated a 4 fold higher ARR after one year of withdrawal in 73 natalizumab quitters compared with 35 continuers; however no rebound activity was observed in this cohort [89]. Lo Re et al. [100] observed 132 MS patients treated with

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natalizumab, whose 37 patients remained therapy-free; a significant higher risk of both clinical and radiological relapses were observed in natalizumab quitters when compared with natalizumab continuers after a 1 year. On the other hand, a French observational study [101] on a very large population of 4055 MS patients reported data on 198 patients who remained therapy free for at least 1 year after natalizumab withdrawal; their ARR was lower than before natalizumab start. A recent study [102] compared two different modalities of natalizumab discontinuation, taper protocol versus the standard immediate discontinuation, evaluating the following 1-year disease activity; a statistically significant higher relapse rate was observed in the standard protocol group in comparison to the tapering protocol group.

Some studies [85], [87], [88] did not observed a beneficial effect on disease activity by switching from natalizumab to a first-line therapy in comparison to the therapeutic holiday; thus, also in consideration of the cost/benefit ratio, it could be more appropriate to stop any treatment in case of decision not to continue with other second-line treatments. Other studies [103], [104] attempted to evaluate the effect of steroids on disease activity during the natalizumab wash-out period; the results are contradictory and further studies are needed to expand the sample population and clarify the potential utility of steroids in this context.

Recently, Alping and coll. [105] confirmed the potential utility of rituximab in the treatment of patients interrupting Natalizumab for JCV virus positivity; indeed, they observed on a cohort of 256 patients a higher efficacy and safety of rituximab when compared to the other second-line treatment fingolimod.

9) Conclusion

Natalizumab therapy for MS patients has been demonstrated to be highly effective in several clinical trials. The evidence is very strong both for clinical as well for MRI outcomes. Natalizumab was firstly approved by FDA in 2004 and withdrew from the market in 2005 for two cases of PML in MS patients. After its reintroduction into the market in 2006 a strict surveillance plan has been promoted in order to control and to define natalizumab safety profile. In the post-market observation, hepatobiliary disorders of different degrees of liver injury associated with natalizumab have been reported; 22 of them were referred to liver failure and 12 to autoimmune hepatitis [27], emphasizing the attention that need to be paid to liver function before and during natalizumab therapy. At the same time, some reports on the association between the use of natalizumab and the onset of malignancies such as lymphoma, melanoma and cervical dysplasia should arise the attention of clinicians on the surveillance of natalizumab treated patients, although a clear association between natalizumab and malignancies has not been yet demonstrated.

PML represents a potentially fatal SAE and its prevalence is progressively increased since natalizumab returned back to market in 2006. There are very important keys to be considered in PML management starting from the PML risk evaluation for each patient, the clinical and radiological follow up and their timing in order to identify a possible PML as soon as its first manifestation. Furthermore, considering natalizumab PML related as a different disease than those related to other immunosuppressed patients, like those with HIV or cancer, it seems to have a different outcome. Once an MS natalizumab treated patient develops PML, the first step consists in stopping therapy and in observing a "wait and watch" interval of time, until the IRIS has became clinically evident that is the right time to treat patients with high dose of corticosteroids. Nonetheless, natalizumab related PML represents a potentially fatal disease, with frequent and severe neurological sequelae in survivors; given the presence of new highly effective disease-modifying drugs, PML onset should be avoided by accurately balancing benefits and risks for every single patient, and considering the switch to other therapies when patients are at high risk of PML

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10) Expert opinion

In recent years, the progressively greater efficacy of the approved therapy for MS led physicians to shift their therapy outcome toward the "disease activity free" patient [106]. This started to be more affordable since the MAb were introduced in MS cohort of available therapies. Natalizumab was the first approved MAb [9], [10] and the pivotal trials [17], [18] demonstrated about the 70% of reduction in ARR and more then 80% of reduction in MRI activity for natalizumab treated patients compared to placebo treated ones. The post-marketing studies confirm these results stating for a higher efficacy of natalizumab [107], [108].

