Immunotoxicity and Immunogenicity:

Upcoming changes in the preclinical drug approval process

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With the implementation of the guideline ICH-S8 (Immunotoxicity studies for human Pharmaceuticals) the regulatory agencies for drugs and medicinal products will ask for an extensive consideration concerning immunogenic and immunotoxic characteristics of the active compounds of potential drugs, already at the moment of the preclinical studies. The article below describes how results from standard-toxicity studies as well as from investigations on comparable active agents may induce additional studies to specify the immunomodulatory effects of the new drug. Here, we describe which studies, in addition to the standard-toxicity study, should be considered in cooperation with specialized contract research organizations and the regulatory agencies for a successful drug registration.

The drug registration and approval process has become more complex and demanding over the last years. An extensive proposal, the so-called „Common Technical Document" (CTD) must now be submitted to the regulatory agency [1]. In the future, extended considerations will be required before determining possible immunomodulatory characteristics of the active ingredients and formulations. Therefore, it is to be expected that additional preclinical studies regarding immunotoxicity and immunogenicity will become necessary.

The registration process has already been gradually extended in the past years. And in 1995, the regulatory agencies accomplished a first worldwide harmonization of standards/guidelines (the ICH guideline topic S6) for innovative, biotechnologically derived drugs. At present a further guideline (ICH-guideline topic S8) is in the final consultation stage at the EMEA and ICH (Step 3). This guideline will give advice on whether (and which) nonclinical studies should be performed to immunomodulating effects of low molecular weight substances (non-biologicals). Hence, lead agents and their derived drugs will always require verification of the immunological side effects. Interestingly, the EU chemicals guideline (REACH), which presently is also in revision, will prescribe the same for new chemicals.

Therefore, upcoming all active agent groups are investigated regarding the immunological effects (Tab. 1).

The meaning of the new ICH-guidance S8 for the approval process.

Each regulatory affairs manager is familiar with the following standard components of the preclinical registration process:

- "state-of-the-art" knowledge/literature investigation
- physical-chemical properties including stability
- standard toxicity studies (STS) acc. to OECD-guidelines

To date, there was no common guideline at least for the European agencies that required the investigation of possible side effects of drugs to the immune system during the preclinical standard evaluation (exception: immunotherapeutics). This will change significantly with the guideline ICH-S8. After its implementation any regulatory agency may request the investigation on immunological effects right from the beginning.

Accordingly, possible effects will need to be described already within the CTD. The following procedures for their inclusion are recommended:

The regulatory agency will not ask for further investigations if reviewing of all available data and "state-of-the-art" knowledge in literature does not indicate any relevant immunological effects.

If such effects are present, special attention must be paid to possible indications for immunotoxicity during the course of the standard toxicity studies (STS). ICH-S8 emphasizes the following results of the STS:

- Dose-dependency of immunorelevant effects
- Mode of action and cell types effected
- The degree of severity in immunotoxicity and immuno-stimulatory effect

The regulatory agency will enforce further investigations if the results of the STS or of the data survey indicate that immunotoxic effects may occur. At this point, an immunological consultant familiar with drug registration or a specialised contract research laboratory (CRO) should be involved to set up the next steps (follow-up study). Seeking early advice and arrangement with the regulatory agencies about the planned testings should always be a prerequisite for any preclinical program.

In addition, the short time schedule of only 210 days [5] left for the execution of the whole preclinical testing program should be taken into consideration. Providing additional immunological studies in a GLP certified laboratory may easily take eight weeks from the initial studies design up to the final report.

Selection and Design of Additional Immunotoxicity Studies.

The ICH guideline S8 defines the term "immunotoxicity" by either immunosuppressive or immuno-stimulatory adverse effects of a drugs or its compounds (table 2).

GIT Labor-Fachzeitschrift 3/2005, S188-190, GIT VERLAG GmbH & Co. KG, Darmstadt, www.gitverlag.com/go/git

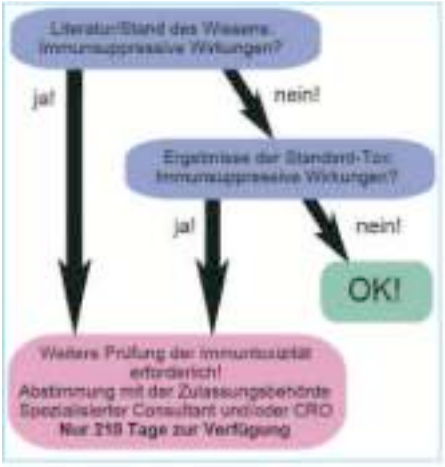

Fig. 1: Flow Diagram for immunotoxicity tests

Fig. 2: PD Dr. Hans-Gerd Pauels and Ms. Simone Brügging

There are two different study designs for follow-up testings, which are performed after STS showed immunotoxic properties of the drug or lead substance:

Functional Assays: In these assays it will be studied "ex vivo" whether different subtypes of immune cells interact or if they are downregulated and do not interact with each other. The T cell-dependent antibody response is mentioned as example in the guideline.

