Application of Interferon Beta-1b in Multiple Sclerosis

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Abstract

Recombinant interferon beta-1b (IFNβ-1b) was the first approved disease-modifying therapy for patients with multiple sclerosis (MS) by the world regulatory agencies for medical drugs and devices. IFNβ-1b significantly decreases the inflammatory component of MS but still has doubtable effect on the neurodegenerative component. This article appraises the beneficial effects of IFNβ-1b on clinical and imaging measures of disease. Upon only briefly discussing the side effects of the drug, it will conclude with some crucial scientific aspects warranting urgent investigations to precisely address the potentials of IFNβ-1b in MS patients.

Multiple sclerosis (MS) is a chronic illness of the central nervous system affecting approximately 2.5 million young people worldwide.[1] Regarded as exclusively a white matter (WM) disease, clinicians are now facing the notion, known to pathologists for over a century, that MS may affect the grey matter (GM) as well.[2]

Currently, MS is an incurable disease. It is, however, to some extent treatable. The past 20 years have witnessed a remarkable expansion of the horizons of pharmacological treatments aimed at delaying disease progression. Recombinant interferon beta-1b (IFNβ-1b) was the first disease-modifying therapy approved in MS by the US Food and Drug Administration (FDA).[3] Today, IFNβ-1b is the drug for which clinicians have the most experience with in chronically treating patients.

This article will attempt to appraise the status of IFNβ-1b application in MS patients today. Upon reviewing the clinical-radiological presentation of the illness and its evolution over time, the effects of IFNβ-1b on clinical and imaging measures of disease, as reported in the largest clinical trials, will be discussed. Then the side effects of the drug will briefly be covered. This review will conclude by addressing some crucial scientific aspects that are still open for investigation to estimate the actual potentials of IFNβ-1b treatment in MS patients.

Two types of IFNβ are currently available for MS treatment, namely IFNβ-1a and IFNβ-1b. Their mechanisms of actions and clinical efficacy almost entirely overlap but, for the purpose of the present review, IFNβ-1b will be focused on.

Clinical-radiological Presentation and Evolution of Multiple Sclerosis

The first clinically manifest event of MS is the relatively acute or subacute occurrence of one or more combined neurological symptoms, lasting at least 24 hours, in the absence of fever and infection, and suggestive of demyelination and inflammation. Such an event, namely clinical relapse, defines the status of clinically isolated syndrome (CIS). This is not considered clinically definite MS (CDMS) until a second attack occurs. Approximately 44% of CIS patients will remain free from a second attack within the following three years.[4] Up to 10% of CIS patients will not convert to CDMS over the course of the subsequent 20 years.[5] Patients not converting to CDMS within the following three years tend to have fewer active lesions during the six months following the first attack (see *Figure 1*) than those who do convert to CDMS.[4]

In 85–90% of CDMS patients (MS hereafter) the disease starts and evolves with a relapsing-remitting (RR) course. RRMS is characterised by repeated and time-interleaved clinical relapses. These relapses may last from a few days to a few months and are sustained by inflammation directed against either the spinal cord or the brain tissue.

Contrast-enhancing Lesions and Clinical Attacks

Magnetic resonance imaging (MRI) mirrors inflammation by showing sharply demarcated focal WM contrast-enhancing lesions (CELs) in T1-weighted (T1-w) images obtained upon the injection of the contrast agent gadolinium-DTPA (see *Figure 2A*). CELs (brain CELs more than spinal cord ones) may be visible even in the absence of a

clinically evident attack, with a clinical flare occurring on average with every 10 CELs.[6] Nevertheless, as seen for CIS patients as well as MS patients, the higher the number of clinically silent CELs over monthly MRIs, the higher the likelihood that a clinical attack will occur later on.[7]

CELs may have different evolution over time. Such an evolution likely depends upon the degree of myelin destruction and axonal degeneration or repair. This may all take place by the time of the first occurrence and continue to evolve upon enhancement resolution. The average life-span of CEL visibility on imaging is nearly one month, with almost all CELs terminating as hyperintense chronic lesions in T2-w images[8] (see *Figure 2B*). Nearly 20% of CELs culminate in persisting hypointensities in T1-w images[9] (see *Figure 2C*).

T2-lesions have poor specificity with respect to the underlying pathological process and may represent areas with any degree of demyelination, axonal loss and repair. Conversely, T1-hypointense WM lesions persisting upon the enhancement resolution, namely persisting black holes (PBHs), are known to be pathologically more severe, since they are sustained by a higher degree of axonal loss.[10] A subset of those PBHs appear as hyperintense lesions in cerebrospinal fluid tissue-specific imaging[11] (CSF-TSI, see *Figure 2D*). Lesions seen by CSF-TSI are likely a later event and are believed to represent cavities filled with CSF-like fluid as a final result of tissue death and loss.

As time progresses, the number of new clinical attacks and WM focal lesions is reduced. Some patients may still demonstrate a slow but constant accretion of physical, cognitive and emotional disability over time. Those patients are the ones shifting towards the secondary progressive (SP) phase of the disease. SPMS is predominantly characterized by WM and GM tissue loss and leads to irreversible clinical deterioration and neurodegeneration. As a result of a long-standing insidious neurodegenerative process, patients also tend to present with cortical thinning[12] and focal cortical lesions, see *Figures 2E* and *2F*.[13]

The Role of Inflammation and Neurodegeneration

In about 10–15% of patients, the disease starts and evolves with a primary progressive (PP) course. PPMS leads patients to disability accretion and rapid brain and spinal cord tissue volume loss, with little inflammation visible by MRI.[14]

Cumulatively, MS presents with two different components (inflammation and neurodegeneration) of variable duration over time within individual patients. An initial phase of inflammation ultimately fades into an ominous and occult neurodegenerative process that leads patients to irreversible disability. At any stage of the disease, the two components may overlap and may be regulated by a complex, yet unknown, interplay. The relationship between inflammation and neurodegeneration in MS is one of the most complicated in medical science and likely regulated by a number of genetic, immunological and environmental factors as yet unknown to clinicians and scientists.