In the pivotal trials [17], [18] the authors did not show significant SAEs in those patients treated with natalizumab compared to those who did not receive natalizumab; in particular, infectious side effects or malignancies were registered in a very low percentage of patients (around 3% of natalizumab treated patients developed infections, other than PML, and less than 1% of natalizumab treated patients developed malignancies). The unique high frequency SAEs, highlighted in the pivotal trials, were the allergic ones and the ones related to a higher fatigue condition. Some different conditions have been pointed out as critical in the post-marketing cohort of studies, in particular those related to hepatobiliary disorders since the FAERS received 628 reports, referring to the period 2009-2014, of different degrees of liver injury associated with natalizumab and 22 of them referred to liver failure and 12 to autoimmune hepatitis [27]. In addition, different lymphoproliferative disorders (mostly PCNSL) have been associated with natalizumab treatment, although the paucity of cases and the absence of EBV positivity raised more than one doubt on the strength of this association.

The PML is a very serious AE, and it has never been reported in MS before natalizumab introduction as one of the possible MS therapies. HIV-1 infection remains the most frequent immunodeficiency setting for PML, accounting for 80% of cases [109]. The occurrence of PML in the context of new therapeutic MAb, which affect certain aspects of leukocyte function, has provided new insights into how JCV reactivates to cause PML and the role of leukocytes.

In MS, after the first two cases of PML, natalizumab was withdrawn from the market and reintroduced later after a Global Risk Management Plan was defined. At the time of December 2016, 698 cases of PML were confirmed on a total of over 150.000 patients who received natalizumab [24]. When PML firstly occurred, in the MS natalizumab treated cohort of patients, neurologists had no specific experience but the one from PML in immunocompromised patients.

The PML management strategy is to be considered both preventive and interventional, once PML has been diagnosed. In order to prevent PML, neurologists should attain to an accurate follow-up plan that EMA has recently redefined [110] on the basis of data on risk stratification groups on about 21.000 natalizumab treated patients. The PML risk has been defined on three different factors: the positivity for anti-JCV antibodies, the number of natalizumab administrations and the JCV index level; after 24 natalizumab administration those patients considered to be at higher risk of PML should undergo an MRI scan every three months, those with a low (<0.9) or intermediate (>0.9 <1.5) index value should repeat the test every six months.

Unfortunately, once PML has been diagnosed there is no antiviral agent demonstrated as being effective against the JCV [69]. Prosperini et al. [82] defined some epidemiological and predictive variable stating that natalizumab related PML evolves in a timeframe from 6 to 12 months, with the peak of disability at 6 months and a variable recovery at 12 months from diagnosis; that the asymptomatic PML and a lower JCV viral load at PML diagnosis are associated with better

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outcomes. De Rossi et al. [84], of the same group, defined as a better outcome is affordable avoiding PLEX and starting steroid not before the IRIS has been initiated with evidence of MRI IRIS characteristics.

Recently, new highly effective drugs have been commercialized for MS treatment but natalizumab remains the most efficacious therapy. However, widening the therapeutic options, MS specialists have the opportunity to administer a patient-tailored therapy depending on the disease features, the patient's preference, and the best risk/benefit drug profile according to the single patient characteristics. Our opinion is that natalizumab should be reserved for patients with a severe form of MS, possibly considering other therapies in case of anti-JCV Ab positivity and/or history of liver dysfunction. A strict monitoring of clinical, laboratory and neuroimaging data is recommended in all patients, in spite of the presence of anti-JCV Ab positivity.

In conclusion, we can affirm that natalizumab remains one of the most efficacious drugs available for the MS treatment; nevertheless, challenging problems of safety have been pointed out during the last years that need particular attention: if PML and its consequences are considered the most relevant safety issues for natalizumab patients, and proved to be not so rare, a continuous surveillance must be maintained in all patients also regarding other possible SAEs like liver diseases and malignancies. In this context, pathologists and clinicians need continual vigilance for "the expected" (PML) as well as ‘‘the unexpected’’ in natalizumab-treated patients.

