CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

WEBINAR TRANSCRIPT

Regulatory FAQs and common concerns for cell and gene therapy raw and starting materials

Kasey Kime, Jerrod Denham & Christopher Bravery

In cell and gene therapy, materials matter. However, misconceptions abound, exacerbating a lack of harmonization and standardization in key areas. For example, uncertainty around quality grades at the various stages of R&D is commonplace, and everyday terms are frequently misinterpreted or misunderstood, with potentially damaging ramifications for advanced therapy development, manufacturing and commercialization. Here, we aim to debunk some popular myths, provide practical guidance based upon long experience in the field, and clarify key regulatory considerations and requirements across the cell and gene therapy raw and starting materials area.

Cell & Gene Therapy Insights 2020; 6(2), 397–406

DOI: 10.18609/cgti.2020.049

BASIC DEFINITIONS

Beginning with definitions of some important common terms that are used for regulatory submissions internationally, the following all come from ICH guidelines:

- Raw materials are described as components or reagents used during the manufacture of a therapeutic product;
- Source or starting materials are raw materials, intermediates, or active substances that are incorporated as a significant structural fragment into the structure of the Active Pharmaceutical Ingredient (API);
- An excipient is an ingredient added intentionally to the drug substance which

should not have pharmacological properties in the quantity used. In other words, an excipient is everything that is used in the final formulation of the therapeutic product except for the active substance and the labelled container closure.

A review article published last year in the *New England Journal of Medicine* provides a high-level example of how this terminology is used in practice [1]. It concerns an *ex vivo* gene therapy product and its manufacture. On day 0, cells are harvested from mobilized peripheral blood using apheresis. $CD34^+$ hematopoietic stem cells are isolated from the apheresis unit using an antibody conjugated to a dextran-coated iron bead and an instrument that comes equipped with a magnet. The isolated cells are cultured overnight in growth medium, supplemented with cytokines, and after overnight culture the cells are transduced with the viral vector that inserts the gene into the DNA of the cells. Following this, the cells are again cultured overnight. On the last day of processing, the transduced cells are harvested, washed, formulated, filled, finished, and cryopreserved.

In this example, the source materials for this product are the apheresis unit and the viral vector to produce the final product. Examples of raw materials are the cell culture medium, cytokine supplements, or even the transduction reagent used. Excipients are the reagents used to formulate the final therapeutic product prior to filling into the final container closure. In this case, this is a bag that is then cryopreserved as the final drug product before being thawed and administered to the patient.

TROUBLESHOOTING MISUSE OF TERMINOLOGY AND ITS REPERCUSSIONS

Firstly, given the differences in basic terms utilized in different regulatory jurisdictions ('ancillary materials' according to the USP, versus 'raw materials' elsewhere, for instance), and the regularity with which internal company-specific terms and acronyms routinely make their way into dossiers for regulatory submission, it is recommended that ICH terminology should be used wherever possible. Broadly speaking, if a cell or gene therapy developer uses the language of the regulators as much as they can, it will facilitate assessment.

A number of specific terms are commonly applied to raw materials inaccurately. One of the chief offenders from the regulatory point of view is "GMP grade". In fact, GMP isn't a grade, it's a quality system (or more accurately, part of a quality system – Good Manufacturing Practices). Suggesting that GMP is a grade is an oxymoron, because a grade is a set of test methods and acceptance criteria that fully characterize the material, i.e. a specification.

There are neither general nor legal requirements in either the EU or the US for raw materials to be manufactured to GMP. The most that can be expected is that they are manufactured to the principles of GMP, because no regulatory agency has the legal remit to inspect a raw materials manufacturer. They may inspect a manufacturer for other reasons – because they are producing licensed materials on the same premises, for instance – but they won't look specifically at the details for other materials manufactured.

It is up to the individual cell or gene therapy developer as to whether they choose to take a risk-based approach to this issue. However, there are a number of reasons why one might want to have a quality system in place for these types of products in particular. For example, there may be a greater need to ensure the traceability of materials that come into contact with the cells or viral particles. Additionally, one may also want assurances regarding material quality. In this instance, GMP does not necessarily need to be the quality system in question. A preferable approach is to consider the nature of the given material and its use, and then consider what level of quality system is adequate for it. This is the typical approach taken by pharma regarding excipients, which are arguably of far

DOI: 10.18609/cgti.2020.049

greater concern because they are administered to humans.

