EUROGTP II GUIDE

Good Practices for evaluating quality, safety and efficacy of novel tissue and cellular therapies and products

GUIDANCE, METHODOLOGIES AND TOOLS

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EURO GTP II
Good Tissue & cell Practices

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Disclaimer:

The Associated and Collaborative Partners of the Good Practices for demonstrating safety and quality through recipient follow up Project (hereinafter referred to as ' EuroGTP II project') developed this guidance, to provide recommendations and to improve the quality of healthcare delivery within the field of human tissues and cells.

This guidance and associated tool represents the views of the EuroGTP II project, which were achieved after careful consideration of the scientific evidence available at the time of preparation. In the absence of scientific evidence on certain aspects, a consensus between the EuroGTP II partners has been obtained.

The aim of the methodologies and tools proposed is to aid tissue bankers and healthcare professionals in the evaluation of safety, quality and efficacy of tissue and cellular therapies and products, therefore providing effective care of their patients.

However, adherence to guidance does not guarantee a successful or specific outcome, nor does it establish a standard of care.

EuroGTP II outcomes do not override national regulations, healthcare professional's clinical judgment and treatment of patients. Ultimately, healthcare professionals must make their own clinical decisions on a case-by-case basis, using their clinical judgment, knowledge, and expertise, and taking into account the condition, circumstances, and in consultation with Competent Authorities.

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Collaborations:

EuroGTP II project is interlinked with:

VISTART Joint Action (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation) – intend to support EU Member States (MS) in developing and strengthening their capacity for monitoring and control of quality, safety and efficacy in the field of blood, tissues and cell transplantation[1].

ECCTR Project (European Cornea and Cell Transplantation Registry) – aims to build a common assessment methodology and establish an EU web-based registry and network for academics, health professionals and authorities to assess and verify the safety quality and efficacy of corneal transplantation.

Collaboration with this project is considered advantageous, as the use of registries is considered an important tool for the evaluation of efficacy and safety of SoHO. The criteria identified by ECCTR are also considered to be a valuable example for the definition of follow up and clinical evaluation principles by EuroGTP II project.

GAPP Joint Action (facilitatinG the Authorisation of Preparation Process for blood and tissues and cells) – having in mind the need for future requirements associated with the clinical evaluation of efficacy and safety performed by national Competent Authorities (CA), and the links needed to assure the coherence between EuroGTP II outcomes and any future tools developed, the Coordinator (Banc Sang i Teixits (BST)) is an Associative Partner in the JA.

These collaborations aim to develop harmonized procedures and Good Tissue and cell Practices (GTPs), for the different European stakeholders: Tissue Establishments (TE), Organisations Responsible for Human Application (ORHA), and national CA.

Acronyms:

AMSTAR – Assessing the Methodological Quality of Systematic Reviews

ART – Assisted Reproductive Technologies

CA – Competent Authority

CBB – Cord Blood Bank

CHAFEA – Consumers, Health, Agriculture and Food Executive Agency

CoE – Council of Europe

CPPs – Critical Process Parameters

CQAs – Critical Quality Attributes

DNA – Deoxyribonucleic acid

EC – European Commission

ECCTR – European Cornea and Cell Transplantation Registry

EDQM – European Directorate for the Quality of Medicines

EIM – European IVF Monitoring

ESSKA – European Society for Sports Traumatology, Knee Surgery and Arthroscopy

EUTCD – European Tissue and Cells Directives

FISH – Fluorescence in situ hybridization

GAPP – facilitatinG the Authorisation of Preparation Process for blood and tissues and cells

GCP – Good Clinical Practices

GRADE – Grading of Recommendations Assessment, Development and Evaluation

GTP – Good Tissue and Cells Practices

GvHD – Graft versus host disease

HLA – Human leukocyte antigen

HPC – Hematopoietic Progenitors Cells

HSC – Hematopoietic Stem Cells

HSCT – Hematopoietic Stem Cell Transplantation

IAT – Interactive assessment tool

ICSI – Intracytoplasmic sperm injection

IVF – In Vitro Fertilization

MED – Minimal Essential Data

MS – Milestones

NICE – National Institute for Clinical Excellence (NICE)

ORHA – Organisation Responsible for Human Application

QC – Quality Control

RCT – Randomized Controlled Trial

ROBIN-S – Risk Of Bias In Non-randomised Studies - of Interventions

SARE – Serious Adverse Reactions and Events

SoF – Summary of findings

SoHO – Substances of Human Origin

T&C – Tissues and Cells

TCD – T-cell depletion

TCTP – Tissue and Cellular Therapy/Product

TE – Tissue Establishment

TESE – Sperm procured via testicular extraction

TNC – Total Nucleated Cell

TUNEL – Terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labelling assay

V&S – Vigilance and Surveillance

VAS – Visual Analogue Scale

VISTART – Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation

WP – Work package

01

Introduction:

Preparation of Tissue and Cellular Therapies and Products (TCTPs), including reproductive cells, intended for human applications must comply with high standards of quality and safety according to the requirements of the European Tissue and Cells Directives (EUTCD)[2–7] in order to ensure a high level of health protection in the Union. This concept becomes even more important with new products that are applied for the first time in humans or are prepared with new and innovative methodologies.

Advances in basic science, technology and medicine continually create opportunities for new and improved TCTPs. These may be wholly new types of TCTPs, or improved methodologies for the preparation of existing TCTPs. While the objective of these changes and novelties is to prepare TCTPs that are safer, clinically more effective and meet the needs of clinicians and patients, there is always a risk that any change in the processing method can result in harm in the recipient. It is therefore vital that an evaluation of the potential risk of a process is systematically evaluated whenever a significant change is made.

To date, no European regulations or standardized methodologies have been established to facilitate systematic evaluation of novel TCTPs prior to introduction into a clinical setting, however the VISTART project has produced a document for Competent Authorities (CAs) of Tissues and Cells (T&C), to introduce the first principles on this topic[1]. This could represent the basis of a future regulatory framework based around the need to gather clinical follow up of recipients as a means of validating the clinical performance of T&C prepared with newly developed processing methodologies, and novel therapies. Furthermore the results of an EU survey of Tissue Establishments (TE) carried out by the EuroGTP II project confirmed the need for safety and efficacy studies, based on risk based assessment.

The European Commission, being conscious of the necessity to strengthen standards for quality, safety and efficacy of TCTPs, especially those related to innovative TCTPs, funded the EuroGTP II project (European Good Tissue and cells Practices II) – "Good Practices for demonstrating safety and quality through recipient follow up". The main objective is to set up good practices with regard to pre-clinical and clinical evaluation of human Tissues, Hematopoietic Stem Cells (HSC) and Assisted Reproduction Technologies (ART) and reproductive tissues and cells products. EuroGTP II will give continuity to the first EuroGTP project[8], which has developed European Good Tissue Practices for the activities carried out in TEs.

By using the systematic approach proposed, the users of this guide will be able to:

a) Evaluate risks resulting from all aspects of T&C supply chain (from donor selection to clinical application) of the final product;

b) Design appropriate studies proportionate to the level of residual/unknown risk to confirm that the TCTP is safe and effective.

The project has developed good practices, principles and reference tools applicable to TCTPs and how to conduct adequate clinical follow up studies. The methodologies proposed in this guide aim to be systematic and consistent, in order to promote a standard approach to practices and recognition amongst the stakeholders.

The methodology defined in this study aims to provide guidance for TEs, Organisations Responsible for Human Application (ORHAs), CAs, and professional societies, and the outcomes will be publicly available.

The good practices proposed do not override or replace national regulations, and authorization procedures defined at national level by the CAs.

Furthermore, the contents developed by the EuroGTP II project only apply to TCTPs and their applications as regulated by the EUTCD. TCTPs that are subject to "substantial manipulation" or that "are not intended to be used for the same essential function or functions in the recipient as in the donor" (as defined in Regulation 1394/2007/EC), are not part of the scope of this project.

1.1. PROJECT RATIONALE AND OBJECTIVES

There are three key outputs from the Euro GTP II Project:

A. Development of a systematic, risk-based mechanism and *Interactive Assessment Tool* (IAT : *http://tool.goodtissuepractices.site*) to:

- Evaluate if a new or changed TCTP has significant novelty
- Determine the overall risk arising from the novelty
- Determine an appropriate level of pre-clinical and clinical evaluations to address and assess the risk
- Implement the result of risk assessment into routine practice and follow up the results

Chapter 2 provides a more detailed methodology for this objective.

B. To create a *Tissue and Cells (T&C) database* (http://db.goodtissuepractices.site) of tissues/cells products, preparation processes, applications and therapies:

- The purpose of this database is the provision of data related to the products and therapies available, and support *end users* in the evaluation of TCTPs for safe and efficacious use. The structure and content of the database was designed to ensure that

the data collected is consistent, to support the collection of efficacy and quality data associated with the clinical use of Substances of Human Origin (SoHO) at European level.

Chapter 7 provides a more detailed methodology for this objective.

C. To put in place mechanisms to ensure the sustainability of the project's outcomes and propose a structure for the development of European accreditation and training programmes for TEs, ART centres and ORHAs:

- The ***GTP's Management Model*** aims to assure the continuity and sustainability of the outcomes of the EuroGTP II Project, and the future update, promotion and harmonization of GTP's standards.

This output does not form part of this guide, as it is an independent deliverable of the EuroGTP II project.

1.2. OVERVIEW OF THE EUROGTP II GUIDE:

The purpose of this document is to provide structured guidance on how to use the tools and methodologies developed by the EuroGTP II project.

This guide has been developed with the collaboration of experts and representatives of EU TEs, OHRAs, scientific associations, universities, CAs, research organizations, and national registries (Partners and Experts of EuroGTP II Project – Annex I).

In order to ensure alignment and coherence with existing documents dealing with patient follow up and quality aspects the above mentioned stakeholders considered the following current guidelines and reference documents in the development of the guide:

- VISTART deliverable regarding regulatory principles for clinical follow up of recipients – Principles for Competent Authorities for the evaluation and approval of clinical follow up protocols for blood, tissues and cells prepared with newly developed and validated processing methodologies[1];
- ARTHIQS (Assisted Reproductive Technologies and Hematopoietic stem cells Improvements for Quality and Safety throughout Europe) recommendations for cord blood banks and ARTHIQS about donor follow up registries[9];
- Outcomes of SoHO Vigilance & Surveillance (V&S) Project[10]
- Deliverables of the European Union Standards and Training for the In-

spection of Tissues Establishments (EUSTITE) Project[11]

- Deliverables of the European Good Tissue Practices (EuroGTP) Project[8]
- Guide to the Quality and Safety of Tissues and Cells for Human Application, 2017, 3rd edition, Council of Europe (CoE), European Directorate for the Quality of Medicines (EDQM)[12]
- The EU Coding Platform – Reference Compendia for the Application of a Single European Code for Tissues and Cells (SEC) for Tissues and Cells
- Notify Library – Global Vigilance and Surveillance Database for Medical Products of Human Origin
- FACT-JACIE (Joint Accreditation Committee – ISCT & EBMT) International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration[13]
- ESHRE (European Society of Human Reproduction and Embryology) Guidelines for good practice in IVF (in vitro fertilization) laboratories[14]

The outputs of the EuroGTP II project will also be used as a basis for the next Joint Action (GAPP) that will develop the criteria for evaluating quality aspects of preparation processes by CAs.

