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Confirmed Progression independent of Relapses activity in Multiple Sclerosis Patients under long-term Natalizumab Therapy

Dissertation

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Summary

"*Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) with a complex, diverse disease course. Clinically, there are two different disease subtypes of MS: relapsing remitting multiple sclerosis (RRMS) and progressive multiple sclerosis (PMS). Natalizumab was early licensed as a disease modifying therapy (DMT) for the treatment of highly active RRMS and its efficacy in reducing the relapse rate has been demonstrated in multiple studies. However, natalizumab failed to meet the primary composite endpoint at two years in a phase-three trial performed in secondary progressive multiple sclerosis (SPMS). To date, the effect of natalizumab on preventing conversion to SPMS is still unclear*" [1] and there is still no consensus regarding the definition of SPMS. "*The objective of this study was to investigate confirmed progression independent of relapse activity (cPIRA) in RRMS patients under long-term natalizumab treatment. We performed a retrospective, cross-sectional study of clinical data captured between 1994 and 2019 at two German MS tertiary referral centers. Data files of all RRMS patients treated with natalizumab for* $\geq$*24 months were analyzed. Confirmed progression independent of relapse activity was defined as* $\geq$*12 week confirmed disability progression on a roving Expanded Disability Status Scale (EDSS) reference score by 1 point in patients with an EDSS score* $\leq$*3 or 0.5 in patients with an EDSS score* $\geq$*3.5 in the absence of a relapse. Cox proportional hazard models were used to analyze the probability of developing confirmed progression independent of relapse activity depending on both disease and natalizumab treatment duration. Among the 184 patients identified, 44 (24%) developed confirmed progression independent of relapse activity under natalizumab irrespective of the EDSS score at natalizumab onset. Time to cPIRA was not affected by EDSS at natalizumab onset (categorized by EDSS score* $\leq$*3.5 versus >3.5) nor by duration of disease nor by duration of therapy. cPIRA occurred earlier in the disease course in patients with an earlier natalizumab therapy onset with regard to disease duration. A stepwise forward regression analysis revealed disease duration as the main factor for cPIRA development (p=0.005). Taken together, cPIRA occurs in a substantial proportion of patients on long-term natalizumab treatment and independent of EDSS score at natalizumab onset. Our findings suggest that patients who are initiated on natalizumab early during disease course, usually in order to treat an aggressive clinical phenotype, have a higher risk of early confirmed progression independent of relapse activity.*" [1]

Zusammenfassung

Multiple Sklerose (MS) ist eine immunvermittelte Erkrankung des zentralen Nervensystems (ZNS) mit einem komplexen, vielfältigen Krankheitsverlauf. Klinisch gibt es zwei verschiedene Subtypen von MS: schubförmig remittierende Multiple Sklerose (RRMS) und progressive Multiple Sklerose (PMS). Natalizumab wurde als krankheitsmodifizierende Therapie (DMT) für die Behandlung von hochaktiver RRMS zugelassen und seine Wirksamkeit bei der Reduzierung der Schubrate wurde in mehreren Studien nachgewiesen. Allerdings verfehlte Natalizumab den primären kombinierten Endpunkt nach zwei Jahren in einer Phase-3-Studie, die bei sekundär progredienter Multipler Sklerose (SPMS) durchgeführt wurde. Bis heute ist die Wirkung von Natalizumab auf die Verhinderung einer Konversion zu SPMS noch unklar und es besteht noch kein Konsens bezüglich der Definition von SPMS. Das Ziel dieser Studie ist es, die bestätigte Progression unabhängig von der Schubaktivität (cPIRA) bei RRMS-Patienten unter Langzeitbehandlung mit Natalizumab zu untersuchen. Wir haben eine retrospektive Querschnittsstudie klinischer Daten durchgeführt, die zwischen 1994 und 2019 in zwei deutschen Zentren für MS erhoben wurden. Daten aller RRMS- Patienten, die $\geq$24 Monate lang mit Natalizumab behandelt wurden, wurden analysiert. Eine Progression unabhängig von Schubaktivität wurde definiert als $\geq$12 Wochen bestätigte Behinderungsprogression auf einer roving Expanded Disability Status Scale (EDSS) um 1 Punkt bei Patienten mit einem EDSS von $\leq$3 oder 0,5 bei Patienten mit einem EDSS $\geq$3,5 ohne Schübe. Cox-Proportional-Hazard-Modelle wurden verwendet, um die Wahrscheinlichkeit der Entwicklung einer bestätigten Progression unabhängig von der Schubaktivität - in Anbetrecht sowohl der Erkrankung als auch der Dauer der Behandlung mit Natalizumab - zu analysieren. Unter den 184 identifizierten Patienten entwickelten 44 (24%) eine bestätigte Progression unabhängig von der Schubaktivität unter Natalizumab, unabhängig vom EDSS-Score zu Beginn der Behandlung mit Natalizumab. Die Zeit bis zur cPIRA wurde weder durch EDSS zu Beginn der Behandlung mit Natalizumab (kategorisiert durch den Score der EDSS $\leq$3,5 versus $>$3,5) noch durch die Krankheitsdauer oder Therapiedauer beeinflusst. cPIRA trat früher im Krankheitsverlauf bei Patienten mit einem früheren Therapiebeginn mit Natalizumab im Hinblick auf die Krankheitsdauer auf. Eine schrittweise Vorwärtsregressionsanalyse ergab, dass die Krankheitsdauer der Hauptfaktor für die cPIRA Entwicklung war (p=0,005). Zusammengenommen tritt cPIRA bei einem erheblichen Anteil der Patienten unter Langzeitbehandlung mit Natalizumab und unabhängig vom EDSS-Score zu Beginn der Behandlung mit Natalizumab auf. Unsere Ergebnisse deuten darauf hin, dass Patienten, die früh im Krankheitsverlauf mit Natalizumab behandelt werden, in der Regel zur Behandlung eines aggressiven klinischen Phänotyps, unabhängig von der Schubaktivität ein höheres Risiko für eine früh bestätigte Progression haben.

List of Abbreviations

ARR: Annualized Relapse Rate
CDP: Confirmed Disability Progression
CIS: Clinical Isolated Syndrome
CNS: Central Nervous System
cPIRA: Confirmed Progression Independent of Relapse Activity
CSF: Cerebrospinal Fluid
DMT: Disease Modifying Therapy
EDSS: Expanded Disability Status Scale
FS: Functional Systems
LMU: Ludwig-Maximilians University
MRI: Magnetic Resonance Imaging
MRZ: Measles Rubella and Varicella Zoster Reaction
MS: Multiple Sclerosis
PPMS: Primary Progressive Multiple Sclerosis
PIRA: Progression Independent of Relapse Activity
RAW: Relapse-Associated Worsening
RPMS: Relapsing Progressive Multiple Sclerosis
RRMS: Relapsing Remitting Multiple Sclerosis
SPMS: Secondary Progression Multiple Sclerosis
SIR: Superimposed Relapses
TOP: Tysabri Observational Program

Contents

1 Introduction

This study focuses on the effects of a long-term natalizumab therapy on preventing from secondary progression in relapsing-remitting multiple sclerosis (MS) patients, and covers the analogous analysis recently published in [1]. In order to set the stage for the development of such discussion, we first of all begin by providing a brief overview about some of the most essential underlying concepts in the following sections. In particular, we present the generalities of the MS in Section 1.1, to then focus in more detail on secondary progression MS (SPMS) in Section 1.2 and on natalizumab in Section 1.3. In Section 1.4, we furthermore outline the aims and the structure of the manuscript.

1.1 Multiple sclerosis

1.1.1 Definition

MS is a chronic, inflammatory, most often immune-mediated disease of the central nervous system (CNS). The CNS inflammation is supposed to be mediated by activated T-Lymphocytes and monocytes causing perivascular infiltrates of inflammatory cells, demyelination, neurodegeneration, tissue damage and gliosis mainly in the white matter with the formation of multiple plaques in the brain and spinal cord.

1.1.2 Epidemiology

MS is the most frequent non-traumatic cause of neurological disability in young adults globally. It usually begins around the age between 20 and 40 years and affects two to three times as many women as men [2]. Current estimates suggest that over 2.5 Million people are affected by MS worldwide with an unevenly prevalence distribution through the world: the frequency of MS increases progressively from the lowest incidence in tropical areas and Asia to the highest in Northern Europe, United States, Canada, New Zealand and Australia [3, 4].

1.1.3 Etiology and pathogenetic

Although the ultimate cause of the inflammation is still unknown, strong evidence suggests that MS is a multifactorial disease with heterogenous etiology which results from complex interactions between susceptibility genes and environmental factors e.g. sunlight and ultraviolet radiation, low blood level of vitamin D, cigarette smocking and infection with Epstein-Barr and other viruses [5]. The multifocal plaques in the white matter are supposed to be the result of a T-cells-mediated immun reaction which leads to activation of macrophages and other inflammatory cells such as cytokine, TNF, chemokine and antibodies [6]. This inflammatory reaction is intimately involved in the process of myelin

destruction which affects the axon and makes it susceptible to further damages through various mechanisms [7]:

- Cytokines-mediated damage of oligodendrocytes and myelin;
- Macrophages-mediated myelin destruction;
- Complement-mediated myelin destruction;
- CD4+ and CD8+ T-cells mediated damage of oligodendrocytes;
- Activated antibodies against oligodendrocytes and myelin.

The described inflammatory pathway may partly explain the relationship between inflammation, demyelination and axonal involvement. Furthermore it has to be kept in mind that MS is not only characterized by acute active demyelinating lesions with pronounced inflammatory activity, but also by inactive lesions and chronic neuroinflammation determining long-term disability and lasting damages [8].

1.1.4 Clinical course

Historically the clinical course of MS was characterized as relapsing-remitting, primary progressive and secondary progressive as follows [9]:

- Relapsing remitting multiple sclerosis (RRMS): represents the most diffuse form of MS, characterized by relapses with full recovery or residual deficit upon recovery, with progression-free periods between the relapses;
- Primary progressive multiple sclerosis (PPMS): form of MS characterized by a disease progression with a gradual worsening of the neurologic imparement from the beginning of the symptoms;
- Secondary progressive multiple sclerosis (SPMS): form of MS characterized by an initial relapsing remitting disease course followed by a progression phase with or without relapse activity, remission phases and plateaus;
- Relapsing progressive multiple sclerosis (RPMS): form of MS characterized by relapses and progression.

This terminology has been re-evaluated and extended on the basis of the increased understanding of MS and its pathology. The new MS phenotypic classification considers the PPMS part of the spectrum of progressive disease, the clinical isolated syndrome (CIS) has been added to the RRMS disease spectrum and the term ”RPMS” was abandoned to due the lack of consensus about its definition. Furthermore the concepts of ”active” and ”not active” have been introduced depending on clinical aspects such as relapses and/or

magnetic resonance imaging (MRI) activity defined as Gadolinium-enhancing lesions or new enlarging T2 hyper intense lesions for relapsing-remitting disease and steadily increasing neurological dysfunction as well as increasing number and volume of T1 hypo intense lesions and brain volume loss for progressive disease. As consequence of the new classification the MS phenotypes are now defined as follows [10]:

- Relapsing remitting disease: active/not active;
- Progressive disease: active with progression, active without progression, not active with progression, not active without progression (stable disease).

Inflammation and degeneration have been both shown to be playing a fundamental role in relapsing and progressive MS courses, with compartmentalized inflammation and degeneration in the CNS particularly important for the latter [11].

1.1.5 Symptoms

Depending on the amount of nerve damage as well as which nerves and/or area of the CNS or spinal cord are affected a variety of different signs and symptoms of MS are possible in the patients. The most common MS manifestation are acute occurring focal neurologic impairments caused by inflammatory and demyelinating activity in CNS and PNS which are commonly referred to as relapses. A relapse is defined as new, focal neurological symptom evolving over days to weeks that occurs with at least a 30 days interval from the last relapse, lasts for $>$24 hours, is not associated with fever or infection, and is typically followed by at least partial recovery of function over time. Depending on the inflammation-affected area, MS can affect the motor system causing numbness or weakness in one or more limbs, tremor, lack of coordination or unsteady gait as well as visual system deficits such as vision loss or double vision. Other very common symptoms may include slurred speech, fatigue, sensory disturbances including paresthesias, dizziness and problems with sexual, bowel and bladder function.

1.1.6 McDonald criteria

Traditionally the MS diagnose is assessed basing on the in 2001 first established McDonald criteria which have been revised multiple times (2005, 2010 and 2017) according to the better understanding of MS and improved MRI techniques. These diagnostic criteria take into account clinical, radiographic, and laboratory parameters and are to be applied primarily to patients experiencing a typical CIS suggestive of MS, or with symptoms consistent with a CNS inflammatory demyelination disease. According to the McDonald criteria, assessing the MS diagnosis is possible based on these five categories:

- *" ≥2 clinical attacks*
  - *with ≥2 lesions with objective clinical evidence*
  - *with no additional data needed*
- *≥2 clinical attacks*
  - *with 1 lesion with objective clinical evidence and a clinical history suggestive of a previous lesion*
  - *with no additional data needed*
- *≥2 clinical attacks*
  - *with 1 lesion with objective clinical evidence and no clinical history suggestive of a previous lesion*
  - *with dissemination in space evident on MRI*
- *1 clinical attack (i.e. clinically isolated syndrome)*
  - *with ≥2 lesions with objective clinical evidence*
  - *with dissemination in time evident on MRI or demonstration of CSF-specific oligoclonal bands*
- *1 clinical attack (i.e. clinically isolated syndrome)*
  - *with 1 lesion with objective clinical evidence*
  - *with dissemination in space evident on MRI*
  - *with dissemination in time evident on MRI or demonstration of CSF-specific oligoclonal bands" [12]*

If T2-hyperintense lesions occur in two or more locations among periventricular (≥3 lesions), cortical or juxtacortical (≥1 lesion), optic nerve (≥1 lesion), infratentorial (≥1 lesion) and spinal cord (≥1 lesion) the definition of dissemination in space is met. On the other hand, a dissemination in time is fullfilled when a new lesion is detected (T2 bright lesion and/or gadolinium-enhancing) compared to a previous MRI (irrespective of timing) or in the presence of both an asymptomatic enhancing lesion and a non-enhancing T2 bright lesion simultaneously [13].