The origin of the raw material has an impact on safety, but its complexity effects how quality is actually defined. The more complex a material is, the greater the need for a robust quality system (i.e. to follow the principles of GMP).

One further example of a potentially misleading term applied to raw materials is "chemically-defined". Taken at face value, this may mean the material is purely a mixture of small molecule chemicals. However, some include highly purified and homogeneous recombinant proteins in this definition, whereas proteins purified from natural sources such as animals, humans, and plants are excluded due to their natural heterogeneity. This is confusing because all biological materials, including all proteins, are inherently heterogenous. Indeed, if one considers the number of potential post-translational modifications for a glycoprotein such as a monoclonal antibody, there is the theoretical possibility of up to 108 different forms of the same protein in a protein mixture. Of course, in practice, the heterogeneity would not be so high because the method of purification used should reduce it, as would other methods that we use during the preparation. Nonetheless, the point is made that both highly purified, homogeneous recombinant proteins and naturally-occurring proteins are isolated by a purification system of some sort, and so it is difficult to make the argument that one is more or less heterogenous than the other. It would very much depend on how each protein was prepared.

Therefore, it is highly questionable whether "chemically-defined" is a particularly useful definition. Equally, there is no particularly compelling reason to use recombinant serum albumin, for instance, over naturally isolated human serum albumin. (Recombinant human serum albumin may be slightly safer as long as suitable viral reduction elimination steps are included).

Actual chemical raw materials have the advantage of fully defined structure and quality. For example, there is a pharmacopeia monograph that fully defines the quality of dimethyl sulfoxide (DMSO). This greatly simplifies the market authorization dossier as it becomes a case of simply citing that the material complies with this monograph. In most case, the monograph will provide compendial test methods, meaning what might be considered the minimum requirement of an identity test is de facto validated. There may be no need to provide further information about the test method or its validation, nor to name the material supplier. It may also be possible to change one supplier for another (of a compendial grade of the given material) without the need to demonstrate comparability or seek regulatory approval. If a chemical molecule isn't covered by a monograph, one may still be able to follow the same approach, to a degree. However, the therapy developer will have to fully define the chemical grade themselves (including potentially developing the requisite test methods) and to justify the particular quality of material chosen.

In contrast, biologically sourced raw materials (or very highly complex chemical materials, such as polymers) will not have a full monograph. They cannot be fully-defined due to their natural heterogeneity, and because the quality (e.g. impurities) is dependent on the manufacturing process used for their preparation, which cannot be envisaged by any pharmacopeia. It is important to bear in mind that pharmacopeia general monographs for materials such as fetal bovine serum do not provide a complete specification. While it is certainly desirable for one's supplier to comply with the monograph, additional testing will be required, particularly to measure the biological activity of the given raw material. The developer may find themselves needing to develop and validate this test, and having to provide all the details for the dossier complete with a full (and fully-justified) specification that includes the tests that the supplier carries out. The developer may also need to assign a shelf-life for the material, which could involve undertaking additional stability studies.

The upshot is that because complex biological materials will vary depending on who manufactures them, one cannot simply substitute for another source without undertaking comparability studies. The extent of those data very much depends on where (in the process) and why the material is used. Additionally, it will be necessary over the course of the product development process to try to understand the critical material attributes (CMA) of any complex materials (not to be confused with critical quality attributes, which relate to the active substance). Batch-to-batch variability will need to be studied and managed, and in some cases, it may be necessary to work with the supplier to improve the material quality if it is insufficient.

Generally speaking, raw materials are not regulated products. Regulatory guidance suggest materials should be made according to GMP, but the type of quality management system is not specified further than an "appropriate quality management system". Therefore, it is important to consider the basis of a supplier's quality claim. A good first step towards understand a supplier's GMP claim is to request evidence of independent quality management systems certification, such as an ISO certificate. This will clarify what particular standard the supplier is certified to, and whether that aligns with the therapy developer's expectations of GMP for raw materials used in cell and gene therapy. To further guard against any misconceptions, it is also recommended to confirm the supplier's GMP claim – for example, by conducting an onsite audit of the supplier to make certain that true alignment of GMP levels or principles exists.

