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Abstract: Multiple Sclerosis (MS) is a heterogeneous disease. Although several EMA approved Disease Modifying Treatments including biopharmaceuticals are available, their efficacy is limited and a certain percentage of patients are always non-responsive. Drug Efficacy Monitoring is an important tool to identify these non-responsive patients early on. Currently, Detection of Anti-Drug Antibodies and quantification of Biological Activity are used as methods of efficacy monitoring for Interferon beta (IFN・) and Natalizumab (NAT) therapies. For NAT and Alemtuzumab treatments, drug level quantification could be an essential component of the overall disease management. Thus, utilization and development of strategies to determine treatment response are vital aspects of MS management given the tremendous clinical and economic promise of this tool.

Keywords: biopharmaceuticals, therapeutic drug monitoring; drug efficacy monitoring; health economics

Introduction

Multiple Sclerosis (MS) is an autoimmune, inflammatory and degenerative disease of the Central Nervous System (CNS) that affects more than 2 million people worldwide. MS is characterized by chronic inflammation leading to CNS damage that results in neurological deterioration along with a multitude of other symptoms.

Depending upon the pattern of the progression of disease, 3 subtypes have been characterized: 1) Relapsing Remitting MS (RRMS): this is the most common disease course, characterized by the appearance of new or increasing neurological symptoms. These attacks, known as relapses, are followed by periods of partial or complete remissions, during which the symptoms may disappear, or may continue and become permanent. However, there is no continuous progression of the disability. Approximately 85% of all patients with MS are initially diagnosed with RRMS. 2) Primary Progressive MS (PPMS): this subtype is characterized by the worsening of neurological functions (accumulation of disability) right from the onset of the symptoms, without early relapses or remissions. Approximately 15 % of patients are diagnosed with PPMS. 3) Secondary Progressive

MS (SPMS) subtype follows an initial relapsing-remitting course. Most patients diagnosed with RRMS eventually transition in to a SPMS which is characterized by progressive worsening of neurological functions with accumulation of disability. Here, evidence of disease activity as indicated by relapses or changes on Magnetic Resonance Imaging (MRI) may or may not be present [1].

Over the past decade, the landscape of care for MS has changed tremendously due to the advent of multiple Disease Modifying Treatments (DMTs). Till date, 15 pharmaceutical formulations have been approved (Tab.1) for RRMS. Amongst these, only Mitoxantrone and IFN・1-b are approved for SPMS as well. These DMTs differ with respect to the efficacy, formulation, method and schedule of administration, possible adverse drug reactions (ADRs) in addition to cost. These latest formulations also include biopharmaceuticals such as different formulations of IFN・, Monoclonal Antibodies (MAbs) against ・4/・1 and ・7 integrin (NAT) and anti-CD52 (Alemtuzumab). Many of these drugs are associated with serious ADRs such as cardiac events, opportunistic infections and secondary autoimmunity [2]. Therefore, the selection of the right drug for the right patient or personalized treatment is highly desirable. Consistent progress has been made towards the identification of pharmacogenomic markers of DMT response [3] in MS. However, limited pharmacogenetic or pharmacogenomic tests are available to predict the efficacy of a treatment till date and as a result, predicting patient response to DMT in advance is very difficult. The general approach is to weigh benefits and risks taking into consideration factors such as the aggressiveness of the disease, the efficacy of the drug and the possibility of ADRs. In addition, several other factors including tolerability, planning of pregnancy, preference and life style of the patient, previous treatments, adherence to treatment, clinical and MRI examinations along with the cost may play an equally important role in the selection of the right drug. In most cases, the neurologists and patients must rely on a “trial and error” approach. This is inadequate and risky because a treatment failure can cause an irreversible damage of CNS functions. Thus, an approach like Drug Efficacy Monitoring is important to enable the physician to detect non-responsive patients as early as possible. Monitoring of drug efficiency can essentially include any biochemical, clinical or genetic evaluations that could aid in modulation of drug type, dosage or schedule of administration to optimally benefit the patient and minimize the possibility of ADRs. On the other hand, the concept of Theraputic Drug Monitoring or TDM essentially involves measurement of the concentration of the drug in the serum. In the context of MS, TDM alone may not be sufficient to provide enough information regarding drug response to enable the physician to effectively individualize the treatment. Therefore, drug efficacy monitoring in MS must include other components such as the quantification of Anti-Drug Antibodies (ADA) (induced by IFN・or NAT) and evaluation of biological activity in addition to TDM in order to predict the efficacy of

biopharmaceuticals. However, the measurement of biological activity can be useful in clinical practice only if a biomarker is specifically up- or down-regulated after the drug administration [4,5].