1.3. STRUCTURE OF THIS DOCUMENT

The guide is structured in 7 principle chapters:

Chapter 1 – General Introduction and overview;

Chapter 2 – Methodology for TCTP characterization, assessment of novelty and risk evaluation;

Chapter 3 – Instructions for the correct use of EuroGTP II methodologies and tools;

Chapter 4 – Specific guidance with regard to using EuroGTP II methodologies and tools for tissue products and therapies;

Chapter 5 – Specific guidance with regard to using EuroGTP II methodologies and tools for HSC products and therapies;

Chapter 6 – Specific guidance with regard to using EuroGTP II methodologies and tools for ART products and therapies;

Chapter 7 – A guide to the structure and use of the T&C database.

1.4. HOW SHOULD THE GUIDE BE USED?

It is suggested that chapters 1, 2 and 3 of this guide be read in their entirety before attempting to use the methodologies proposed by the EuroGTP II Project.

Chapters 4, 5 and 6 are intended to be used as reference, as they provide specific guidance for the use of tools and methodologies applied to the different areas of SoHO.

02

Outline methodology for TCTP characterization, assessment of novelty and risk evaluation

The assessment methodologies proposed by the EuroGTP II project can be applied on paper using the available templates (Annex II and Annex III) or online using the IAT.

Instructions for the correct use of these methodologies can be found in the chapter 3 and/or in the SoHO specific chapters 4 – Tissues, 5 – HSC and 6 – ART *of this guide.*

An overview of EuroGTP II methodologies is available in the Annex IV.

2.1. CHARACTERIZATION OF THE TCTP

Before commencing assessment of novelty and the associated risk, it is important that the TCTP is thoroughly characterised so that the process can be performed accurately. This requires that the following details be documented (the template provided in the Annex II may assist users in this process):

- Justification for the implementation of change, including the key benefits of the innovation.
- How is the TCTP prepared; what, if any, changes have been made to the established preparation or treatment protocol?
- What is the origin of the TCTP (autologous or allogeneic, or in case of ART concerning partner or non-partner donation)?
- In what format is it presented for clinical application (e.g. packaging, methodology and preservation technique)?
- What, if any, excipients or other reagents or residues could be transferred through the clinical application with the TCTP (such as carriers or preservatives)?
- What are the critical process parameters applied to the TCTP preparation protocol?
- What are the critical quality attributes necessary for the TCTP to deliver its intended result?
- What clinical indication is the TCTP to be used for?

Additionally, prior to the implementation of changed/new processes, the template provided in the Annex II should be completed with a description of the factors that justify the developments. This may include for example the following information:

- Existence of prior clinical data reported by other centres (if applicable);
- Quality control measures and any other quality indicators evaluated;
- Overview of the intended clinical effect of the TCTP;
- Bibliographic evidence that supports the implementation of changes;
- In house data generated to justify the process.

2.2. EVALUATION OF NOVELTY (STEP 1)

It is important that the definition of 'novelty' within the context of this process is clearly established. It is not intended to encompass every change to a product or process, regardless of how minute the change is; rather it intends to capture any change that could significantly affect the quality and/or safety of the TCTP and/or the safety of recipients. This is the first step of the novelty and risk evaluation process (Figure 2.1. below)

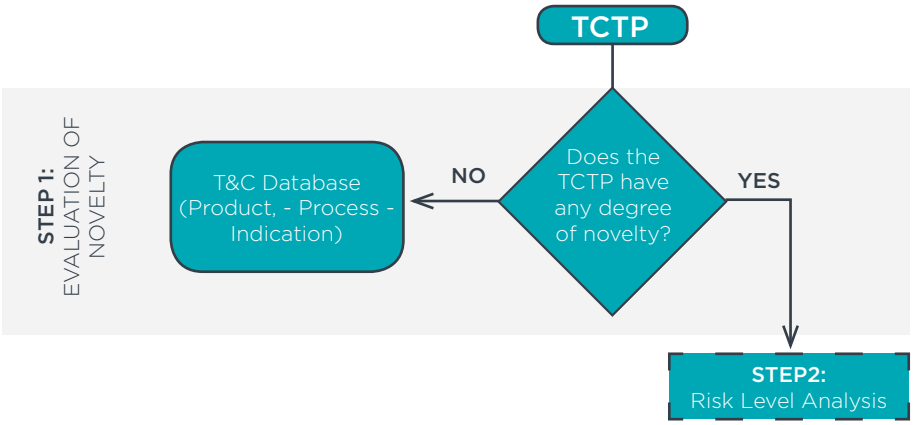

Figure 2.1.: Evaluation of Novelty – This assessment involves answering a series of seven questions, covering all aspects of TCTP provision from donor selection to clinical application of the final product. If no novelty is identified (This process is discussed in detail in the Chapters 3 – Generic methodologies and tools, 4 – Tissues, 5 – HSC and 6 – ART), it can be concluded that there is no significant change or innovation in the TCTP being assessed and exercise ends at this point. Users are encouraged to add their established product to the *T&C Database* (A guide to the structure and use of the *T&C database* in provided in the Chapter 7 of this document).

2.3. OVERVIEW OF THE RISK ASSESSMENT PROCESS – LEVEL RISK ANALYSIS (STEP 2)

If step 1 establishes that a new or changed TCTP has significant novelty, a sys-

tematic risk assessment must be undertaken to identify and quantify the risks associated with it. This must be a comprehensive process that considers all aspects of TCTP supply chain: from donor selection through to implantation or clinical application of the product or therapy. This is the second step of the novelty and risk evaluation process (Figure 2.2. below)

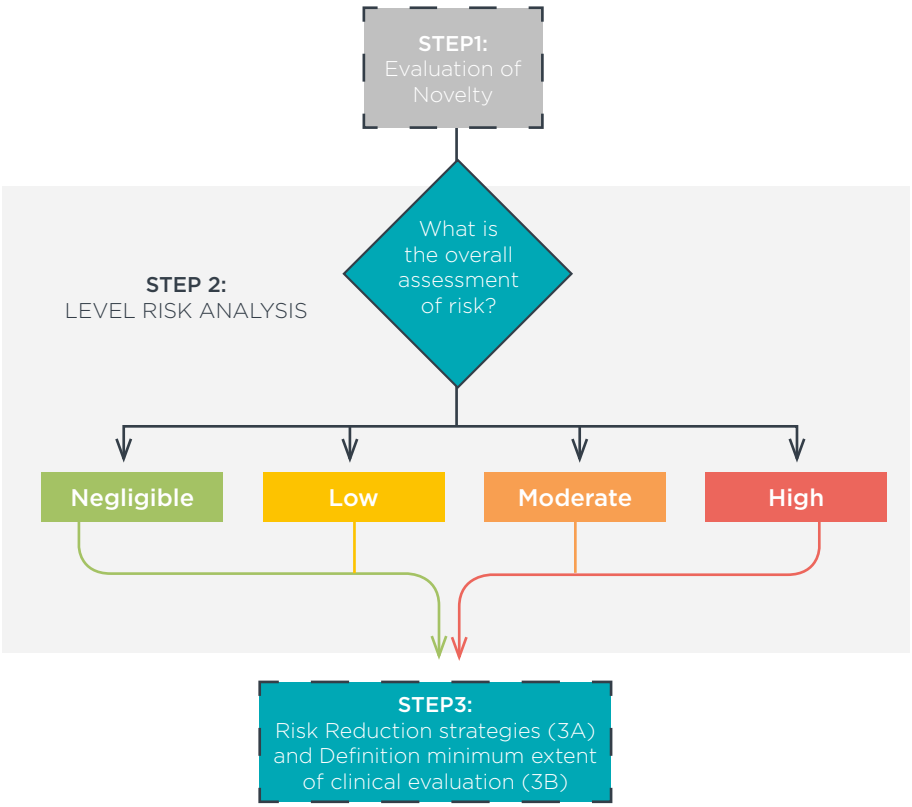

Figure 2.2.: The risk assessment process – The risk profile is determined through the identification of potential risks factors (Figure 2.3.) and analysis of risks consequences (Figure 2.4.). This is further explained in Chapter 3, and in subsequent specific chapters (4 – Tissues, 5 – HSC, and 6 – ART), with some examples.

The overall process requires that firstly, specific risks relating to the potential risk factors (Figure 2.3) and risks consequences (Figure 2.4.) be identified. Each of these must be individually risk assessed to determine the residual risk of implementing the change, assessed by considering:

i) The probability of the **risk occurring.**

ii) The **severity of the consequences** should the risk occur.

iii) The probability that **the source of the hazard for the risk consequences will be detected** before the TCTP is applied. This does not refer to detection of the consequences of the risk post implantation.

iv) Any existing **evidence that can be used to mitigate the risk.**

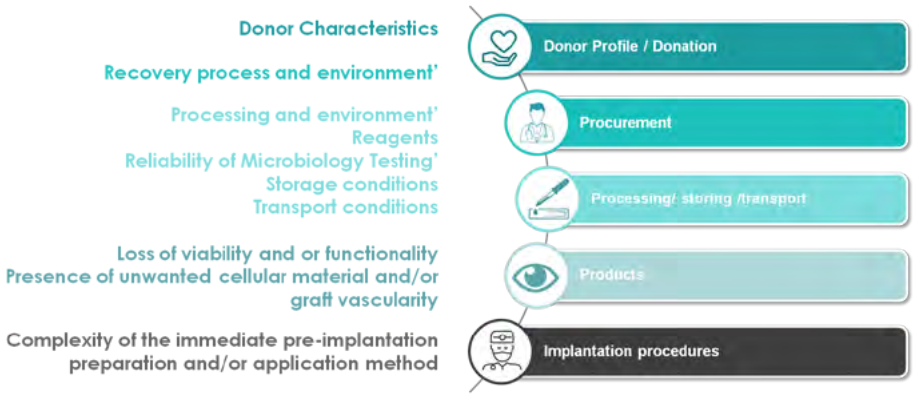

Figure 2.3.: Risk factors

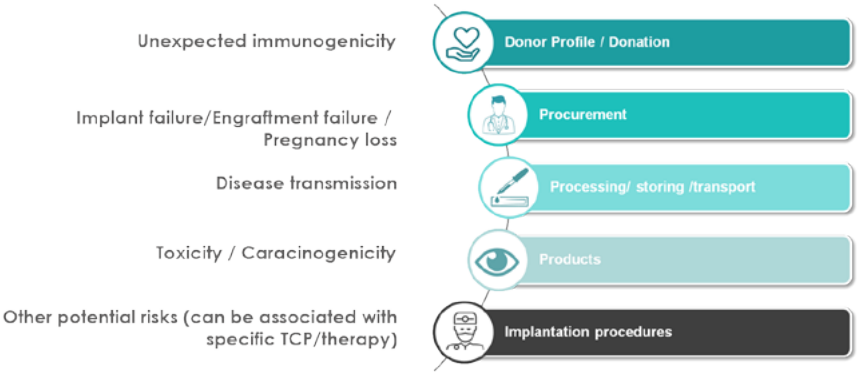

Figure 2.4: Potential Risk Consequences

The outcome of this exercise will be a single, overall risk score (in a scale of 0 to 100) – ***Final Risk Score*** – that can be used to inform the definition and extent of pre-clinical and clinical evaluation, necessary to support the proposed novelty or change (EuroGTP II Algorithm for the calculation of ***Final Risk Score*** detailed in the Annex V).