WHERE DO YOUR MATERIALS COME FROM AND WHY IS IT IMPORTANT TO KNOW?

Understanding where materials come from is vital for knowing the right questions to ask of a supplier.

There are numerous examples from the wider world of the unsuspected presence of materials of animal origin in everyday items – new plastic banknotes (and other plastic objects) containing animal fats, for instance. DMSO is a by-product of the paper industry. Various amino acids are isolated from sources such as hair, feathers, hides or skins, and even basal culture media is likely to contain amino acids. Plant extracts may seem harmless, but they may have been grown in locations where they are open to interaction with rodents or birds. Whilst there may well be nothing inherently risky about these materials, providing they are correctly prepared, it is nevertheless important to know where they come from in order to know which questions to ask.

One of the key questions relating to source is whether or not it is acceptable to use raw materials that may contain human/animal origin components. Firstly, it is important to note that there are several levels of animal origin, including at the product level, which means there might be animal materials present within the raw material, and at the production level, which means animal/human origin materials might have been used during the manufacturing process (but not intended to be present in the final raw material). In some cases, it may be necessary to go further back. While the general advice is to avoid human/animal origin components when possible, it is not always possible to do so. Therefore, a risk-based approach to the selection of raw materials is critically important. For one thing, viral reduction/elimination steps cannot be applied to cell and gene therapy products, making it absolutely essential to mitigate risk as far as possible and identify any human/animal-derived materials.

If a raw material composition or manufacturing process does utilize human/animal origin materials, items to consider include:

- Country of origin. (This is important to consider for ruminant-derived components due to transmissible spongiform encephalopathies risk in certain countries, as well as for some viral and parasitic disease risks related to human blood-derived materials);

DOI: 10.18609/cgti.2020.049

- Whether viral inactivation is feasible for the given material or process;
- Material grade (quality);
- Where in the process the material is being used (e.g. in upstream or downstream processing);
- Whether a lower risk option is a possibility (e.g. could a biologically derived protein be replaced by one from a recombinant source; and would that be preferable, e.g. viral risk, performance?);
- Available supplier traceability and testing documentation to help support risk assessment.

However, while it may seem a very straightforward decision to switch from a human/animal-derived to a human/animal-free material, there are a number of potential issues to consider.

For example, manufacturers of mesenchymal stromal cells (MSC) have been keen to move away from fetal bovine serum (FBS), and some have begun to consider human platelet lysate. From a viral safety perspective, they both pose a risk and arguably, FBS may actually pose a lower viral risk because the viruses are animal not human. Furthermore, in some jurisdictions such as the EU, it is basically mandatory to irradiate FBS, whereas many sources of human platelet lysate have no viral inaction steps. (FDA guidance and the USP also suggest that irradiating FBS is desirable, although recent experiences suggest that this is not enforced by the FDA to the same extent that it is in the EU – a point of regulatory disharmony which can lead to an unwanted requirement for unnecessary comparability work for some US developers). A further consideration for using human platelet lysate is that it is preferable to use pooled platelets rather than individual platelets to reduce batch to batch variability. However, this brings with it the question of how many platelets to pool together from how many donors? (The Paul-Ehrlich-Institut in Germany suggests it should be fewer than 16 [2], unless a viral reduction step is to be implemented, but even this number might be considered risky with market authorization in mind).

Pooled human AB serum provides an example of the importance of knowing about the preparation of a material. It is pooled from multiple donors and not usually subjected to viral reduction elimination steps in its manufacture, meaning it will also likely need to be irradiated, or similar. Furthermore, one must consider that the human serum may have been made from plasma, which necessitates use of an anticoagulant. There have been recent examples of (non-medicinal grade) heparin being used as the anticoagulant in this application – a material derived from pig gut.

The key lesson here, in addition to knowing a material's source, is to ensure any likely material changes are identified and made as early in development as possible.