Numerous drugs have been tested and proven to be effective in defeating or at least controlling the inflammatory phase of MS, with little or unknown independent effect on the neurodegenerative component. IFNβ-1b belongs to this group of drugs.
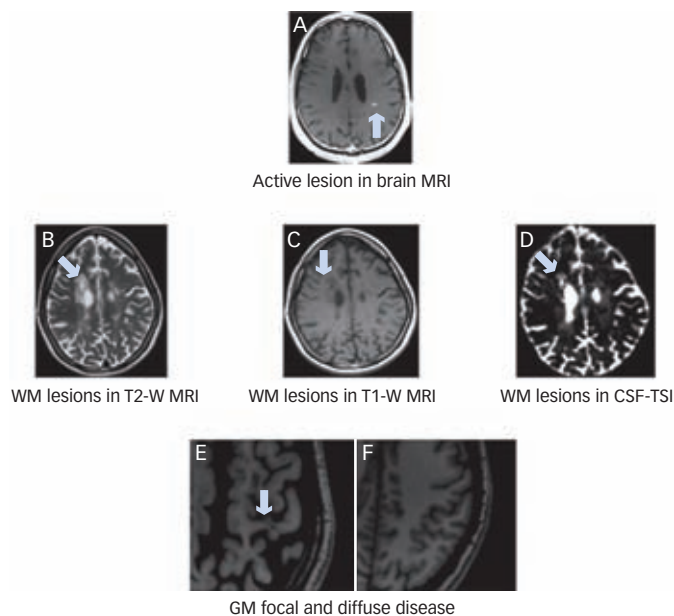
Figure 1: Monthly Contrast-enhancing Lesion Activity in Clinically Isolated Syndrome Patients Converting and Non-converting to Multiple Sclerosis within Three Years
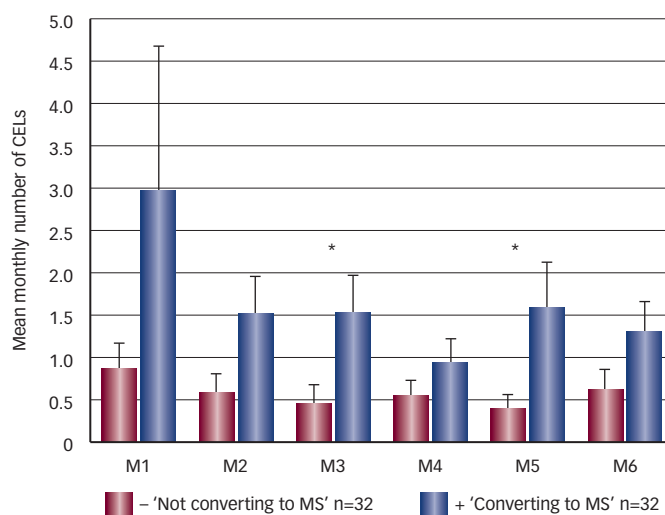
Although a clear trend towards higher contrast-enhancing lesions (CELs) number in clinically isolated syndrome (CIS) patients converting to multiple sclerosis is seen on a monthly basis, differences are statistically significant* at months three and five (by student t-tests). M = month.
Data presented in this figure are courtesy of Professor Carlo Pozzili, La Sapienza University of Rome, Italy.

Figure 2: Active and Chronic Multiple Sclerosis Disease Visible by Magnetic Resonance Imaging

Active lesion in brain MRI

WM lesions in T2-W MRI   WM lesions in T1-W MRI   WM lesions in CSF-TSI

GM focal and diffuse disease

The arrow indicates an active brain contrast-enhancing lesion (A); in a patient with relapsing–remitting multiple sclerosis (RRMS), 7.5 years of MS and an Expanded Disability Status Scale (EDSS) score of 2.5. T2-weighted (T2-w) fast-spin echo (B); in a T1-w spin echo image (C); cerebrospinal fluid tissue specific imaging (CSF-TSI; D) showing persistent black hole (arrow in D) that is visible as in T2-w image (arrow in B) and as a CSF-TSI lesion (arrow in D). The image was acquired in a RRMS patient with an EDSS score of 6 and 33 years of disease. The T1-w inversion recovery spoiled gradient-recalled image of a 35-year-old secondary-progressive MS female patient with 18 years of disease and an EDSS score of 6 (E) and a 35-year-old healthy volunteer (F). The following three important details need to be derived from E and F: (1) an overall enlargement of the cerebrospinal fluid spaces at the expense of shrinking of the entire brain tissue is visible; (2) the magnetic resonance image (MRI) signal is reduced in the cortex of the patient (E), which also appears thinner compared with the cortex of the healthy volunteer (F); and (3) juxtacortical and cortical lesions are visible in the patient's MRI as indicated by the arrows. The MRIs presented in this figure were acquired using a 3Tesla GE scanner equipped with an eight-channel coil. GM = grey matter; WM = white matter.
Images and clinical data are courtesy of Drs Jeff Duyn and Henry McFarland, National Institutes of Health, Bethesda, MD. Image reconstruction of CSF-TSI was facilitated by Dr Vasiliki Ikonomidou, George Mason University, Fairfax, VA.