The tool used to quantify risk (described in detail in the next chapter) takes into account the number of individual risks assessed when calculating the proportional risk value. Thus, a process where multiple minor risks are identi-

fied could generate the same *Final Risk Score* as a process where only a single major risk is identified.

The quantity and quality of the available evidence, such as published data in peer reviewed literature and internal validation reports, can be used to reduce this overall risk score. The whole risk assessment process is explained in more detail in Chapter 3.

2.4. DEFINITION OF STUDIES EXTENT (STEP 3):

The *Final Risk Score* generated by the risk assessment process determines the corresponding extent of studies required to ensure the safety and efficacy of the TCTP, in terms of the pre-clinical (*in vitro* and/or *in vivo*) and clinical evaluation. The specific, individual risks consequences identified further determine the precise test criteria indicated. The methodology proposed (detailed in chapters 3 – Generic methodologies and tools, 4 – Tissues, 5 – HSC and 6 – ART of this guide), will assist users in designing these protocols.

Step 3A: Risk reduction strategies – Use pre-clinical studies (*in vitro* and *in vivo*) to mitigate the identified risks

After the risk assessment exercise, users should consider if the given risk score can be mitigated by performing pre-clinical studies.

In some scenarios, the initial risk may be negligible and the TCTP may be used in humans without additional pre-clinical studies. However, if risk is higher than negligible, it may be possible to perform additional *in vitro* and *vivo* pre-clinical studies (if not already done) to mitigate and potentially reduce the level of risk prior to clinical application (example of pre-clinical evaluations proposed in chapters 4 – Tissues, 5 – HSC and 6 – ART of this guide).

Step 3B: Extent of clinical evaluation

In situations when the risks cannot be reduced sufficiently with pre-clinical studies, an internal risk-benefit exercise should be done in collaboration with the clinicians, to assess if it is justifiable to use the TCTP in a clinical setting.

The requirements of the clinical evaluation should be proportional to the remaining level of risk. Details on how to design and implement these studies are listed below, and described with more detail in the specific chapters (4 – Tissues, 5 – HSC and 6 – ART) of this guide.

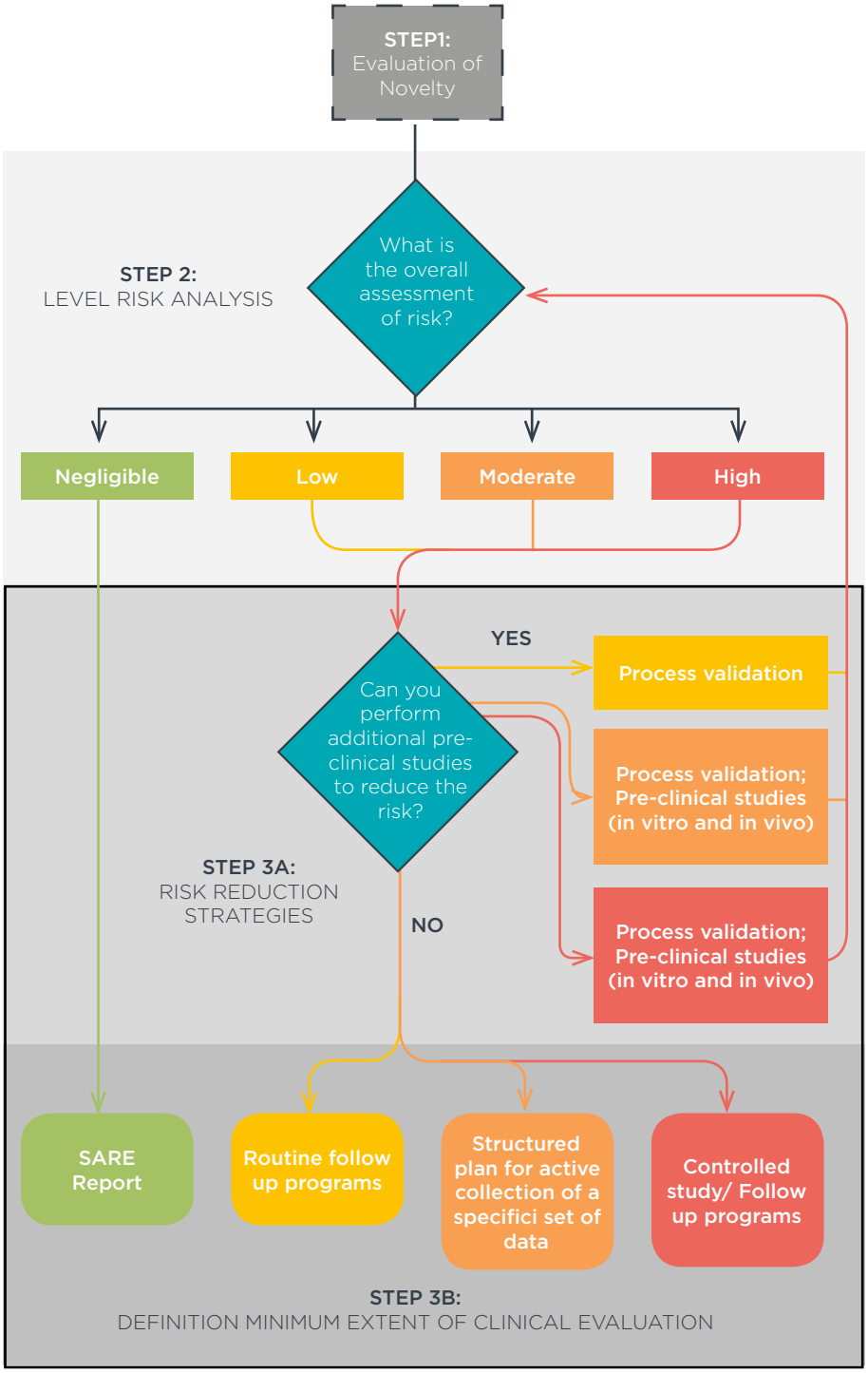

Figure 2.5.: The risk reduction and determination of the extent of studies required

Table 2.2. Extent of studies according the level of risk determined in the assessment*

Level of Risk	Proposed studies extent
NEGLIGIBLE:	**Step3A: Risk reduction strategies** The assessment indicates that the TCTP is safe and efficacious for clinical use and very unlikely to cause harm to recipients, however, it may be advisable to conduct a validation of the process, if not already done. If the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application.
	Step 3B: Extent of clinical evaluation No clinical follow up over and above what is the mandatory requirement, such as serious adverse reaction and event (SARE) reporting.
LOW	**Step3A: Risk reduction strategies** The assessment indicates that the TCTP is safe and efficacious for clinical use and unlikely to cause harm to recipients, however, a validation of the process, if not already done, should be performed. If the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application.
	Step 3B:Extent of clinical evaluation In addition to the mandatory requirement for serious adverse reaction and event (SARE) reporting, feedback from immediate post clinical application monitoring (routine clinical follow up) may be collected for a defined period or number of procedures. Clinical audit** may also be used after an appropriate period of use.
MODERATE	**Step3A: Risk reduction strategies** The assessment indicates that more evidence is needed to support safe and effective use of this TCTP and mitigate risk. Process validation should be performed, however if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. Pre-clinical in vitro evaluation studies, specific to the identified risks, should be performed if not already done. Pre-clinical *in vivo* evaluation using an animal model should be considered if applicable (and if not already done). *Please refer to specific chapters of this guide (4 – Tissues, 5 – HSC, and 6 – ART) for additional details.*

*(Process Validation, Pre-clinical *in vitro* and *in vivo* studies, and Clinical Evaluation)

**In the context of this guide clinical audit refers to retrospective or prospective evaluation of routinely collected clinical data.

Level of Risk	Proposed studies extent
MODERATE	**Step 3B:Extent of clinical evaluation** A structured plan for active collection of a specific set of data relating to the safety and efficacy of the TCTP should be put in place, in addition to routine clinical follow up. Ethical approval may be required and the principles of Good Clinical Practices (GCP)[15] adhered to. Consideration should be given to restricting provision of the TCTP to a limited number of patients and/or centres until the risks have been adequately mitigated. *Please refer to specific chapters of this guide (4 – Tissues, 5 – HSC, and 6 – ART) for additional details.*
HIGH	**Step3A: Risk reduction strategies** The assessment indicates that significantly more evidence is needed to support safe and effective use of this TCTP and mitigate risk. Process validation should be performed, however if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. Pre-clinical *in vitro* evaluation studies, specific to the identified risks, should be performed if not already done. Pre-clinical *in vivo* evaluation using an animal model should be considered if applicable (and if not already done). *Please refer to specific chapters of this guide (4 – Tissues, 5 – HSC, and 6 – ART) for additional details.*
	Step 3B:Extent of clinical evaluation The TCTP should only be used clinically in the context of an ethically approved, controlled (where applicable) clinical evaluation until the residual risks have been adequately mitigated. The principles of GCP[15] must be adhered to. Clinical evaluation and follow up programs should be implemented and safety and efficacy must be continuously monitored. If available national and international registries are recommended for gathering follow up data. *Please refer to specific chapters of this guide (4 – Tissues, 5 – HSC, and 6 – ART) for additional details.*

Design considerations for clinical evaluations adapted to T&C products/ therapies

The design of clinical evaluation programs must be planned in close cooperation between the TEs and the clinicians responsible for the clinical application of the TCTP. The collaboration between TEs and *end users* is critical to identify suitable design parameters, clinical indications, number of patients, type of follow up proportionate to the residual risks identified, and to ensure that comprehensive data is gathered to evaluate efficacy.

The design of the clinical evaluation should consider:

a) The nature of the risk (e.g. if sudden graft failure is a significant risk, then patient recruitment should allow for sufficient observation time between one patient and the next to be enrolled);

b) The number of patients required to obtain statistically significant data, where applicable. If the number needed is too high because the disease is a rare disease or the follow up period is very long then alternative solutions must be proposed.

The design of clinical evaluation should take into consideration the requirements of GCP[15], including independent ethical committee opinion, and any other national or regional specific regulations.

Specific guidance relating to design of clinical evaluation for different types of TCTP will be provided in Chapters 4 – Tissues, 5 – HSC and 6 – ART. However, certain features relating to design of clinical evaluation protocols' are common to all types of TCTP. These are fundamentally two types of evaluation;

i) A single arm study (case series/registry approach);

ii) A controlled study, where the TCTP is directly compared to a control treatment.