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Drug summary box

Drug name	natalizumab
Phase	Launched
Indication	Multiple sclerosis
Pharmacology description	Alpha4beta1 integrin antagonist Integrin antagonist Alpha4beta7 integrin antagonist Alpha4 integrin antagonist
Route of administration	Injectable
Chemical structure	Immunoglobulin G4, anti-(human integrinalpha4) (human-mouse monoclonal AN100226 gamma4-chain), disulfide with human-mouse monoclonal AN100226 light chain, dimer [CAS]
Pivotal trial(s)	AFFIRM: the Natalizumab Safety and Efficacy in Relapsing Remitting Multiple Sclerosis study. A two-year phase 3 randomized, double-blind, placebo-controlled study to confirm the efficacy of natalizumab in relapsing multiple sclerosis and to evaluate the safety of long-term treatment. Enrolled patients were randomized in a 2:1 ratio to receive an intravenous infusion of natalizumab 300 mg or placebo every 4 weeks for up to 116 weeks. SENTINEL: Safety and Efficacy of Natalizumab in Combination With Interferon Beta-1a in Patients With Relapsing-Remitting Multiple Sclerosis. A two-year phase 3 randomized, double-blind, placebo-controlled study. Enrolled patients were randomized to receive an intravenous infusion of natalizumab 300 mg or placebo every 4 weeks in addition to an intramuscular injection of interferon β-1a (30 µg) once weekly for up to 116 weeks.

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Table 1: Adverse events other than PML

Adverse event	Frequency	Bibliography
Serious infection	3,2% 2,7% 4% 1,9%	AFFIRM Trial [13] SENTINEL Trial [14] STRATA Study [21] TOP Study [22]
Malignancies	<1% 1% 2% 0,5%	AFFIRM Trial [13] SENTINEL Trial [14] STRATA Study [21] TOP Study [22]
Fatigue	27%	AFFIRM Trial [13]
Infusion reactions (hypersensivity)	24% (4%) 24% (1,9%) 5% 0,5% (0,5%)	AFFIRM Trial [13] SENTINEL Trial [14] STRATA Study [21] TOP Study [22]
Gastrointestinal disorders	2%	STRATA Study [21]
Hepatobiliary disorders Significant liver injury	<1% <1% 12 case reports	AFFIRM Trial [13] SENTINEL Trial [14] Antezana A. et al [23]
Persistent natalizumab antibodies	6% 6% 3% 3%	AFFIRM Trial [13] SENTINEL Trial [14] STRATA Study [21] TOP Study [22]

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Table 2: PML and IRIS

PML diagnosis	The gold standard for PML diagnosis is the histopathology examination of biopsy material. Clinical diagnosis is currently made by clinical presentation, MRI findings, and detection of JC virus DNA in CSF.
Symptoms	Cognitive changes; behavioral, motor and language symptoms; visual symptoms (especially visual field deficit); seizures.
MRI	**T2/FLAIR**: areas of hyperintensitity in the white matter with a peripheral, bilateral and asymmetric distribution. Small punctate T2 lesions may be in proximity to the lesions. Subcortical rather than periventricular location, affecting U-fibers.
	Early asymptomatic stage: focal hyperintense T2 signal in the juxta-cortical white matter, most commonly unilobar in the frontal lobe, involving U-fibers and also frequently the adjacent grey matter. Border ill-defined toward the white matter and sharp toward the cortical grey matter (GM).
	T1: lesions become increasingly hypointense, as white matter destruction occurs. **T1-contrast-enhancement**: 30-40% at the diagnosis; pattern of enhancement variable (patchy, linear, nodular, peripheral or perivascular).
	DWI: varies depending on the stage of the disease and can be negative in asymptomatic patients.
IRIS	Sub-acute progression and exacerbation of earlier symptoms of PML within days to weeks after natalizumab withdrawal and/or plasma exchange therapy, due to recovery of the CNS immune system after a period of immunosuppression.
	IRIS diagnosis is performed by MRI evidence of enlarging and/or gadolinium enhancement of previous CNS lesions (both PML and previous MS lesions).
Therapy	**PLEX** doesn't improve clinical outcome (36.5% of good outcome versus 44% without PLEX; 84% of survival versus 88% without PLEX).
	Medications selective for 5-HT2A receptor (mirtazapine, risperidone): conflicting results.
	Anti-malarial drug mefloquine: only case reports and case series.
	Inhibitors of DNA replication (Brincidofovir, Ganciclovir, Leflunomide), **immune-response modulators** (IL-2 and IL-7) and **passive or active immunization**: case reports or small case series.
	Corticosteroids: used in PML-IRIS patients to reduce the inflammation; 2 different approaches (pulse corticosteroids alone or prolonged corticosteroid course with slowly tapering regimens alternating with pulse corticosteroid). Corticosteroids administered out of the IRIS onset are associated to a negative disability progression in PML patients.
Prognostic factors for	• Younger age; • Lower EDSS before PML onset;

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PML better outcome	• Lower JCV-DNA load in CSF; • Unifocal MRI lesion; • Early PML diagnosis.

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