Critical aspects regarding the application of GPL principles to new compounds such as biotechnology products

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Summary. The safety evaluation of new products such as the biotechnology-derived pharmaceuticals (biopharmaceuticals) requires a less standardised and more flexible approach. This is basically due to the characteristics of each product, especially so as regards species specificity and immunogenicity activity. Thus, it is necessary to select the relevant animal species for toxicity testing, to evaluate the effects on the immune system, and then to develop new types of tests (*e.g.*, *in vitro* tests and analytical methods). Nevertheless, also regulatory authorities (RAs) recognise that some studies/tests may be part of the registration dossier, although good laboratory practice (GLP) compliance cannot be fully claimed. Non-compliance issues need to be clearly identified as well as their impact on the overall safety evaluation. The application of GLP principles to new tests/methods always requires their re-interpretation and adaptation and, as usual, new doubts and questions arise, *e.g.*, the availability, need and extension of the characterisation of the reference items, including the blank matrices, as well as the feasibility, need and extension of the validation of any new test/method developed. Difficulties may well arise if the same level of application of the GLP principles to traditional analytical methods is compulsorily requested also for new test/method, especially in the case of the non-quantitative ones. Therefore, it seems necessary to evaluate the issue more in depth in order to establish a dialogue among all involved parties for a harmonised understanding and application of the GLP principles in this field.

Key words: good laboratory practice, *in vitro* studies, biotechnology, analytical methods.

Riassunto *(Aspetti critici nella applicazione dei principi di BPL a nuovi composti quali i prodotti biotecnologici).* La valutazione della sicurezza di nuovi prodotti, quali prodotti farmaceutici di derivazione biotecnologica, richiede un approccio non standard e più flessibile. Questo è dovuto principalmente alle caratteristiche di ciascun prodotto, specialmente per quanto riguarda la specie-specificità e l'attività immunologica. Quindi diventa necessario selezionare la specie animale adeguata per la valutazione della risposta immune e di conseguenza sviluppare nuove tipologie di saggio (ad esempio, saggi *in vitro* e metodi analitici). Tuttavia, anche le autorità regolatorie sembrano riconoscere che studi/saggi possono entrare a far parte del fascicolo registrativo sebbene presentino delle deviazioni rispetto ai principi di buona pratica di laborastorio (BPL). Tali deviazioni dovranno essere chiaramente identificate e ne dovrà essere stimato l'impatto nell'ambito della valutazione complessiva della sicurezza. L'applicazione dei principi di BPL a nuovi saggi/metodi comporta una loro reinterpretazione e, come sempre, sorgono nuovi dubbi e domande, quali la necessità, disponibilità ed estensione della caratterizzazione della sostanza di riferimento, inclusa la sostanza di controllo (matrice bianca) e la necessità, fattibilità ed estensione della convalida di nuovi saggi/metodi. Difficoltà possono insorgere se lo stesso livello di applicazione dei principi di BPL richiesto per i metodi analitici tradizionali viene considerato obbligatorio anche per la convalida dei nuovi metodi analitici, specialmente nel caso di quelli non quantitativi. Di qui la necessità di discutere più in dettaglio tale materia stabilendo un dialogo tra tutte le parti coinvolte al fine di raggiungere una interpretazione armonizzata dei principi di BPL applicabili.

Parole chiave: buona pratica di laboratorio, studi *in vivo*, biotecnologia, metodi analitici.

INTRODUCTION

The safety evaluation of new products such as biotechnology-derived pharmaceuticals (biopharmaceuticals) requires a less standardised and more flexible strategic approach, to be defined on a case-by-case basis. In fact, the conventional approach to toxicity testing is not appropriate. This is due basically to the biological and structural properties that are specific for each product, especially regarding aspects like species specificity, immunogenicity and

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pleiotropic activities. New products are in general macromolecules (proteins, complex biopolymers) and are biological/biotechnology derived, whereas conventional compounds are in general small molecules obtained by organic synthesis. The main difference between the two categories, small and large molecules, are mostly due to the size and origin along with the fact that small molecules are in general xenobiotics while macromolecules are endogenous and/or structurally similar to the endogenous counterparts. The above structural differences between the two types of molecules also explain the great diversity in terms of immunological properties. This fact explains why, for new products, it is necessary to select the relevant animal species for toxicity testing to evaluate the effects on the immune system, considering both the humoral and cell-mediated immune responses, and also the necessity to develop new types of tests (*e.g.*, *in vitro* tests, analytical methods).

