Bundesgesundheitsbl 2010 · 53:24–29
DOI 10.1007/s00103-009-0991-5
Online publiziert: 27. November 2009

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Regulatory requirements for clinical trial and marketing authorisation application for cell-based medicinal products

The new era of regenerative medicine has led to rapid development of new innovative therapies especially for diseases and tissue/organ defects for which traditional therapies and medicinal products have not provided satisfactory outcome. Skin replacement products [1] and cartilage repair using articular chondrocytes [2] have been standard care already for a decade; however, the ability to isolate, expand and differentiate various stem cells has been the real fire-lighter for new innovative therapies and their applications.

Cell-based medicinal products (CBMPs) include somatic cell therapy products and tissue engineered products, manufactured from viable autologous, allogeneic or xenogeneic cells. All CBMPs can also contain non-cellular components (chemical/biological compounds, matrices, scaffolds etc.) either as raw materials or as part of the active substance. Somatic cell therapy medicinal products are intended for treatment or prevention of diseases, or to make a diagnosis, via a pharmacological, immunological or metabolic mode of action of the cells. Most common product types of somatic cell therapies are cancer immunotherapy products (cancer vaccines). Tissue engineering products (TEPs) are developed for structural and functional repair of tissue/organ defects and their mode of action is to repair, restore or replace tissue structure/function. The first use of tissue engineering products has provided treatment for defects of cornea, skin, liver, bone and cartilage.

The legal requirements for CBMPs are set in the Regulation 1394/2007/EEC [3] and in the revised Annex I of Directive 2001/83/EEC [4]. The technical requirements are further addressed in the guideline for human cell-based medicinal products (EMEA/CHMP/410869/2006) [5].

Risk-based approach to define the amount of data for a MAA

The wide variety of cell-based products and the foreseen limitations (small sample sizes, short shelf life) vs. particular risks (microbiological purity, variability, immunogenicity, tumourigenicity) associated with CBMPs have called for a flexible, case-by-case regulatory approach for these products. Consequently, a risk-based approach can be applied to define the amount of scientific data needed for a Marketing Authorisation Application (MAA) of each CBMP. The initial risk analysis performed by the developers should identify the risks related to the product, its production and clinical use and the evaluation should cover the whole product development. The issues to be considered include cell origin (autologous versus allogeneic), ability to proliferate/differentiate, ability to initiate an immune response, level of cell manipulation, route of administration, duration of exposure, use of combination products etc. The design of the preclinical studies can also be justified on the basis of the risk analysis. The initial risk evaluation should also serve as a basis for the preparation of a risk management plan [6]. Further guidance for the initial risk analysis is currently under development.

Quality requirements for CBMPs

Cells are fragile, complex, living systems and highly dependent on their micro-environment. Whenever the environment changes, the cells tend to change. This makes the quality control of CBMPs very challenging. Poorly controlled product and production processes may have a direct impact on the safety and efficacy of the product.

The major risks related to a cell-based product are microbiological contamination, dedifferentiation/loss of cell function, cell transformation/malignancies, immunogenicity and ectopic engraftment of the cells to non-target tissues. These risks can be mitigated to some extent through a proper quality management system, where the CBMPs are manufactured using good-quality starting materials and a validated, aseptic manufacturing process. Whenever there are limited possibilities for batch release testing, the missing information should be complemented through proper product characterisation and process validation data. Definition and characterisation of the product are of outmost importance, as these data provide the tools for proper process validati-

on, in-process testing and release testing. In order to ensure reliability of all quality controls, the test methods should be validated and suitable for their intended use.