The type of clinical evaluation protocol selected will depend on a number of considerations, specifically:

- The level of risk – if risk is high, a controlled study is more suitable, provided that it is feasible for the TCTP in question.
- The availability of a suitable control treatment.
- The length of time that patients need to be followed up for; if long term follow up is required, a controlled study may not be practical, and a registry approach may be considered.

In addition to these considerations, TEs should endeavour to collaborate with fellow TEs to set up multicentre studies, to ensure that sufficient patients are recruited. Collaborations with clinical trial units should also be pursued to ensure that the requisite skills and resources are available to manage studies.

2.5. ETHICAL PRINCIPLES AND CONSIDERATIONS

Innovative and experimental therapies are often the place where scientific research and clinical practice meet. Understanding and applying of basic ethical principles (autonomy, non-maleficence, beneficence and justice) is essential for the clinical implementation of novel treatments.

Thus, clinical application of novel TCTPs must always follow the *Ethical Principles for Medical Research Involving Human subjects*, determined in the Declaration of Helsinki[16], namely in what concerns the careful assessment of predictable risks and burdens to the individuals, the procedures associated with informed consent of recipients and donors, and the considerations and approval of Research Ethics Committees including the (impact of) procurement, and source of SoHO.

03

Instructions for the correct use of EuroGTP II methodologies and tools

There are three distinct phases of the risk assessment process, as explained in the previous chapters. To facilitate this process, an online *Interactive Assessment Tool* (IAT) has been developed. The IAT addresses the first two of these phases: evaluation of novelty, and analysis of risk. This generates individual risk scores for each risk consequence identified, plus a *Final Risk Score* for the TCTP as a whole. The output from the analysis of risk is used to inform the third phase of the process, to determine whether or not the TCTP can be made generally available for clinical application on request, or if further pre-clinical and/or clinical evaluations are required.

3.1. ACCESSING THE IAT

The IAT is accessible on-line (http://tool.goodtissuepractices.site/).

Due to the significant volume of data that can be introduced in the IAT for each individual assessment, and the need to reassess data (as described in section 3.2), the **tool allows users to save their data**:

To do this, users need to use the "save" option available in the report page of IAT (results). After selecting this option, a file (.gtptool) will be downloaded. This document can be further used to "restore" the assessment in a new session.

3.2. KEY PRINCIPLES FOR EFFECTIVE USE OF THE EUROGTP II METHODOLOGIES AND IAT

The value of the outputs from the IAT will be determined by the accuracy, comprehensiveness and relevance of the information that is put into it. It is therefore advised that:

i) The **process should be treated as a long term exercise**: The intention is that the IAT will provide the framework for a detailed assessment of risk. It is important that the **rationale for these decisions is recorded and documented.**

ii) It is unlikely that a single individual will have sufficient knowledge and expertise to complete the whole process at one go with no support. Ideally, the **assessment should be performed by a group of individuals selected for their knowledge and experience** who will consider all available information to generate an accurate assessment of risk. The process should be performed by a team selected to provide the requisite knowledge and experience to fully identify and evaluate all potential risks. This may include all professionals involved in the SoHO activities, namely:

- Operational staff;
- Scientists and embryologist developing TCTPs;
- Quality control personnel;
- Health care professionals
- Please note that this list is not exhaustive.

iii) **The IAT may be used at any point in the process/product development cycle:** The initial process can be performed at an early stage in the development of new or revised TCTPs; this may identify areas of high risk that could be addressed by pre-clinical development work. The exercise can be repeated at different stages of the development and implementation of the TCTP, in order to re-evaluate the risks based on the information recollected (by the studies performed and/or relevant references). Much of the potential risk inherent to a new product or process can generally be eliminated or ameliorated by well- planned and focussed pre-clinical studies. It can therefore be useful to use the IAT at a very early stage, where it can pinpoint areas where there is a high level of risk that could be addressed with pre-clinical in vitro studies, or sourcing the appropriate literature. Often at this stage, potential risk must be assessed as high, purely due to lack of data. The IAT can be re-run during the development cycle to evaluate how ongoing work is contributing to ameliorating the overall risk, and identify areas where further effort should be focussed. If used in this manner, the final use of the IAT prior to providing products for clinical use will identify the residual risk that can only be addressed with clinical evaluation or follow up. This final output, along with all associated documentation and evidence, can be used to support submissions to CAs to seek approval to provide the TCTP for clinical use, either in a routine or restricted setting as indicated by the level of residual risk.

iv) There must be a clear **understanding of the critical parameters of the TCTP which will contribute to its safety and efficacy,** to enable the risk assessment to be performed accurately.

Note also that the IAT should only be **used to assess new risks resulting from the novelty**. It is assumed that for existing TCTPs, which are being provided for clinical use, the existing risks have been evaluated and are adequately controlled.

Specific guidance applicable to the use of EuroGTP II methodologies and tools for different TCTPs is described in the chapters 4 – Tissues, 5 – HSC, and 6 – ART.

3.3. STEP 1: EVALUATION OF NOVELTY

The first stage of the tool is the assessment of novelty. This involves answering a series of seven questions, shown in Table 3.1 below, covering all aspects of the T&C supply chain from donation to clinical application. This stage is intended to generate a simple 'yes' or 'no' answer; there is either novelty or not, irrespective of the degree of novelty.

Additionally, a third option – 'Not Applicable / Not relevant' (NA) – is provided to cover situations that are not addressed for the TCTP under evaluation.

If no novelty is identified, it can be concluded that there is no significant change or innovation in the TCTP being assessed; in this case, there is no need to proceed with the rest of the IAT, and users are invited to add their TCTPs in the *T&C Database*.

Specific examples and explanations regarding the interpretation of these questions are provided in the specific chapters (4 – Tissues, 5 – HSC and 6 – ART).

Table 3.1: Evaluation of novelty (Step 1)

		YES	NO	NA
A.	Has this type of TCTP previously been prepared and issued for clinical use by your establishment?			
B.	Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?			
C.	Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?			
D.	Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?			
E.	Will this TCTP be packaged and stored using a protocol and materials used previously in your establishment for this type of TCTP?			
F.	Will this type of TCTP provided by your establishment be applied clinically using an implantation method used previously?			
G.	Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site before?			

3.4. STEP 2: LEVEL RISK ANALYSIS – IDENTIFICATION AND QUANTIFICATION OF RISK

If, after completing the step 1, you determine that there is some novelty resulting from your proposed change, you then proceed to step 2 to identify and quantify the potential risks resulting from this novelty. There are a number of stages in this process:

Step 2A: Identification of risk factors

This step involves identifying the potential risk factors that are relevant to the change. The global risks that should be considered during this assessment are listed in Table 3.2 below. Specific risk factors, examples and explanations regarding the interpretation of these risk factors, are provided in the next chapters (4 – Tissues, 5 – HSC and 6 – ART).

Table 3.2 – Identification of Risk Factors

Process	Specific risk factors	Guidance notes
Donation	Donor Characteristics	Consider whether the donor population you intend to obtain the TCTP from could impart any risk, for example if the TCTP is sourced from an allogeneic donor, there may be risks that immunogenicity could impact on the clinical performance of the TCTP, and risks of disease transmission'
Procurement	Procurement process and environment'	Consider where and how the TCTP is collected, procured or recovered, and if this process could have an influence on the TCTP. How long does the process take, how complex is it, and what is quality of the environment - for example, these factors may impact on the probability that the TCTP becomes contaminated, or damaged during recovery

Process	Specific risk factors	Guidance notes
Processing/ storing /transport	Processing and environment'	Consider where and how the TCTP is processed, namely how long does the processing take and how complex is it(including all physical and chemical treatments applied to the product) – this may impact on the risk of contamination, or that it may not be prepared to consistent specifications and quality. Also consider the quality of the processing environment, which may also affect the risk of contamination. (Please notice that risks associated to reagents are considered in the following specific risk factor 'Reagent').
	Reagents	Consider any reagents used during processing, decontamination, preservation, storage and transport of the TCTP. Could they damage the TCTP in any way, or could residual traces of reagent remain in the TCTP that could cause toxic or immunogenic effects in recipients?
	Reliability of Microbiology Testing'	Consider the risk that the nature of the TCTP, the testing methodology and/or the presence of residual processing reagents such as antibiotics in the finished TCTP may impact the accuracy of any microbiology tests. Note: this refers specifically to bacteriology/mycology testing of the TCTP, not any blood tests performed on the donor.
	Storage conditions	Consider any potential risks arising from how the starting material and TCTP are stored, between procurement and processing, during processing, and between processing and clinical application.
	Transport conditions	Consider any potential risks arising from how the starting material and TCTP are transported, for example between the sites procurement and processing, and between the sites of storage and clinical application.

Process	Specific risk factors	Guidance notes
Product	Presence of unwanted cellular material and/or graft vascularity	This risk must be considered from the perspective that for some TCTPs, the presence of intact vital cells is desirable, although it may also increase risks of, for example, immunogenicity or disease transmission This presence might affect to tumour formation, immunogenicity and disease transmission risks. Vascular tissues may be more at risk to infiltration by pathogens or malignant cells than avascular tissues
	Loss of viability and/or functionality	Consider the risk that the changes in procedures of processes can have on the viability or functionality of the TCTP
Clinical Application procedures	Complexity of the immediate pre-implantation preparation and/or application method	Consider how complex the method of clinical application will be for this TCTP. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be? Highly complex methods of application could influence the risks of implant failure and/or disease transmission.

Step 2B: Identification of risk consequences

Consider the potential consequences for the risk factors identified above.

The potential risks consequence associated with the clinical use of TCTP comprise:

- Unexpected immunogenicity
- Implant failure / engraftment failure / pregnancy loss
- Disease transmission
- Toxicity / Carcinogenicity
- Other potential risks (can be associated with specific TCTP)

Examples of the combination of risk factors and specific consequences that may need to be considered are provided in the TCTP type specific chapters (4 – Tissues, 5 – HSC and 6 – ART). The purpose of the exercise is to systematically consider each risk factor and risk consequence in turn against the change. Note that for certain combinations of risk factor and risk consequence, there may be no relevant examples. It is recognised that the IAT cannot anticipate all potential types of risk; the specific risk consequences listed for each TCTP type are those which it is generally agreed will be most commonly related to that type. For any risks not covered by these risk consequences, an open, 'other' category is provided and it is highly recommended to use during the assessment.

Step 2C: Quantification of Risk

The next step is to perform the risk assessment by determining the probability, severity and detectability for each risk factor identified for each risk consequence. When calculating Probability, Severity and Detectability, you should consider the following sources of information:

- Internal development and validation reports
- Previous experience and existing knowledge originating within your establishment
- Quality related data (trend analysis, indicators, product or process quality reviews, etc.)
- Internal process validation studies, pre-clinical in vitro studies, pre-clinical in vivo studies, clinical evaluation protocols.

Note that:

- There may be more than one risk consequence associated with each risk factor. If this is the case, the quantification of risk should be performed for all the risk consequence-risk factor combination, in order to be able to address each risk specifically in future risk reduction strategies.

An explanation of the rationale behind the performed analysis should be recorded and included in the exercise. This will allow the user to keep record of the risks consequences and risk factors evaluated.