NEW PRODUCTS: APPLICABLE PRINCIPLES OF GOOD LABORATORY PRACTICE AND SCIENTIFIC GUIDELINES

From the good laboratory practice (GLP) point of view, changes in developing strategies as well as the set-up of *ad hoc*, non-standard experimental design for classic *in vivo*/*in vitro* studies, do not have a real impact on the interpretation and application of the GLP principles [1, 2]. The difference resides in the analytical aspects. Product-specific tests need to be set up for the evaluation of the immunological aspects as well as for the analyses required to characterise the product, the analyses of doses prepared during pre-clinical *in vivo*/*in vitro* studies and the analyses of the product in the biological matrix performed for toxicokinetic purpose.

Any test performed for the safety evaluation of a new product shall be carried out in compliance with the GLP principles. Nevertheless, also regulatory authorities (RAs) appear to recognise that some studies/tests may be part of the registration dossier, even if not fully GLP-compliant. In this case the non-compliance areas/aspects need to be clearly identified as well as their impact on the overall safety evaluation.

In Italy the applicable GLP principles include the OECD GLP principles and the national acts on GLP [1, 3]. Since the Italian acts adopted the OECD GLP principles, these can be considered as just one set of GLP principles to be followed [3]. As regards the application of GLP to the analytical tests/methods for new products, the most important guidance is the OECD Advisory Document No. 14 issued in order to facilitate the proper application and interpretation of the GLP principles in the organisation and management of *in vitro* studies [2]. It should be emphasized that new tests, although often performed as part of *in vivo* tests (*e.g.*, analyses of doses, TK analyses, and still others) should be considered as stand-alone tests in terms of application of the GLP principles. Thus, the advisory document No. 14 is the best document when dealing with new tests as it really provides clarification and guidance in this field.

Regarding guidelines, guidance documents, scientific articles, and the like, it should be pointed out that, starting working in a new field, a quality assurance (QA) person should always assess such documents in order to better understand the requirements of RAs. Meeting these requirements is the final goal of a company performing studies/tests, so that the point of view of RAs can have an impact on the interpretation and application of the GLP principles.

The ICH Topic S 6 of 1998 deals primarily with the preclinical safety evaluation of biotechnology-derived pharmaceuticals [4]. At the beginning of the guidance, in section 3.1 (general principles), it is recognised that, although GLP compliance is always required, some tests/studies employing specialised test systems may not be able to fully comply with the GLP principles. Nevertheless it is also clearly stated that such non compliance does not necessarily mean that the studies/tests cannot be included in a dossier to support clinical trials and marketing authorisations. What is required is that the areas of non compliance are clearly identified and discussed in order to evaluate their impact on the overall safety assessment.

The *Guidance for industry on pharmacogenomic data*, issued by FDA in 2005, provides assistance in the submission of valid biomarkers data and clarifies that pharmacogenomic data must always be submitted where used to make decisions (*e.g.*, selection of species for preclinical studies) [5]. Furthermore, it also encourages the voluntary submission of exploratory pharmacogenomic data for which submission is not mandatory. In section IV D, compliance with 21 CFR Part 58 is addressed. It is stated very clearly that the GLP principles also apply to non-clinical pharmacogenomic data/studies that must be submitted. On the other hand, it is also said that, for data used to support safety findings and/or to support regulatory decision-making, if fully GLP compliance cannot be assured, the non-compliant parts must be clearly identified in the study report.

The *CHMP guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins* has been issued very recently, April 2008 [6]. This guideline deals with the impact of unwanted immune response that can also exert an influence on the interpretation of results from non-clinical studies. This is a good example to support the view that this field is rapidly evolving and that official guidelines/guidances cannot be the only reference documents because the procedure to issue such documents cannot keep up the pace.

Another critical document for anyone involved with new quantitative bioanalytical tests/methods, such as the ligand-binding assays, is the FDA guidance related to the bioanalytical method validation (issued in 2001 [7]. In this guidance the need of flexibility

is recognised by RAs. For example, in section III is stated that for each reference standard, besides other information, a certificate of analyses is needed, when available, and/or evidence of identity and purity generated internally or externally should be provided.

A more specific document to be used for the validation of new methods/assays is the workshop/conference report on best practices for quantitative bioanalytical methods, published in 2007, derived from the 3rd American Association of Pharmaceutical Scientists (AAPS)/FDA Bioanalytical Workshop [8]. This document sets forth new requirements in bioanalytical field and approaches specific issues related to non-chromatographic assays. For example, it takes into account that, in general, higher variability is expected for non-chromatographic methods when compared to conventional ones and thus different and more flexible acceptance criteria are needed.

The last document addressed was published in 2004 [9]. It deals with tests intended to determine unwanted immuno-response against biotechnology products. This issue is another example which highlights the novelty and rapid evolution of scientific knowledge in this field. As a consequence, no official guidelines/guidances are available for immuno-tests, because the issue of standard guidelines appears to be not useful due to such very rapid changes. A guideline might be obsolete as soon as it is issued and even before it is published; thus, scientific articles remain the only reference documents.