Tissues and cells, used as starting materials for CBMPs, should comply with the requirements set in Directive 2004/23/EEC [7] and the technical directives drawn from it [8, 9]. Furthermore, if animal-derived materials are used in the production of CBMPs, TSE and viral safety of such materials should be verified prior to their use. Manufacture of living cells does not allow terminal sterilisation of the product or removal/inactivation of microbial contaminants. Thus, appropriately tested and qualified starting materials and a validated aseptic manufacturing process are the key factors to ensure microbiological purity of the product. Sterility testing according to current European Pharmacopoeia (Ph.Eur.) requirements may not be possible for all products, especially when the shelf-life of the product is very short. In such a case, alternative methods with shorter read-outs could be used [10]. Testing for the absence of bacteria, mycoplasma and fungi should be conducted at release, whenever possible.

Product characterisation as a minimum should include tests for identity, purity, impurities/sterility, potency, viability and cell number. Additionally, tumourigenicity and biocompatibility testing should be performed, where appropriate.

Identity parameters should be established for all components of the product and the test methods should be specific for each component. For cells, identity testing should be based on phenotypic and/or genotypic markers; phenotypic markers include various assays related to, e.g. cell surface molecule expression, bioactivity, production of specific biomolecules. For adherent cells, morphological analysis may be a useful tool, e.g. as an in-process control in conjunction with other tests. However, most adherent primary cells tend to take an elongated, spindle-shaped form on the plastic culture surface. By conventional light microscope, the morphological phenotype, e.g. of chondrocytes, osteoblasts, adipocytes and skeletal myocytes, is very difficult, if not impossible, to distinguish. Furthermore, depending on the stage of individual cells (attaching, mo-

Abstract · Zusammenfassung

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Abstract
The new era of regenerative medicine has led to rapid development of new innovative therapies especially for diseases and tissue/organ defects for which traditional therapies and medicinal products have not provided satisfactory outcome. Although the clinical use and developments of cell-based medicinal products (CBMPs) could be witnessed already for a decade, robust scientific and regulatory provisions for these products have only recently been enacted. The new Regulation for Advanced Therapies (EC) 1394/2007 together with the revised Annex I, Part IV of Directive 2001/83/EC provides the new legal framework for CBMPs. The wide variety of cell-based products and the foreseen limitations (small sample sizes, short shelf life) vs. particular risks (microbiological purity, variability, immunogenicity, tumourigenicity) associated with CBMPs have called for a flexible, case-by-case regulatory approach for these products. Consequently, a risk-based approach has been developed to allow definition of the amount of scientific data needed for a Marketing Authorisation Application (MAA) of each CBMP. The article provides further insight into the initial risk evaluation, as well as to the quality, non-clinical, and clinical requirements of CBMPs. Special somatic cell therapies designed for active immunotherapy are also addressed.

Keywords
ATMP · Regulatory · Cell-based therapy · Regenerative

Regulatorische Anforderungen an die klinische Prüfung und Marktzulassung von zellbasierten Arzneimitteln

Zusammenfassung
Eine neue Ära der regenerativen Medizin führte zur schnellen Entwicklung von neuen, innovativen Therapien insbesondere für solche Krankheiten und Gewebe- beziehungsweise Organschäden, für die die traditionellen Therapiekonzepte und Arzneimittel keine befriedigenden Ergebnisse liefern können. Obwohl die Entwicklung und der klinische Einsatz von zellbasierten Arzneimitteln (cell-based medicinal products, CBMP) bereits seit einer Dekade bekannt sind, ließen stabile wissenschaftliche und regulatorische Vorgaben bis heute auf sich warten. Die Verordnung (EG) 1394/2007 über Arzneimittel für Neuartige Therapien bildet nun zusammen mit dem überarbeiteten Annex I, Part IV der Richtlinie 2001/83/EG einen neuen rechtlichen Rahmen für CBMP. Ihre große Heterogenität sowie die offensichtlichen Begrenzungen (wie kleine Stückzahlen und kurze Haltbarkeit) auf der einen und spezifische Risiken der CBMP (wie mikrobiologische Reinheit, hohe Variabilität, Immunogenität, Tumorrisiko) auf der anderen Seite erfordern für diese Produktgruppe einen flexiblen, den Einzelfall bewertenden regulatorischen Ansatz. Deshalb wurde ein Ansatz entwickelt, mit dem der Umfang der wissenschaftlichen Daten für den Zulassungsantrag für jedes CBMP im Einzelfall festgelegt werden kann, basierend auf der Einschätzung des Risikos, das vom Arzneimittel ausgeht (Risiko-basierter Ansatz, risk-based approach). Dieser Artikel vermittelt einen vertiefenden Einblick sowohl in die initiale Risikobewertung von CBMP als auch in die Anforderungen an Qualität, nichtklinische und klinische Daten der CBMP. Auch somatische Zelltherapeutika für die aktive Immuntherapie werden in die Betrachtungen eingeschlossen.