RAW MATERIALS QUALIFICATION & CERTIFICATION

When it comes to the testing of raw materials, ultimately the user is responsible for the quality of the materials used in their process, but they need to work in cooperation with the supplier to achieve this. An end user may choose to accept the supplier's Certificate of Analysis (COA), if the raw material is fully characterized and the COA is sufficiently detailed. However, if the end user is qualifying a material intended for research use, they may need to perform additional quality control (QC) testing to determine suitability, and if the material is considered suitable, may also need to implement some routine testing.

For biological raw materials, sterility, residual host cell DNA, endotoxin, mycoplasma, and 9 CFR–compliant, species-specific adventitious agent testing may all be recommended.

We have already established the importance of traceability and regulatory documentation to support raw material risk assessment. Some common examples of key documents

for risk assessment include the COA, Certificate of Origin (CO), Material Safety Data Sheet (MSDS), Certificates of Compliance (if available), and whether or not the supplier provides access to regulatory support files or master files. Some suppliers may offer master files, which are confidential documents filed directly with the regulatory agency. However, not all regions will have the ability for suppliers to submit raw material master files, as master files are not available for the end user to review. In those situations, suppliers may offer regulatory support files, often under a Confidential Disclosure Agreement (CDA). Therefore, the regulatory support mechanism will depend on the level of propriety information and the region in which the supplier is operating.

A raw material supplier can significantly reduce the end user's qualification burden by designing highly characterized products, meeting the various pharmacopeia requirements, CMC guidelines, and ISO requirements as applicable.

RAW MATERIAL RISK ASSESSMENT FROM ANY EARLY STAGE

Acting early and decisively is key when evaluating raw material risk, with the ultimate goal being development of a material qualification program. The purpose of this type of program is to establish the source, identity, purity, biological safety, and overall suitability of a specific raw material. As part of this qualification program, a structured risk assessment strategy should be employed to evaluate overall safety risk of using the raw material in a given manufacturing process.

A failure modes and effect analysis (FMEA) approach considers severity, probability, and detection of failure related to a raw material. This allows for prioritization of what and when to mitigate. This is often useful, as one can evaluate risk pre- versus post-implementation of any mitigations that have been put in place, thus showing how overall risk will be reduced once these mitigations or hazard controls have been implemented

Regarding the identification of worst-case residual levels related to a certain raw material, it is often possible to gauge these early in product development by initially using worst-case estimations of process related residuals (process-related impurities) that are essentially calculated using simple wash-out numbers. However, the need to formally characterize through testing (depending on the nature of the raw material used) must be kept in mind, even for early-phase products. For example, a gene editing step may involve raw materials that could have a significant effect on the final product even at very low levels.

Depending on the raw material, mitigations might include the therapy developer's raw material process intermediates, or final drug product specifications and testing, as well as any material supplier information: for example, the supplier's production processes, their own specifications and testing, quality systems, or overall policies. Within the US, while some groups do still use USP-1043, which does have information related to materials for cell and gene therapy products, this is a general guide only (not a monograph, just guidance).

While many developers tend to focus on safety risks, it is important not to forget about business and supply chain risk. Changes to raw materials are generally easier to implement earlier in development, in part because the extent of evidence for comparability is lower earlier in development.

Last, but not least, it is necessary to communicate clearly and frequently with the raw material supplier from the earliest stages of developing of a material qualification program.

SOURCE OR STARTING MATERIAL TESTING

For source/starting materials, a general rule of thumb is that for straightforward cases

DOI: 10.18609/cgti.2020.049

- for example, an apheresis unit for autologous collection – it is simply a case of looking up the regulations. With respect to donor cells, two key regulations that describe the requirements are 21 CFR Part 1271 from the US FDA, and the European Tissues and Cells Directive (2004/23/EC). Both of these texts outline the key requirements for controlling donor cellular materials and include information such as procurement, donor eligibility, screening, processes, and other requirements that are necessary to control donor cellular material. However, for a source material such as an established human embryonic stem cell (hESC) line, which is used as the starting material for a product that is going to be used to treat many patients, it is often more complicated. For this type of more complex case, additional testing may be required that can often exceed what is considered standard for typical biologics cell substrates.