Other documents that are relevant from the QA point of view, when applying GLP principles to a new field, are findings from RAs, if available. Very useful are those published by FDA due to either the details provided or the up-to-date status. A review of FDA warning letters could help to understand the expectations of RAs and, as a consequence, their interpretation of the GLP principles. Unfortunately no findings are available as regards new analytical tests performed during development of new products, although there are many findings related to standard analytical tests (*e.g.*, HPLC or MS/MS methods). These findings can provide advice for new quantitative tests to which most requirements related to classic analytical tests are also applicable.

APPLICATION OF GLP PRINCIPLES TO NEW TESTS/METHODS

The application of GLP principles to new tests/methods requires their re-interpretation and this can lead to new doubts and questions. Difficulties are well evident, *e.g.,* if the same level of application of the GLP requirements applied to traditional analytical methods is considered as mandatory also for these new tests/methods, especially for the non-quantitative ones. For instance, the reference item can be an antibody prepared in house through an *in vivo* study. To decide how to manage the preparation phase, how to characterise this antibody and which is the minimal information required is not easy nor foreseeable, especially keeping in mind that such reference item could be used only for one analytical test/method carried out as part of a toxicology *in vivo* study in order to get supporting information.

Case history that includes the examples mentioned above is discussed below in order to provide examples of the difficulties encountered in applying the GLP principles to new tests/methods.

Case 1

Study details

- type of study: 13 weeks in rodents;
- test item: protein (an antibody to be used as a vaccine);
- study plan: including the evaluation of immunogenicity in responsive species.

Difficulties encountered

- reference item (standard) to be prepared in-house;
- *ad hoc* quantitative immunogenicity analytical method (ELISA test) to be set up in-house, to be validated and then to be used for analyses.

The difficulty in this instance is the evaluation of immunogenicity which is one of the study plan requirements. No specific test (*e.g.*, ELISA test) and no reference item were available on the market. Hence, there was the need to develop both of them in-house.

Reference item. Three steps can be identified in the process of in-house preparation of the reference item (an antibody), namely: preparation, purification, and characterisation (quantification included). For each phase the basic question is whether it is mandatory to carry out such a phase in a GLP environment. The preparation phase of the reference item consists of an *in vivo* study where rabbits are treated in order to produce immuno-serum. Subsequently it should be decided whether such a study needs to be GLP-compliant. For conventional products the reply will be no, whereas for new product the decision is not so easy. The preparation phase should be traceable although this does not necessarily mean that the study needs to be performed in compliance with the GLP principles.

As regards the purification phase, it is self-evident that no GLP-compliance is required as it is in the case of a conventional product.

The third phase, in turn, requires in Italy that the characterisation of a product is performed in compliance with the GLP principles as it is done for conventional products. Nevertheless, doubts can arise regarding the meaning of such phase for new products and regarding the required extension of the characterisation. According to the GLP principles a reference item should be characterised and appropriately labelled. For labelling it is clear that information on identity, concentration, storage conditions, preparation date, and expiry/retest date should be available. On the other hand, it is unclear whether

the quantification of a reference item (*e.g.*, the protein contents determined by a Lowry method) along with labelling information can be considered to be sufficient. According to the FDA guidance [7] the information needed for a standard (reference item) prepared in-house includes source and lot number, expiry date, certificate of analysis (when available), and/or evidence of identity and purity. Then, accordingly, it appears that quantification could be enough as characterisation, provided that the preparation phase is also traced (source and lot number) as discussed above.

ELISA test. Also regarding the ELISA test three steps can be identified: set up, validation and analyses. Again the basic question to address is if it is mandatory to carry out such phase under GLP. Regarding the set up phase it is clear that no GLP compliance is required. Regarding the validation phase regulatory authorities, with few exceptions, states very clearly that GLP compliance is not required, although it is a requirement that a method/test should be validated. Regarding the third phase, analyses, it is clear that GLP compliance is mandatory.

Case 2

Study details

- type of study: *in vivo* studies;
- test item: biopharmaceutical product;
- study plans: including evaluation of immunogenicity for which a new test needs to be developed (ELISA or another type of test).

Difficulties encountered

- Control item (matrix) to be prepared in-house.

This is a more general case than Case 1 as it does not related to a single study. Rather, it can be applied to any study where samples need to be analysed. The problem here is about the control matrix. From the GLP point of view, a control matrix can be regarded as a control item; hence, the requirements of reference item should also hold for a control matrix. Furthermore, there is a need to clarify how to prepare and how to manage a control matrix prepared in-house as well as to specify what is required in order to characterise and appropriately label it. As is discussed above for a test item, also for a control item the preparation phase should be traceable and this may well supply most of the information required (source and lot number as well as evidence of identity and purity). The expiry date should be also given as additional information.