Tab. 1: Different groups of pharmacologic agents and corresponding guidelines

Group of active agents	Guidelines
High molecular agents	ICH-S6: Preclinical Safety of Biotechnology-Derived Pharmaceuti-cals [2]
Agents with a high or intermediate MW	ICH-S8: Immunotoxicity Studies for Human Pharmaceuticals. Step 2: Note for Guidance on Immunotoxicity Studies for Human Pharmaceuticals [3]
Chemicals	EU REACH: Registration, Evaluation and Authorisation of Chemicals [4]

It is to be expected that regulatory agencies will refer to the ICH S8 most frequently.

Table 2: Immunomodulatory effects can be divided into two subgroups

Adverse immunosuppressive effects:
• Decreased host resistance to bacteria, fungi and viruses • Increased incidence of opportunistic infections • Decreased host resistance to tumor cells
Adverse immunostimulatoric effects
• Allergic reactions • Dermal sensitization

Table 3: List of testing methods that quantify the immunogenic and immunotoxic properties of drugs and lead structures

- Determination of the distribution of of different immunglobulin isotypes (IgM, IgG, IgA, IgE)
- Immunophenotyping: FACS analysis of surface markers expressed by cells of the immune system
- Identification of cells affected by immunomodulatoric effects.
- Phagozytosis assay: Quantification of the ability of macrophages to take up substances in order to present them after processing
- Mixed Lymphocyte Reaction (MLR): It is analyzed if T cells can be activated by antigen presenting cells.
- TDAR T-cell dependent antibody response
- Determination of the activity of Natural Killer (NK) cells
- Infection models quantifying susceptibility to bacterial and viral infections
- Determination of the CTL activity against allogenic tumor cells

"Surface-markers": Depending on their function and maturation state, cells of the immune system express specific surface markers. Immunotoxicity can lead to the down-regulation of these specific markers in a defined subpopulation of immune cells. This can be easily detected by a FACS analysis using protein-specific antibodies. As an example one might mention dendritic cells. Drugs can influence the maturation of these cells resulting in the downregulation of the maturation markers CD83, CD86, MHC I and MHC II.

The guideline provides detailed instructions concerning the study design: application of the testing substance to mice or rats for 28 consecutive days is accepted in general. Species, dose, duration and application should be consistent with the STS in which the adverse effect on the immune system was observed.

Additional Immunotoxicity studies

An extensive panel of specific tests is available for the characterization of immunotoxic effects. It is not necessary to carry out all assays in order to test a „Lead"-Substance. As a general rule it is sufficient to perform tests basing on the STS results in order to quantify immunotoxic effects.

Summary

The most Regulatory affairs managers are not yet familiar with the guidance ICH-S8 and its demands on the preclinical, immunological characterization of a drug. The majority of the specialized service companies does not offer precisely focused tests to quantify drug specific immunotoxic side effects. Therefore PARA BioScience stated its core business to tests that quantify immunotoxic and immunogenic side effects of drugs. Therefore the company is the first central european GLP-accredited CRO completely covering this test panel.

These additional requirements, described in the article above, will make it necessary in the near future to prepare for requests from the regulatory agencies concerning testing of immunotoxic side effects even in the early preclinic phase of drug development.

Keeping this in mind the applicant will be able to contact a CRO early enough to perform recommended testings in time. This not only counts for authorization procedure of new drugs and medicinal product but also for authorization procedure retrospectively.

Literature

[1] CTD: Common Technical Document; Ergebnisnotiz der Sitzung der BfArM-Arbeits-gruppe „Qualität" am 15. 04. 2003, 10:00 bis 12:15 Uhr im Bundesinstitut für Arzneimittel und Medizinprodukte, Bonn.

[2] ICH-S6: ICH Topic S6, Preclinical Safety of Biotechnology-Derived Pharmaceuticals, Step 4, Consensus guideline, 16 July 1997. Note for Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. (CPMP/ICH/302/95).

[3] ICH-S8: Immunotoxicity Studies for Human Pharmaceuticals. Step 2: Note for Guidance on Immunotoxicity Studies for Human Pharmaceuticals. (EMEA/CHMP/167235/2004)

[4] EU REACH: Registration, Evaluation and Au-thorisation of Chemicals

[5] Gesetz über den Verkehr mit Arzneimitteln, Arzneimittelgesetz §27 Absatz 1: Die zuständige Bundesbehörde hat eine Entscheidung über den Antrag auf Zulassung innerhalb einer Frist von 7 Monaten zu treffen.

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