Although the McDonald criteria facilitate the diagnostic process and allow an early MS diagnose they are not MS specific and can be observed also in other non-MS idiopathic inflammatory demyelinating diseases. A MS-similar clinical presentation can also occur in the course of infections as well as metabolic, vascular and neoplastic diseases. For this reason the MS diagnosis requires not only the McDonald criteria to be met but also that alternative explanations for the clinical presentation (differential diagnosis) are considered and excluded [14].

1.1.7 EDSS

The Expanded Disability Status Scale (EDSS) is a score for rating impairment in MS patients. This method examines eight Functional systems (FS) (Pyramidal, Cerebellar, Brain Stem, Sensory, Bowel & Bladder, Visual, Cerebral, and other) and rates each of these systems from 0 (=normal) to 6 (=maximal impairment) based on the degree of neurological deficit. The combination of the FS-rating scale and the evaluation of the patients walking distance gives an EDSS Value between 0 (= normal neurologic exam) and 10 (= death due to MS). It is to be noted that an EDSS value of $\leq$4.5 describes patients with full ambulation (ability to walk without aid or rest for some 500 meters) and that for EDSS values up to 4.5 the walking disability becomes increasingly relevant [15]. A graphical representation of this scale is shown in Fig. 1.

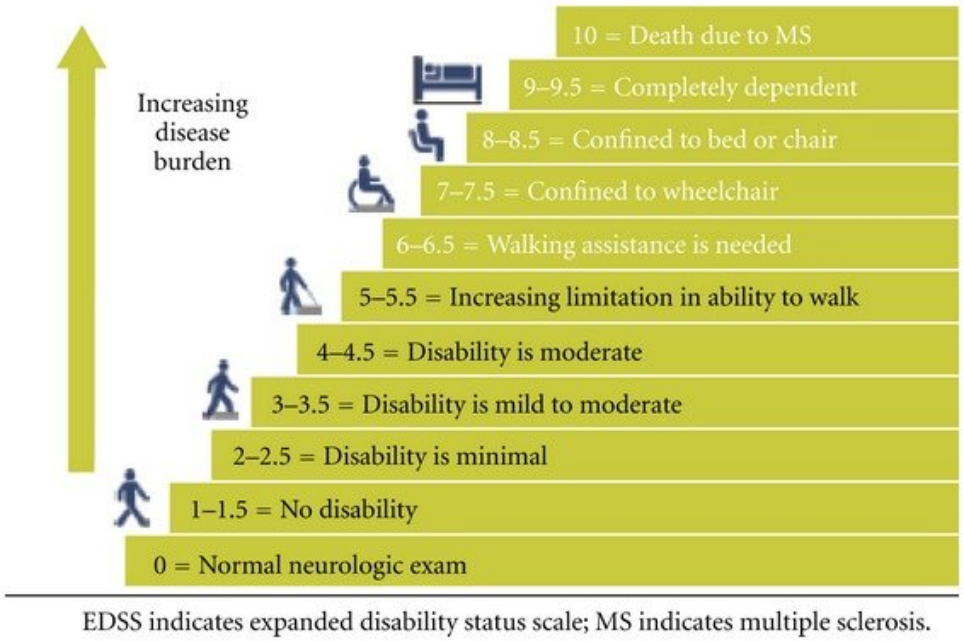


Figure 1: Simplified graphical representation of the EDSS. Figure adapted from [16].

1.1.8 Therapy

Because of the epidemiological relevance of MS, this disease and its treatment has historically been the focus of intense, worldwide research effort. Nevertheless, it has to be kept in mind that the current MS treatmet is just symptomatic, with the purpose of reducing the relapse activity and the disease progression. A curative approach is not available. The MS therapy can be divided into three groups:

- Symptomatic therapy
- Relapses therapy
- Disease modifying therapy

The aim of the symptomatic therapy is to eliminate or reduce the symptoms impairing the functional abilities and quality of life of the affected patients. Symptomatic therapies includes not only medicaments but also alternative medical measures such as physiotherapy, physical training, multimodal rehabilitation, intrathecal drug application, etc [17]. The established standard therapy for acute relapses consists in high-dose, short-term, intravenous corticosteroids (methylprednisolone 1g/day for 3-5 days) aiming at speeding the recovery from attacks [18]. Alternatively the plasma exchange is a valid therapeutic option in the case of severe, steroid-unresponsive relapses. [19, 20]. Disease modifying therapies are long-term treatments which interfere in the disease pathophysiology and have an immunmodulatory and immunsuppresive effect. They includes injectable, oral and infusion drugs which cannot cure MS but can decrease the relapse frequency, reduce lesions activity and accumulation in NS and may slow progression of disability [21]. The most suitable DMT is to choose according to - among others - the disease activity (i.e. relapse rate and severity, disease progression, MRI-findings and therapy response), symptomatic, patient age, intrathecal IgG or IgM synthesis [22]. The DMTs can be grouped according to the application modus as follows:

- Self-injected agents:
  - daclizumab (Zinbryta®)
  - glatiramer acetate (Copaxone® and Glatopa®)
  - interferon beta 1-a, subcutaneous (Rebif®)
  - interferon beta 1-a, intramuscular (Avonex®)
  - interferon beta 1-b (Betaseron® and Extavia®)
  - pegylated interferon beta-1 a (Plegridy®)
- Oral agents:
  - dimethyl fumarate (Tecfidera®)
  - fingolimod (Gilenya®)
  - teriflunomide (Aubagio®)

- Intravenous agents:
  - ocrelizumab (OcrevusTM)
  - alemtuzumab (Lemtrada®)
  - mitoxantrone (Novantrone®)
  - natalizumab (Tysabri®)

1.2 SPMS

As discussed in the previous sections, the SPMS has been defined as an initial relapsing remitting disease course with gradual worsening with or without superimposed relapses, plateaus and partial neurological imperriment remission. Moreover, SPMS is mostly a retrospective diagnosis characterized by an irreversible disability progression independent of relapse activity, although SPMS patients can still experience superimposed relapses in the progressive phase of the disease. Nevertheless, a commonly accepted definition of secondary progression has not been reached yet and in particular the duration of the progression required for the SPMS diagnosis divides the literature [23–26].

Some the most popular guidelines for the diagnosis of SPMS are the Lublin criteria [27]. These underline the importance of standardized clinical course descriptors to be defined in a specified time frame in order to avoid confusion and facilitate accurate identification of patient populations in the clinical practice. The identified terms are described as follows:

- Active disease: phase of the disease carachterizied by relapse activity and occurance of new neurologiscal impairments, with full or partial recovery, which occure without any fever, infection, gadolinium-enhancing lesions or new enlarging T2 MRI lesions;
- Progressing disease: disability progression, irrespective of relapse activity, during the progressive phase of MS;
- Worsening disease: any neurolocical disability progress independent of previous relapse activity or (increasing) progressive disability during the progressive phase of the illness.

Another widely used SPMS definition is based on the EDSS and information about preceding relapses with a short confirmation period of 3 months, and seems to enable a timely and reproducible SPMS diagnosis [28]. In similar studies based on EDSS, SPMS has been defined as clinical worsening in EDSS of 1.5, 1.0, or 0.5 points (or greater) from a baseline EDSS = 0, 1.0-5.0, or 5.5 or higher, respectively, wih a yearly EDSS assestment. An EDSS worsening between the two annual visits during a year with relapse activity was considered ”short-term worsening”, while an EDSS worsening maintained for

two consecutive annual visits was referred to as "confirmed disability". Furthermore, the long-term disability progression seems to be largely independent of relapses and correlates mostly with diffuse brain atrophy and brain volume loss, which can occur gradually and early in the disease course without clinical correlate such as EDSS worsening [29].

1.2.1 cPIRA

Based on these observations, the concept of progression independent of relapse activity (PIRA) has been brought forward [30]. In the same reference, "*in particular the confirmed progression independent of relapse activity (cPIRA) has been identified as a common indicator of SPMS conversion, and has been defined as a period of confirmed disability progression (CDP) indepeniseaseent of relapse activity for a period longer than 12 weeks.*" [1] This disability progression was formally defined (in the aformentioned reference as well as in this study) as a worsening of 1 or 0.5 EDSS points in patients with a baseline EDSS lower than 3 or larger than 3.5, respectively.

1.3 Natalizumab

1.3.1 Pharmacodynamic effects

Natalizumab is a monoclonal antibody which targets a protein called $\alpha 4\beta 1$ integrin, as graphically depicted in Fig. 2 (adapted form [31]). This integrin is highly expressed on human leukocytes and is a key player in cell adhesion. It acts as a mediating agent for the recruitment and transmigration of immune cells into inflamed tissues. By binding to the $\alpha 4$-subunit of human integrins natalizumab inhibits the interaction between $\alpha 4\beta 1$-expressing immune cells and their ligands VCAM-1 (vascular cell adhesion 1) in endothelium. This process leads to an interruption of the leukocytes migration across the blood-brain barrier. The migration of activated T-Lymphocytes across the blood-brain barrier in the CNS is one of the mechanisms believed to cause the MS lesions. Under normal conditions, VCAM1 is not expressed in the brain tissue. Its expression on the endothelial cells is upregulated in the presence of inflammation. By blocking the process of leukocytes migration, natalizumab stops the perpetuation of the MS-typical inflammatory cascade [32].

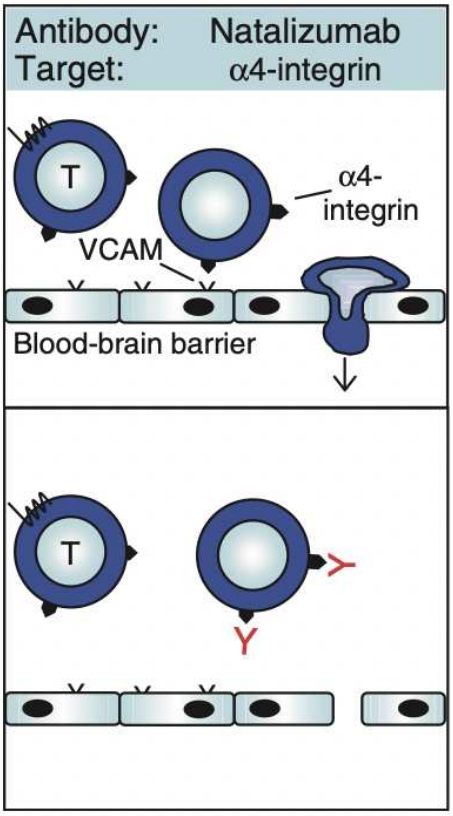


Figure 2: Simplified graphical representation of natalizumab mechanism of action. Figure adapted from [31].

1.3.2 Clinical effects

"*Natalizumab was licensed in 2006 as a disease modifying therapy (DMT) for the treatment of highly active RRMS and its clinical efficacy has been the focus of multiple studies.*" [1] Among others, two randomised, multi center studies showed a 68% relapse rate reduction compared to placebo (AFFIRM Study) [33] and a 55% relapse rate reduction compared to a Therapy with IFN-ß-1a (SENTINEL Study) [34]. Furthermore also a reduction of Gadolinum enhancing lesions and a positive effect on the disease progression was detected.

However, in the more recent ASCEND study, natalizumab failed to meet the primary endpoint at year two, as it did not significantly reduce disability progression in SPMS patients. Nevertheless, a potential benefit has been identified for the function of the upper extremities, and a subcessive extension of the study has shown an efficacy on the primary outcome at year three [35]. "*A possible explanation for the difference in efficacy of natalizumab in relapsing and progressive MS may be the inability to reach the compartmentalized pathology.*

To date, the effect of natalizumab on preventing conversion to secondary progressive MS is still unclear. In a prospective cohort published in 2026, the continued natalizumab therapy for over four years was associated with a decrease of relapse rate and no dis-

ease progression, but no association was found with the accumulation of lesion burden or the magnitude of brain volume loss, suggesting an uncertain benefit of prolonged natalizumab use on clinical and MRI outcomes of disease progression [36]. Another large real-world evidence study demonstrated that early natalizumab treatment during disease course reduced the risk of conversion to secondary progressive MS [37]." [1]

1.3.3 PML

The use of natalizumab has been associated with an increased risk of rare and often letal brain infection called progressive multifocal leukoencephalopathy (PML). The PML is an opportunistic viral infection caused by the John-Cunningham (JC). An infection with the JC-Virus tipically affects people with severe immune deficiency leading to a quickly destruction of the white matter in CNS [38]. It has been observed that the PML-risk is associated to the following risk factors: I) natalizumab treatment duration >2 years; II) immunosuppressant use prior to natalizumab therapy onset; III) presence of anti-JCV antibodies in blood. For this reason the indication for a treatment with natalizumab has to be constantly individually reconsidered and the regular testing for serum anti-JCV antibodies is recommended [32].