Turning to viral vectors, many groups are using the FDA guidance for gene therapies. Key messages from this guidance include the importance of an understanding of the impurity profile of your viral vector, and the need to characterize the biological activity early. It is also important to note that viral vectors have their own starting material, including both plasmid DNA and the cell line or cell bank that is used to generate the viral vector. For plasmids, it is recommended to avoid beta-lactam antibiotic resistance even early in development. (The majority of groups today have either already transitioned or are going to transition to kanamycin). Furthermore, testing of the bacterial bank should not be overlooked. Finally, as characterization is key, one must have complete understanding of the identity of the plasmids as well as the viral vector. Regarding cell line and cell banks, a good understanding of the cell line's history is important, but so is a strong focus on viral safety, as this information is critical even for early stage programs.

Many cell lines in use today were derived prior to establishment of the current regulations. (In the US FDA example, that is prior to 2005 when 21 CFR Part 1271 was established). Consequently, not all donor eligibility or testing was performed according to the formal regulations that are in place now. In addition, because of the nature of the material that is used to derive hESC lines, the donors are often not tested exactly according to the established donor eligibility or screening regulations, because that is not required for the donors' own IVF needs. This issue typically only comes into play once the parents decide to donate the embryo for research purposes. As a result, in these more complex cases, it is vital to establish a strategy that allows for additional testing on the back end to make up for any tests that may be excluded upstream.

Additionally, there are difficulties when one begins to delve into the fine details around the testing done on such cell banks. For example, on one level, there is an agreed testing regime across ICH regions. However, supplementary to this are the various texts from individual regional and national jurisdictions, some of which may be guidelines from the regulatory agencies, while others could be texts within pharmacopeia. It is easy to assume that they all add up to the same thing (and to some extent, this is true), but careful study reveals many discrepancies between the various texts. For example, US FDA guidance relating to *in vivo* testing in suckling mice states a preference for 28 days, whereas the EU is happy with 14 days. Similar differences arise around the *in vitro* indicator cell lines. To some degree if you're working globally you may have to test to the highest standard. Both US and European agencies state they are happy for cell and gene therapy developers to suggest non-*in vivo* cell bank testing, despite the fact that most existing guidelines for what does need to be included feature *in vivo* testing. Although it may seem a somewhat difficult conversation to have with the regulator, it is nonetheless encouraged.

In between the two extremes of autologous cell therapy and cell therapy based on pluripotent cells, lie allogeneic cell products.

Some are much nearer to autologous, in that each allogeneic donor can only be manufactured into a few doses. Others can be used to prepare thousands or hundreds of thousands of doses. This raises the question, is it really necessary to do full ICH testing of all allogeneic banks? There is arguably room for maneuver here at the present time. The degree of risk carried by the allogeneic cell therapy in question, which may be defined by the number of patients who will be treated by the given cell donor, should dictate whereabouts on the spectrum one should aim for: from ICH-level full testing through to very minimal autologous therapy testing. Of course, the eventual degree of testing required will be the result of case-by-case negotiations with the agencies.

PARTING ADVICE

Kasey Kime

I would reiterate the importance of taking your time, and really focusing on your raw materials risk assessment upfront. This will save a huge amount of time and energy later, and help you avoid any kind of clinical hold due to an inadequate raw material safety issue. And when possible, choose highly characterized raw materials with strong evidence of GMP manufacture, which are specifically designed for use in cell and gene therapy manufacturing.

Jerrod Denham

Even for the most complex raw materials and reagents, try to keep the description, explanation and analysis as simple as possible, especially initially. It makes it far easier to provide that information to the regulators and they can then get a hold of what is really important. As you then advance further and further, develop and enhance your programs – both as the reviewers deem necessary, but actually more importantly, as you gain a greater understanding of what you're trying to do with regard to manufacturing and controlling your product.

Christopher Bravery

When you write a document and then you re-read what you've written, you find some mistakes. If you hand it to someone else when you think it's perfect, they immediately spot typographical errors, missing punctuation, spelling errors, almost immediately. It's the first thing they see. The same is true of your dossier, and the same will be true of your risk assessments. Make sure you've got a diverse array of people looking at these documents, including some people from outside of the group developing the product. That final point is really key - one of the things with risk assessments is it's very easy to talk yourself out of risks. "Oh no, that will be fine because..." Get a third party to have a look as well, and make sure you've thought of everything.