These registers can be recorded by entering the information directly in the IAT or using the templates available in Annex II, Annex III (Tissues, HSC and ART Templates).

Assessment of Probability

This assessment requires estimating the probability of any risk occurring. There are five levels of probability

Table 3.3 – Probability levels*

Level of probability	Definition
1 - Rare	Difficult to believe it could happen
2 - Unlikely	Not expected to happen but possible
3 - Possible	May occur occasionally
4 - Likely	Probable but not persistent
5 - Almost certain	Likely to occur on many occasions

Assessment of Severity

This assessment requires that you estimate the severity of the consequences of the risk, should it occur. There are four levels of severity.

Table 3.4 – Severity levels**

Level of severity	Definition
1- Non-serious	Mild clinical or psychological consequences for the recipient, however with no hospitalisation, or anticipated long term consequences/disability
2- Serious	Hospitalisation and/or: • Persistent/significant disability or incapacity • Intervention to preclude permanent damage • Evidence of a serious transmitted infection • Significant decrease in the expected treatment success • Birth of a child with an infectious or genetic disease following ART with donor gametes or embryos
3- Life-threatening	• Major intervention necessary to prevent death • Evidence of a life threatening transmissible infection • Birth of a child with life threatening genetic disease following ART with donor gametes of embryos
4- Fatal	Death of the patient

***Definitions from V&S SoHO Project, 2009[10]

****Definitions adapted from V&S SoHO Project, 2009[10]

Assessment of Detectability

This assessment requires that you estimate the probability that, the source of the hazard for the risk consequences will be detected before the TCTP is applied. This does not refer to detection of the consequences of the risk post implantation.

Table 3.5 – Detectability levels

Level of detectability	Definition
1 - Very high	The potential defect will almost certainly be detected before clinical application in the recipient
2 - Moderately high	There is a reasonable chance that the potential defect will be detected before clinical application in the recipient
3- Low	There is a low chance that the potential defect will be detected before clinical application in the recipient
4 - Very low	It is unlikely that the potential defect will be detected before clinical application in the recipient
5 - Cannot be detected	The potential defect will be detected only after clinical application in the recipient

Step 2D: Assessment of Risk Reduction

Having calculated probability, severity and detectability, and thus an overall risk score based on 'internal' knowledge and data, it may be possible to adjust this score by taking into account other external sources of information.

This external data is not used to specifically reduce probability, severity or detectability, rather it is used to calculate a general reduction in the overall risk score.

Data that should be taken into account when calculating risk reduction may include:

- Published data in peer reviewed literature;
- Unpublished data from external sources;
- Advice and information from external experts;
- Clinical outcome data from external sources (e.g. registries).

When calculating the risk reduction factor, it is important that the quality and reliability of the data be considered; for example a large scale clinical trial in

a high impact, peer reviewed journal would be considered of high quality and reliability, whereas unpublished clinical data with limited follow up in a small number of patients less so.

An objective assessment of the quality of evidence is recommended. Available data should be reviewed in an explicit, systematic and transparent process that can be applied to both quantitative (experimental, observational and correlational) and qualitative evidence[17]. The key aim of any review is to provide a summary of the relevant evidence to ensure that assessments are performed based on adequate information.

Several methodologies are available to perform an objective evaluation of the quality and reliability of scientific data:

- To assess the risk of bias for individual studies/reviews: Assessing the Methodological Quality of Systematic Reviews (AMSTAR)[18], Risk Of Bias In Non-randomised Studies - of Interventions (ROBIN-S)[19] The Cochrane Collaboration's tool for assessing risk of bias[20], and other quality assessment methods or checklists

- Grading of Recommendations Assessment, Development and Evaluation (GRADE) summary of findings (SoF) tables [21,22], or NICE (National Institute for Clinical Excellence) guidelines: the Manual[17].

Although this step does include some subjectivity and should be a team exercise (as referred in section 3.2), the evidence used to justify the risk reduction should be accurately described in the rationale of the assessment. It is advisable to keep the references/documents associated with the risk assessment report (provided by IAT or registered in the templates of the Annex II, and Annex III (Tissues, HSC and ART Templates) in order to easily justify the rationale behind each risk assessment.

Based on the assessment of the data, different levels of risk reduction can be applied. This is accomplished by a applying a percentage reduction to the overall risk score (probability x severity x detectability) calculated in the first three steps of the risk assessment. These levels are shown in Table 3.6 below:

Table 3.6. – Percentage risk reduction definitions

Percentage risk reduction		Definition
0	None	There is no relevant data available to support reducing the calculated risk score
25	Limited	There is a moderate relevant data available to support reducing the calculated risk score, based predominantly on unpublished data
50	Moderate	There is moderate amount of good quality relevant data available to support reducing the calculated risk score, including published and unpublished data from external sources, and some data which has been through an independent peer review process
75	Substantial	There is high quality relevant data to support reducing the calculated risk score, including data that has been peer reviewed and published
95	Extensive	There is an extensive amount of high quality relevant data, including multiple peer reviewed publications, that demonstrates that the probability of the risk occurring, having a significant impact, and/or being undetected is negligible

On completion of this step, a *Final Risk Score* is calculated, which will determine if the risk is **negligible**, **low**, **moderate** or **high**.

The level of residual risk will inform whether or not (and what level of) pre-clinical (*in vitro & in vivo)* evaluation is indicated for the TCTP, and what level of clinical evaluation and/or follow up will be proportional to the level of risk estimated. Note that after the preliminary use of the IAT, the *Final Risk Score* may be in a higher risk category due to insufficient information. It may be possible to perform further pre-clinical (*in vitro/in vivo*) studies to gather new data to reduce probability/severity/detectability scores (as discussed in section 3.5) before making a final decision to determine the level of clinical follow up required.

3.5. STEP 3: DEFINITION OF EXTENT OF STUDIES NEEDED BASED ON THE RISKS QUANTIFIED

The output from step 2 (A: Identification of risks factors, B: Identification of risks; C: Quantification of risks, D: Assessment of risk reduction) will result in the identification and quantification of one or more residual risk consequences; these can be expressed in the standard format:

'There is a risk that the TCTP will ..... due to ..... resulting in.....'

E.g. – There is a risk that the TCTP will **be immunogenic** due to **the inadequate removal of donor cells** resulting in **an unwanted localised and systemic immune response**

Or: - There is a risk that the TCTP will **fail** due to **biomechanical damage caused by the processing protocol** resulting in **sudden mechanical failure**

The purpose of step 3 is to provide users of this guide with guidance as to how to evaluate and mitigate these risks through the sequential application of pre-clinical (*in vitro*, *in vivo)* and clinical assessments:

Process validation

Process validation is a mandatory activity under the EUTCD, to ensure that a process is reliable achieving its stated objective. Guidance for performing process validation can be found in the Guide to the Quality and Safety of Tissues and Cells for Human Application[12], and is not within the scope of this document. If the final, overall risk is determined to be negligible, no further risk mitigation is necessary, however, it may be advisable to conduct a validation of the process. If the final overall risk is determined to be low, it is necessary that as a minimum the process is revalidated. However, if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application.

Pre-Clinical – In vitro Studies

Generally, *in vitro* assessments will be performed prior to other pre-clinical (*in vivo)* studies. This category may also incorporate routine process validation studies. Where the overall risk is low, it is likely that it can be mitigated purely with *in vitro* assessments.

Pre- Clinical – In vivo Studies

In vivo assessments will usually only be considered where the risk cannot be sufficiently mitigated with *in vitro* studies, for cost and ethical reasons. There may however be criteria that can only be accurately evaluated with *in vivo* models. The specific chapters give guidance on how to define which tests could be used for the different types of novel TCTP regarding specific risk consequences (4 – Tissues, 5 – HSC and 6 – ART)

Clinical Evaluation Protocols

If the risk cannot be mitigated to 'negligible' or 'low' levels by *in vitro* or pre-clinical studies, and when ethically accepted, clinical evaluation protocols may be necessary before the TCTP is made generally available.

Guidance for the correct definition of protocols to address the specific risk categories referred in the step 2 is presented in chapters 4 – Tissues, 5 – HSC and 6 – ART of this document.

In the context of this guide, ***Clinical Evaluation*** is defined as: Clinical follow up studies for monitoring predefined clinical outcome indicators to evaluate quality, safety and effectiveness/efficacy of tissue or cell product for a defined number of patients

The studies proposed in the specific chapters and relevant appendices **are for guidance purposes, and are not intended to be an exhaustive, authoritative or mandatory list of tests that must be performed.** These should be considered in conjunction with any tests already performed by the TE.

04

Tissues specific chapter – Specific guidance for the use of EuroGTP II methodologies and tools

Define which type of TCTP you are evaluating

First it is important to define for which type of TCTP you are going to use the tool, as this will generate specific risk factors. In case of Tissues, choose 'Tissues' and subsequently which type of tissue is the subject of the process under evaluation.

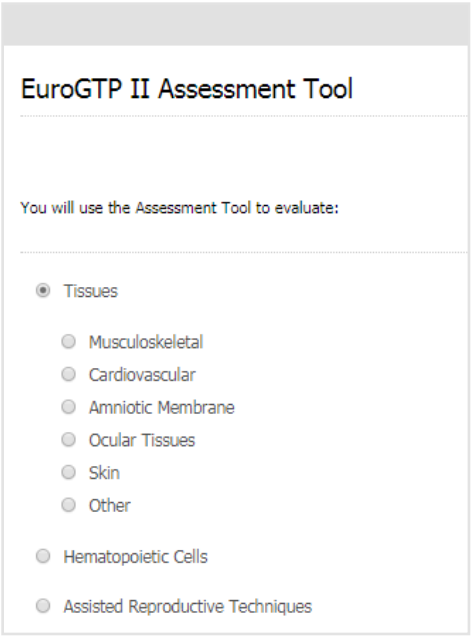

FIG 4.1: Diagram of IAT: different types of tissues

If selecting Tissues, you will be asked to choose a specific anatomical type of Tissue:

- Musculoskeletal
- Cardiovascular
- Amniotic Membrane
- Ocular tissue
- Skin
- Other

4.1. EVALUATION OF NOVELTY (STEP 1)

This section outlines the questions asked when the tool is being used, a brief explanation of the information that the question is intended to elicit, and some examples to demonstrate when novelty may or may not be present, are shown in Table 4.1. below.

When performing this exercise please note the following definitions:

"this type of TCTP" should be interpreted as the type of TCTP (example: Pulmonary valve, Amniotic Membrane, Skin, etc.) aims to ask if despite the novelty your TE has experience handling this TCTP.