Schlüsselwörter
ATMP · Regularien · Zelltherapie · Regenerativ

ving, detaching), the morphology of the cells may vary considerably and by microscopic evaluation it is very difficult to identify putative cellular impurities during the in vitro cell culture. Therefore, microscopic analysis of the cells alone is not sufficient as an identity test. Methods used for evaluation of cell purity should also be carefully chosen and the need for a homogeneous or heterogeneous cell population for the intended indication should be defined as part of the product characterisation and development. Where a homogeneous cell population is required for the intended indication, other cells are considered as impurities and their amount in the final product needs to be controlled. When a product is based on allogeneic cells, evaluation of histocompatibility markers might be necessary. The strategy to evaluate the amount of impurities (cellular and process-related) and their removal during the manufacturing process can be addressed in the risk analysis. Only clinically significant impurities should be tested and/or their removal demonstrated through validation.

Potency testing is one of the key parameters of a cell-based product and, when properly designed, most valuable tool to control functionality/biological activity of the cells throughout the lifetime of the product. The potency test should be based on the intended biological effect of the product and its main objective is to identify clinically significant changes in the product. The test methods may comprise in vitro or in vivo assays or assays based on surrogate markers (e.g. protein expression profiles, flow cytometric immunoassays). In some cases, there may be a need to establish multiple assays to cover both the needs of characterisation and process validation and, on the other hand, the limitations related to batch release testing. As the cell-based assays may exhibit quite wide variability inherent to the material itself, the assays should be kept as basic as possible and combination of evaluation for both potency and cell purity in the same assay should be avoided.

Tumourigenicity testing is required in cases of an increased risk of cell transformation. Whenever stem cells are used or the manufacturing process predisposes the cells to karyotypic changes, genetic stability of the cells should be evaluated. Guidance related to the testing strategy can be found in ICH Q5D [11].

Biocompatibility testing is required for combination products and it should confirm that the system maintains the desired cell differentiation, functionality and genotype during production. Furthermore, when the non-cellular components are based on biodegradable materials, testing for harmful/toxic components may be necessary to ensure proper growth environment for the cells. Important characteristics of structural components (e.g. topography, surface chemistry, strength) should be evaluated through the quality control of the component itself. Whenever this data is evaluated by a Notified Body, the results of the assessment should be included in the MAA.

The manufacturing process should be carefully designed and validated to ensure consistent production. For CBMPs, comparability testing will be very challenging and knowing that even small changes in the culture conditions may result in dramatic changes in cell phenotype (and perhaps, also in genotype), the need for scale-up activities should be considered already at the beginning of the development.

Non-clinical requirements for CBMPs

General aspects

The objectives of the non-clinical studies are to demonstrate the proof of principle for the medicinal product and to define the pharmacological and toxicological effects that are predictive of the responses in humans. Further objectives include the establishment of safe doses for subsequent clinical studies and to support the route of administration of the cell-based medicinal product. Moreover, the non-clinical studies should also identify target organs for toxicity and should allow the definition of parameters to be monitored in the patients. Non-clinical studies should be performed in relevant animal models, meaning that the animals should allow the human response to the CBMP to be predicted. Therefore, the animal model should display similar characteristics as humans in terms of cell biology, anatomy, biomechanics and pathophysiology. The potential and limitations of the animal model(s) should be identified and addressed as part of the risk evaluation.

