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Reflection paper on design modifications of gene therapy medicinal products during development

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Reflection paper on modifications of gene therapy medicinal product design during its development

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1. Introduction (Background)

The concept of using protein encoding genes or nucleic acid sequences as medicinal products for the treatment of human diseases covers different product types (e.g. plasmid DNA, non replicative RNA, recombinant viral vectors and genetically modified cells). As for any medicinal product, modifications to the product design are often introduced during development. It is desirable, from a consistency point of view, to have minimal changes to the product during its development; however this might not always be feasible for the developers, especially when more knowledge of the product features has been gained during development. Consequently, in order to maximise the efficacy/safety profile of a gene therapy medicinal product (GTMP), changes to its design in order to obtain new improved product characteristics could be required during its development. Examples of such modifications include a change of promoter, introduction of tissue specific enhancers, addition of insulators including Locus Control Region elements, or other changes in the genetic construct that will change the characteristics of the GTMP. The consequences of these changes in terms of regulatory compliance are somewhat complicated. While improvement of a product profile during its development is welcomed, it can be difficult to fully predict the impact of the modifications on the safety/efficacy profile established with the previous product. A timely evaluation of the impact that such changes might have on the safety and efficacy profile of the product is beneficial for the developers as it may reduce the number of additional studies that might be requested to address this concern at the time of a new clinical trial or marketing authorisation application.

Where on a case by case basis it is established that a modified product shares many properties with the previous one, for example pharmacology of the encoded gene product(s) and tissue tropism of the vector, the evaluation of the specific alteration in the modified product can be accomplished in the light of data from the previous product, complemented with new studies as implicated from the specific change (e.g. pharmacological toxicity caused by increased expression of the therapeutic gene).

In addition to modifications to the product design changes can be made to the manufacturing process. For biologicals such changes typically impose a comparability exercise (ICH Topic Q5E). In this respect the requirement for demonstrating comparability of GTMPs does not differ from other biologicals.

As such, this paper focuses only on changes made to the design of the product with a purpose to improve the efficacy and/or safety of the product.

Presented here is a collection of regulatory considerations given for specific GTMPs where the characteristics have been changed at various stages during clinical development. The intention of this paper is to give some insight into the types of studies that are likely to be required in an application dossier to support the modification in the product design introduced during development.

Note: This reflection paper has been developed to communicate the current status of scientific discussions within EMA committees/working parties. Reflection papers are not guidelines. They provide a framework for discussion or clarification particularly in areas where scientific knowledge is fast evolving or experience is limited, such as in the area of gene therapy medicinal products.

2. Discussion

Change of splice donor/acceptor sequences

In a given vector, during clinical development, the therapeutic gene is found to contain a cryptic splice site which, if used, could render the product inactive. Prior to phase III studies a silent mutation is

introduced in the coding sequence of the therapeutic gene in order to 'inactivate' the cryptic splice site, ensuring only active protein is translated.

In a situation such as this the characteristics of the active substance would be considered to be changed and comparability aspects of the vector, pre and post- change, would need to be considered prior to phase III trials.

If the vector in question was a retroviral vector required for ex-vivo transduction of a target cell population, that was manufactured using a stably transfected packaging cell line, the generation of a new packaging cell line would be required. In this case the comparability evaluation would need to address the quality of the virus supernatants and the transduction effectiveness of the vectors, as well as the quality/comparability of the transduced ex vivo cells pre- and post-product change, to ensure the ex vivo cells re-infused to a patient would not adversely impact safety or efficacy between the Phase I/II and Phase III trials. Comparable data from in-process control and release testing (virus harvest, drug substance and drug product) would also be needed. In addition, to address the comparability of the final product data on immunophenotyping, specific sequencing, viability, endotoxin, vector integrity, copy number, RCR, transgene expression, level and product stability, adventitious viruses, mycoplasma, specific spliced forms, and sterility would be required.

Although the product characteristics are changed it is considered likely that the comparison conducted on the quality level will allow concluding that the change does not impact on the safety and efficacy profile of the vector. Thus there is possibly no requirement for additional non clinical studies in order to bridge the safety package obtained using the original vector with that of the 'modified' vector.