Clinical and Imaging Parameters to Measure Disease Progression and Drug Efficacy in Multiple Sclerosis

Clinical Measures

The main clinical metrics used thus far for measuring IFNβ-1b's effect in MS are:

- relapse rate;
- proportion of relapse-free patients;
- severity of relapse;
- disability progression according to the Expanded Disability Status Scale (EDSS);[15]
- proportion of patients free from EDSS change; and
- change in MS functional composite (MSFC) score.[16]

The meaning of clinical relapses was clarified earlier in this article. With respect to severity, the following measures are considered indicative of disease severity.

- extent of functional recovery;
- need to use steroid medication and/or hospitalise the patient; and
- number of days of hospitalisation.

Numerous clinical scales are used to measure physical, cognitive and emotional disability of MS patients. Nevertheless, the ones commonly employed thus far are the EDSS and the MSFC scale.

The EDSS scale ranges from 0 to 10 with 0.5-step increases and quantifies MS-induced disability in the pyramidal, cerebellar, brainstem, sensory, bowel, bladder, visual and cerebral functional systems. EDSS steps 1 to 4.5 are indicative of MS patients who have independent walking ability. EDSS steps 5.0 to 9.5 are defined by severe impairment to ambulation. A sustained progression is defined as any change ≥1 for EDSS scores ≤5 and any change ≥0.5 for EDSS scores ≤5.5. The variables most commonly used to identify drug effects are: the proportion of patients who remain free from disability progression over three- or six-month time-windows; time to sustained disability progression; group- and/or within-person differences over time in median EDSS scores.

The MSFC is a 3D scale that measures cognitive function, leg function/ambulation, arm/hand motor and co-ordination functions. The scale produces values that are translated into z-scores in reference to normative values. These scores are used for group comparisons or for within-person comparison over a set time period.

Imaging Measures

The use of MRI provides a number of measurable disease parameters by means of which disease progression can be monitored. This is achieved with the use of conventional and non-conventional MRI techniques.[17] Conventional MRI techniques include T1-w and T2-w spin echo images. They permit the identification and computation of acute and chronic WM lesions (see details later). These measures are currently mostly employed in clinical trials. WM lesion identification and computation is a sensitive method for assessing disease progression and at the same time is highly reproducible across different centres. Non-conventional MRI techniques allow one to obtain quantitative measurements of brain and spinal cord normal-appearing WM (NAWM), normal-appearing GM and lesions. One such technique is magnetisation transfer imaging (MTI).

Quantities derived from MTI, namely magnetisation transfer ratios (MTRs), are considered to indirectly reflect the amount of demyelination and axonal loss within visible lesions as well as in regions of normal-appearing tissue and lesions.

Compared with conventional MRI techniques, non-conventional ones offer several challenges when one attempts their application in large multicentre clinical trials. First, scanning time may considerably increase. Second, results may suffer inter-centre variability, therefore being poorly reproducible. Third, sophisticated image post-processing may be required for some of the non-conventional techniques, rendering their application in large clinical trials unpractical. To date, besides limited applications, which will be discussed later in the course of this review, no studies have attempted to investigate the effects of IFNβ-1b on MRI quantities derived from measurements on normal-appearing tissue and lesions.

A third image technique that is considered a non-conventional MRI technique but reflects the application of advanced post-processing analysis in conventional sequences, is the measurement of brain volume and its changes over time. Such a measurement is achieved by quantifying the so-called brain parenchyma fraction (BPF). This is given by the ratio of the sum of the WM and GM volumes over the volume of the entire brain, i.e. (WM+GM)/(WM+GM+CSF). The concept behind the application of BPF is that as the ventricles enlarge due to brain tissue shrinkage, the BPF decreases. BPF measurements have been successfully applied in large clinical trials.[18]

Types of Study Design

Different types of study design have been used in MS to detect the effect of IFNβ-1b. Study type depends upon two main factors:

- the number of subjects needed to treat in order to detect a significant effect on the chosen primary outcome measure; and
- ethical motivations that over time limit the possibility of assigning patients to the placebo control arm.

Two main types of study designs have been used to identify the effect of IFNβ-1b in MS thus far: randomised controlled trials (RCTs) and baseline versus therapy (BVT) studies.

Radomised Controlled Trials

In RCTs patients are randomly allocated to the 'intervention' or placebo group and both investigators and patients are blind with respect to the compounds administered. This type of study design provides important data regarding the efficacy of IFNβ-1b compared with placebo in reducing the number and severity of clinical relapses as well as newly-formed lesions on MRI.

RCTs led to the approval of IFNβ-1b by regulatory agencies for medical drugs and devices worldwide. They were subsequently slightly modified by substituting the placebo with another approved disease-modifying therapy. This type of 'active comparator' study allows the superiority of IFNβ-1b versus other standard-of-care or experimental compounds to be measured.

Baseline versus Therapy Studies

A BVT study is where each patient receives exposure to the drug after a natural history evaluation period. The main advantages offered by this type of study versus the RCTs are:

- each patient serves as his/her own control, thus minimising the effect of inter-patient variability in measuring drug effect; and
- it generally requires a smaller number of subjects.

However, at the same time BVT studies eliminate the blinding component of the RCT, thus potentially introducing the so-called Pygmalion type of bias. In MS, studies of this led to the initial identification of IFNβ-1b's efficacy in decreasing the number of active lesions or CELs within individual patients.

Presently, in patients with MS, placebo-controlled trials can still ethically be prepared, but with restrictions. Specifically, for MS patients for whom established effective therapies exist, placebo-controlled trials should only be offered with rigorous informed consent if the patients refuse any of the existing therapies, have not responded to them, or if these treatments are not available for other reasons, such as cost.[19]

Interferon Beta-1b Effects on Clinical and Imaging Disease Parameters

This section discusses the effects of IFNβ-1b on clinical and imaging measures of disease seen in the main clinical trials. Clinical and imaging findings will be presented separately. For each type of finding, evidence obtained in each patient subgroup, i.e. CIS, RRMS and SPMS with and without superimposed relapses and PPMS, is discussed.