Such relevant models may comprise knock-in or knock-out animals or even homologous models. In a homologous model, cells or tissues from the respective animal (instead of the original human product) are harvested, isolated, manipulated and manufactured similar to the clinical product. While a homologous model mimics the environment in the patient to the maximally possible extent, it also comprises several uncertainties: the respective cells in the model animal and/or their components such as cell-surface receptors and cell effector proteins, may be less well characterised than their human homologues. The homologous cell preparations may have different physiological functions or may be regulated differently in the animal. In addition, a similar manufacturing process may lead to different impurities (depending on the starting material), which may lead to a pharmacological and toxicological profile that is different from the human preparation. However, for certain preclinical studies, such as the biodistribution of cells, the homologous model might provide more valuable information than xenogeneic models, e.g. human cells in immune-suppressed or immunodeficient animals.

The criteria for choosing a particular animal model should be scientifically justified. Although the number of animals may vary depending on different factors such as the disease model, the test species, delivery system, the total number of tested animals per study group has to be sufficient to ensure a statistical and biologically significant interpretation of the results. It is also important to use animals of both genders where possible and to provide adequate positive and negative controls. For the latter, sham treatment or vehicle might be used and the rationale for each functional test needs to be provided. In cases where relevant animal models cannot be developed, in vitro studies may replace the animal studies, but the underpinning rationale to use the in vitro studies also need to be justified. It is also important to lay out the ratio-

nale for the chosen time points, frequency and the overall duration of the monitoring to detect possible adverse effects. Depending on the intended duration of the treatment with a given product, the studies may take longer than would be required for classical medicinal products, up to several months or in small animal models even for the rest of the life of the animals, because the cells may persist in the animal for longer times or it may induce long-term effects.

It is also necessary to provide safety, suitability and biocompatibility data for any additional substances that are administered together or as part of the cell-based medicinal product, such as cellular products, biomolecules, biomaterials, and/or chemical substances. Especially for scaffolds, the physical, mechanical, biological and chemical properties should be considered. The selection of materials should be justified based on biocompatibility. Scaffolds may reside permanently in the host or they might be desorbed; they may be two-dimensional or three-dimensional. All these aspects influence their interactions with the cells and should be addressed.

Pharmacology

The primary pharmacological studies for the cell-based medicinal product should be adequate to demonstrate proof-of-principle in a relevant model of disease or injury, as described above. The markers of biological activity need to be reasonably justified. The interaction of the applied cells with non-cellular component(s) of the product and the interaction/integration of the product with surrounding tissue should be addressed using imaging techniques such as MRI.

Animal studies should also be used to determine the effective dose, i.e. the amount of the cell-based medicinal product that is needed to achieve the desired effect, and where appropriate, the frequency of dosing should be determined as well. The secondary pharmacology studies should be performed to identify potential undesirable physiological effects that are not related to the desired therapeutic effect of the product, e.g. migration of stem cells to unintended locations followed by proliferation or the unintended differentiation of cells.

Pharmacokinetics

Conventional pharmacokinetic studies are usually not relevant for cell-based medicinal products, because absorption, metabolism and excretion are not taking place, unless the mode of action of a CBMP is based on biomolecules expressed by the cells (e.g. encapsulated cells). Instead, investigators should focus on parameters like viability, (bio-)distribution, growth, migration and differentiation of the cells over time to evaluate the risk of the CBMP based on the interaction of the body with the cells.

Toxicology

As outlined in the Guideline on Human Cell-Based Medicinal Products [5], the need for toxicological studies depends on the specific cell-based medicinal product. Conventional study designs may not be appropriate, but scientific justification should be provided for the models used as well as for the omission of studies. Toxicity of CBMPs may arise from unknown cellular alterations that take place during the manufacturing process such as altered secretion of chemokines or unintended differentiation of the cells. Immune toxicity arising e.g. from allogenic use of the product or the interaction with components that were used during the manufacturing or that are part of structural components might be an issue. Therefore, the toxicology studies should be performed with the finished cell-based medicinal product in order to include any of these potential hazards.