The principle outline here could be extended to the alternative retroviral manufacturing strategy using transient transfection.

E4 substitution in chimeric non-human adenoviral vectors

Adenovirus vectors that are commonly used in gene therapy are mostly based on human adenoviruses. Neutralizing antibodies against human adenoviruses are generally highly present in human populations due to regular adenovirus infections. Consequently, effectiveness of gene therapy vectors based on human adenoviruses may be hampered. To overcome this problem, vectors based on adenoviruses that rarely infect the human population have been developed, against which a pre-existing humoral immunity (neutralizing antibodies) is likely to be absent. In this context, adenoviruses from non-human sources are interesting candidates, as it is believed that the absence of pre-existing immunity against these vectors will improve their efficacy and safety profile compared to human adenoviral vectors. However, efforts to develop a diverse repertoire of serologically distinct adenovirus vectors may be hindered by the necessity to generate complementary cell lines capable of supporting the propagation of vectors to sufficiently high titres. To solve this, chimeric adenoviral vectors can be developed.

In order to propagate E1 deleted non-human adenoviral vectors, human packaging cell lines that constitutively express human Ad5 E1 proteins can be used (Fallaux et al, 1998). It has been suggested that productivity of non-human adenoviral vectors can be further improved by substitution of E4 ORFs 3 and 4 with the human E4 variants.

A situation may arise where during clinical development a vector containing non-human E4 ORF, is modified by substituting the human E4 ORF to improve vector yields. In this case an extensive study to compare the two constructs would be required. The two adenoviral vectors lots would be evaluated by

assays designed to demonstrate their comparability in terms of identity, purity, potency in cell culture and in vivo potency. However, such studies on quality attributes are unlikely to address the effects of the structural changes on i) the immunogenicity profile of the adenoviral vector itself, and ii) the transgene expression cascade and the consequential immunological events. Therefore to avoid repetition of clinical trials with the newly constructed vector, non-clinical data, bridging the old and new vector, is considered crucial.

Non-clinical bridging studies would comprise safety and efficacy of the drug product, and would characterise the in vivo immunological potency of the modified vector relative to the original. Furthermore, non-clinical studies including pharmacokinetics, distribution and repeat dose toxicology may be needed.

It should also be noted that although the vector is replication defective, in vivo replication might occur in the presence of complementing wild type adenoviruses. Complementing wild type adenoviruses might be present in patients but not in animals used in non-clinical studies. Therefore risk of complementation events will most likely not be addressed in non-clinical studies but should be taken into consideration for the forthcoming clinical trial situation. Furthermore, the nature, localisation and expression levels of adenoviral receptors differ between patients and commonly used non-clinical animal models. These host related aspects might influence the outcome of pharmacokinetic and distribution data. Therefore a conservative approach has to be taken when assessing the predictive value of those non-clinical bridging results, and the need to repeat clinical trials with the "humanized vector" will depend on the predictability of the chosen animal model when compared to the clinical situation.

Change of selection marker in plasmid vector

Special attention is given to the use of antibiotic resistant selection markers incorporated into the backbone of naked plasmid DNA vectors, as these may adversely impact on other clinical therapies in the targeted population. When feasible, the use of such markers should be avoided (EMEA Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products CPMP/BWP/3088/99, European Pharmacopoeia (Ph. Eur., Monograph 5.14). However, if an antibiotic resistance gene is required, this gene should preferably carry resistance to an antibiotic that is not used in current clinical practice. Due to this regulatory requirement, it is possible that an ampicillin resistance selection marker, initially used in the design and construct of the plasmid vector, may have to be replaced prior to the first use in man.

This design modification would result in a change in the molecular characteristics of the medicinal product. Concerning the intrinsic characteristics of the target medicinal product, if the expression levels in human cells are comparable before and after the change in the selection marker, most of the animal studies that have been carried out using the plasmid DNA carrying the previous selection gene, may not have to be repeated (e.g. pharmacodynamic studies). However, the newly introduced marker gene may influence the capability of the DNA to chromosomally integrate. Therefore integration studies may have to be considered unless a lack of integration can be supported by other information such as sequence analysis.