In the present review, attempts have been made to explore the available literature with respect to two of the most commonly used biopharmaceuticals in MS, namely, IFN・ and NAT in addition to a newer drug such as Alemtuzumab and delineate the available methods for drug efficacy monitoring in detail.

1. IFN・

Mechanism of Action of IFN・

Natural IFN・, the type I IFN, is secreted by fibroblasts. It binds to the IFN receptor (IFNAR) and activates the JAK/STAT pathway to phosphorylate STAT1 and STAT2 [6]. These factors dimerize and associate with IFN regulatory factor-3 and bind to IFN-stimulated response elements in the cell nucleus. This in turn activates hundreds of IFN-stimulated genes (ISG) and leads to the production of antiviral, anti-proliferative, and anti-tumor products [7]. The mechanism of action of IFN・ is complex. It balances the expression of anti-inflammatory and pro-inflammatory cytokines, reduces the trafficking of inflammatory cells across the blood-brain-barrier and increases the production of nerve growth factor. Moreover, in the peripheral blood, it increases the number of natural killer cells, which are producers of anti-inflammatory mediators. In MS, IFN・ acts via decreasing Annualized Relapse Rate (ARR), the risk of sustained disability progression, reducing MRI lesion activity and brain atrophy. It might also delay the onset of clinically definite MS after the first appearance of neurological symptoms [8].

Drug Level

To evaluate IFN・ serum level an "antibody sandwich" ELISA has been developed, which involves coating the plates with a mouse monoclonal anti-human IFN・ antibody [9,10]. However, drug level has never been used as a parameter to monitor the efficacy of any form of IFN・, because of relatively short half-lives (range: 5-78 hours).

A pharmacokinetic study carried out in a group of six MS patients receiving 6 MU of non-PEGylated IFN・-1a intramuscular (IM) once a week, demonstrated that the IFN・-1a levels

become detectable at 4 hours, and peak at 8 hours post injection. IFN・-1a levels became undetectable in serum 24 hours post-injection. Peak serum levels range from 92 to 102 IU/mL, with a mean of 94.8 IU/mL [9]. Additionally, other recent studies performed on a new formulation of PEGylated IFN・-1a (PEG- IFN・..have shown that the concentration peak, measured using an ELISA, occurs later in this form of IFN as compared to the non-PEGylated IFN・-1a (~36 hours) [11]. After subcutaneous doses of PEG-IFN・-1a in MS patients, the mean Cmax is 280 pg/mL and the peak of serum concentration occurs between 1 and 1.5 days. The pharmacokinetics (PK) profile of PEGylated form in a study involving 1512 RRMS patients was consistent with that in healthy subjects. In healthy volunteers, the median Area Under the Curve (AUC) from time 0 to 168 h post-dose (AUC(0,168 h)) was reported to be 27.2 ng/ml h, while in MS patients the same AUC (0,168h) ranged from 23.5 to 32.0 ng/ml h. [12]

A dosing regimen of PEG-IFN・-1a once every 2 weeks provides 4.5-fold higher cumulative AUC, as compared with non-PEGylated IFN・-1a administered weekly. Although definitive exposure−efficacy relationships are yet to be established, the increased cumulative exposure potentially explains the maintained efficacy of PEG-IFN・-1a despite its reduced dosing frequency. However, such pharmacokinetic studies have only helped to define the best route and frequency of administration and have not been utilized so far in the individualization of the treatment.

Pharmacogenomics: Identification of Biomarkers

Quantification of biological activity of IFN・ allows the early identification of patients that are not responsive to the treatment. The biological activity of IFN・ is investigated by evaluating a number of ISGs, induced by IFN・ injection, including Myxovirus-resistance protein A (MxA) at the level of protein or mRNA, b2-microglobulin, oligo-adenylate-synthetase, TRAIL, viperin, IFI27, CCL2 and CXCL10 [13]. A strong risk of relapses in the absence of biological activity has been found [14]. The European Recommendations suggest the combined evaluation of MxA mRNA and ADA to assess the continuing efficacy of IFN・ therapy [15].