1.4 Aims and outlook

In light of these previous results and considerations, "*in this study we aim at assessing the relevance of relapse-independent disease progression as an indicator for secondary progressive MS conversion in two independent real-world cohorts of MS patients under long-term natalizumab treatment.*" [1]

We do so by describing the methodology employed to select and describe the patients as well as the statistical setup used to evaluate the cohorts in Section 2. We then present the resulting quantitative findings in Section 3, which we place in the more general landscape of the literature on natalizumab in Section 4 together with a summary and closing remarks.

2 Materials and Methods

2.1 Patients and recruitment

In the context of this study, we performed a retrospective chart review based on data gathered during clinical routine visits at the Multiple Sclerosis Centers of the Heinrich-Heine-University Düsseldorf and of the Institute of Clinical Neuroimmunology, LMU Hospital, Ludwig-Maximilians University, Munich. Concretely, the necessary epidemiological, clinical and paraclinical information has been collected via the hospital information system (MEDICO, Cerner/CGM [Düsseldorf]) and clinical charts (LMU Hospital) and regards, among others, age, sex, disease duration, relapses, EDSS, MRI, previous and current therapies. In order to qualify for the study, the following inclusion criteria needed to be passed by the patients:

1. the presence of a diagnosis of RRMS according to the McDonald criteria 2010 (see Section 1.1.6),
2. a continuous natalizumab therapy with a duration of more than 24 months, and
3. the availability of longitudinal EDSS and relapse data (with a minimum of three EDSS and documentation on relapse dates) for a period of more than 24 months.

On the other hand, these two exclusion criteria were also enforced:

1. that the cPIRA (see Section 1.2.1) onset happened before the start of the natalizumab treatment, and
2. that other causes than MS (like for instance a stroke, polyneuropathy or any other neurodegenerative disease) have lead to a disability change, clinical impairment or deficit.

In light of the discussion introduced in Section 1.2.1, here we also used cPIRA as an indicator for SPMS conversion. Concretely, cPIRA has been determined following a $\geq$12 week CDP independent of relapse activity and evaluated in all patients including those who discontinued the natalizumab therapy in the follow-up. "*Disability progression was defined as a worsening of 1 point on the EDSS in patients with a baseline EDSS $\leq$3 or 0.5 EDSS steps in patients with a baseline EDSS $\geq$3.5 in the absence of a relapse using a roving EDSS reference score. We chose to define EDSS progression based on a cut-off value of 3.5 as EDSS steps up to 3.5 are mainly dominated by single functional system scores and rather sensitive to interrater variability, while from scores above 3.0 relevant disability in more than one system is required and above 4.0 the walking disability becomes increasingly relevant. The relapse-free interval relevant for cPIRA was defined as a time interval without relapses for a minimum of 12 consecutive months. All patients with an*

EDSS worsening according to the aforementioned definition of disease progression were included in the cPIRA group when the relapse unrelated EDSS worsening (PIRA) could be confirmed in the next clinical follow-up at least 12 weeks later (cPIRA). Relapses occurring after the development of cPIRA were classified as superimposed relapses (SIR). To include a maximum of data and to analyze the relevance of events outside of the natalizumab treatment interval, we did not limit the follow-up length, but instead included all available EDSS and MRI data from the MS first diagnosis to the last documented visit. Therefore, cPIRA evaluation began with the first EDSS documented, e.g. at the time of MS diagnosis.

Relapse data were extracted from the clinical databases and by chart review. Relapses have been identified and classified during the clinical routine by experienced MS specialists at our tertiary referral centers based on patient interviews and clinical examination. Relapses were defined as a neurologic deficit compatible with an acute central nervous system inflammatory demyelinating event lasting at least 24 hours in the absence of fever. Disability progression observed in visits with a relapse in between was considered as relapse-associated worsening (RAW) and not considered for analysis of cPIRA. Furthermore, in order to avoid the risk of carry over EDSS progression resulting from prior relapses, all follow-up intervals with relapse activity within one month prior to the baseline examination were excluded from the cPIRA analysis.

MRI activity was defined as presence of gadolinium enhancing lesions on T1 imaging or the development of new or enlarging T2 lesions in comparison to the previous MRI. MRI data and findings were collected retrospectively during the observational period. Due to impaired comparability of different and non-standardized MRI protocols performed on different scanners in the clinical routine, we had to limit our analysis to the occurrence of inflammatory lesions and were not able to analyze brain volume and/or brain atrophy patterns." [1]

2.2 Ethics approval and consent to participate

"*The study was approved by the local ethics committee at the Heinrich Heine University of Düsseldorf (registry number 6083R) and at the Ludwig-Maximilians University Munich (Nr. 19116). Due to the retrospective design of the study, informed consent was not necessary according to the local ethics committee.*" [1]

2.3 Statistical analysis

"*Statistical analyses were performed using SPSS 20 (IBM, Armonk, New York) and were run for all 184 patients included even if some of them stopped the natalizumab treatment and switched to another therapy during the follow-up.*

A Mann-Whitney U test was used to identify significant differences between the groups and a power analysis was conducted to define the correlation coefficient r. A Kruskal-Wallis test with Bonferroni post-hoc test was used for to identify significant EDSS differences between groups. p-values below 0.05 were considered significant. A logistic regression analysis was performed to analyze which factors could have had an influence on cPIRA development in the overall cohort as well as in the single Munich and Düsseldorf cohorts separately. We opted for this regression model despite the risk of overfitting in order to decrease the chance of missing a signal from our defined variables.

The following factors were included in the regression model:

1. *age at natalizumab onset,*
2. *sex at natalizumab onset,*
3. *number and class of DMTs prior to treatment with natalizumab, and*
4. *duration of natalizumab therapy.*

DMTs were classified as first or second line as followed: as first line treatments we considered beta-interferon, glatiramer acetate, teriflunomide and dimethyl fumarate while second line treatments were mitoxantrone, alemtuzumab, fingolimod, natalizumab, rituximab, ocrelizumab and azathioprine.

For the comparison of the demographic and clinical characteristics between the Düsseldorf and the Munich cohorts we used the Mann-Whitney U two-sample rank-sum test. The probabilities of developing cPIRA were estimated using a Kaplan-Meier analysis. In order to facilitate interpretation and presentation of the results we divided the patients in equally sized subgroups based on the natalizumab treatment onset ($\leq$8.6 years and $>$8.6 years), the EDSS score ($\leq$3.5 and $>$3.5), the number of DMTs prior to natalizumab therapy onset ($\leq$2 and $>$2) and the number of relapses that had occurred prior to the natalizumab treatment onset ($<$1, 1-2 and $>$2). Cox proportional hazard models correcting for age and sex reporting the hazard ratios (HR; Exp(B)) were used to compare the probability to develop cPIRA for these subgroups. Reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline." [1]

3 Results

3.1 Dependence of the cPIRA rate on baseline parameters

"*Out of the 271 RRMS patients identified with natalizumab therapy at the two investigating centers (Figs. 3 & 4, Tables 1 & 2), 184 patients met the inclusion criteria, while 87 were excluded from the data analysis due to lack of EDSS and relapse data (27) or insufficient follow-up data (≤24 months of natalizumab therapy) (50). Furthermore, 10 patients were excluded due to cPIRA onset outside a natalizumab treatment interval, e.g. before initiation of natalizumab or development of cPIRA during a pause of natalizumab treatment. A more in-depth analysis dedicated to this group of patients is performed in Sec. 3.4. Of these 184 patients, 140 patients remained relapsing remitting (76%), while 44 developed cPIRA as an indicator for SPMS (24%). Under the 140 relapsing remitting patients, 16 patients (9%) presented a RAW with relevant EDSS increase. The median time on natalizumab therapy until cPIRA occurred was 10±1 years.*" [1]

3.2 Investigation of factors responsible for time to cPIRA

"*Information on MRI activity was available for all patients but not for all follow-up intervals due to the heterogeneity of follow-up in the real-world setting. Approximately half of the 184 included patients had neither MRI nor relapse activity, and patients with relapses and MRI activity were less common (Fig. 3). In the No-cPIRA under natalizumab group, 70 of 140 patients (50%) had neither relapse nor MRI activity, as compared to 26 of 44 patients (59%) in the cPIRA under natalizumab group. On the other hand, 20 of 140 patients (14.3%) in the No-cPIRA under natalizumab and 2 of 44 patients (4.5%) in the cPIRA under natalizumab group had both MRI and relapse activity. Overall, disease duration was significantly longer, the number of hospital visits prior to natalizumab and the increase of EDSS in the relapse-free period were significantly higher in patients with cPIRA under natalizumab compared to No-cPIRA patients (Table 1).*

The EDSS remained stable and showed a tendency to improvement in No-cPIRA RRMS patients without RAW (mean change of -0.2±0.6 over a mean of 5.5 years) while patients who developed cPIRA with and without SIR as well as RRMS patients with RAW (Fig. 3) presented a significant deterioration of EDSS with a mean of 1.5±0.9 over a mean of 5.8 years (Fig. 4). A significant difference with regards to MRI and CSF parameters was not detected (Table 2). The regression analysis revealed disease duration as the main factor for cPIRA development (p=0.005). Other factors (like sex, age, class of treatment and number of prior therapies as well as natalizumab therapy duration) had no additional influence on the development of cPIRA.

Cox proportional hazard models were used to compare the cPIRA-free survival between subgroups over time (Figs. 5-9), and the number of remaining patients under

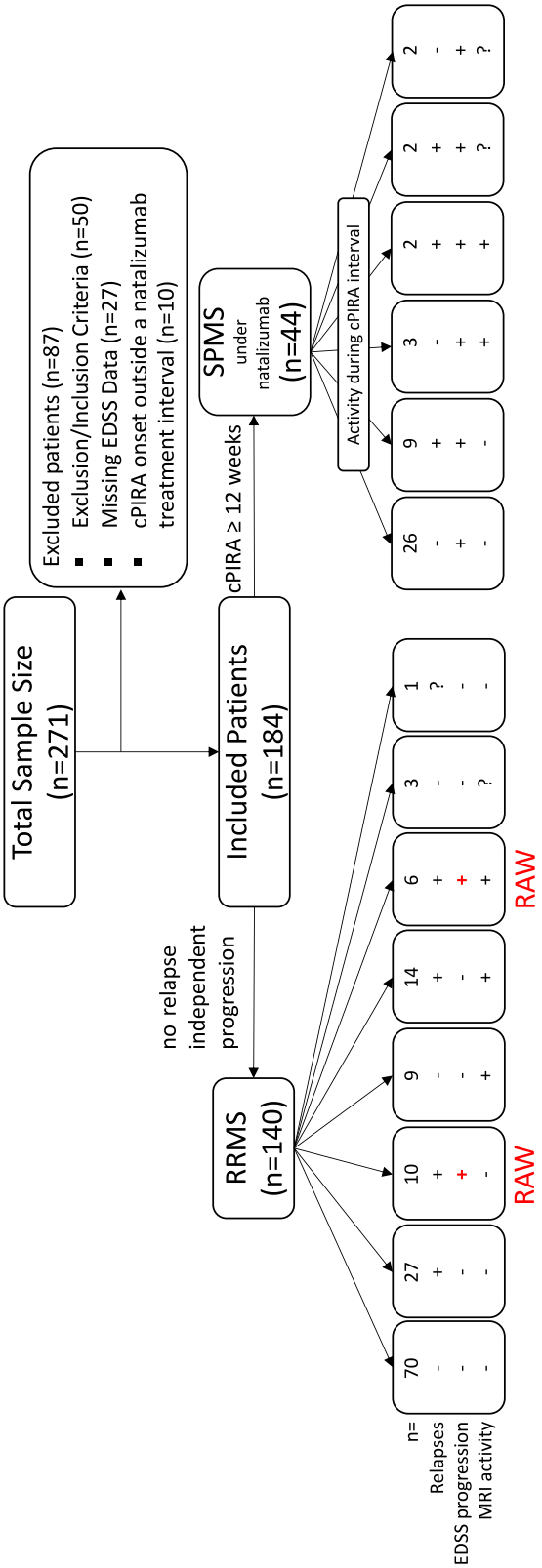


Figure 3: "*Overviewing flowchart of the total cohort. In the confirmed progression independent of relapse activity (cPIRA) group, the '+' indicates the presence of relapse and MRI activity before or after the cPIRA defining interval.*" [1] Figure from [1].