In the majority of clinical trials and post-marketing studies, IFNβ-1b has been administered at a dosage of 8 million international units, equivalent to 250μg, subcutaneously every other day (EOD). For the purpose of this review, this dosage is always referred to as the as standard dose unless otherwise indicated. In addition, when specifying that the effects of the drug were found to be significant, a p-value ≤0.05 is always referred to. Readers are advised to consult the individual papers referred to for additional details regarding the magnitude of alpha error of each study.

Clinical Measures

Frequency and Severity of Clinical Relapses

When looking at differences in clinical relapse rate between patients treated with IFNβ-1b and patients in the placebo group, a statistically significant reduction was seen and maintained over the course of two-year observational periods in patients with CIS,[20] RRMS[21] and SPMS with superimposed relapses.[22] On average, one in every seven RRMS[21] or one in every nine CIS[20] patients will remain relapse-free for two years if treated with 250μg IFNβ-1b. It is important to highlight that when a greater proportion of CIS patients remain relapse-free, ultimately a greater proportion have a delay in time to conversion to MS.[20]

Such reductions, to some extent dose-dependent,[21] are attributable to an average two-fold decrease in the frequency of moderate and severe attacks in treated versus untreated patients. Subcutaneous administrations of 250μg IFNβ-1b EOD had greater efficacy than 50μg EOD.[21] On clinical relapse, 250μg IFNβ-1b was not inferior to:

- 500μg subcutaneous IFNβ-1b EOD;[23]
- 22μg IFNβ-1a subcutaneously once weekly;[24]
- 44μg IFNβ-1a subcutaneously three times per week; or[25]
- 20mg subcutaneous glatimerar acetate (GA) daily[23] or EOD.[26]

The greater efficacy of standard doses of IFNβ-1b over 30μg IFNβ-1a given intramuscularly once weekly has been proven.[25,27] In SPMS patients without superimposed relapses, IFNβ-1b 250μg or 160μg/m^{2} body surface area administered EOD significantly decreased the number of clinical attacks over a time period of up to two years.[28]

Observational periods longer than two years have provided controversial results. In CIS patients, early treatment with 250μg IFNβ-1b maintained a reduced annualised relapse rate for only one additional year, but not from year four of therapy onwards versus patients starting therapy two years later.[29] In RRMS patients, a one-third reduction in exacerbation rate was maintained for up to five years versus placebo.[30] Differences in mean yearly exacerbation rate between patients treated with a standard dose of IFNβ-1b and those treated with either 50μg subcutaneous IFNβ-1b EOD or placebo only remained statistically significant until the end of the second year of therapy.[30] Conversely, a lower rate of severe versus moderate relapses was seen throughout five years in the standard-dose group.[30] In SPMS patients with superimposed relapses, a statistically relevant decrease in mean relapse rate was sustained for up to four years in treated patients.[31]

Disability Progression

When one looks at the effect of a given drug in reducing disability progression, two important considerations need be kept in mind. First, disability changes during therapy might be linked to the indirect effect of a given drug in reducing the number and severity of clinical relapses, thereby leading to slower or lower accumulation of disability over time. Second, changes in disability might be due to a direct effect of the drug in promoting faster and more efficient remyelination or relenting demyelination. This will favor slower and less pronounced disability accumulation independently from the effect of reducing clinical relapse.

Compared with placebo, 250μg subcutaneous IFNβ-1b EoD over a two-year time period significantly reduced the proportion of patients experiencing a sustained progression in disability among those with CIS, RRMS and SPMS with superimposed relapses.[20,21,22] As seen with relapse rate, 250μg was superior to 50μg administered EOD[21] but was not inferior to the following subcutaneous regimens for remaining free from disability progression or having relevant beneficial changes in EDSS during therapy: 500μg IFNβ-1b EOD,[23] 22μg IFNβ-1a once weekly[24] and 44μg IFNβ-1a three times per week.[25] Interestingly, on measures of disability progression by EDSS scores, 250μg subcutaneous IFNβ-1b EOD was superior to 20mg subcutaneous GA daily[23] or EOD[26] and 30μg intramuscular IFNβ-1a weekly.[24,27] None of the effects of IFNβ-1b on physical disability were seen in patients with SPMS without superimposed relapses[28] or in those with PPMS.[32] It is important to highlight that although IFNβ-1b keeps a higher number of patients free from disability progression, it does not necessarily imply that the EDSS scores of treated versus untreated patients or patients before and after therapy are statistically different.

Few studies have looked at the effect of IFNβ-1b on MSFC score. Over a two-year observational period, a significant improvement in MSFC score was seen in patients with PPMS treated with IFNβ-1b compared with those taking placebo.[32] This improvement was not maintained beyond three months after drug discontinuation. Nor was MSFC score paralleled by improvement in other cognitive tests evaluating verbal fluency and processing speed. Conversely, in