Anti-IFN・ Antibodies

Several studies have reported the occurrence of binding antibodies (BAbs) and neutralizing antibodies (NAbs) against IFN・ during the treatment [15]. Majority of the patients develop BAbs, however, only NAbs interfere with the biological activity of IFN・ and they are present in a smaller proportion of patients with ADA. NAbs inhibit the binding between IFN・ and IFNAR, abolishing its biological activity and consequently the therapeutic effect. The development of BAbs occurs during the first months of IFN・ treatment whereas the occurrence of NAbs requires several months. Most patients become positive for NAbs during the first 18 months of therapy and rarely during the second or third year of treatment as well.

The importance of quantification of the NAbs and of the biological activity of IFN・ in the management of MS patients is underlined by the European and Italian National Guidelines [16,17] and by international expert consensus [17] that provide recommendations for timing of measurement and therapeutic consequences of NAbs against IFN・ and of absence of biological activity (Fig. 1).

ELISA, both with or without a capture antibody, is the most commonly used method for BAbs measurement [18]. For NAbs measurement, 3 methods are used based on the antiviral MxA protein: i) Cytopathic Effect Assay, considered as “gold standard” and recommended by both the World Health Organization and European Guidelines [18]; ii) MxA Protein assay [19] and iii) MxA gene expression assay [20]. Another type of assay based on the evaluation of luciferase expressed after sera incubation on cells transfected with an IFN regulated luciferase reporter-gene construct has been proposed [21].

ADA abolishes the biological activity of IFN・, but also other factors such as non-compliance and soluble circulating IFN・ receptors could contribute to the lack of biological activity [13]. Many evidences indicate that NAbs reduce or abolish the therapeutic efficacy of IFN・ in preventing relapses, independently of the type of IFN used [16,17,18,22]. In fact, MRI, clinical disease activity [22] and the risk of disability progression are higher in NAbs-positive patients [23]. The risk of development of NAbs varies between <1% to 31% for different IFN・ formulations [24,25]. This immunogenicity difference is intensely influenced by excipients, route and timing of administration and drug composition that differ among the various formulations.

Neurologists face two options during MS management in patients. Multiple weekly injections offer more clinical efficacy than once a week injection. However, in this approach, many more patients are at risk of becoming NAbs positive than patients treated once a week. As a result, they will lose the clinical benefits of IFN・˜ Moreover, they must be switched to another category of DMT, as NAbs are cross-reactive against all the types of IFN・ [26].

2. Natalizumab

Mechanism of Action of Natalizumab

NAT is a humanized MAbs that binds to the α4-subunit of integrins, also called CD49d antigen, which is highly expressed on all leukocytes, except neutrophils. Specifically, after binding to the α4β1 integrin, NAT blocks the interaction of this integrin with its receptor, vascular cell adhesion molecule-1 (VCAM-1), and other ligands. Disruption of these interactions avoids transmigration of leukocytes across the brain-blood barrier and recruitment of activated T-lymphocytes into inflamed tissue and may suppress inflammation in the CNS. Normally, VCAM-1 is not expressed in the brain. However, in the presence of pro-inflammatory cytokines, it is upregulated in endothelial cells and possibly in glial cells close to the sites of inflammation.

A phase III placebo-controlled study [27] showed the efficacy of NAT in reducing ARR and preventing disability progression which might be higher or comparable to IFN・. These findings were confirmed by another independent trial that compared NAT plus IFN・-1a against IFN・-1a alone [28].