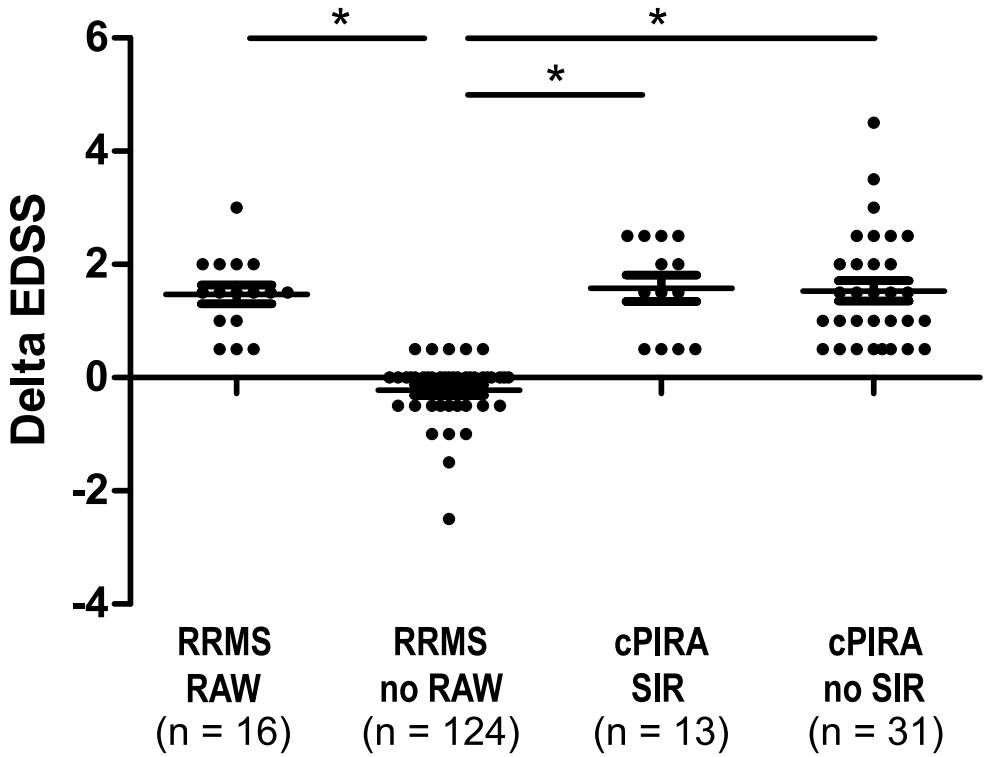


Figure 4: "*Disability change of the total cohort: EDSS change of confirmed progression independent of relapse activity (cPIRA) patients [with and without superimposed relapses (SIR)] and relapsing remitting MS patients [with and without relapse-associated worsening (RAW)]. The $*$ refers to a $p < 0.05$ value obtained via a Kruskall–Wallis with Bonferroni post hoc test.*" [1] Figure from [1].

observation at a given timepoint are indicated as 'patients at risk' below the x axes in Figs. 5-9. cPIRA occurred earlier in the course of disease in patients with an earlier onset of natalizumab therapy (≤8.6 versus >8.6 years, p <0.001, HR Exp(B)=0.060, 95%CI=0.021-0.174) but considering only the time on natalizumab the onset of cPIRA did not differ between both groups (p=0.250, HR Exp(B)=1.470, 95%CI=0.763-2.835). Time to cPIRA did not differ between patients with EDSS ≤3.5 and >3.5 neither considering the duration of disease (p=0.303, HR Exp(B)=0.696, 95%CI=0.350-1.387) nor the duration of therapy (p=0.969, HR Exp(B) = 0.987, 95%CI= 0.495-1.966).

Furthermore, patients with >2 DMTs before natalizumab show no difference in the development of cPIRA over the disease course with respect to patients with ≤2 DMTs (p=0.640, HR Exp(B) = 1.170, 95%CI= 0.607-2.255). However, considering only the period of natalizumab therapy, patients with >2 DMTs prior to natalizumab developed cPIRA significantly earlier than patients with ≤2 DMTs (p=0.031, HR Exp(B) = 1.996, 95%CI = 1.064-3.744). The annualized relapse rate (ARR) did not differ between patients who developed cPIRA and those who did not. However, the mean EDSS deterioration was significantly higher in natalizumab treated patients who developed cPIRA (Fig. 4)." [1]

	Medians (interquartile range)		
Baseline characteristics	Group 1: No-cPIRA[a] under natalizumab[2] ($n = 140$)	Group 2: cPIRA under natalizumab[2] ($n = 44$)	p-values[3]
Age at natalizumab onset - yr	33.5(27; 42)	38.5(29; 45)	n/s
Female sex - no (%)	92(65.7)	26(59.1)	n/s
Therapy duration natalizumab[4] - yr	4.8(3; 7.6)	5(3; 9)	n/s
Disease duration since first manifestation - yr	14(10; 19)	18(14; 25)	0.004 $r < 0.3$
Disease duration since first diagnosis - yr	12(9; 17)	15.5(12; 19.8)	0.004 $r < 0.3$
Disease duration between first manifestation and natalizumab onset - yr	6(3; 11)	8(3; 17)	n/s
Disease duration between first diagnosis and natalizumab onset - yr	5(2; 8)	4.5(2; 12.5)	n/s
Number of other therapies[5] - no at study inclusion	3(2; 4)	3(2; 4)	n/s
Number of DMTs[b] prior to natalizumab - no	2(1; 3)	2(1; 3)	n/s
Annualized Relapse Rate under natalizumab[6] - no	0(0; 0.3)	0(0; 0.3)	n/s
Number of visits[7] - no	12(8; 16)	16(12; 22)	0.009 $r < 0.3$
EDSSc-change[8] in relapse free interval - no	0(-0.5; 0)	1.5(0.5; 2.5)	< 0.0001 $r > 0.5$

Table 1: "***Demographic and clinical characteristics of the patients***[1]
[1] *Data include only patients who have been treated with natalizumab for a minimum of 2 consecutive years (main inclusion criterium) and whom EDSS values were available. All patient information is from the electronic database MEDICO (for the Düsseldorf Cohort) and from the patient files, which include clinical examinations and investigations results such as MRI findings, CSF and blood tests that have been collected before 01.01.2018 for the Düsseldorf cohort and before 07.08.2019 for the München cohort. Metric variables are reported following the notation: median (interquartile range). Categorical variables are reported as counts of patients with available data and percentage.*" [1]
(Caption continues in following page)

Table 1: "[2] *The patient groups were defined as follows: patients who still experienced relapses during the observation time without EDSS worsening were included in the Group1; Patients who developed a secondary progression under the natalizumab treatment were assigned to Group2. The secondary progression was defined as an EDSS worsening of* $\leq$*1.0 point from the baseline EDSS score for patients with baseline score of 3.0 or less, or* $\leq$*0.5 for patients with baseline score of 3.5 or more that cannot be attributable to recent relapse activity. For each variable we provide the median of a given group with the corresponding interquartile range.*
[3] *p-values reflect Mann-Whitney U test. A power analysis was conducted to obtain the correlation coefficient r. p-values for the comparison of Group 1 with Group 2 showed significant differences (*$p < 0.05$*) for the following variables: Disease duration since first manifestation, Disease duration since first diagnosis, Number of visits and EDSS-Change in relapse-free interval.*
[4] *The minimal natalizumab therapy duration is 2 years according to the inclusion criteria.*
[5] *This category includes all documented therapeutic measures from relapse-treatments to DMTs which have been taken since the first MS manifestation.*
[6] *Recorded between the first and the last recorded relapse in the period of time under natalizumab treatment.*
[7] *Meant is the number of visits which took place in the Universitätsklinikum Düsseldorf (UKD) or in the Universitätsklinikum München (LMU).*
[8] *Recorded between first and last recorded EDSS value during the relapse-free period.*
[a] *Confirmed Disability Progression independent of Relapse Activity*
[b] *Disease Modifying Therapies*
[c] *Expanded Disability Status Scale*" [1]
Table from [1].

3.3 Düsseldorf vs Munich

"*An overview of the Düsseldorf and Munich cohorts including separate sub-analyses is provided in Table 3 (for the subgroup of cPIRA patients under natalizumab) and Figs. 10-11. Comparing the Düsseldorf and Munich cohorts revealed significant differences regarding the time between first manifestation and first diagnosis, current therapy, Measles Rubella and Varicella Zoster (MRZ)-reaction and natalizumab discontinuation, but no differences regarding age, sex, EDSS at first diagnosis and disease duration. A stepwise forward regression analysis revealed that no variable influenced the occurrence of cPIRA in the Munich cohort while for the Düsseldorf cohort age and prior therapies had a significant influence on SPMS development. In the Munich cohort, 14 of 70 included patients (20%) and in the Düsseldorf cohort, 30 out of 114 included patients (26.3%) developed SPMS according to our cPIRA definition (Fig. 10). We observed no significant difference regarding the EDSS change between the Munich and Düsseldorf cohorts.*" [1]

	Medians (interquartile range)		
Baseline characteristics	Group 1: No-cPIRA[a] under natalizumab[2] ($n = 140$)	Group 2: cPIRA under natalizumab[2] ($n = 44$)	p-values[3]
Age at first manifestation - yr	25(20 ; 31)	26(23 ; 32)	n/s
Age at first diagnosis - yr	28(21 ; 35)	30.5(23 ; 36)	n/s
Time between first manifestation and first diagnosis RRMSa[4] - yr	4(0 ; 22)	5(1 ; 23)	n/s
Time betweeen RRMSa-first diagnosis and SPMSb-first diagnosis[5] - yr	n/a	10(5 ; 17)	n/s
Time between first manifestation and first dose Natalizumab - yr	6 (3;11)	8(3 ; 17)	n/s
Time between first diagnosis and first dose Natalizumab - yr	5(2 ; 8)	4.5(2 ; 12.5)	n/s
Annualized Relapses[6] outside Natalizumab Therapy - no	1(0.6 ; 1.8)	1(0.4 ; 2)	n/s
Visits during relapse-free period[7] - no	7(4 ; 11)	8(7 ; 13.5)	n/s
Duration first relapse-free period[8] - yr	4(2 ; 7)	5(3 ; 7)	n/s
Duration second relapse-free period[8] - yr	2(2 ; 3)	3(2.5 ; 4)	n/s
Number of Optic neuritis	1(0 ; 1)	0 (0;1)	n/s
EDSSc at RRMSa-first diagnosis - no (%)			
0,0	4 / 37(10.8)	1/12 (8.3)	0,931
1,0	5 / 37(13.5)	2 / 12(16.7)	
1,5	9 / 37(24.3)	3 / 12(25)	
2,0	7 / 37(18.9)	2 / 12(16.7)	
2,5	3 / 37(8.1)	2 / 12(16.7)	
3,0	2/37 (5.4)	2 / 12(16.7)	
3,5	4 / 37(10.8)	0/12 (0)	
4,0	1 / 37(2.7)	0/12 (0)	
4,5	1 / 37(2.7)	0/12 (0)	
5,5	1 / 37(2.7)	0/12 (0)	
Spinal lesions - no (%)	99/126 (78.7)	31/40 (77.5)	0,746

	Medians (interquartile range)		
Baseline characteristics	Group 1: No-cPIRA[a] under natalizumab[2] ($n = 140$)	Group 2: cPIRA under natalizumab[2] ($n = 44$)	p-values[3]
Current MRI lesions load[9] - no (%)			
< 3	5/98(5.1)	1/27 (3.7)	
3 - 9	5/98 (5.1)	1/27 (3.7)	0,662
> 9	88/98 (89.8)	25/27 (92.6)	
Previous therapies prior to Natalizumab - no (%)	130/140 (92.9)	42/44 (95.5)	0,583
Kind of previous therapies prior to Natalizumab[10] - no (%)			
First Line Therapies	112/140 (80)	33/44 (75)	0,228
Second Line Therapies	17/140 (12.1)	9/44 (20.5)	
Current therapy[11] - no (%)			
Fingolimod	1/124 (0.8)	2/40 (5)	
Natalizumab	68/124 (54.8)	17/40 (42.5)	
Rituximab	14/124 (11.3)	11/40 (27.5)	
Alemtuzumab	5/124 (4)	1/40 (2.5)	
Dimethylfumarat	1/124 (0.8)	0/40 (0)	0,888
Mitoxantron	0/124 (0)	1/40 (2.5)	
Teriflunomid	1/124 (0.8)	0/40 (0)	
Daclizumab	4/124 (3.2)	1/40 (2.5)	
Ocrelizumab	21/124 (16.9)	4/40 (10)	
Azathioprine	0/124 (0)	0/40 (0)	
Previous therapies with Natalizumab - no (%)	28/134 (20.9)	16/43 (37.2)	0,060
Natalizumab paused - no (%)	27/139 (19.4)	11/44 (25)	0,072
Natalizumab discontinued - no (%)	62/137 (45.3)	23/40 (57.5)	0,058
Natalizumab side effects - no (%)	35/139 (25.2)	14/44 (31.8)	0,614
PMLd - no (%)	1/140 (0.7)	2/44 (4.5)	0,185
Positive Oligoclonal bands - no (%)	104/117 (88.9)	35/38 (92.1)	0,569
Positive JCVe - no (%)	94/139 (67.6)	26/43 (60.5)	0,597
Positive MRZf reaction - no (%)	13/58 (22.45)	5/13 (38.5)	0,148

Table 2: "***Other Demographic and clinical Characteristics of the Patients at Baseline***[1]
[1] *and* [2] *same as in Table 1.*
[3] *p-values reflect Kruskal-Wallis test. Significance p-values have been adjusted by the Bonferroni correction for multiple tests.*
[4] *RRMS diagnoses were made according to McDonald criteria 2010.*" [1]
(Caption continues in following page)

Table 2: [5] *The secondary progression was defined as a relapses-independent increase of $\leq$1.0 point from the baseline EDSS score for patients with baseline score of 3.0 or less, or $\leq$0.5 for patients with baseline score of 3.5 or more. Only patients with a confirmed disability progression (CDP) $>$ 12 weeks were considered to be secondary progressive. The CDP was defined as the time in weeks between the date on which the considered clinical diagnostic criteria for SPMS were first met and the date on which a clinical confirmation of the diagnosis was documented.*
[6] *Recorded between the first and the last recorded relapse in the period of time respectively outside and under Natalizumab treatment.*
[7] *Meant is the number of visits which took place in the Universitätsklinikum Düsseldorf (UKD) or in the Universitätsklinikum München (LMU).*
[8] *Defined as the longest recorded period without relapse activity. Only relapse-free intervals longer than 1 year have been considered.*
[9] *Reported is the last performed T1-, T2-weighted and FLAIR MRI with documented radiological evaluation of the lesion load.*
[10] *First line treatments = Beta-Interferon, Glatiramer acetate, Teriflunomide, Dimethyl fumarate; Second line treatments = Mitoxantrone, Alemtuzumab, Fingolimod, Natalizumab, Rituximab, Ocrelizumab, Azathioprine.*
[11] *Reported is the last documented therapy which has been recorded before 01.01.2018 for the Düsseldorf Cohort and before 07.08.2019 for the München Cohort.*
[a] *Relapsing Remitting Multiple Sclerosis*
[b] *Secondary Progressive Multiple Sclerosis*
[c] *Expanded Dysability Status Scale*
[d] *Progressive Multifokal Leukoencephalopathy*
[e] *John Cunningham Virus*
[f] *Measles, Rubella and Varicella Zoster Virus Reaction*" [1]
Table from [1].