"this TCTP" refers to the specific product or therapy under evaluation (Example: Decellularised heart valve, Amniotic Membrane Extract, Demineralised Bone)

Table 4.1: Exercise for assessing novelty

	YES	NO	NA
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?			
Explanation: The purpose of this question is to determine if your establishment has previously banked or provided the specific, anatomical type of TCTP for clinical application. It does not require that this TCTP has been banked using the same process. **Examples:** A1 – Your establishment already banks pulmonary and aortic heart valves, but you intend to start processing them in a different way. In this case, you would answer 'Yes' to this question, and there is no novelty. A2 – Your establishment already banks Achilles tendon, and you intend to start banking peroneus longus tendons. In this case you would answer 'No'; although you already bank tendons, you do not bank this particular anatomical type of tendon, so there is novelty. A3 – Your establishment provides pericardial graft as a dural patch, and you intend to start banking fascia lata for the same purpose. In this case, you would answer 'No'; although the graft is to be used for the same purpose for which you already provide another type of graft, you have not banked this type of tissue previously, so there is novelty.			

	YES	NO	NA
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?			

Explanation:

This question aims to elicit if there may be differences in the TCTP resultant from the donor population. Examples of changes that would create novelty are changing the age limits for donors of the TCTP, or changing specific aspects of the donor selection criteria applicable to the TCTP. Note that this does not apply to generic changes to donor selection criteria; for example if screening requirements for blood borne infections are amended, rather it should be considered when making specific changes to donor selection criteria that impact on specific TCTPs

Examples

B1 – Your establishment wishes to raise the age limit for donors of tendons from 65 to 70. In this case, you are clearly changing your donor population, so you would answer 'No'; there is novelty.

B2 – Your establishment implements routine screening of your donor population for a new tropical virus that has become endemic in your country. In this case, it is a systematic change which will affect donors of all tissues; whilst you may technically be impacting on your donor population by implementing a new test, you are not changing the overall makeup of the donor population. You would therefore answer 'Yes' to this question, and there is no novelty.

	YES	NO	NA
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?			

Explanation:

The question is to determine if a change in the way in which the TCTP is procured from the donor (or patient) may impact on its safety or quality

Examples

C1 – Your establishment currently banks skin allografts, which are procured from donors using an electric dermatome. In order to improve the quality of your grafts, you are proposing to change to a different type of dermatome. In this case, there may be novelty; you would need to take a view, based on your knowledge of the process, as to whether or not this could introduce significant change

C2 – Your establishments currently procure hearts for valve donation from deceased donors within 24 hours of death; you are considering expanding this time limit to 48 hours. In this case, there is definite novelty, as there would clearly be risks relating to contamination of the tissue and deterioration of the tissue quality resulting from the increased post-mortem retrieval time.

	YES	NO	NA
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?			

Explanation:
This question covers a wide range of protocols, essentially covering all processes applied to the graft between retrieval and preservation

Examples:
D1 – Your establishment currently banks tendon allografts which are terminally sterilised with gamma irradiation; you are considering changing to gas plasma sterilisation. There would clearly be novelty here, as you are introducing a novel process which could have significant implications for graft safety and quality.

D2 – Your establishment currently used buffered saline in many of your routine tissue processing protocols. Your current supplier has discontinued this product, and you intend to switch to a new supplier who provides the reagent to the same specification. In this case, there is unlikely to be novelty; you are not proposing to make a change to the fundamental process, just replacing 'like with like'.

	YES	NO	NA
E. Will this TCTP be packaged and stored using a protocol and materials used previously in your establishment for this type of TCTP?			

Explanation:
This question seeks to elicit whether there are any significant changes in how the TCTP is packaged, stored, and distributed prior to transplantation.

Examples:
E1 – Your establishment currently stored bone allografts prior to distribution at -40°C; you are considering changing this to -20°C. In this case, there is novelty as you are making a change that could clearly affect the safety and quality of your grafts.

E2 – Your establishment currently provides morsellised bone allografts in 20g pack sizes. You are considering changing this pack size to 40g. In this case, there is unlikely to be novelty; the change must be one that could significantly affect the quality and/or safety of the graft.

	YES	NO	NA
F. Will this type of TCTP provided by your establishment be applied clinically using an implantation method used previously?			

Explanation:
This question seeks to elicit whether there are any significant changes in how the TCTP is clinically applied.

Example:
F1 – A graft that is being used with an open surgical procedure for implantation is now to be implanted using a minimal invasive technique (e.g. Arthroscopic). You need to consider if the change in the implantation method could impact on the properties/performance of the graft. In this case there is novelty, and your answer would be "No".

F2 – Your TE have been preparing cold storage corneas, and is currently implementing the procedures to prepare "cultured corneas". In this case there is no novelty in the implantation method, and your answer would be "Yes".

	YES	NO	NA
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site before?			

Explanation:
This question seeks to elicit whether the TCTP will be implanted into a different anatomical site to which it has been implanted previously

Examples:
G1 – You have been providing decellularised skin to treat leg ulcers, and the surgeons wish to utilize the graft for breast reconstruction. In this situation the properties required for performance of the graft have changed, you now need to consider if the graft is biomechanically suitable for this indication. In this case there is novelty, and your answer would be "No".

G2 – You have been providing heart valves for transplant, and now your TE aims to prepare decellularised heart valves for the same type of pathology. In this case there is no novelty, because the anatomical site will be the same, and your answer would be "Yes".

4.2. LEVEL RISK ANALYSIS (STEP 2)

Step 2A: Identification of risk factors

If, after completing part 1 of the IAT, you determine that there is some novelty resulting from your proposed change, you should now proceed to step 2 to

identify and quantify the potential risks resulting from this novelty. The risks have been subdivided into 9 factors:

I) Donor Characteristics.

II) Procurement process and environment.

III) Processing and environment.

IV) Reagents.

V) Reliability of Microbiology Testing.

VI) Storage Conditions

VII) Transport Conditions.

VIII) Presence of unwanted cellular material and/or graft vascularity.

IX) Complexity of the immediate pre-implantation preparation and/or application method.

You must first determine which of these risk factors are relevant to the aspect or aspects of your proposed change which result in novelty. Worked examples are provided later in this document to demonstrate how the process works.

Step 2B: Identification of risks

Having identified the appropriate risk factor(s), you should then determine which specific risk consequences are applicable. A standard set of risk consequences is applied to each factor, with an open, 'other' category for any risks not covered in the four main categories.

a) Unwanted immunogenicity

b) Implant failure

c) Disease transmission

d) Toxicity/Carcinogenicity

e) Other

Examples of the combination of risk factors and specific risk consequences that may need to be considered are provided in the table 4.2. The purpose of the exercise is to systematically consider each risk factor and risk consequences in turn against the nature of the change. Note that for certain combinations of risk factor and specific risk, there may be no relevant examples. It is recognised that the IAT cannot anticipate all potential types of risk; the four specific risks consequences listed are those which it is generally agreed will be most commonly related to TCTPs. For any risks not covered by these four categories, an open, 'other' category may be used, and is provided in the IAT.

Table 4.2. Identification and interpretation of the risk factors and risk associated with tissues

	Risks Factors	Examples and Explanation	Risk	Examples and Explanation
Donation	Donor Characteristics	This factor requires that you consider whether the donor population you intend to obtain the TCTP from could impart any risk	Unwanted immuno-genicity	If your TE decide to stop Human leukocyte antigen (HLA) matching your donors for a specific TCTP, you should consider if this could impact in the clinical outcome of the recipient?
			Implant failure	i) If you increase the age of your donor population, this could impact on the quality of your graft? ii) Certain aspects of a donor's medical history may impact on the suitability of certain grafts for transplantation; changes should be considered in this light.
			Disease transmission	If a change is made so that a graft that was previously only obtained from heart beating donors will now be obtained from deceased donors, this may affect the risk of graft contamination and disease transmission?
			Toxicity/Carcinogenicity	This consequence is unlikely to be applicable to this risk factor, however changes in donor selection criteria relating to poisoning for example, may create a risk.
			Other	Consider other risks if applicable.
Procurement	Procurement process and environment	This factor requires that you consider where and how the TCTP, or the material used to manufacture it, are recovered. For example, how long does the process take, how complex is it, and what is quality of the environment?	Unwanted immuno-genicity	Could changes to the procurement process result in elevated quantities of immunogenic material being present in the graft?
			Implant failure	Could changes to the procurement process result in the grafts being damaged during procurement?
			Disease transmission	i) Could changes to the procurement process result in an increased risk of donor-recipient disease transmission? ii) Could changes to the procurement process result in an increased risk of the graft being contaminated with environmental organisms?
			Toxicity/Carcinogenicity	Could any chemicals (e.g. disinfectants) used in the procurement process be transferred to the graft?
			Other	Consider other risks if applicable

	Risks Factors	Examples and Explanation	Risk	Examples and Explanation
Processing/ storing /transport	Processing and environment	This factor requires that you consider where and how the TCTP is processed. For example, how long and how complex is processing, and what is the quality of the processing environment?	Unwanted immunogenicity	Could changes in cleaning or washing protocols lead to the graft retaining more residual donor cell content.
			Implant failure	i) Could the length of the process result in the quality of the graft deteriorating? ii) Could the environmental conditions applied during processing (e.g. heat, pressure, humidity etc. affect the graft quality?
			Disease transmission	Could the length, complexity or environment where processing (e.g. heat, pressure, humidity, etc.) takes place affect the risk of environmental contamination?
			Toxicity/Carcinogenicity	Could the TCTP degrade during processing, generating toxic compounds?
			Other	Consider other risks if applicable
	Reagents	This factor requires that you consider any reagents used during recovery, processing, decontamination and storage of the TCTP. For example, could they damage the TCTP in any way, or could residual traces of reagent remain in the TCTP that could cause toxic or immunogenic effects in recipients?	Unwanted immunogenicity	Could any of the reagents you use of which residual traces could remain in the final product, generate immunogenicity?
			Implant failure	Could any of the reagents alter the essential biomechanical properties of the product?
			Disease transmission	Are quality control procedures applied to reagents sufficient to avoid the risk of contamination?
			Toxicity/Carcinogenicity	Could residual traces of any of the reagents you use remain in the final product, generating toxicity/carcinogenicity?
			Other	Consider other risks if applicable
	Reliability of Microbiology Testing.	This factor requires that you consider the risk that the nature of the TCTP, the testing methodology and/or the presence of residual processing reagents such as antibiotics in the finished TCTP may impact the accuracy of any microbiology tests. Note this refers specifically to bacteriology/ mycology testing of the TCTP, not any blood tests performed on the donor.	Unwanted immunogenicity	It is unlikely this combination of risk and risk factor could occur associated with tissues
			Implant failure	Could undetected microorganisms damage the graft, leading to implant failure?
			Disease transmission	Could undetected microorganisms result in disease transmission?
			Toxicity/Carcinogenicity	It is unlikely this combination of risk and risk factor could occur associated with tissues
			Other	Consider other risks if applicable