RAW MATERIAL SUPPLIER SELECTION & PARTNERING STRATEGY

It is important for an end user to calculate their likely future demand and select an experienced raw materials supplier that can scale its manufacturing to meet it. Security of supply is also desirable, as is the ability to customize product configurations and testing based on an individual end user's manufacturing needs - for example, the ability to adjust pack sizes of certain buffers or reagents, or to conduct additional characterization testing on a custom basis. In order to help facilitate the end user's risk assessment and set them up for clinical manufacturing success, securing a supplier offering highly characterized raw materials with quality manufacturing evidenced by independent quality management system certification is optimal.

The supplier-end user relationship is also key. There will be numerous instances where it is critical for the end user to work closely with their supplier – for instance, when requiring the supplier's support regarding detailed raw material traceability questions.

REFERENCES

1. High KA, Roncarolo MG. Gene Therapy. *N. Engl. J. Med.* 2019; 381:455–64.

2. Reinhardt J, Stühler A, Blümel J. Safety of bovine sera for production of mesenchymal stem cells for therapeutic use. *Hum. Gene Ther.* 2011; 22(6): 775.

DOI: 10.18609/cgti.2020.049

AFFILIATIONS

Kasey Kime
Thermo Fisher Scientific

Jerrod Denham
Dark Horse Consulting

Christopher Bravery
Consulting on Advanced Biologicals Ltd

VIEW RESOURCES

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *This article has been created from a previously aired webinar that can be found here.*

Webinar recorded: *Mar 18 2020;* ***Publication date:*** *Apr 8 2020.*

Regulatory FAQs and common concerns for cell & gene therapy raw & starting materials: **Dos & Don'ts**

DO look at all the supplier documentation that comes with the product

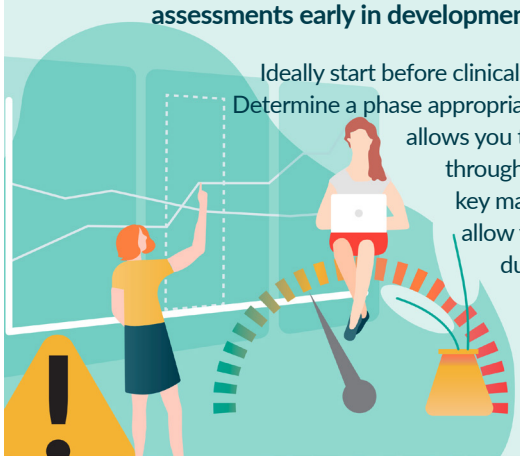
Compare it to the regulatory guidance to check if there are any gaps, or there is any further evidence you might need from your supplier. For example, the supplier may mention that a product has been virally inactivated, but you might need evidence of that viral inactivation report.

DON'T take product marketing claims for granted

For example, if a supplier claims animal origin freedom, research and verify that claim before choosing a raw material. Investigate multiple levels of processing when assessing risk associated with the use of a material of animal origin.

DO start doing material qualification and risk assessments early in development

Ideally start before clinical manufacturing. Determine a phase appropriate strategy that allows you to start working through mitigations for key materials. This will allow you to show reduced risk in your supply chain going into your clinical process.



DO educate yourself about where materials come from and the true impact of that origin

For example, plant origin may not be 'harmless' compared with animal origin when you consider the conditions under which the plant was produced.

DON'T use internal company jargon/terminology in your dossiers - use the language of the regulators

DO get a third party to review your dossier before submitting it

This should preferably be someone from outside of the core project team.

DOI: 10.18609/cgti.2020.052

Driving performance through collaboration

Biologics are complex and every process is unique. That's why you want a partner who can respond to your needs—even if what you need is a custom or open architecture approach. With a proven portfolio of solutions that spans from discovery through large-scale commercial production, our specialists have the know-how to adapt to what's next. It's our commitment to you, and **it's what we call Bioprocessing by Design**.

Let's move forward together.
Find out more at **thermofisher.com/bioprocessing**

ThermoFisher SCIENTIFIC