Case 3

Study details

- type of study: *in vivo* study (repeated dose in rodents);
- test item: recombinant protein;
- study plan: including:
  1. kinetic analysis (through a non-chromatographic method). For this evaluation a new test needs to be developed (ELISA, or another type of test);
  2. evaluation of immunogenicity (in terms of immuno-response present/absent). For this evaluation a new test needs to be developed (ELISA, or another type of test).

Difficulties encountered

Two type of tests to be set up, validated and used for analyses:

- kinetic (test item + reference item – biosimilar), *ad hoc* quantitative method;
- immunogenicity (test item + reference item – biosimilar), *ad hoc* qualitative/semiquantitative method.

Here two types of tests had to be developed in order to fulfil the study plan requirements. For kinetic analyses a quantitative method was needed. In terms of GLP requirements the situation is the same as for Case 1. On the other hand, the method for immunogenicity evaluation was not quantitative, but rather qualitative/semiquantitative. The analytical output is just as simple as a yes or no, this meaning that a cut point is defined, samples above the cut point being considered positive while those below the cut point are regarded as negative. It is unclear whether this type of test/method should be validated or what does validation mean in such a case. For example, parameters like linearity and accuracy cannot be evaluated. Therefore, the guidance document on validation cannot be entirely applied [7]. For non-quantitative methods the testing performed in order to support that the method is fit for purpose appears to be more a qualification than a validation of the method. It should be ascertained whether this approach is also acceptable to RAs.

Case 4

Study details

- type of study: repeated dose *in vivo* study;
- test item: DNA vaccine;
- study plan: including
  1. evaluation in responsive specie of biodistribution through a PCR test (to be carried out at an external site *not* GLP compliant);
  2. evaluation of immunogenicity for which a new test/s need to be developed (ELISA, or another type of test).

Difficulties encountered

Two type of tests to be set up, validated and used for Immunogenicity evaluation:

- humoral immunogenicity (a non-quantitative test in which modified cells needed to be used);
- cell mediated immunogenicity (an *in vivo* study).

Again, two types of tests need to be developed in order to fulfil the study plan requirements. For the humoral immunogenicity assay a semiquantitative test was set up. The discussion for the immuno-

genicity test of Case 3 holds here as well, although an additional point should be considered. This test requires the use of modified cells (Balb 3T3 cells expressing a specific antigen) prepared at a university site and supplied by the sponsor. A full characterisation of such cells was unavailable and therefore it was checked through FACS whether the cells expressed the gene of interest. It should be clarified whether this checking procedure is also acceptable for RAs.

For cell mediated immunogenicity it was necessary to develop and carry out an *in vivo* test. Primary cells (splenocytes), conjugated or not with peptide plus fluorochrome, were inoculated in treated mice and samples obtained from mice were then analysed through FACS in order to measure fluorescence. In this case it was not possible to validate the test following the standard requirements of methods for quantitative bioanalyses. A preliminary test was carried out in order to support the assumption that the test was fit for purpose. It is unclear whether such a preliminary test should be performed in compliance with the GLP principles. This could be regarded as the validation phase of the test and then it should be considered sufficient to trace it and to store all related documents.

Biomarkers case

Difficulties arise also in the case of determination of biomarkers. These tests can be of several types, quantitative as well as qualitative (*e.g.*, ELISA, PCR, FACS, Multiplex). For tests performed during preliminary and exploratory phase GLP compliance is not a requirement, while it is a requirement when biomarkers are taken into account for regulatory purposes or decisions regarding GLP-compliant preclinical studies performed during the development of a new product (such as the selection of the responsive species to be used). Under such circumstances the conclusions reached for Cases 1 through 4 are still applicable depending on the type of tests performed.

Personnel qualification and training

Based on the selected cases discussed above, an additional consideration can be made as regards a critical aspect that must be taken into account when working in this field. GLP principles always ask for adequate qualification and training of personnel involved in GLP-compliant studies. Qualification and training of personnel is of a paramount importance for scientific personnel as well as for technicians. The scientific personnel, study director (SD) in the first place are responsible for the set up of *ad hoc* tests, specific for each new product for which no clear, detailed scientific guidelines are available. To design a test in a wrong way will lead only to wrong results. The technicians involved with these tests, especially with those where the manual part is of importance, can have a direct impact on the reliability of results.

CONCLUSIONS

As always for new investigation areas, test facility (TF) personnel as well as RAs, should not disregard common sense and should be ready to be more flexible in the interpretation and application of the GLP principles. It must be recognised that, although deviations can occur, the test results can still remain valid and acceptable for regulatory purposes. It is therefore needed to assess in more depth the mentioned issues with the participation of all involved parties, namely, industry, contract research organizations (CROs) and RA representatives, in order to establish a dialogue from different points of view for a harmonised understanding and application of the GLP principles in this new field.

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