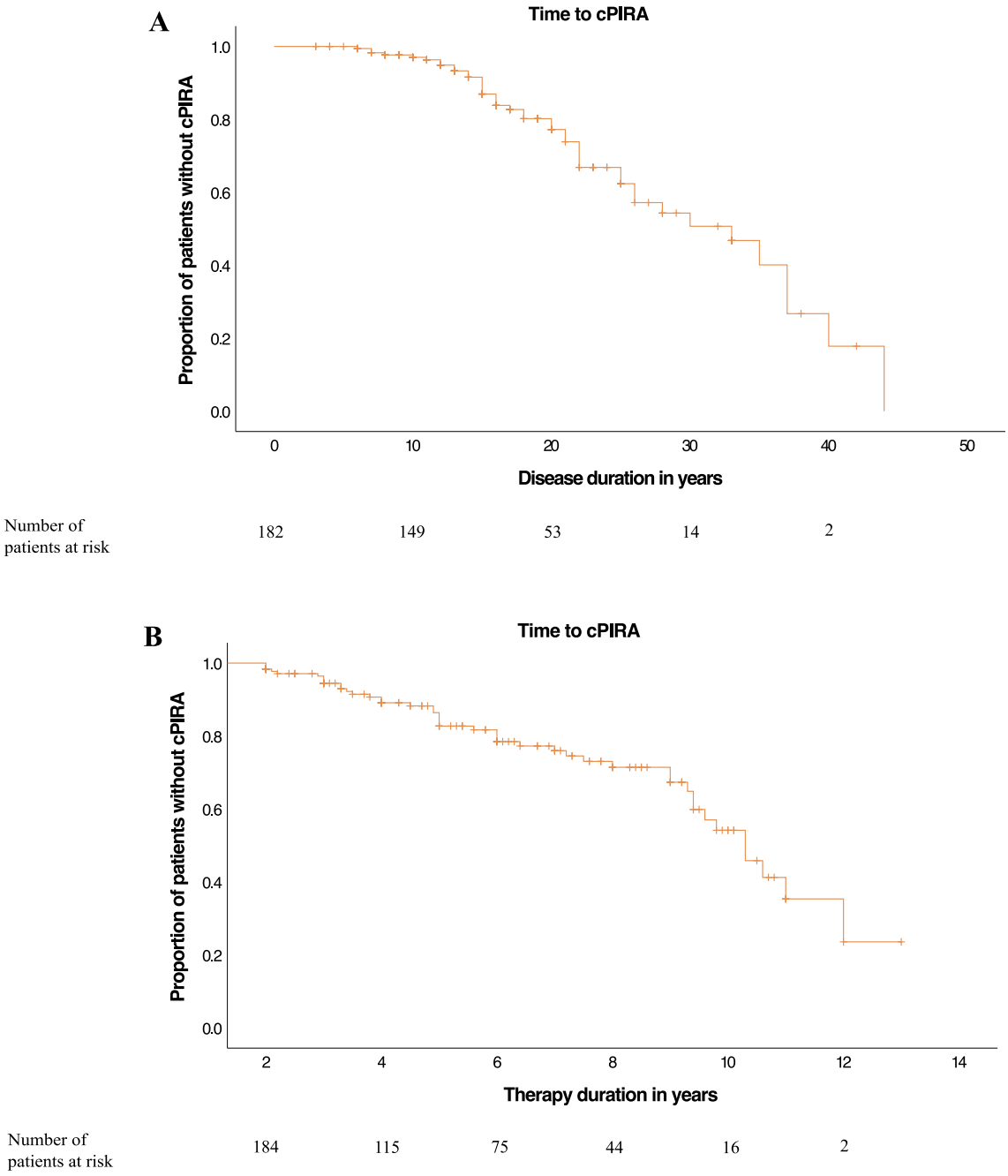


Figure 5: "***Kaplan-Meier curves of the total cohort.*** *A) Kaplan-Meier curves for the time (in years) of disease duration from the first MS manifestation and B) natalizumab treatment duration until the outcome confirmed progression independent of relapse activity (cPIRA) occured. cPIRA was defined as an EDSS increase of ≥1.0 point from the baseline EDSS score for patients with baseline score of 3.0 or less, or ≥0.5 for patients with baseline score of 3.5 or more.*" [1] Figure adapted from [1].

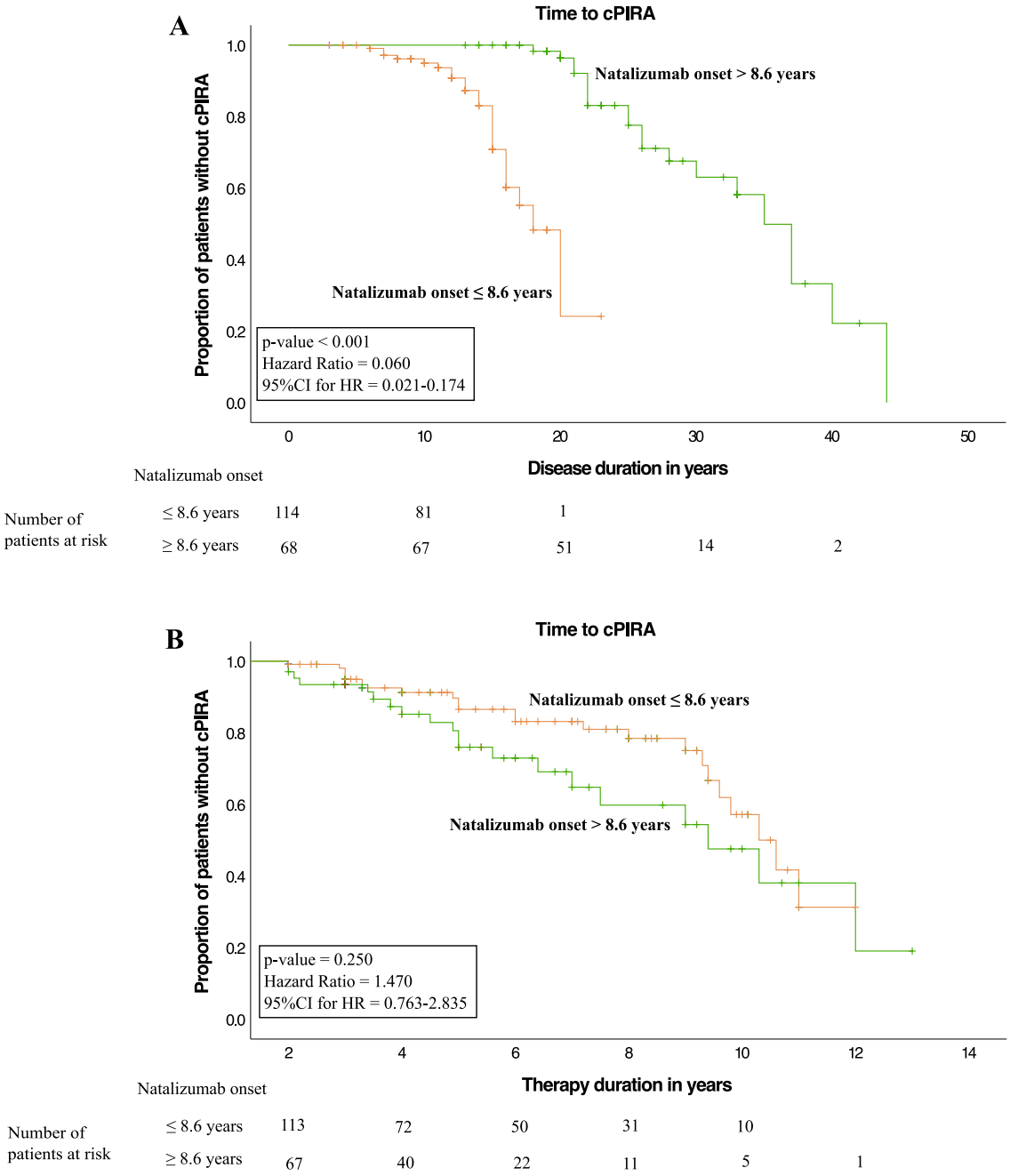
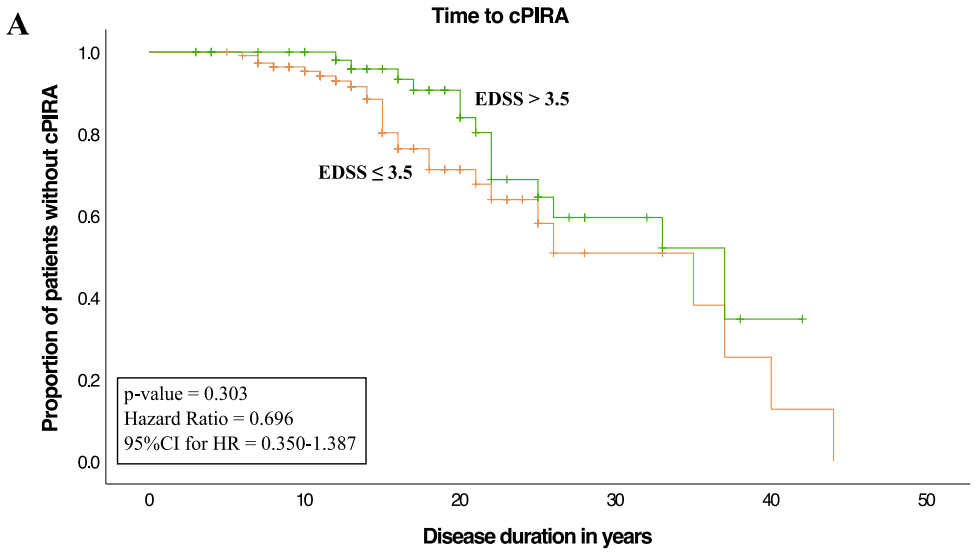
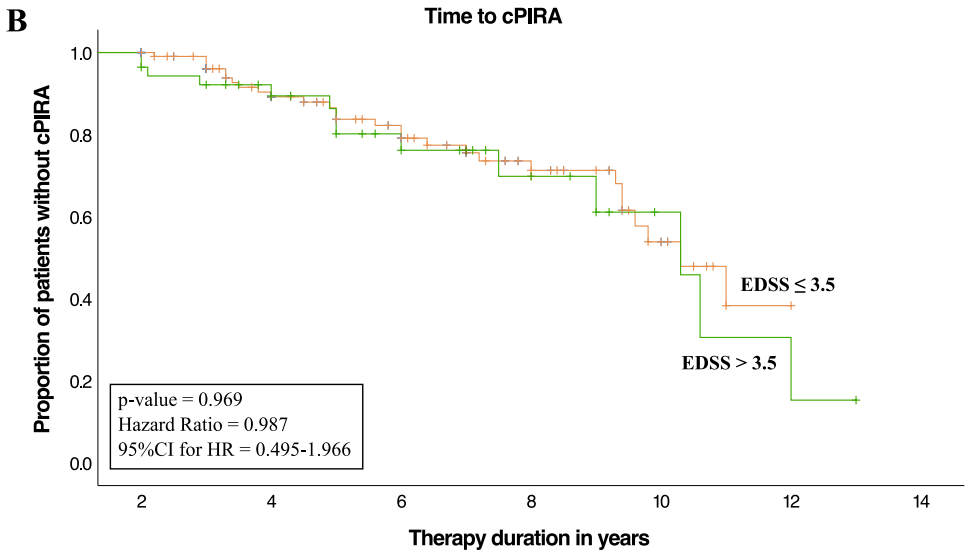


Figure 6: "***Kaplan-Meier curves of the total cohort, disease duration subanalysis***. *A) Kaplan Meier curve for the time of disease duration and B) natalizumab therapy duration (in years) to onset of confirmed progression independent of relapse activity (cPIRA). Progression free survival in the patients with natalizumab therapy onset is compared within (orange curve) and after (green curve) 8.6 years from MS first manifestation to first natalizumab dose. The discriminatory value of 8.6 years corresponds to the mean duration between the first MS manifestation and the first received natalizumab dose of the total cohort. p-values for the comparison of the two groups were obtained with Cox proportional hazard models correcting for age and sex.*" [1] Figure adapted from [1].