	Risks Factors	Examples and Explanation	Risk	Examples and Explanation
Processing/ storing /transport	Storage Conditions	This factor requires that you consider any potential risks arising from how the starting material and TCTP are stored, between procurement and processing, during processing, and between processing and implantation.	Unwanted immunogenicity	Changes in storage temperature may preserve immunogenic factors more effectively
			Implant failure	Consider how storage conditions (e.g. temperature, time) may impact on the important properties of the graft.
			Disease transmission	Consider how storage conditions (e.g. temperature, time) impact on the risk of the graft being contaminated due to for example, changes in the primary packaging.
			Toxicity/Carcinogenicity	Could, packaging material degrade due to time and/or temperature, generating toxic compounds? Or could the graft itself degrade due to storage conditions?
			Other	Consider other risks if applicable
	Transport Conditions	This factor requires that you consider any potential risks arising from how the starting material and TCTP are transported, for example between the sites of procurement and processing, and between the sites of storage and implantation.	Unwanted immunogenicity	Changes in transport temperature/time may preserve immunogenic factors more effectively
			Implant failure	Consider how transport conditions (e.g. temperature, time) may impact on the properties of the graft.
			Disease transmission	Consider how transport conditions (e.g. temperature, time) impact on the risk of the graft being contaminated due to for example, changes in the primary packaging.
			Toxicity/Carcinogenicity	Could solutions, packaging material, or the graft itself degrade due to transport conditions (e.g. due to changes in the temperature), generating toxic or carcinogenic chemicals?
			Other	Consider other risks if applicable

	Risks Factors	Examples and Explanation	Risk	Examples and Explanation
Product	Presence of unwanted cellular material and/or graft vascularity	This factor requires that you consider the risk that for some TCTPs, the presence of intact vital cells is desirable, although it may also increase risks of, for example, immunogenicity or disease transmission. Consider also if the risk that vascular tissues may be more at risk to infiltration by pathogens or malignant cells than avascular tissues.	Unwanted immunogenicity	Grafts that contain donor material that is not intended to be present may be more immunogenic
			Implant failure	Could donor cell material impact on the clinical performance of the graft, perhaps by delaying integration?
			Disease transmission	Consider if the presence of donor cells could increase the risk of transmission of intracellular viruses, or malignancy. The degree of tissue vascularity may also increase the risk that the tissue could harbour donor derived infections.
			Toxicity/ Carcinogenicity	It is unlikely that this risk factor could apply to this risk, however each situation must be considered on a case by case basis.
			Other	Consider other risks if applicable
Clinical application procedure	Complexity of the pre-implantation preparation and/or application method	This factor requires that you consider how complexity the method of pre implantation will be for this TCTP. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?	Unwanted immunogenicity	Consider if the pre-implantation preparation procedure (e.g. washing of the graft immediately before implantation) is sufficiently robust to ensure that immunogenic reagents or donor derived components present in the graft are removed prior to implantation.
			Implant failure	Consider the complexity of the pre-implantation and application methods and how critical are these for the clinical performance of the graft. Are they complex and potentially liable to error?
			Disease transmission	Consider if the pre-implantation and application methods may increase the risk of disease transmission due to the length and complexity of the procedures (e.g. long period of exposure to the environment during the preparation and implantation)
			Toxicity/ Carcinogenicity	Consider if the pre-implantation preparation procedure (e.g. washing of the graft immediately before implantation) is sufficiently robust to ensure that reagents or donor derived components present in the graft that could cause toxicity/carcinogenicity, are removed prior to implantation.
			Other	Consider other risks if applicable

Step 2C: Quantification of risks consequences

When the risk factors and the potential risk consequences have been identified, the potential impact of this risk analysis needs to be determined according to the definitions presented in section 3.4 (and summarized in Annex IV).

By entering the information into the IAT users will generate a report detailing the assessment performed, which will include the identification and quantifications of individual risks consequences, the *Final Risk Score*, and risk classification (detailed algorithm is described in the Annex V).

Step 2D: Assessment of risk reduction

Having calculated probability, severity and detectability, and thus an overall risk based on 'internal' knowledge and data, it may be possible to adjust this score by taking into account other external sources of information. This external data is not used to specifically reduce probability, severity or detectability, rather it is used to calculate a general reduction in the overall risk. (More details related with risk reduction are described in the section 3.4 of this guide)

4.3. INTERPRETATION OF THE OUTCOMES OF THE RISK ANALYSIS AND DEFINITION OF EXTENT OF STUDIES NEEDED BASED ON THE RISKS QUANTIFIED (STEP 3)

The *Final Risk Score* informs the overall level of risk inherent in the TCTP. Based on this, further actions to reduce risk may or may not be necessary as described in the table 4.3.

Step 3A: Risk reduction strategies – Use pre-clinical studies (***in vitro*** and ***in vivo***) to mitigate the identified risks

If the *Final Risk Score* is "low", "moderate" or "high" further studies may be performed, if not already done, to provide additional information to re-evaluate the level of risk (using step 2).

Additional guidance to facilitate the implementation of Step 3A (Risk reduction strategies) is provided in Annex VI. In this annex information is provided for each type of tissue in the form of matrices that can be used to select *in vitro* and *in vivo* tests appropriate to mitigate the risk previously identified in step 2.

The methodology proposed by EuroGTP II, suggests this be done by reference to matrices and tables. The matrices suggest a number of different test criteria, which are again specific for different types of TCTP, each of which are also subdivided into specific tests. It then suggests which of these tests could be applied to address specific risk consequences (Annex VI).

Tests listed in the matrices of Annex VI are for guidance only and not intended to be an exhaustive list of mandatory tests.

Step 3B: Definition of extent of clinical evaluation

In situations when the risks cannot be further reduced with pre-clinical studies, the TCTP may be used in humans subject to authorization by the CA, with the provision that appropriate clinical evaluation protocols (monitoring, follow up or evaluation appropriate to the level of remaining risk) are put in place.

Table 4.3. Extent of studies** according the level of risk determined in the assessment***

Level of Risk	Proposed studies extent
NEGLIGIBLE:	**Step3A: Risk reduction strategies** • The assessment indicates that the TCTP is safe and efficacious for clinical use and very unlikely to cause harm to recipients. You should conduct a validation of the process, if not already done. If the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application.
	Step 3B: Step 3B: Extent of clinical evaluation • Adverse reaction and event (SARE) reporting.

***** (Process Validation, Pre-clinical in vitro and in vivo Studies, and Clinical Evaluation)

Level of Risk	Proposed studies extent
LOW	**Step3A: Risk reduction strategies** • The TCTP is safe and efficacious for clinical use and unlikely to cause harm to recipients. A validation of the process, if not already done, should be performed. If the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. *Please refer to Annex VI for additional details.*
	Step 3B: Extent of clinical evaluation • Serious adverse reaction and event (SARE) reporting; • Feedback from immediate post transplant monitoring (routine clinical follow up) may be collected for a defined period or number of procedures. • *Clinical audit*[******] may also be used after an appropriate period of use. *Please refer to Annex VI for additional details.*
MODERATE	**Step3A: Risk reduction strategies** • The assessment indicates that more evidence is needed to support safe and effective use of this TCTP and mitigate risk. Process validation should be performed, however if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. • Pre-clinical *in vitro* evaluation, specific to the identified risks, should be performed if not already done. • Pre-clinical *in vivo* evaluation, specific to the identified risks, using an animal model should be done if applicable (and if not already completed). *Please refer to Annex VI for additional details.*
	Step 3B: Step 3B: Extent of clinical evaluation • A structured plan for active collection of a specific set of data relating to the safety and efficacy of the TCTP should be put in place, in addition to routine clinical follow up. Ethical approval may be required and the principles of Good Clinical Practices (GCP)[15] adhered to. • Consideration should be given to restricting provision of the TCTP to a limited number of patients and/or centres until the risks have been adequately mitigated. *Please refer to Annex VI for additional details.*

****** In the context of this guide clinical audit refers to retrospective or prospective evaluation of routinely collected clinical data.

Level of Risk	Proposed studies extent
HIGH	**Step3A: Risk reduction strategies** • The assessment indicates that significantly more evidence is needed to support safe and effective use of this TCTP and mitigate risk. • Process validation should be performed, however if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. • Pre-clinical in vitro evaluation, specific to the identified risks, should be performed if not already done. • Pre-clinical *in vivo* evaluation, specific to the identified risks, using an animal model should be done if applicable (and if not already completed). *Please refer to Annex VI for additional details.*
	Step 3B: Extent of clinical evaluation • The TCTP should only be used clinically in the context of an ethically approved, controlled (where applicable) clinical evaluation until the residual risks have been adequately mitigated. The principles of GCP[15] must be adhered to. • Clinical evaluation and follow up programs should be implemented and safety and efficacy must be continuously monitored. If available national and international registries are recommended for gathering follow up data. *Please refer to Annex VI for additional details.*

A worked example demonstrating the whole process from novelty assessment to the definition of extent of studies is provided in the Annex VII.

05

Hematopoietic Stem Cell Specific Chapter – Specific guidance for the use of EuroGTP II methodologies and tools

Define which type of TCTP you are evaluating

At first it is important to define for which TCTP you are going to use the tool, as this will generate specific risk factors. In case of Hematopoietic Stem Cells (HSC), choose which type of cells is the subject of the process under evaluation.

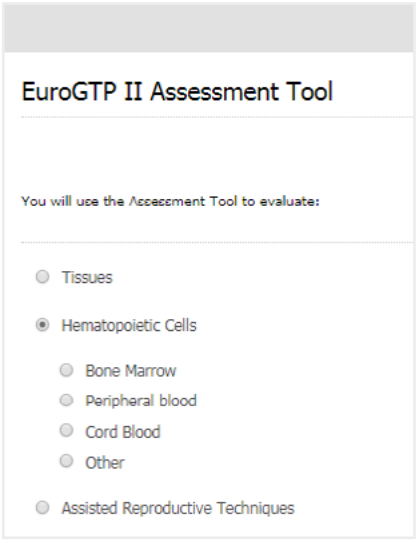

Figure 5.1.: Diagram of IAT: different types of HSC

If selecting HSC, you will also be asked to choose a specific type of Cells under evaluation:

- From Bone Marrow
- From Peripheral Blood
- From Cord Blood
- From other sources

5.1. EVALUATION OF NOVELTY (STEP 1)

This chapter presents the questions as asked when the tool is being used, a brief explanation of what the question is intended to elicit, and some examples to demonstrate when novelty may or may not be present. The questions as they appear in the IAT are shown in Table 5.1 below.

Table 5.1: Exercise for assessing novelty

	YES	NO	NA
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?			

Explanation:

The purpose of this question is to determine whether your institution has previously prepared, and issued the specific, anatomical type of TCTP in clinical application for a specific indication. It does not require that the TCTP has been issued and administered before for a different indication.

Examples:

A1 – Your establishment is already performing T-cell depletion on hematopoietic grafts, but you intend to revise the processing. In this case you would answer 'Yes' to this question, and there is no novelty.

A2 – Your establishment is performing hematopoietic stem cell transplantation (HSCT) using bone marrow (BM) and peripheral stem cell (PBSC) grafts. It is decided to start a cord blood transplantation programme. In this case you answer 'No'; you have no experience in handling and issuing cord blood.

	YES	NO	NA
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?			

Explanation:

This question aims to elicit possible differences in the characteristics of the TCTP caused by a change in the donor population. Examples of changes that would create novelty are changing the age limits for donors of the TCTP, or changing specific aspects of the donor selection criteria applicable to the TCTP. Note that this does not apply to generic changes to donor selection criteria; for example if screening requirements for blood borne infections are amended. It rather should be considered when making specific changes to donor selection criteria that has an impact on specifications of the TCTP's.

