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**BIOLOGICS WORKING PARTY**
**MEETING on 11-13 February 2008**
**Chairman: Prof. J-H Trouvin**

**BWP report to the CHMP**

# Beta-interferons and neutralising antibodies (in multiple sclerosis)

1st phase of the project: Development of a standardised assay methodology for the determination of neutralising antibodies

Background information

Currently three interferon (IFN) beta preparations are registered for the treatment of certain stages of multiple sclerosis (MS). Two preparations (Rebif and Avonex) are produced from mammalian cells (CHO) and one preparation (Betaferon) from bacteria (*E. coli*). The two CHO-derived beta interferons are glycosylated products. The *E. coli* derived product, which is not glycosylated and differs in two amino acid residues from the CHO expressed products, has about 10 % of the specific activity of the CHO-derived products. All three products differ in formulation, dosing schedule and route of administration. All three products have been reported to induce neutralising antibodies (NABs) in MS-patients (from 5 to more than 50% after one year of treatment). There have been reports suggesting that these antibodies may be associated with a loss of efficacy of treatment.

With respect to the effect of NAB on the safety and efficacy of Interferon beta used for the treatment of MS, there are two separate, but related issues to be addressed:

- the incidence of NAB formation
- if and at what level do these antibodies have real biological effects such as inhibition of efficacy

At its July 1999 meeting, the CPMP appointed Dr. Huub Schellekens (NL) as the co-ordinator for the first phase of the project dedicated to the development of a standardised assay methodology for measuring neutralising antibodies. Given the nature of this topic, the BWP was asked to provide the CPMP with recommendations on the proposals to be developed in collaboration with the 3 Marketing Authorisation Holders (MAHs).

On 17 February 2000, a BWP/CPMP adopted recommendation was sent out to the 3 Marketing Authorisation Holders (Ares-Serono, Biogen and Schering AG) with the following proposals:

- the MAHs were asked to introduce as a common assay the viral cytopathic effect (CPE) inhibition based assay analysed according to the Kawade principle.
- the MAHs were asked to collaborate in the blind panel testing approach.

During the development of the common assay, the progress was closely monitored and discussed at:

- April 2000 BWP (session with MAHs to agree upon a common approach)
- December 2000 BWP (report from co-ordinator and start of exchange of sera)
- June 2001 BWP (report from co-ordinator on first results on serum titers using in-house assays)
- July 2001 BWP (adoption of BWP report on the report of the co-ordinator dated June 2001)

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- July 2002 BWP (report from co-ordinator on further blind exchange of sera and advantages of the MxA assay; adoption of BWP report)
- November 2002 BWP (session with MAHs to report on the MxA assay as a suitable common assay and to agree on a time table to finalise the development and validation of the common assay)
- December 2002 BWP (adoption of BWP report on the MxA assay and the outstanding issues)
- July 2004 BWP (session with MAHs to report on the validation of the MxA assay and the blind panel testing)
- September 2007 BMWP/BWP Workshop on Immunogenicity Assessment of Therapeutic Proteins

Common assay methodology

Following a recommendation from the CHMP/BWP, the MAHs agreed to validate a common bioassay based on viral CPE inhibition and the Kawade principle in addition to their established in-house methods. The assays used by Serono and Biogen (both viral CPE-assays) were shown to be dependent on the interferon used in the assay. The assay used by Schering/Berlex (MxA- ELISA assay) however, seemed independent of the interferon used. Although this observation could not be confirmed by further experiments in the laboratories of the different MAHs, based on this initial finding the MxA-assay was selected for further development as the common assay and it has been decided to continue to develop this assay, because of its advantages, i.e. no need for viruses, easier to standardise, possibility to automate and safer methodology. In addition, practical problems were raised regarding the distribution and importation of the viruses in the required different geographical areas for the CPE assay.

Briefly, in the MxA-assay, A549 cells[1] seeded in well plates are incubated with a mixture of sera and challenge IFN. The IFN stimulates the intracellular production of the MxA protein. The cells are then lysed and the amount of MxA is measured with an ELISA using a rat monoclonal antibody (MAb) to capture and a biotinylated mouse MAb for detection. The titer is calculated using the Kawade equation, defining the titer as the dilution of serum that reduces the amount of Laboratory Units (LU) of IFN by 90% (1 LU = $EC_{50}$ i.e. 50% MxA induction).