Figure 3: Contrast-enhancing Lesion Decrease During Therapy with Interferon Beta-1b
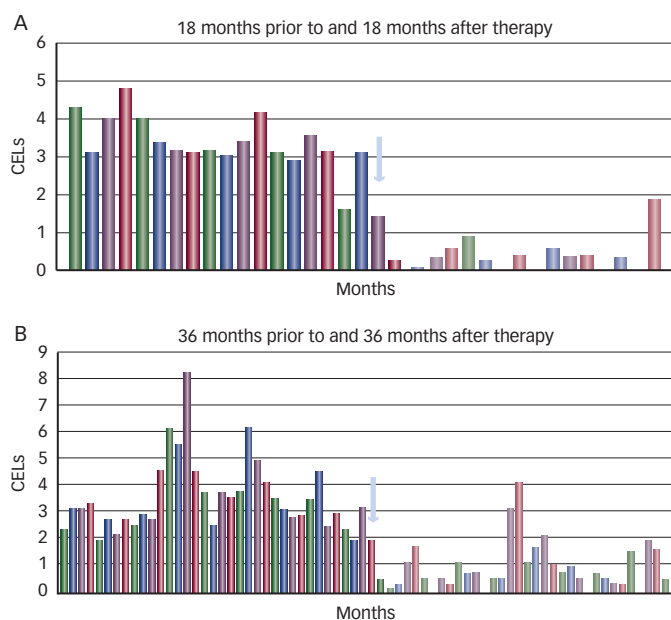
A: Mean monthly contrast-enhancing lesion (CEL) activity 18 months before and 18 months after therapy with interferon beta-1b (IFNβ-1b) in a cohort of 10 patients with multiple sclerosis (MS). The drug significantly reduced the mean number of CELs;[40] B: Mean monthly CEL activity 36 months before and 36 months after therapy with IFNβ-1b in a cohort of six patients with MS. The drug significantly reduced the mean number of CELs.[41] The arrow indicates therapy start.

patients with CIS a non-significant MSFC score improvement was seen between early- and late-treated patients after five years.[29] Interestingly, such improvement was driven by the cognitive component of MSFC score that, more than any other component, may be affected by patient training.[33]

In an attempt to understand the long-term effect of IFNβ-1b in MS patients (i.e. beyond five years) following completion of the pivotal trial, 372 RRMS participants were contacted by physicians and enrolled in a 16-year survey. Of the 372 patients initially enrolled, 260 (69.9% of the original group) had EDSS evaluations after 16 years of follow-up. These patients were taking various medications, as recommended by their physician. Although there is no statistical analysis in this long term follow-up, descriptive results indicate that continuous administrations of 250μg IFNβ-1b tended to reduce the proportion of patients converting to SPMS and progressing to an EDSS >6. Conversely, patients who were exposed to 50μg IFNβ-1b progressed faster and more frequently to SPMS and were more likely to end up with an EDSS score >6.[34]

Imaging Measures

Magnetic Resonance Imaging Measures of Active Inflammation

In addressing the question of whether IFNβ-1b has an effect on blood–brain barrier (BBB) breakdown and inflammation, one should consider two important pathogenetic points. First, it is crucial to investigate and assess whether IFNβ-1b or any other given drug can prevent BBB breakdown. Such an assessment permits the anti-inflammatory and immunomodulating property of a given drug to be established. Second, it is important to examine the ability of IFNβ-1b to halt the WM changes preceding CEL occurrence before BBB breakdown and, later, promoting faster and better CEL recovery. The latter would ultimately provide evidence of the indirect effects of IFNβ-1b as neuroprotective agent.

With respect to the effect of IFNβ-1b before BBB breakdown, two main imaging outcome measures have been used thus far, namely number of CELs in T1-w post-contrast MRI and number of newly-formed lesions in T2-w images. From a statistical stand-point, defining new lesions as either active T2-lesions or CELs as MRI end-point does not seem to be substantially different for monitoring MRI activity in MS clinical trials.[35] However, biologically the advantage of CELs over newly-formed T2-lesions is given by the fact that CELs directly visualise the BBB breakdown while newly-formed T2-lesions may be only an indirect harbinger of an ongoing inflammatory process or inflammation that has occured previously.

The effect of IFNβ-1b on CELs has not been investigated in all clinical trials, mainly due to the cost of studying this. Monitoring CEL activity not only adds the costs of the contrast agent itself to the study, but also requires numerous monthly scans that certainly further increase costs. The effect of IFNβ-1b beyond BBB resolution and quality of inflammatory activity has only been assessed sporadically. This has been carried out by obtaining pre-therapy versus therapy-phase but also treated versus untreated patient comparisons in:

- the proportion of CELs converting into PBHs;
- the size of CELs formed during the different pre-therapy and therapy-phases; and
- the evolution of CEL by MTR measurements.

Interferon Beta-1b Effect on Lesion Quantity

That IFNβ-1b reduces the number of CELs, newly-formed and growing T2-lesions over a two-year time period has been extensively demonstrated in large RCTs performed in patients with CIS,[21] RRMS,[21,23,24,27,36] SPMS with[22] and without superimposed relapses[28] and PPMS.[32] Such an effect was preserved on a yearly basis for up to four years in CIS patients,[29] five years in RRMS patients[30] and four years in SPMS patients.[29] The effect of 250μg IFNβ-1b administered subcutaneously EOD on T2-lesions was similar to hat of 500μg subcutaneous IFNβ-1b EOD or 20mg subcutaneous GA daily.[23]

Using both a fixed and a random effect model to investigate the distribution of active T2-lesion reduction over a three-year follow-up of SPMS patients with superimposed relapses, it has consistently been shown that between-patient response to IFNβ-1b in T2-lesion reduction is highly heterogeneous.[37] Reduction in T2 lesions account for only 57% of the of the treatment's effect on disability progression and 68% of the effect on relapse rate reduction.[38] Patients receiving treatment had >65% chance of showing ≥60% T2-lesion reduction but also a 7% probability that there would be no reduction or even an increase in T2-lesion size.[37]

When looking at the results of the small open-label BVT studies performed thus far, IFNβ-1b administrations significantly decreased the number of new and total CELs for up to 19 months of follow-up.[39] Similarly, a significant overall reduction in monthly CEL frequency was detected during therapy phases of 18[40] (see Figure 3A) and 36 months[41] (see Figure 3B) in comparison to a natural history phase of equal time.