Promoter exchange in genetically modified encapsulated cells

The encapsulation of genetically modified cells using a semi-permeable membrane provides a mechanism of insulating cells from the receiving environment, thereby avoiding the risk of immune

rejection and/or migration of the cells. The therapeutic protein is secreted from the genetically modified cells and can pass through the encapsulation membrane to affect the target tissue.

In order to increase expression of the therapeutic protein, the transgene promoter and/or enhancer can be changed during product development. Additionally, the capsule matrix can be also changed to improve cell viability and longevity. Since the active substance consists of a cell clone stably transfected with the expression cassette encoding the therapeutic protein of interest, a new master cell bank will also be generated.

In order to demonstrate that the secretion level of the therapeutic protein is increased while otherwise the product characteristics are unaltered, a full toxicology programme will be required.

Considering the increased survival of the cells in the new capsule matrix, the potential increased period of exposure of the target tissue to the secreted protein compared with the previous product would also be addressed by the toxicology testing.

Since differences in the impurity profile between the two master cell banks can be anticipated but are difficult to evaluate by physico-chemical and/or biological means, this issue may need to be addressed in the toxicology studies.

The fact that cells are encapsulated would obviate any need for new biodistribution studies. Nevertheless, the integrity of the encapsulation matrix (including capacity for cell containment) and its compatibility would need to be demonstrated using a relevant cell line.

AVV vector serotype changes

So far, more than 60 clinical trials have been undertaken using AAV vectors, the majority of which have used the AAV2 serotype. However, other serotypes are currently under evaluation in preclinical studies and several have already entered clinical trial phase such as AAV1/SERCA2a for heart diseases (Periasamy and Kalyanasundaram, 2008), AAV6/CAP for cystic fibrosis (Chen, 2008), AAV2.5/Dystrophin for Duchenne muscular dystrophy (Wellstone Center for muscular dystrophy research – Projects: Project 3R, Jude Samulski,PhD ttp://cfx.research.unc.edu/wellstone/projects.cfm) and AAV2.8/FIX for haemophilia B (Cunningham et al. 2008).

Important issues related to AAV vectors include tissue specificity and immunogenicity of AAV capsids. All of the known AAV serotypes can infect cells from multiple diverse tissue types. This may present a significant challenge if the aim is to specifically target AAV vectors to desired tissues and compromise both the safety and efficacy of products. Immunogenicity of AAV capsids, in particular AAV2, has been another major issue in developing medicinal products using AAV. More and more studies indicate that different AAV serotypes have preferential tissue-tropisms, for example, AAV8 has an increased tropism to the liver, AAV1, 6 and 7 are more susceptible to skeletal muscles and a hybrid of AAV1 and AAV2 has shown lower immunogenicity than AAV2. Therefore, it is plausible for the serotypes of some products to be changed during development to improve tissue or cell targeting and hence improve the safety and/or efficacy profile. Indeed, a number of attempts have been made so far, for example, replacing AAV2/FIX with AAV2.8/FIX for haemophilia B and replacing AAV2/CAP with AAV6/CAP for cystic fibrosis or AAV2.5 for muscular dystrophy gene therapy (for more information on clinical gene therapy trials see http://www.wiley.co.uk/genmed/clinical/). Serotype changes may positively influence vector transduction efficiency while reducing the risk of any immune-associated pathology.

Potentially, a serotype change of vector capsids may result in partial changes of product genetics and quality attributes, considerable changes in the production process, significant changes (ideally improved) in product toxicity due to tissue tropism/biodistribution/immunogenicity differences, and

some changes (increased) in pharmacology/potency. As a result, a full package of quality data will be required, including also the risk-assessment of RCV generation and a suitable assay for RCV detection of the changed serotype. A complete non-clinical evaluation of the new serotype vector, in terms of biodistribution and immunological responses (if applicable) would be needed. Based on the results from biodistribution studies, toxicological studies could focus on product immunogenicity, immunotoxicity and a reduced pharmacological data package together with bridging data may be considered sufficient.