	EDSS					
Number of patients at risk	≤ 3.5	118	89	22	5	1
	> 3.5	56	51	26	9	1



	EDSS						
Number of patients at risk	≤ 3.5	119	77	52	31	12	
	> 3.5	55	33	19	10	3	1

Figure 7: "***Kaplan-Meier curves of the total cohort, diseases severity subanalysis***. *A) Kaplan Meier curve for the time of disease duration and B) natalizumab therapy duration (in years) to onset of confirmed progression independent of relapse activity (cPIRA). The analysis was performed after the patients have been divided into two groups according to the EDSS Score performed at the time of natalizumab therapy onset. The green curve represents the patients with an EDSS score greater than 3.5, while the orange curve the patients with an EDSS score of 3.5 or less. p-values for the comparison of the two groups were obtained with Cox proportional hazard model correcting for age and sex.*" [1] Figure adapted from [1].

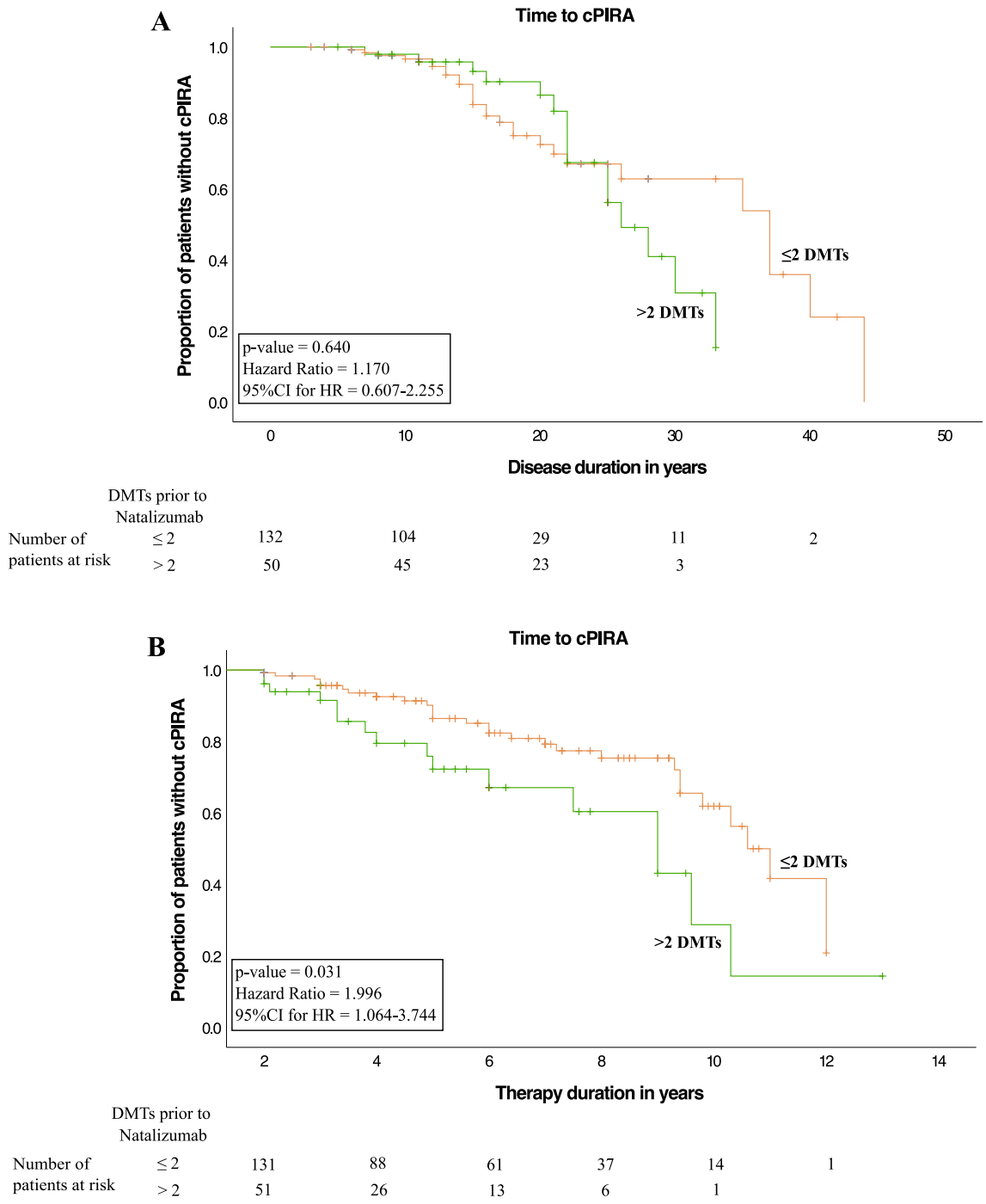
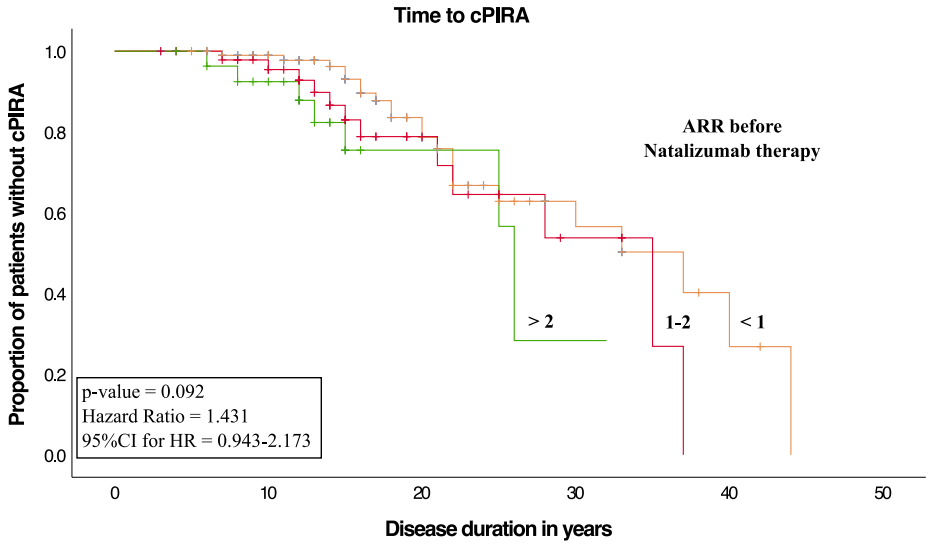
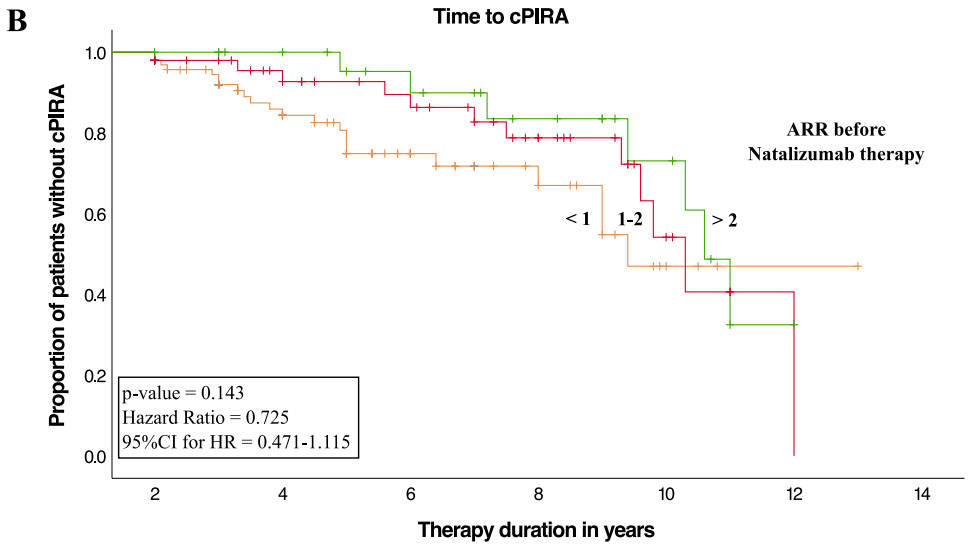


Figure 8: "***Kaplan-Meier curves of the total cohort, previous treatment subanalysis.*** *A) Kaplan Meier curve for the time of disease duration and B) natalizumab therapy duration (in years) to onset of confirmed progression independent of relapse activity (cPIRA). Compared is the progression free survival in the patients with less (orange curve) and more (green curve) than 2 DMTs prior to natalizumab therapy onset. p-values for the comparison of the two groups were obtained with Cox proportional hazard model correcting for age and sex.*" [1] Figure adapted from [1].



	ARR before Natalizumab therapy					
Number of patients at risk	< 1	103	83	33	9	2
	1 - 2	48	40	11	3	
	> 2	28	21	6	1	



	ARR before Natalizumab therapy					
Number of patients at risk	<1	102	55	28	14	3
	1 - 2	48	34	27	17	5
	>2	27	22	17	11	6

Figure 9: "***Kaplan-Meier curves of the total cohort, relapse rate subanalysis.*** *A Kaplan Meier curve for the time of disease duration and B natalizumab therapy duration (in years) to onset of confirmed progression independent of relapse activity (cPIRA). The survival analysis was performed for subgroups stratified based on the rate of annualized relapse rate (ARR) prior to natalizumab treatment onset. p-values for the comparison of the groups were calculated with Cox proportional hazard model correcting for age and sex.*" [1] Figure adapted from [1].

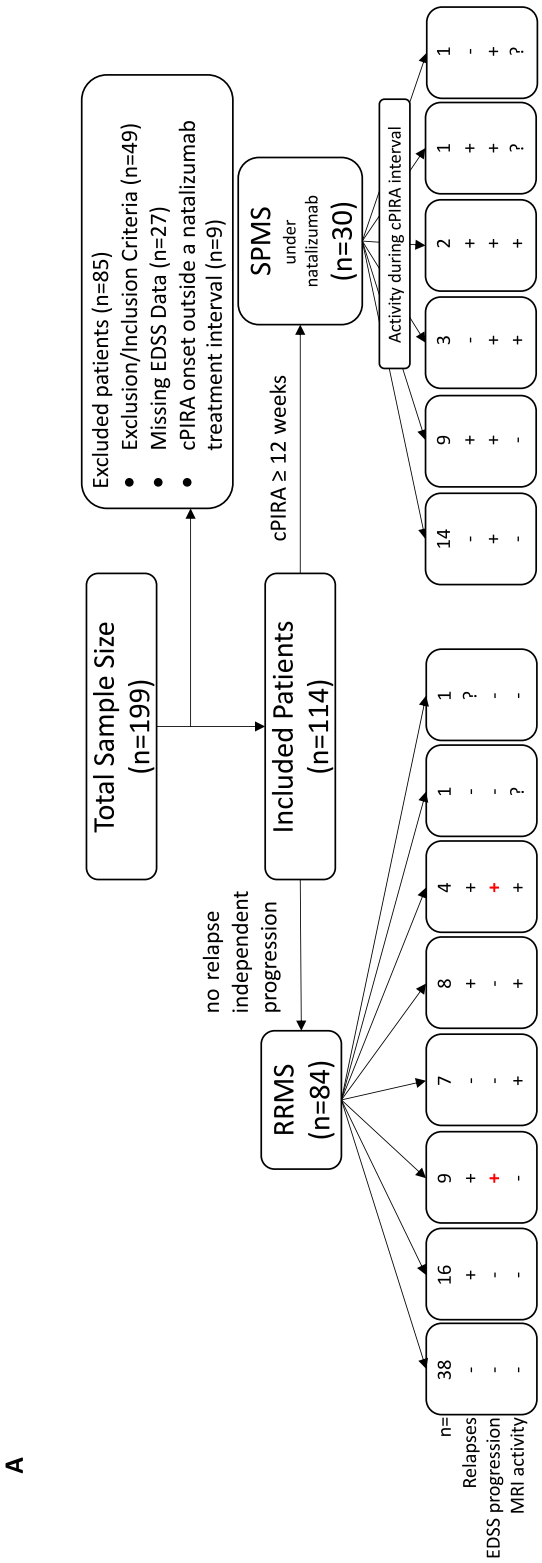


Figure 10: Same as in Fig. 3, but for the Düsseldorf cohort alone. Figure from [1].