As seen for the distribution of active T2-lesion reduction in SPMS, individual variability and month-to-month fluctuations in drug effects were also noted in these small studies, which relied upon CEL counting.[42] Specifically, ≥60% reduction in CEL activity was achieved

and remained constant over three years in slightly more than 50% of patients. An additional 13.7% of individuals had a 60% reduction in CEL activity after less significant changes initially. The remaining patients never reached ≥60% reduction in CEL frequency.

Interferon Beta-1b Effect on Contrast-enhancing Lesion Quality

To evaluate the effect of IFNβ-1b on CEL quality, several imaging measures were considered. The first such measure was the proportion of CELs evolving into PBHs. Studies performed by the National Institutes of Neurological Disorders and Stroke (NINDS) group on RRMS patients yielded contradictory findings depending on the group of patients studied. In a 72-month open-label BVT study performed on six patients with RRMS, patients were observed to have a significantly reduced likelihood of forming PBHs from CELs forming during therapy than during the pre-therapy phase.[41] No similar significant effect was observed in a shorter 36-month follow-up study including 10 MS patients (eight RRMS and two with SPMS).[43]

The ability of IFNβ-1b to reduce the likelihood of PBH formation from CELs in RRMS has recently been confirmed by Filippi and co-authors in a large RCT whereby patients were assigned to receive either IFNβ-1b or GA.[44] The drugs were found to be equally significantly effective in protecting patients against PBH formation during therapy. Conversely, when patients with SPMS and superimposed relapses were examined, Brex and co-authors failed to find any significant decrease in the proportion of CELs converting into PBHs 18 months later compared with placebo.[45] A more extensive and detailed description of IFNβ-1b reducing the likelihood of CEL progression to PBH has been reported by the NINDS group.[46]

A second imaging marker used to identify the effect of IFNβ-1b on CEL quality is CEL size. The size of a given CEL is known to be an indirect indicator of the underlying disease pathology. Larger CELs have a greater tendency to evolve into PBHs, have lower MTRs and tend to be present in SPMS more than in RRMS patients. A relatively large study performed in the NINDS group during a short-term (six-month) time window, has shown that the effect of IFNβ-1b in reducing the likelihood of CEL formation is not paralleled by its ability to reduce the size of CELs once formed.[47] IFNβ-1b reduced the number of CELs by 76.4% over 18 months of therapy. This decrease however, was statistically greater for small lesions enhancing only once during therapy (82.3%) than for larger ones enhancing multiple times (57.4%).[40]

A third imaging measure used to reliably track CEL evolution over time and investigate the ability of IFNβ-1b to promote faster recovery is monthly measurements of MTR. The effect in CEL MTR is rather limited. As early as 12 months before the occurrence of enhancement, MTR starts to decrease. Such a pre-lesional decrease in MTR is not affected by IFNβ-1b. In addition, the extent of CEL MTR recovery over time appears to be more evident if steroids are used in association with IFNβ-1b.[48]

Magnetic Resonance Imaging Measures of Neurodegeneration

In addressing the question of whether IFNβ-1b has an effect on imaging measures of neurodeneration, two important measures need to be considered. First, one should keep in mind measures of focal chronic lesions, such as lesion volume (LV) in T1-w (i.e PBHs) and T2-w images. Second, one should consider the effect of IFNβ-1b

in measures of global brain and spinal cord degeneration. These measures include brain volume loss (or BPF changes), whole-brain MTR, NAWM-MTR and cervical cord area measurements at the level of the second cervical vertebra or C2.

Interferon Beta-1b Effect on Focal Chronic Lesions

The role of IFNβ-1b in reducing T2-LV is accompanied by a dual component. First, changes in T2-LV may reflect changes in chronic T2-lesions present prior to therapy. Second, those changes may only reflect the effect of the drug on newly-formed lesions. In general, as previously stated, patients receiving 250µg subcutaneous IFNβ-1b EOD tend to form fewer new lesions (as seen in T2-w images). As a result, the difference in total T2-LV between treated and untreated patients at the end of the study may reach statistical significance. Such results were seen in patients with CIS,[20,29] RRMS,[23,30,36] SPMS with[22,31] and without[28] superimposed relapses and in those with PPMS.[32] Interestingly, in patients with CIS and RRMS, group differences remained significant throughout years four and five, respectively. As stated earlier, however, these results do not necessarily imply that IFNβ-1b affects, by decreasing their size, the number of chronic lesions in T2-w images present at the time of the therapy start. Similarly, when one looks at the LV of PBHs, there are no reports to date that have sought to determine the role of IFNβ-1b in preventing the formation of new PBHs or in decreasing the quantity of pre-existing PBHs, separately.

Kappos and co-authors measured the volume of PBHs in CIS patients on therapy with IFNβ-1b for up to five years and failed to demonstrate a drug effect.[29] Conversely, Barkhof and co-authors measured the PBH volume changes over a three-year observation period in a subgroup of 95 patients with SPMS and superimposed relapses and found that the IFNβ-1b-treated group had a significantly slower accumulation of PBHs at 36 months.[49] These results were confirmed by Montalban and co-authors, who performed the same type of analysis in a cohort PPMS patients treated for 24 months with the same therapy regimen.[32] O'Connor and co-authors found the same in RRMS patients.[23] With respect to the latter study, it is interesting to highlight that 250µg subcutaneous IFNβ-1b EOD had a superior effect on T2-lesions but not on T1-lesions compared with 20mg subcutaneous GA administered daily.[23] In previous open-label BVT observational studies performed at the the National Institutes of Health, a decrease in the number of PBHs forming *de novo* during therapy has been shown. Nevertheless, the longevity of PBHs arising during the natural history phase is no shorter than those formed during therapy. Consequently, according to these findings the ability of IFNβ-1b to promote the formation of less aggressive CELs or a faster recovery over time should be questioned.[41]