For some of the toxicology studies, the use of homologous models may be reasonable.

Single and repeated dose toxicity studies may be necessary depending on the intended clinical use of the CBMP. In these studies the application route and the dosing regime should reflect the intended clinical use.

Conventional carcinogenicity and genotoxicity studies are usually not required for CBMPs products. However, the CBMP may have an intrinsic tumourigenic potential due to their source (e.g. stem cells), or they might become tumourigenic upon ex vivo manipulation. In cases where it can scientifically be justified, tumourigenicity studies might be omitted.

Clinical requirements for CBMPs

In general, the whole clinical development of a CBMP should follow the same regulatory requirements as established for other medicinal products (Dir.2001/83/EC) [4] and the existing general and specific guidelines available for the conditions to be treated. The clinical development plan should be clearly focused on effective and safe use of the CBMP in the target condition and population.

The initial risk evaluation should be used to design the entire clinical development plan. Part of the risks can be mitigated during quality and non-clinical development; however, certain risks remain to be alleviated during the clinical development, both pre- and post-marketing. Similar to the development of classical pharmaceuticals, the main clinical phases of exploratory and confirmatory clinical studies are needed to substantiate the MAA for CBMPs. In general, the clinical development plan should include pharmacodynamic studies, pharmacokinetic (PK) studies (if applicable), mechanism of action studies, dose finding studies and randomised pivotal clinical trials.

External experience with similar products may provide valuable information for these products. This experience may provide supportive evidence for a MAA or even earlier – during the clinical development phase for exploratory purposes. However, special attention should be paid on interpretability of the data available from previous experience using particular CBMPs. There may be major drawbacks not taken into account, e.g. underestimation of impact of manufacturing changes, including scale-up, taken place during clinical development or overestimation of observational and/or non-randomised study results.

During the exploratory phase of clinical studies, special attention should be paid to pharmacodynamic and pharmacokinetic studies (by monitoring of viability, proliferation/differentiation, body

distribution/migration and functionality during the intended viability of the CBMP). Pharmacokinetic features from relevant animal models could replace part of necessary clinical PK studies. One of critical features in exploratory studies is the definition of the dose. Challenges in using classical dose finding strategies by choosing dose for confirmatory study from several tested ones raise the need to elaborate alternative reasonable approaches to at least define minimally effective dose.

Efficacy should be established using clinically meaningful primary endpoints, supported by secondary endpoints, and both structural and functional measurements should be considered. It is expected that classical approaches of confirmatory study designs are used and all deviations are justified. As with other medicinal products, a prospective, randomized concurrently controlled pivotal study is expected, unless otherwise justified. Any foreseen omission should be addressed before study design as well as unresolved issues in the MAA by proper assessment of the impact of these gaps on final data and their interpretability. In case a single pivotal study approach is used, it should comply with the requirements of the Points to Consider on Applications with One Pivotal Study [12]. In such a case, the strength of evidence should be compelling considering the success to provide evidence of internal consistency, in terms of important endpoints and subpopulations, and degree of statistical significance observed. The margin selected for the assessment of delta (e.g. for noninferiority, in %) should be appropriate to exclude all differences of clinical significance. More than one pivotal clinical study should be considered, if (1) the mechanism of action is unknown, (2) there is a new pharmacological principle, (3) phase I and II data are too limited or (4) there is a new therapeutic area with a history of failed studies.

In the field of regenerative medicine, proper comparator treatments/products are not always available, which is one of the main clinical challenges. However, a proper reference product or technology (e.g. surgical, sham control) suitable as an established therapeutic alternative, as well as suitable statistical analysis plans should be used, whenever possible. In case an open-label approach is unavoidable, the best attempts to mitigate biases should be discussed prospectively and appropriate measures be implemented, such as thorough stratification, employment of an independent and blinded endpoint adjudication committee, using of properly validated/reasonable protocols or decision tree algorithms, or representative and comprehensive sensitivity analyses based on updated confounding factors.