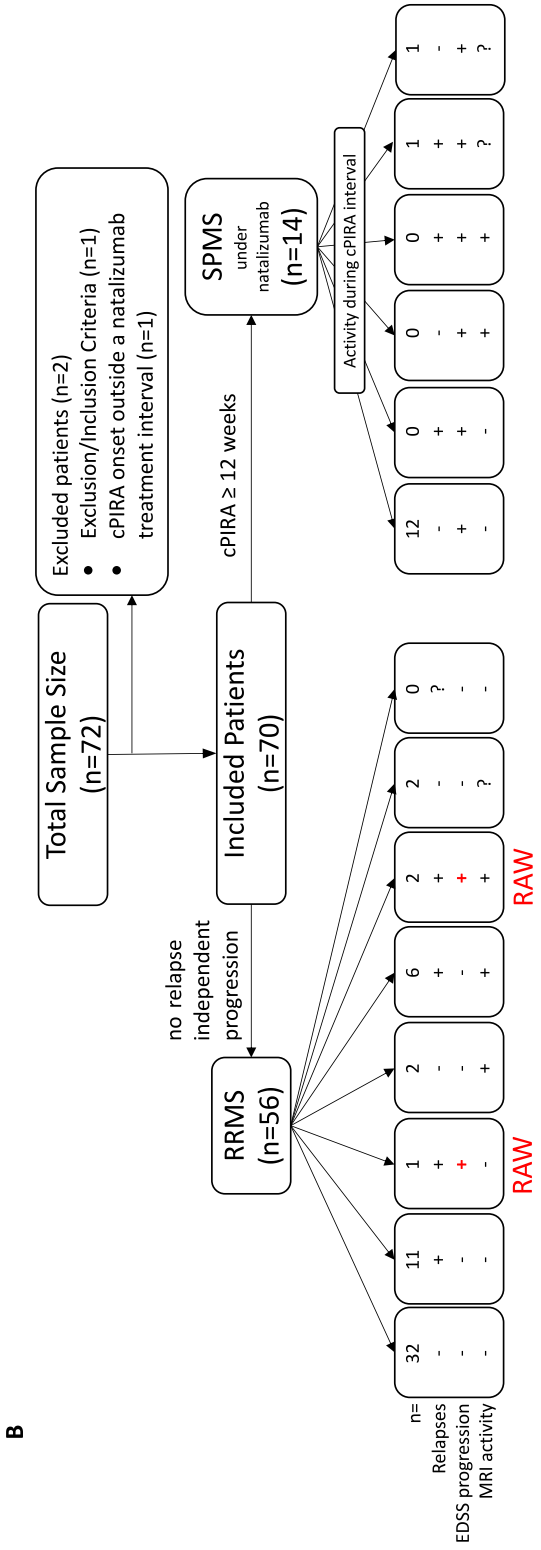


Figure 11: Same as in Fig. 3, but for the Munich cohort alone. Figure from [1].

Baseline characteristics	Cohort	N	Mean rank	Sum of ranks	Asymp. sig.
Sex	Düsseldorf	30	22.70	681.00	n/s
	Munich	14	22.07	309.00	
Age	Düsseldorf	30	22.73	682.00	n/s
	Munich	14	22.00	308.00	
Age at first manifestation	Düsseldorf	29	22.05	639.50	n/s
	Munich	14	21.89	306.50	
Age at first diagnosis	Düsseldorf	30	22.90	687.00	n/s
	Munich	14	21.64	303.00	
Time between first manifestation and first diagnosis RRMS - yr	Düsseldorf	29	15.41	447.00	<0.001
	Munich	14	35.64	499.00	
EDSS at RRMS-first diagnosis	Düsseldorf	9	7.44	67.00	n/s
	Munich	3	3.67	11.00	
Time between RRMS-first diagnosis and SPMS-first diagnosis - yr	Düsseldorf	29	15.41	447.00	n/s
	Munich	14	35.64	499.00	
Disease duration since first manifestation - yr	Düsseldorf	29	22.76	660.00	n/s
	Munich	14	20.43	286.00	
Disease duration since first diagnosis - yr	Düsseldorf	29	22.47	674.00	n/s
	Munich	14	22.57	316.00	
Spinal lesions	Düsseldorf	26	21.92	570.00	n/s
	Munich	14	17.86	250.00	
Current MRI lesions	Düsseldorf	13	13.92	181.00	n/s
	Munich	14	14.07	197.00	
No other therapies	Düsseldorf	30	22.65	679.50	n/s
	Munich	14	22.18	310.50	
Previous therapies prior to Natalizumab	Düsseldorf	30	22.03	661.00	n/s
	Munich	14	23.50	329.00	
Kind of previous therapies prior to Natalizumab	Düsseldorf	30	22.03	661.00	n/s
	Munich	14	23.50	329.00	
Number of DMTs prior to Natalizumab	Düsseldorf	30	23.52	705.50	n/s
	Munich	14	20.32	284.50	
Current Therapy	Düsseldorf	27	17.46	471.50	0.013
	Munich	13	26.81	348.50	
Previous Therapies with Natalizumab	Düsseldorf	30	23.82	714.50	n/s
	Munich	13	17.81	231.50	
Time between first manifestation and first dose Natalizumab - yr	Düsseldorf	29	23.74	688.50	n/s
	Munich	14	18.39	257.50	

Baseline characteristics	Cohort	N	Mean rank	Sum of ranks	Asymp. sig.
Time between first diagnosis and first dose Natalizumab - yr	Düsseldorf	30	24.12	723.50	n/s
	Munich	14	19.04	266.50	
Therapy duration Natalizumab	Düsseldorf	30	21.55	646.50	n/s
	Munich	14	24.54	343.50	
Natalizumab paused	Düsseldorf	30	22.87	686.00	n/s
	Munich	14	21.71	304.00	
Natalizumab discontinued	Düsseldorf	27	17.15	463.00	0.002
	Munich	13	27.46	357.00	
Natalizumab side effects	Düsseldorf	30	22.10	663.00	n/s
	Munich	14	23.36	327.00	
PML	Düsseldorf	30	21.50	645.00	0.036
	Munich	14	24.64	345.00	
Infections	Düsseldorf	30	23.07	692.00	n/s
	Munich	14	21.29	298.00	
Annualized Relapses outside Natalizumab Therapy	Düsseldorf	29	20.81	603.50	n/s
	Munich	12	21.46	257.50	
Annualized Relapses under Natalizumab Therapy	Düsseldorf	30	24.83	745.00	n/s
	Munich	14	17.50	245.00	
Number of visits	Düsseldorf	30	17.77	533.00	n/s
	Munich	6	22.17	133.00	
Visits during relapse-free period	Düsseldorf	30	18.23	547.00	n/s
	Munich	6	19.83	119.00	
Duration first relapse-free period - yr	Düsseldorf	29	22.26	645.50	n/s
	Munich	14	21.46	300.50	
Duration second relapse-free period - yr	Düsseldorf	6	5.25	31.50	n/s
	Munich	4	5.88	23.50	
Delta EDSS in first relapse-free period - yr	Düsseldorf	26	22.13	575.50	n/s
	Munich	13	15.73	204.50	
Fatigue	Düsseldorf	26	22.15	576.00	n/s
	Munich	14	17.43	224.00	
Optic neuritis	Düsseldorf	28	17.36	486.00	0.001
	Munich	13	28.85	375.00	
Number of Optic neuritis	Düsseldorf	29	16.84	488.50	<0.001
	Munich	12	31.04	372.00	
Oligoclonal bands	Düsseldorf	26	18.81	489.00	n/s
	Munich	12	21.00	252.00	

Baseline characteristics	Cohort	N	Mean rank	Sum of ranks	Asymp. sig.
JCV	Düsseldorf	30	19.03	571.00	n/s
	Munich	12	28.85	357.00	
MRZ	Düsseldorf	3	4.50	13.50	0.005
	Munich	10	7.75	77.50	

Table 3: **Comparison of the demographic and clinical characteristics between Düsseldorf Cohort and Munich Cohort.** The considered demographic and clinical variables are the same as in Tables 1 and 2. These were compared using the Mann–Whitney U two-sample rank-sum test. Significant differences (Asympt. Sig. <0.05) between the two cohorts are found for the variables "Time between first manifestation and first diagnosis RRMS", "Current Therapy", "Natalizumab discontinued", "PML", "Optic neuritis" and "MRZ". Table from [1].

3.4 Impact of patients with cPIRA outside of natalizumab

Among the different groups considered in the study the patients who developed cPIRA outside of natalizumab therapy are of particular interest as they might in principle display differences with respect to the analysis performed in the previous sections. For this reason, we performed a second analysis of the total cohort including those patients to investigate whether their presence has a statistically significant impact on the results of the study.

This group of patients is composed of those that developed cPIRA either before the natalizumab therapy or during a pause between two phases of treatment. In our total cohort we count 10 such patients. The flowchart graphically illustrating how these patients are distributed within the classifications discussed in the previous sections is shown in Fig. 12 (to be compared to Fig. 3 where the case without these 10 patients is presented). As can be seen from the figure, a total of 194 patients is now included, among which 140 remained relapsing-remitting (72.2%), as before, while now 54 developed cPIRA (28%). The 54 patients identified to be progressive were further characterized depending on whether or not cPIRA occurred during the natalizumab treatment. Out of those patients, 44 (22.7%) showed worsening progression while receiving natalizumab, while, of the 10 patients labeled as "cPIRA outside natalizumab", 4 developed cPIRA before natalizumab therapy and 6 during a natalizumab pause after having received natalizumab for 24 months or longer.

With this new cohort we perform the same analysis already conducted above and find no relevant difference between the two, suggesting that the choice made for the patients who developed cPIRA outside natalizumab are not biasing our conclusions. Nevertheless, for sake of completeness we show in Figs. 13-14 the same Kaplan-Mayer curves already shown in Figs. 5-9 but with the new extended cohort.

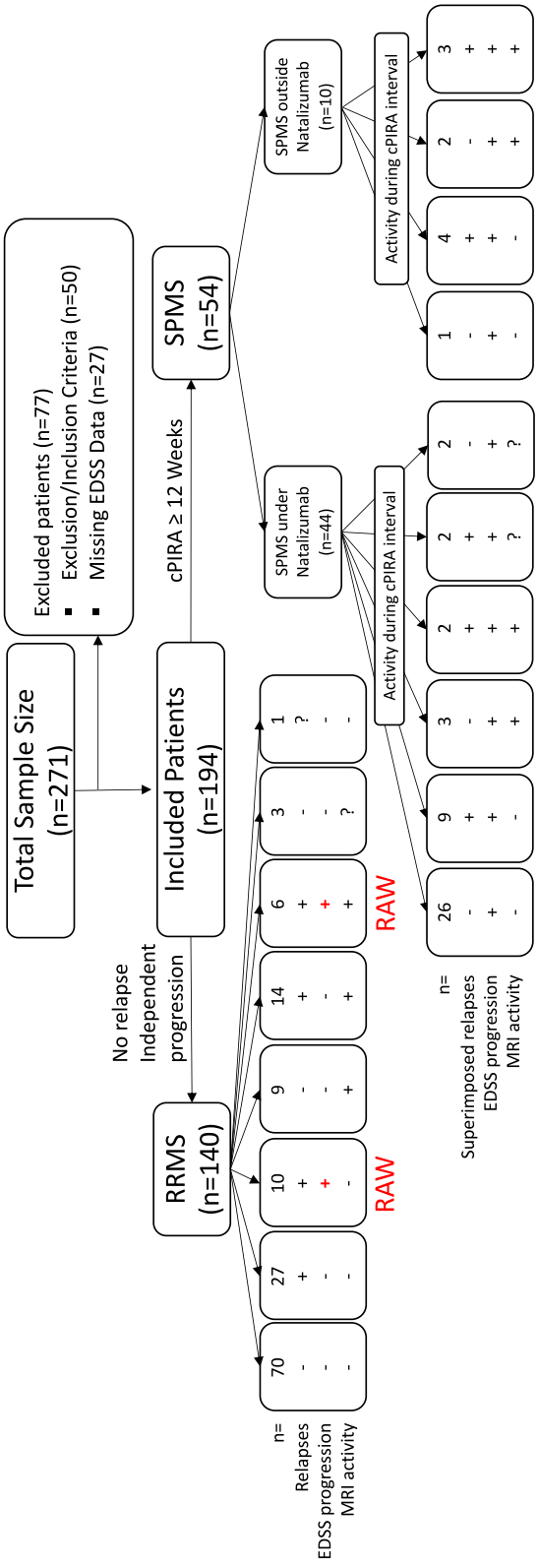


Figure 12: Same as in Fig. 3, but including in the analysis the patients that developed cPIRA outside of natalizumab therapy.