Interferon Beta-1b Effect on Global Disease – Brain Parenchyma Fraction and Magnetisation Transfer Ratios

The reduction of brain atrophy accumulation over time with IFNβ-1b is controversial. At variable observational time periods, no significant differences in loss of brain volume were seen in treated versus untreated CIS (five years),[29] SPMS with superimposed relapses (three years)[50] or PPMS (two years).[32] Conversely, IFNβ-1b seemed to protect the brain from atrophy better than GA in patients with RRMS.[23] Similarly, over a three-year time period, an open-label study conducted in RRMS patients showed that IFNβ-1b successfully decreased brain volume loss with respect to pre-therapy phase.[51]

Figure 4: Relation Between Contrast-enhancing Lesions Decrease and Atrophy Increase in a Treated Multiple Sclerosis Patient
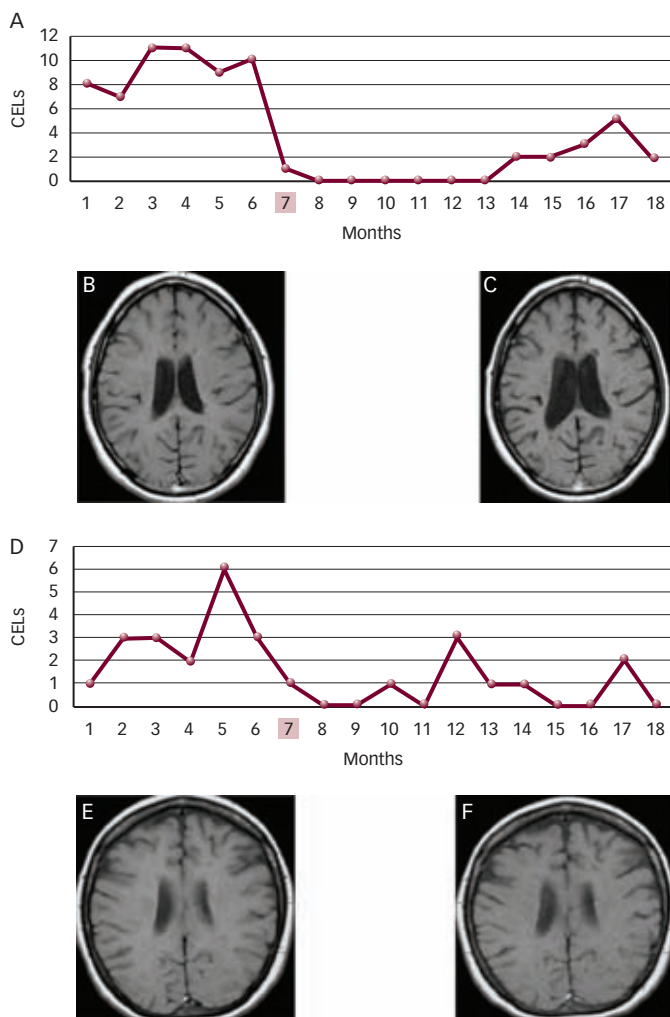
The chart represents monthly contrast-enhancing lesions (CELs) in a responder (A) and non-responder (D) patient. The red box in the chart indicates the start of therapy. The responder is defined on the basis of a monthly reduction by ≥60% in the number of CELs during the first 12 months of therapy compared with the six-month pre-therapy phase. For the same patients, baseline (B–C) and 10-year (E–F) T1-weighted (T1-w) spin echo magnetic resonance images are shown. One can see relatively larger ventricle enlargement in the responder patient by CEL of A–C, whose annualised rate of brain atrophy decrease was 0.55% compared with the non-responder patient by CEL in D–F, whose brain atrophy annualised rate of decrease was 0.33% (Bagnato F, unpublished data).
The magnetic resonance images presented in this figure were acquired using a 1.5Tesla GE scanner equipped with a standard head coil.
The magnetic resonance images presented in this figure are courtesy of Joseph Frank and Henry McFarland, National Institutes of Health, Bethesda, MD. Image post-processing was facilitated Ms Jailan Hanafy and Carolyne Bavan, NIH, Bethesda, MD.

As seen with BPF, no significant effect of the whole brain and NAWM regions was seen to be exerted in MTR by IFNβ-1b in SPMS patients with superimposed relapses[52] or PPMS[32] after three and two years of therapy, respectively. Finally, no significant changes in spinal cord area measurements were seen to be exerted by IFNβ-1b in treated versus untreated patients over the course of two years of therapy.[32]

The effect of IFNβ-1b in reducing neurodegeneration, as measured by brain atrophy, is still being debated and is controversial. When visible, such an effect has been shown to be dependent on two main factors: time and the CEL-status of the patient. With respect to time, it is hypothesised that in the short term, IFNβ-1b may induce accelerated, non-tissue-related brain volume loss (i.e. pseudoatrophy) due to the reduction of CELs and inflammation-related oedema.[18,50] The actual

biological substrate of the effect of IFNβ-1b in reducing brain atrophy accumulation may become visible later on, once the oedema is resolved.