As it is expected that CBMPs do have long-lasting effects, the risks of late events, both from an efficacy and safety point of view, should be addressed during clinical development and in a risk management plan. The risk minimisation might be sought through better standardisation and optimisation of surgical, physical, and/or rehabilitation procedures, such as employing technical protocols, reflecting pre/intra/post surgical interventions, training of investigators and setting up the SOPs with minimal requirements for practitioners. Another set of risks emerges from long-term use of CBMPs. Consideration should be given to the possible presence of a Plato effect for efficacy (no increase in efficacy despite further time flow or dosage increases) and/or unsafe features (e.g. in case of signs of local hypertrophy), as well to the loss of the beneficial effect due to natural aging and lifestyle, to the monitoring programme for late adverse events such as malignancies.

Cell-based immunotherapy

There are several immunotherapeutic approaches aiming to mobilize the immune system for therapeutic benefit. Immunotherapy products that have successfully been developed and licensed during recent years comprise chemical or biological products, such as tyrosine kinase inhibitors or monoclonal antibodies (mAb), respectively. While some cell-based immunotherapy products are already in advanced clinical development [13], none has been licensed so far in the EU.

While therapies utilising e.g. mAb are used for passive immunization, cell-based products that can be used for active immunization are available. This is obviously important where a durable therapeutic effect is desired, e.g. for the treatment of cancer. During recent years it has been shown that active therapeutic immunization utilizing cancer-associated self antigens is feasible [14]. Moreover, other non-infectious diseases such as Alzheimer disease, nicotine abuse, and increased blood pressure have also been treated in clinical trials via active immunization [15, 16, 17]. Even established infectious diseases such as HIV infection are currently being treated using active therapeutic immunization regimens [18].

Dendritic cells (DC) are central for the induction of cellular and humoral immunity by (i) taking up antigens, (ii) migration to draining lymph nodes, and (iii) presentation of antigenic peptides in an HLA-restricted manner to T cells [19], thereby priming an immune response. After it was possible to generate antigen-loaded DC ex vivo, several clinical trials have been initiated. Since T cells are especially important to fight cancer, adoptive transfer of ex vivo generated tumor-specific T cells to cancer patients constitutes another promising cell-based therapeutic approach.

Guidelines dealing with cell-based immunotherapies have been published by EMEA with respect to potency assessment [20]. The legal basis for the latter guidance is Directive 2001/83/EC [4], since antigen-loaded DC and adoptively transferred T cells are classified as somatic cells. Further general guidance is not available for cancer immunotherapeutic products (cancer vaccines) due to the wide variability of the products (peptide-based, gene therapy products and cell therapy products). The current definition of vaccines provided in EU Directive 2001/83 applies to infectious agents only. The same is true for the vaccine definition given in the European Pharmacopoeia. Especially from the public point of view the term "vaccine" might be misleading in that cancer vaccines could be envisaged as a means of cancer prophylaxis. Prophylactic cancer vaccines, however, will presumably only be successful in cases of virus-induced cancers such as cervical cancers mediated by Papilloma virus infection.

Taken together, cell-based immunotherapeutic products (e.g. dendritic cells) and other cell-based medicines like antigen-specific T cells are, amongst others,

immunotherapy products that will have an important role in the treatment of diseases for which currently an urgent medical need exists.

Disclaimer

The views expressed in this article are personal views of the authors and may not be understood or quoted as being made on behalf of the Committee for Advanced Therapies (CAT) or Committee for Human Medicinal Products (CHMP) or reflecting the position of the CAT/CHMP. However, the regulatory requirements described in the article are based on the Regulation 1394/2007/EC, on technical requirements laid down in the revised Annex I, Part IV of Directive 2001/83/EC and on the EMEA/CHMP guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006).

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