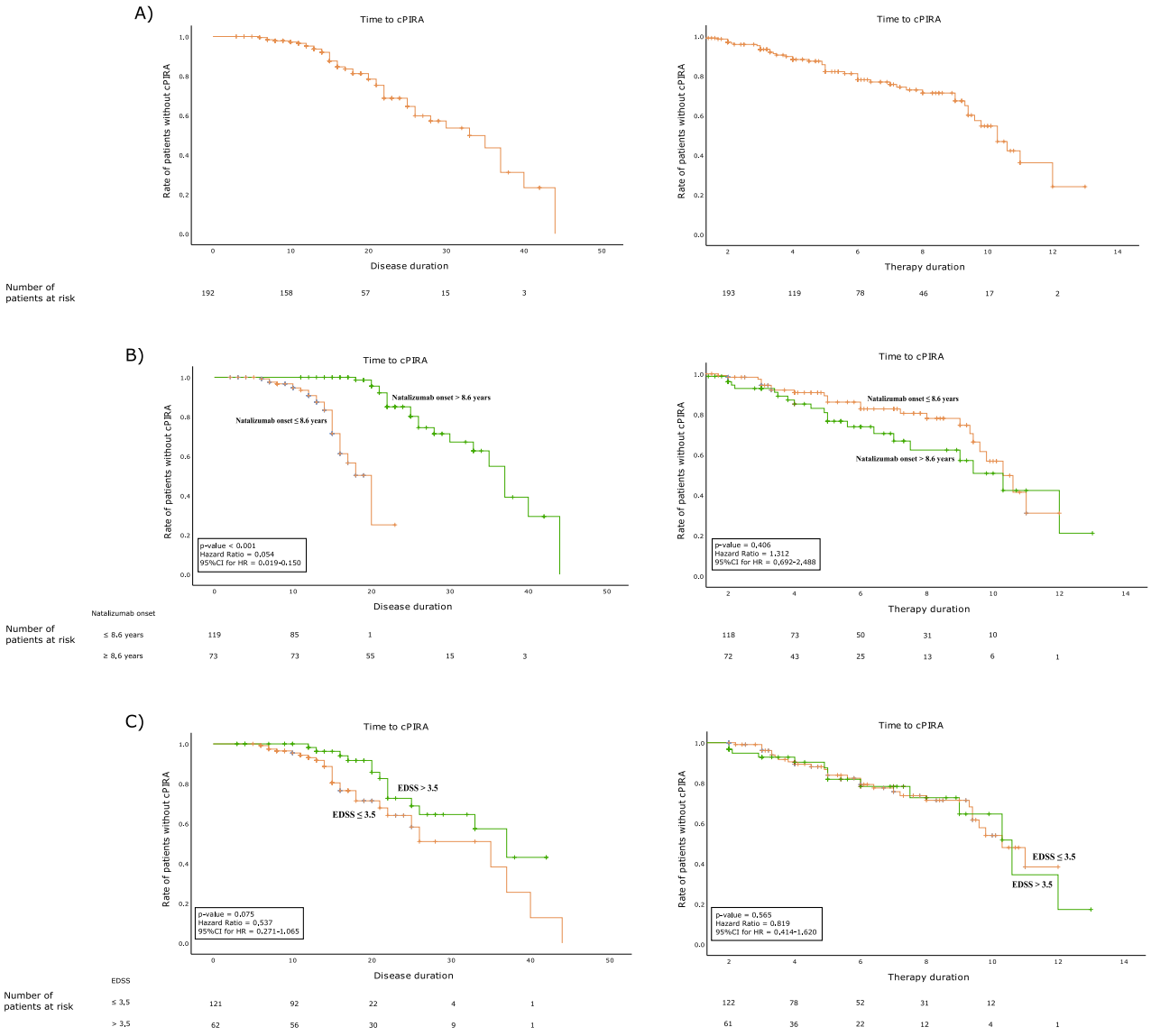


Figure 13: Same as in Figs. 5-7, but including in the analysis the patients that developed cPIRA outside of natalizumab therapy.

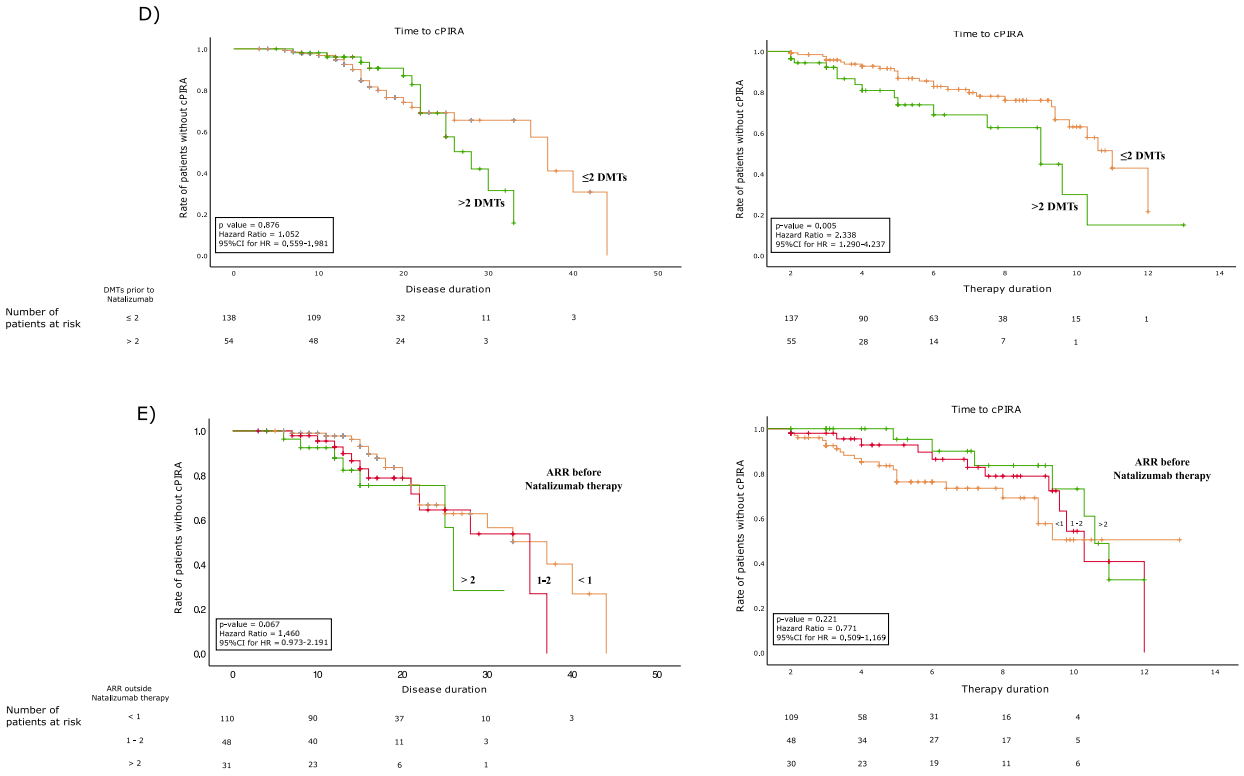


Figure 14: Same as in Figs. 8-9, but including in the analysis the patients that developed cPIRA outside of natalizumab therapy.

4 Discussion and conclusions

Since after approximately 20 years of the natural disease course, more than 50% of patients develop SPMS [26, 39, 40], the relevance of diagnosing and treating SPMS has become more and more urgent. Over the years, a lot of highly active DMTs for RRMS have been introduced to the market and patients have been treated for increasingly long periods of time. Considering that no clear moment of transition between RRMS and SPMS could been found and defined so far, not only the effects on the relapse rate, but also on conversion to SPMS become very important in evaluating the efficacy of a DMT. With over a decade of experience treating MS patients with natalizumab, it is now possible to explore the long-term effects not only on relapses but also on disability progression and SPMS conversion.

Multiple analyses suggest that there are a few factors which seem to increase the risk of developing SPSMS. Among these are of particular relevance a shorter time period between the first two relapses [24,41], higher age, disease duration, disability progression and number of relapses increase the risk of developing SPMS [42]. The beneficial effect on preventing a conversion from RRMS to SPMS has been evaluated in mild to moderate DMTs. 65% of patients treated with glatiramer acetate had not progressed to SPMS after 15 years of observation in the ongoing patient cohort study [43]. Furthermore, in a beta-interferon follow-up trial, the SPMS conversion risk was slightly lower in the early- versus delayed treatment group (4.5% vs. 8.3% respectively) [44]. In another prospective study of RMS patients, 18.1% (95% CI 13.5–22.5%) developed SPMS [45].

"*Our data suggest that a remarkable rate of almost 80% of RRMS patients do not convert to SPMS and remain with a stable EDSS despite a mean disease duration of 15.6±7.5 years. Of note, natalizumab was not completely ineffective in a phase 3 trial in SPMS patients as it may positively influence upper limb function [35].*" [1] On the other hand, however, more than 20% of MS patients on long-term natalizumab treatment in our cohort do develop a confirmed, relevant and relapse-independent disease progression (cPIRA). The mean delta EDSS of patients who developed cPIRA under natalizumab was significantly increasing with 1.4±0.9, underlining the relevance of this observation. In this aspect, our data are in line with data from the Tysabri® Observational Program (TOP) [46].

"*When comparing our results with published analyses of the natural disease course (>50% of RRMS treatment-naïve patients convert to SPMS, see above), the SPMS conversion rate may be reduced by about a half under natalizumab [46]. However, the SPMS conversion rate of the natalizumab treated patients in our study was not inferior to the rates reported in studies on other DMTs (22.7% in our natalizumab cohort versus 35% in glatiramer acetate, 4.5%/8.3% in beta-interferon, and 18.1% in the EPIC study) [43–45]. As natalizumab failed to reduce EDSS progression in a phase 3 SPMS trial, its effective*

mechanism of preventing leukocyte trafficking may not be of sufficient relevance for halting progression. Moreover, the relevance of preventing relapses in order to prevent secondary disease progression may not be as relevant as expected." [1] This is in line with our finding that the ARR before natalizumab onset was not associated with an increased risk of developing cPIRA. Of note, those patients who remain stable on natalizumab in our cohort remained stable or even improved for the duration of our observation.

A recent study [37] included 82 RRMS patients with an initial natalizumab treatment. In these patients, conversion to SPMS was significantly lower compared to untreated patients after 5 years (19% versus 38%) and 6 years (34% versus 48%). "*Considering our data, this effect may not only be associated with the number of prior therapies, but rather with disease duration. MRI and basic CSF parameters do not seem to differ between our groups and may not be suitable to determine the patients' risk of developing cPIRA. Neuroimaging studies suggest that the estimated rate of lesion growth [47] and of atrophied brain T2 lesion volume [48] are associated with SPMS conversion risk. However, as our study is limited by its retrospective way of conduct and certainly by an indication bias favoring more active patients for natalizumab treatment*" [1], the different patient cohorts described in the above-mentioned studies cannot be compared, and direct cause-effect relationships cannot be drawn.

"*As disease duration was the only factor influencing cPIRA in our natalizumab cohort, we cannot postulate a clear treatment associated mechanism.*" [1] Early natalizumab treatment may be more beneficial for RRMS patients than late natalizumab treatment with regard to long-term outcomes. Currently, consensus criteria for the diagnosis of SPMS are not available. The concept of cPIRA in combination with the use of a roving EDSS reference score may be an adequate approach for this unmet need. In any way, large, prospective observational studies are necessary in order to better define SPMS. "*In our cohort, the risk of developing cPIRA was not different between the EDSS $\leq$3 and $\geq$3.5 group. Therefore, a bias due to the EDSS scoring system seems rather unlikely.*" [1] Furthermore, it has to be kept in mind that EDSS based cPIRA-definitions have a poor sensitivity for patients with progression in such functional domains like fatigue and cognition because these are not assessed by EDSS. On the other hand, EDSS-based definitions have a good specificity [49].

"*A strength of our study is the real-world setting and the long follow up times as well as the fact that the observations could be made in two independent cohorts.*" [1] Limitations of our study are mainly linked to its retrospective design and include the lack of standardized follow up intervals, biomaterials, additional functional test and of standardized MRI protocols precluding an in-depth analysis of possible new predictive factors for the development of cPIRA. Furthermore, "*we acknowledge that a comparison to patients on long-term treatment with other therapeutics would be of highest interest.*" [1] However, sufficient data for such an analysis were not available for this study.

"*All in all, our data suggest that considering the disease duration, patients with early onset (≤8,6 years) natalizumab therapy seem to be more likely to develop secondary progression than those with late onset of the treatment (see left panel of Fig. 6). However, since the natalizumab therapy duration and the timing of natalizumab initiation seem to have no influence on the progression (see right panel of Fig. 6), we interpret this finding to be in correlation with the severity of the disease.*" [1] Patients with a more severe disease course and thus a higher risk for SPMS conversion are more likely to receive an early natalizumab treatment. This reasoning can in principle also explain the fact that in our cohorts patients with >2 DMTs prior to natalizumab therapy seem to develop cPIRA significantly earlier then patients with ≤2 DMTs (see right panel of Fig. 8).

In our cohort, more than 20% of patients on long-term natalizumab treatment developed cPIRA independently of the EDSS at natalizumab onset and ARR before natalizumab therapy initiation. This suggests that natalizumab may be more effective in reducing the relapse rate than preventing from conversion to SPMS. However, despite the great success of the DMTs in reducing relapse frequency, their efficacy in preventing secondary progression has been challenging to demonstrate. This fact may suggests that targeting peripheral inflammation is not enough to avoid the disease progression and underlines the different pathophysiologies between RRMS and SPMS [49]. The necessity to also target the chronic inflammation in the CNS has became more and more relevant. For instance, the EXPAND study has found siponimod to slow disability accumulation compared with placebo in patients with SPMS [46]. Siponimod – a selective, newer-generation sphingosine 1-phosphate receptor 1,5 modulator – has been approved in 2020 as a treatment for the therapy of relapsing forms of MS and active secondary progressive disease.

Currently there are many other pharmacological approaches under development and testing. Among those the Bruton's Tyrosine Kinase inhibitors seem to be one of the most promising by targeting the CNS compartmentalized inflammation [50]. By reducing the disability worsening and MRI disease progression in the secondary progressive form, this may be a valid therapeutic option against MS.

In conclusion, therefore, a lot of effort has been invested in the past in the development of treatments to prevent MS secondary progression and this has been followed by a number of very important steps forward. Nevertheless, a lot is still left to be done in order to reach a deeper consensus around a definition of cPIRA and SPMS conversion rate as well as an effective course of therapy, and the multitude of on-going studies testifies to the relevance of this field of research.

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