Drug Level

Population-based modeling of the relationship between dose, concentration and effects, i.e. PK and pharmacokinetics-pharmacodynamics (PK-PD) of NAT, could help to precisely quantify individual sources of variability based on dynamic biomarkers and considering the onset of adverse events. Readers are encouraged to see chapter 6 of this TDM special issue and the article by Ternant et al. [29] for the rationale of developing PK-PD modeling of monoclonal antibodies in TDM of inflammatory diseases. From an initial phase I study [30], it was concluded that doses from 0.03 to 3 mg/kg were safe, despite minor side effects. The approved 300 mg dose every 4 weeks leads to a mean half-life of 16±4 days and a mean clearance of 13.1±5 ml/h (file EMA/H/C/000603), depending on weight and anti-NAT antibodies. This dose was chosen to achieve 70% of α4-integrin saturation throughout the 28-day dosing interval. In MS221 study from Biogen (reported in FDA clinical pharmacology and biopharmaceutics review, application number 125104) cytometry analysis of receptor occupancy was nearly saturated at all tested doses ranging

from 1 to 6 mg/kg, however the duration of saturation increased with increasing dose level. As the non-compartmental analysis performed in this study does not adequately describe the non-linear elimination of PK and therefore receptor saturation, it could be relevant to describe PK profile by compartmental approach, using for example Michaelis-Menten type elimination, to address this problem. However, PML was found to be a major safety concern. Khatri et al. [31] developed a plasma-exchange strategy to swiftly reduce concentrations of circulating NAT to restore immune surveillance in the brain; VLA-4 desaturation appears to take place below 1 µg/ml of circulating NAT. However, the rate of wash-out may vary considerably between patients, which suggests that measurement of NAT concentrations may be helpful to guide plasma exchange strategy [32]. Evaluation of serum NAT concentrations is complicated since NAT can exchange Fab arms with endogenous human IgG4 [33]. Several immunoassays were developed to quantify serum NAT concentrations accurately [34,35], without interference by Fab arm exchange nor IgG4 Fc interactions. Interestingly it has been shown that both low NAT concentration, below 1 µg/ml, and high antibody titers, are associated with a lack of therapeutic efficacy [36].

Utilizing paired CSF and serum samples, a recent study shows that it would be helpful to measure free and cell-bound NAT to determine the optimal individual NAT dosing regimen for patients [37]. DELIVER study [38] suggests that NAT will probably lead to similar efficacy whatever the administration route (intravenous IV, subcutaneous SC or IM). PK profiles were quite similar with variations in Cmax: subcutaneous SC and intramuscular IM were about 40% lower than IV and mean bioavailability relative to IV was about 50% with SC or IM administration. Mean trough serum concentrations were lower with IM administration.

Pharmacodynamics of Natalizumab

Apart from ADA, current data available in the literature do not allow clinicians to design a personalized dosing regimen. However, Defer et al. [39] found a 55% decrease of CD49d expression on circulating T and B lymphocytes after NAT infusion. This low level remained stable for the entire period of treatment, except for patients ADA positive, in which CD49d levels reverted to pre-treatment levels. Thus this antigen expression could be used to monitor the effectiveness of NAT. Millonig et al. [40] confirmed this finding, suggesting that CD49d is decreased on T-cells, but also on B-cells and NK-cells. Moreover, they showed a significant decrease of serum sVCAM-1 concentration in ADA negative patients. sVCAM-1 concentration reverts to pre-treatment levels in case of ADA development. CD49d and sVCAM-1 could be useful in establishing a personalized timing of NAT administration.

Anti-Natalizumab Antibodies

Clinical trial with NAT have demonstrated the possibility of ADA generation with this treatment. [41]. ADAs induce a loss of efficacy with a higher risk of adverse events [27,36,41]. The proposed

mechanism of loss of clinical outcomes is the formation of NAT-ADAs immune complexes that lead to enhanced clearance and decreased functional serum concentration of NAT [36]. As per current data, 9-12% of NAT treated patients develop ADA, out of which 6% remain persistently positive and 3-6% are transiently positive for ADA [41]. The treatment is discontinued if the measures reveal persistent ADAs. Patients with infusion reactions or with disease activity should be tested for ADAs. The assay currently used to evaluate the presence of Anti-NAT antibodies is a standardized bridging ELISA method developed by Biogen Idec (Cambridge, MA, USA); protocol "Assay procedure to determine Natalizumab (Tysabri) immunogenicity (CST02-180AP-R.2)" [41]. The combined measurements of ADA, NAT serum level and CD49d could be utilized to tailor a personalized infusion regimen. These measurements could also be useful to determine the withdrawal of NAT in patients with persistently high level of ADA.