RV to RV-SIN in monogenic inherited diseases

Gene therapy of inherited monogenic disease aims at adding a functional allele of the non-functional gene to human somatic cells which are relevant for disease development or address the most serious disease effects. To allow a stable, possibly life-long correction of the genetic defect in dividing cells, retroviral vectors that integrate the therapeutic gene into the patient's chromosomes are being used. Several examples of such applications have concerned gene transfer into hematopoietic stem cells. Most commonly, replication-incompetent vector particles derived from murine leukaemia virus (MLV) have been applied.

Reported results with MLV-derived vectors for severe immunodeficiencies have shown sustained efficacy (Fisher et al., 2010). However, some patients developed post treatment lymphoproliferative disorders, which were regarded as unexpected serious adverse reactions induced by the gene therapy medicinal product (Marshall, 2003). In detailed analyses the MLV transfer vector was found integrated close to or within known proto-oncogenes (Hacein-Bey-Abina et al, 2003), which resulted in genetic modification and led to malignant transformation and expansion of these genetically abnormal cells. It was suspected that the strong promoter/enhancer within the 5´ Long Terminal Repeat (LTR) of the MLV vector induced deregulation of the neighbouring genes, resulting in over-expression of proto-oncogenes as a consequence of MLV vector insertion.

One way to minimise the insertional mutagenesis effect is to change the MLV vector design by deleting the strong transcriptional promoter/enhancer, resulting in self-inactivating LTRs. The viral promoter/enhancer is functionally replaced by a weaker internal promoter/enhancer to mediate transcription of the therapeutic gene.

If those modifications are introduced in the GTMP during clinical development, several points would be considered:

- Comparability of in vitro transduction of human haematopoietic cells using the re-designed vector e.g. a MLV-SIN vector and the expressed therapeutic protein levels following the transduction, as the re-designed vector will most likely result in a different transcriptional activity.
- The hypothesis that the SIN vector would be less prone to induce insertional onocogenesis will have to be tested experimentally. Assessing insertional mutagenesis only by means of in vitro models might not address adequately the real safety risk, in terms of its insertional oncogenesis. Although no animal model has currently been validated to predict the risk of insertional oncogenesis, tumour-prone animal models have been shown to be sensitive to insertional oncogenesis. They are therefore useful for comparison of the risk for insertional oncogenesis between an MLV and an MLV-SIN vector containing an identical transgene. With more data indicating a reduced probability of insertional oncogenesis, platform studies comparing the probability for insertional oncogenesis of different vector design may be sufficient to support the use of a retroviral vector re-designed to a SIN vector.

- Non-clinical testing in a suitable animal model and/or a relevant in vitro model to prove that the intended mechanism and disease correction have not been changed as a result of the re-designed MLV-SIN vector.
- Preferably the gene dose (i.e. vector copies per cell) used to transduce cells in non-clinical studies may need to exceed the anticipated clinical gene dose in order to evaluate the safety risk of a vector in the worst possible situation.

Finally, it is desirable to demonstrate that the characteristics and quality attributes of the re-designed RV product are comparable to the parental RV product in terms of vector titre, vector impurity profile, transduction efficiency and copy number per cell and potency of the redesigned RV particles in the transduced cells, in agreement with the general principles laid down in ICH Q5E.

3. Conclusion

As presented above, significant modifications in the product design can be, and have been made during product development of gene therapy medicinal products. Pertinent regulatory issues identified include the stage of development at which bridging studies should be envisaged, the extent of additional non-clinical studies required in conjunction with the introduced change, the relevance of non-clinical models used and the need for additional non-clinical data to support bridging of the new product design before further clinical trials. For some specific situations bridging with previous clinical studies may be obtained based solely on characterisation studies. This latter outcome is dependent of availability of appropriately developed analytical methods to test the expected effect of the modification made to the product design.

Altogether, these cases demonstrate that for each modification to the product design the influence on the already established efficacy and/or safety profile of the product and the potential risk for the patient should be evaluated. However, in all cases, the developers are strongly advised to seek product-specific scientific advice from the regulatory authorities:
http://www.ema.europa.eu/htms/human/sciadvice/Scientific.htm

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