With respect to CEL patient status, controversies and uncertainties still dominate. Exploratory subgroup analyses of the SPMS cohort of treated patients indicated that patients without CELs prior to therapy had a significantly greater reduction in cerebral volume in the placebo group compared with those with CELs prior to therapy. There was a trend towards greater reduction in cerebral volume, however, if the patient was on therapy. Although those differences did not reach a statistically relevant threshold, the trend suggests that in untreated patients no relation exists between inflammation and neurodegeneration. Conversely, when patients are taking IFNβ-1b, the reduction in inflammatory activity is paralleled by halting of brain volume loss. Similarly, monthly MRI analyses of individual RRMS cases performed in the NINDS group have shown that a decrease in the accumulation of brain atrophy is generally associated with decreases in the number of CELs.[53]

The fundamental question unanswered by all these studies is to what extent reduction in brain atrophy accumulation is merely sustained by CEL reduction or whether there is an additional effect independently exerted by IFNβ-1b. To this end, two patients who exemplify the complexity of the relationship between inflammation and neurodegneration when IFNβ-1b is active are discussed here (see *Figure 4*). Both patients were monitored monthly with six MRIs prior therapy and 12 MRIs during the first 12 months of therapy and one MRI at year 10 of therapy. The patient represented in *Figure 4A–C* had a substantial (≤60% decrease) monthly decrease in CEL number, but his atrophy progressed at a rate of -0.55% yearly. Such a progression rate is similar to that observed in untreated MS patients. Over the 10-year follow-up period, he shifted from RRMS the SPMS with an EDSS increase of 3.5 (from 2.5 to 6.0). Conversely, the patient in *Figure 4D–F* was not an optimal CEL responder during the first year of therapy but her atrophy progressed at a rate of -0.33% per year, which is similar to what is observed in healthy subjects. Over the 10-year treatment period, her disease remained RRMS and she had an EDSS increase of 0.5 (from 1 to 1.5).

Interferon Beta-1b Side Effects, Tolerability and Resistance

IFNβ-1b is a relatively well-tolerated drug. Its side effects are generally manageable with temporary drug discontinuation, although discontinuation is rarely necessary due to side-effects. The most common side effects include lymphopenia, injection-site reaction, flu-like syndrome, headache, pain and thyroid dysfunctions.

Very few patients exhibit depression and suicidal ideas. Compared with IFNα which circulates for longer than IFNβ-1b in the blood, depression does not occur *de novo* in patients treated with IFNβ-1b. Despite this, care needs to be taken in those patients with depressive symptoms prior to therapy, since these patients may exhibit suicidal ideas as therapy starts. This topic is only briefly expanded upon since numerous extensive reviews and post-marketing studies have been compiled on the topic.[54,55,56] Specifically, it is recommended that readers consult an important long-term (16-year) follow-up study on the safety and tolerability of IFNβ-1b very recently published[57] and several informative reports on the effect of IFNβ-1b on the quality of life of MS patients.[58,59,60] Today there is class III evidence that RRMS

patients taking IFNβ-1b 50µg or 250µg subcutaneously EOD for up to five years, with subsequent unspecified treatment, have fewer deaths after 16 years of follow-up than similar patients on placebo for up to five years, with subsequent unspecified treatment. An important problem associated with therapy with IFNβ-1b is the development of neutralising antibodies (NABs). NABs may occur after as little as six months of therapy and persist for a few years. Whether NABs are the cause or epiphenomenon of therapy failure is still a controversial question that, similarly to the topic of side effects, has been extensively debated in the literature.[61,62,63]

Conclusions

IFNβ-1b is one of the most widely used and effective therapies for preventing disease progression in MS. Despite this, several uncertainties remain in the clinical community regarding the actual therapeutic potential of IFNβ-1b. Undoubtedly, the combined effort of basic and clinical scientists is warranted to provide insights into the several still-unanswered questions.

Interferon Beta-1b Beside Blood–Brain Barrier Breakdown

Both imaging and clinical evidence thus far prove that when the inflammatory component of MS still plays the major visible pathogenetic role, IFNβ-1b seems to have a key effect in preventing the occurrence of new flares. However, the effect of IFNβ-1b on neurodegeneration and disability accretion remains controversial. In addition, it is unclear whether the effect of IFNβ-1b in halting neurodegeneration progress is independent of its indirect effect on inflammation or is an epiphenomenon. Studies designed to elucidate this topic have never been conducted and are certainly warranted.

Interferon Beta-1b Before Blood–Brain Barrier Breakdown and Beyond its Resolution

Limited and discouraging evidence is available on the (in)ability of IFNβ-1b to affect NAWM pre-lesional or outside-lesion MRI quantities. Further knowledge on such topic would ultimately allow one to understand:

- to what extent IFNβ-1b alters the disease course before CELs become visible; and
- the need to treat patients without visible CELs.

Serial imaging studies in large patient cohorts examining the effect of IFNβ-1b on the changes occurring in NAWM before a lesion develops and once its enhancement resolves are crucial.

Interferon Beta-1b and Grey-matter Disease

No evidence exists thus far on the role of IFNβ-1b in affecting focal and diffuse GM disease in MS. Lack of such knowledge impedes elucidation of the neuroprotective properties of the drug. Nowadays numerous quantitative and non-quantitative MRI techniques are available to identify part of the cortical lesions and topographically measure selective damage to the GM. Using these techniques in the context of clinical trials is the next step.

Interferon Beta-1b Beyond Clinical Relapses and Physical Disability

Although the effect of IFNβ-1b in decreasing the occurrence of new clinical relapses and relenting physical disability progression is established, no evidence is available on its ability to halt other disease-associated symptoms. Among these symptoms, cognitive and emotional disability play a substantial role in affecting the life of MS patients. Exploring the interplay between IFNβ-1b and these symptoms is hence crucial in order to define the actual potential of the drug.

Current knowledge yields the conclusion that in patients with MS, IFNβ-1b partially affects the quantity of disease but probably leaves its quality unaltered. Conversely, quality of disease leads patients to irreversible disability as much as, if not more than, its quantity.

The combined effort of clinicians and basic scientists is required to disentangle this topic, which will ultimately permit understanding the role of IFNβ-1b, if any, in the complex interplay between inflammation and neurodegeneration in MS. ■

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