3. Alemtuzumab

Mechanism of Action of Alemtuzumab

Alemtuzumab is a MAb of the IgG1 subclass that selectively binds to the CD52 protein, present in large amounts on the surface of T and B cells and to a lesser extent on other cells. The treatment with this drug induces the depletion of circulating B and T cells, followed by repopulation. The repopulation phenomenon is faster for B cells and slower for T Lymphocytes. Alemtuzumab action in MS is therefore attributable not only to the destruction of T and B-cells, but also to the way in which the repopulation occurs. This treatment has minimal impact on other immune cells, ensuring the protection of the innate immune system. Clinical studies [42,43] comparing Alemtuzumab and IFN・ sub cutaneous 3 times a week, demonstrated that the former reduces both ARR and disability progression more efficiently than IFN・ .

Drug Level

From the EMA approval of Alemtuzumab for leukemia in 2001 till its approval for MS in 2014, all pharmacokinetic studies have been carried out in leukemia patients only. ELISA and FACS have been the assays used in these studies for the assessment of the Alemtuzumab serum concentration [44]. In MS, the approved treatment strategy is 12 mg IV daily for 5 consecutive days and 12 mg IV daily for 3 consecutive days administered 12 months after the first treatment course. This treatment regimen results in a mean Cmax of 3014 ng/ml on Day 5 of the initial treatment course, and 2276 ng/ml on Day 3 of the second treatment course. The half-life of this drug is approximately 4-5 days and is comparable between courses. The serum concentration of Alemtuzumab reaches low or undetectable levels within approximately 30 days following each treatment course [45]. In addition, attempts have been made in patients with chronic lymphocytic leukaemia (CLL) to delineate the pharmacokinetics of Alemtuzumab. A two-compartment model

with nonlinear elimination has been proposed by Mould et al. In this study performed in 2007, they demonstrate that the maximal trough concentrations range from 3.6–21.0 mg/ml with a mean of 10.2 mg/ml in responders and below the limit of quantification to 26.8 mg/ml with a mean of 5.9 mg/ml in non-responders. Additionally, a direct relationship between maximal trough concentrations and clinical outcomes was also described, with increasing Alemtuzumab exposure resulting in a greater probability of positive tumour response [46]. Data from any such studies in MS patients are so far unavailable. Therefore, it would be interesting to design future prospective studies in MS to model dose-concentration-effects relationship of Alemtuzumab and investigate if indeed it is similar to that observed in CLL. Such studies of Alemtuzumab pharmacokinetics in MS patients would also aid in the implementation of TDM strategies and further individualization of treatment with this drug.

Anti-Alemtuzumab Antibodies

Alemtuzumab-binding antibodies have been shown to be present in 29% of patients immediately before the second course of treatment and in 86% of patients 1 month after the second course of treatment [42]. The percentage of patients whose test results were considered positive for antibodies to Alemtuzumab using an ELISA and confirmed by a competitive binding assay. The presence and concentration of anti-Alemtuzumab antibodies do not seem to influence either the efficacy or the safety of the MAb [42] nor the pharmacodynamics at the beginning of treatment courses. However, their impact after many doses remains to be established.

It has been shown that, during the first 5 years of treatment, almost one third of the patients develop a secondary autoimmunity, in particular thyroid autoimmunity (30%) and idiopathic thrombocytopenic purpura (2%). Some studies have suggested that the pre-treatment evaluation of IL-21 serum level could predict the development of post-treatment autoimmunity. However, currently available ELISA kits to evaluate IL-21 level seem to fail as predictive tests to evaluate this potential biomarker of secondary autoimmunity.[47]

Economic Impact of Drug Efficacy Monitoring

Very few studies have investigated the economic impact of drug efficacy monitoring in MS, and all of them have so far focused only on IFN・. An Austrian study showed that testing for ADA against IFN・, according to the European guideline, is cost effective because it reduces total direct costs by approximately 34 million € in 5 years. Translated to the whole of Europe the reduction of total direct costs would amount to be approximately 594 million € [48].