The MxA assay validation has been finalised and showed good inter- (1.9 – 5.3%) and intra-lab (0.4 – 4.4%) reproducibility of the assay, estimated by the Coefficient of Variation (CV %). As part of the validation, the relative sensitivity of the assay was tested in the labs of the three MAHs using a limiting dilution assay with the international reference preparation for IFN beta. The use of Betaferon as a challenge antigen produced consistently lower titers than Avonex, Rebif or the natural interferon beta standard. Furthermore, the detection limit was shown to be dependent on the challenge antigen, with Avonex and Rebif as a challenge antigen being more sensitive.
As a last step in the assay development and validation, a panel of sera were sent by the MAHs to NIBSC (November 2003), which were subsequently blinded and distributed (62 samples) to the MAHs by NIBSC (January 2004). The panel was tested against all three authorised interferon beta preparations and the natural interferon beta standard. Testing showed 100% concordance in terms of positive and negative assay results across the three MAHs with the natural interferon beta standard as a challenge antigen. A high concordance was also demonstrated when the CHO derived beta-interferons were used as a challenge antigen. Also the titres obtained for all sera were largely concordant when the natural interferon beta standard or the CHO derived beta-interferons were used as a challenge antigens; the use of *E. coli* derived interferon-beta (Betaferon) resulted in lower titres.

Because of the limited supply of the natural interferon beta standard, it cannot be chosen as a challenge antigen for future routine testing; NIBSC was requested to undertake a statistical analysis so a decision could be made on which of the three beta-interferons should be chosen as a challenge antigen for the common assay. Following timely provision of the data by the MAHs to NIBSC, a summary of this statistical analysis for discussion by BWP was submitted on 28 August 2007. The statistical analysis comparing the viral CPE assay and the MxA assay was also performed. The statistical analysis conducted by NIBSC shows that the CHO derived interferon beta as a challenge

[1] Continuous cell line derived from human alveolar carcinoma.

should be the standard antigen in both assays. The MAHs agreed in principle to publish the MxA assay method after the statistical analysis has been finalised.

Previously, it was highlighted that discussions concerning the use of the common assay identified two main goals that could be considered:

- Harmonise the section on antibodies in the different SPCs by expressing titres and incidence of development of antibodies based on the common assay. This can be achieved by retesting (a representative part of) the sera collected during the clinical trials on which the SPCs are based or by using samples from more recent studies using preparations and formulations that are currently being used.
- Correlate titres obtained by the common assay with clinical effects.

Availability of materials and reagents

Novartis has confirmed that the MxA ELISA assay and the MxA antibody are no longer protected by patents in Europe. NIBSC indicated that they will conduct a collaborative study in order to compare the antibody produced by Novartis with the previously used antibody made by Biogen. Initially NIBSC will also produce and make available a first batch of antibody produced from hybridoma. EDQM indicated that, in principle is willing to take over the larger-scale production of the antibody reagent in the future, provided that the hybridomas are made available.

**Conclusions**

A potential common assay methodology for the determination of neutralising antibodies was successfully developed. When using CHO derived antigen the MxA assay correlated well with the CPE method. The final development of an antigen independent assay necessitated overcoming difficulties with the availability of viral stocks for the initial assay method. The availability of the test methodology and reagents is resolved.

It was also remarked that sponsors may be able to use other methods that utilise updated technologies for the quantification step of the assay (e.g. mRNA quantification). However, it should be stressed that in case the sponsors use those new technologies, they will have to demonstrate how the new assay compares to the agreed upon common assay, so as to guarantee standardisation in the expression of the results in antibody formation and incidence rate (to be reported in product literature).

The first phase of the project as requested for the CHMP has been completed.

**BWP recommendation to the CHMP**

- In view of the satisfactory outcome of the statistical analysis of the validation results, the MxA assay has been developed on the understanding that the description of the method and reagents are publicly available. The MAHs should be encouraged to publish the method.
- The statistical report presented by NIBSC confirmed that the MxA assay is a suitable standardised test method for the assay of interferon beta neutralizing antibodies, and CHMP may consider:
  -the harmonisation of the section on antibody formation in the SPCs of the respective products by expressing titres of antibody formation and incidence rate based on the common assay;
  -consider pathways to achieve the second goal, i.e. correlating titres obtained by the common assay with clinical consequences[2].
- Agree that this report is copied to the MAHs and to make it public on the EMEA website.

[2] Please note that in the new EC Framework Programme there is a call in the area of biopharmaceuticals, which emphasises the collaboration between scientists, industry, regulatory authorities and others.