An Italian study has estimated the annual cost of managing RRMS patients with and without NAbs. The results have shown an increase of 3,100 € per patient-year as the consequence of the onset of NAbs. Considering the MS patients treated with IFN · in Italy and the percentage of NAbs development, the evaluation of ADA could allow a better allocation of approximately 10 million €/year [49].

For the other DMTs, no study related to the drug efficacy monitoring exists to date, although considering their cost, relapses and disability progression in young patients it would be surprising if drug efficacy monitoring strategies would not be more cost-effective.

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Figure legend

FIG. 1 Clinical and biological flow-chart for identification of subsets of IFN-β responders and non-responders patients using pharmacogenomics and anti-INF・ ADAs quantification [48].

Table 1. EMA and FDA approved DMTs for Multiple Sclerosis

Treatment	Brand name	Type of MS	Posology and route of administration
IFNβ-1a	Avonex	RRMS	30 ug weekly, IM
IFNβ-1a	Rebif 22 Rebif 44	RRMS	22 or 44 µg three times a week, SC
IFNβ-1b	Betaferon Extavia	RRMS SPMS	250 µg every other day, SC
PEG-IFNβ-1a	Plegridy	RRMS	125 ug every 2 weeks, SC
Glatiramer Acetate	Copaxone 20 mg Glatopa 20 mg Copaxone 40 mg	RRMS	20 mg once a day or 40 mg three times a week, SC
Natalizumab	Tysabri	RRMS	300 mg every 28 days, IV infusion
Fingolimod	Gilenya	RRMS	0.5 mg once a day, PO
Mitoxantrone	Novantrone	RRMS SPMS	12 mg/m^2 every 3 months, IV infusion with a lifetime cumulative dose of no more than 140 mg/m^2
Teriflunomide	Aubagio	RRMS	7 or 14 mg daily, PO
Dimethyl Fumarate	Tecfidera	RRMS	120 mg twice a day for 7 days, PO; after 7 days, 240 mg twice a day, PO
Alemtuzumab	Lemtrada	RRMS	First course: 12 mg/day on 5 consecutive days, IV infusion Second course after 1 year: 12 mg/day on 3 consecutive days, IV infusion

PO = per os

SC = Sub-cutaneous

IV = Intra-venous

IM = Intra-muscular

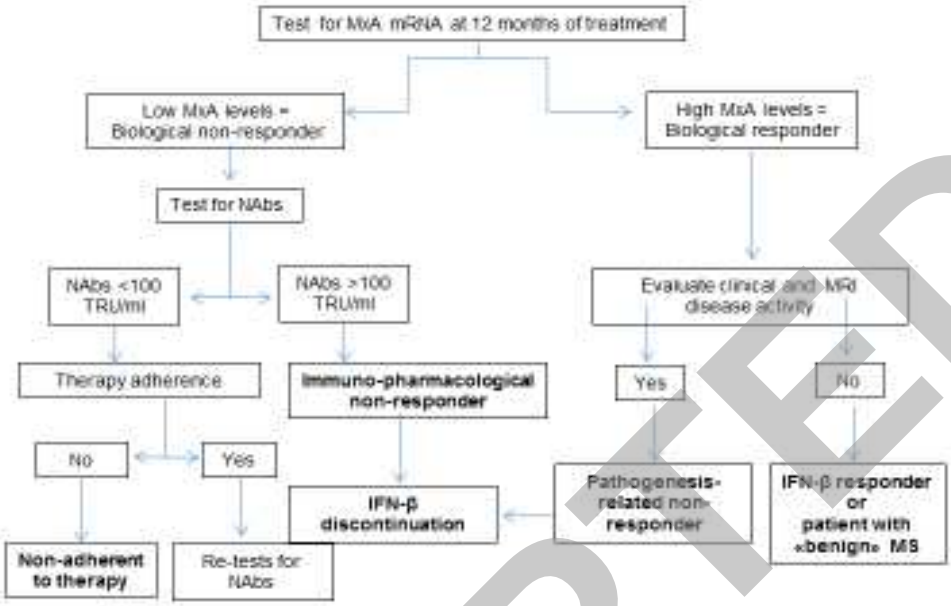

FIG. 1 Clinical and biological flow-chart for identification of subsets of IFN-β responders and non-responders patients using pharmacogenomics and anti-IFN‧ ADAs quantification [50].