Examples:

B1 – Your establishment wishes to raise the age limit for donors of hematopoietic stem cells from 70 to 75. In this case, you are clearly changing your donor population, so you would answer 'No'; there is a novelty.

B2 – Your establishment implements routine screening of your donor population for a new virus that has become endemic in your country. In this case, it is a systematic change which will affect donors of all tissues; whilst you may technically have an impact on your donor population by implementing a new test, you are not changing the overall makeup of the donor population. You would therefore answer 'Yes' to this question, and there is no novelty.

B3 – Your organisation decides to start immunization of donors prior to stem cell donation. This is a specific change directed at the immune system of donor and recipient, which will result in change to your donor population characteristics. You would answer 'No' to this question; there is a novelty.

	YES	NO	NA
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?			

Explanation:
The question is to determine whether a change in the way in which the TCTP is procured from the donor (or patient) may impact on its safety or quality.

Examples:
C1 – Your establishment is currently administering filgrastim (G-CSF) for the mobilization of hematopoietic stem cells in donors. It is decided to start using a biosimilar for this purpose. In this case, there may be a novelty; because the nature of the cells and composition of the graft could have been changed in a way that it influences the quality and efficacy.

C2 – Your establishment decides to change the apheresis kits/system from brand A to brand B. Both devices have CE marking for collection for stem cells and are used in other establishments. The collection technique is based on the same principles. This is not a novelty , because the procedure has shown to be suitable for the purpose and the technique is not new in your hands

	YES	NO	NA
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?			

Explanation:
This question covers a wide range of protocols, essentially covering all processes applied to the graft between retrieval and preservation.

Examples:
D1 – Your establishment currently stores autologous PBSC grafts in liquid nitrogen storage, after controlled-rate freezing. You are considering changing to mechanical freezing and storage. There would clearly be novelty here, as you are introducing a novel process which could have significant implications for graft safety and quality.

D2 – Your establishment currently uses buffered saline in many of your routine cell processing protocols. Your current supplier has discontinued this product, and you intend to switch to a new supplier who proved the reagent to the same specification. In this case, there is unlikely to be a novelty; you are not proposing to make a change to the fundamental process, just changing 'like with like'.

	YES	NO	NA
E. Will this TCTP be packaged and stored using a protocol and materials used previously in your establishment for this type of TCTP?			

Explanation:
This question seeks to elicit whether there are any significant changes in how the TCTP is packaged and stored, and distributed prior to transplantation.

Examples:
E1 – Your establishment currently transports BM grafts by room temperature. You are considering to change the procedure and transport all HPC-BM and A products cooled (4-10°C). There would clearly be a novelty, as you are making a change that could affect the safety and quality of your grafts.

E2 – Your establishment is adding a tempex box to protect the stem cell bag during transport. There is no novelty because the box does not influence the essential characteristics of the product.

	YES	NO	NA
F. Will this type of TCTP provided by your establishment be applied clinically using an implantation method used previously?			

Explanation:
This question seeks to elicit whether there are any significant changes in how the TCTP is clinically applied.

Examples:
F1 – Your establishment currently administers cord blood stem cells intravenously. It is considered to start direct intra-bone infusion. In this case there is a novelty, the safety and efficacy of the changed method has to be proved.

F2 – Your establishment has infused cord blood from related donors. They consider to start using cord blood from unrelated donors. There is no novelty in the infusion method, and your answer would be 'Yes'.

	YES	NO	NA
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site before?			
Explanation: This question seeks to elicit whether there are any significant changes in how the TCTP is clinically applied. **Examples:** G1 – Your establishment currently provides the TCTP for patients suffering from hematological malignancies via intravenous infusion. It is considered to start a programme for the use of this TCTP for cardiovascular repair by direct infusion into affected areas of the heart muscles In this case you answer is 'No', there is a novelty G2 – Your establishment currently provides stem cells for hematological malignancies via intravenous infusion. It is considered to start a program to treat patients with hemoglobinopathies. Stem cells are administered via intravenous infusion. Your answer would be 'Yes', there is no novelty.			

5.2. LEVEL RISK ANALYSIS (STEP 2)

The 2nd exercise aims to determine the risk associated with the novelties attenuated in the process being evaluated.

Every modification in the processes associated with the donation, procurement, testing, processing, storage, and distribution of TCTP may have potential consequences for the quality of these products and safety of recipients. Moreover, different levels of novelties represent different risks and distinct impact on the quality and safety of the tissue and cell products. The evaluation of the different levels of these risks can be performed using the methodology proposed in the current rationale.

Step 2A: Identification of risk factors

At first, the risk factors associated with the changes in the process are selected. There are nine risk factors that could apply to HPC when hematopoietic cells are concerned (table 5.2).

You must first determine which of these risk factors are relevant to the aspect or aspects of your proposed change which result in novelty. Worked examples are provided later in this document to demonstrate how the process works.

Step 2B: Identification of risks consequences

Having identified the appropriate risk factor(s), you should then determine which specific risk consequences are applicable. A standard set of risk consequences is applied to each factor, with an open, 'other' category for any risks not covered in the four main categories.

a) Unwanted immunogenicity

b) Engraftment failure

c) Disease transmission

d) Toxicity/Carcinogenicity

e) Other

Examples of the combination of risk factors and specific risk consequences that may need to be considered are provided in the table 5.2. The purpose of the exercise is to systematically consider each risk factor and risk consequence in turn against the nature of the change. Note that for certain combinations of risk factor and specific risk consequences, there may be no relevant examples.

Table 5.2. Identification of the risk factors and risks associated with HSC

	Risks Factors	Explanation	Risk	Examples
Donation	Donor Characteristics	Consider whether the novelty in your process has an impact at the moment of the donation. This factor requires that you consider whether the donor population you intend to obtain the TCTP from, could cause any risk for the recipient	Unwanted immunogenicity	Could adjustment of donor selection criteria (age, HLA match grade), induce (severe) Graft versus Host Disease?
			Engraftment failure	Could increasing the age of the donor population impact the quality of the graft? Could certain aspects of a donor's medical history impact the number of HPCs before transplantation?
			Disease transmission	Is the risk for transmission of infectious diseases increased if you accept donors who travelled in endemic areas
			Toxicity/Carcinogenicity	Could the use of a new type of bag to collect the graft induce toxicity?
			Other	Will the use of a new apheresis device affect the number of hematopoietic progenitor cells collected?
Procurement	Procurement process and environment	Consider where and how the TCTP is recovered currently and whether the changes proposed with the novel method changes recovery time, complexity, quality of the environment? For example, how long does the process take, how complex is it, and what is how does the procurement devices affect the quality of the HPC?	Unwanted immunogenicity	Could changes to the procurement process result in elevated quantities of immunogenic material being present in the graft?
			Engraftment failure	Could the use of new hematopoietic growth factors affect the composition of the graft, and resulting in poor engraftment?
			Disease transmission	Could changes to the procurement process result in an increased risk of donor-recipient disease transmission?
			Toxicity/Carcinogenicity	Could any chemicals (e.g. disinfectants) used in the procurement process be transferred to the graft?
			Other	Does a different collection needle influence the number of specific type of cells?

	Risks Factors	Explanation	Risk	Examples
Processing/ storing /transport	Processing and environment	Consider the current processing method for the TCTP how the novelty in processing can affect the product. How long does the novel preparation process take and how complex is it – this may have an impact on the risk of contamination, or cell characteristics that may not be consistent with product specifications. Also consider the quality of the processing environment, which may also affect the risk of contamination.	Unwanted immunogenicity	Could the process change lead to the introduction of unwanted cellular components.
			Engraftment failure	Could the length of the process result in the quality of the graft deteriorating? Could the environmental conditions applied during processing (e.g. temperature, pressure, humidity) affect the graft quality?
			Disease transmission	Could the length, complexity or environment where the processing takes place affect the risk of environmental contamination Could changes to the processing result in an increased risk of the graft being contaminated with environmental organisms?
			Toxicity/ Carcinogenicity	Could the TCTP degrade during processing, generating toxic compounds?
			Other	Can the devices use in the processing influence the quality of the HPC?
	Reagents	Consider any reagents used during recovery, processing, decontamination, and storage of the TCTP. Could they damage the TCTP in any way, or could residual traces of reagent remain in the TCTP that could cause toxic or immunogenic effects in recipients.	Unwanted immunogenicity	Could change of cryoprotectant induce an unwanted immunogenic reaction?
			Engraftment failure	Could change of cryoprotectant affect engraftment?
			Disease transmission	Could the use of reagents lead to decontamination of the graft?
			Toxicity/ Carcinogenicity	Could the use of reagents cause toxic effects in the recipient?
			Other	Could the use of reagents cause any other effects in the recipient?

	Risks Factors	Explanation	Risk	Examples
Processing/ storing /transport	Reliability of Microbiology Testing.	Consider the risk that the testing methodology and / or presence of residual processing reagents such as antibiotics in the finished TCTP may impact the accuracy of any microbiology/ mycology testing of the TCTP. This risk factor is not about blood tests on the donor.	Unwanted immunogenicity	Could the residual presence of antibiotics lead to anaphylactic/allergic reactions?
			Engraftment failure	Could the reaction to the presence of microbiological agents lead to non-engraftment of rejection of the graft?
			Disease transmission	Could the change of processing medium mask a positive outcome of current microbiology testing?
			Toxicity/Carcinogenicity	Could unwanted presence of toxin producing bacteria cause reaction in the recipient?
			Other	Consider other risks if applicable
	Storage Conditions	Consider any potential risks arising from how the starting material and TCTP are stored, between procurement and processing, during processing, and between processing and implantation.	Unwanted immunogenicity	Can a change in the plastics of primary packaging cause enhanced immunogenic material in the grafts
			Engraftment failure	Could the storage temperature affect the viability of the cells?
			Disease transmission	Could the storage temperature affect the risk of contamination?
			Toxicity/Carcinogenicity	Can the cryoprotectant cause toxic reactions in the recipient of the graft?
			Other	Could storage conditions cause any other risk to the recipient?
	Transport Conditions	Consider any potential risks arising from how the starting material and TCTP are transported, for example between the sites of procurement and processing, and between the sites of storage and implantation.	Unwanted immunogenicity	Unlikely that this factor could apply risk.
			Engraftment failure	Can the duration of the shipment influence the number of relevant cells present in the graft ?l
			Disease transmission	Could the duration of the transport induce the risk of contamination?
			Toxicity/Carcinogenicity	Could transport conditions (e.g. heavy shaking) lead to damage of the packaging and chemical contamination of the product.
			Other	Can shaking and mechanical movements caused by a new transport method hamper the integrity of the packaging?

	Risks Factors	Explanation	Risk	Examples
Product	Presence of unwanted cellular material.	Consider the risk of s the presence of inactivated cells, debris or cell components which may cause, immunogenicity or disease transmission.	Unwanted immunogenicity	Do centrifugation forces during apheresis cause the presence of cell debris?
			Engraftment failure	Could the presence of inactivated cells lead to engraftment failure?
			Disease transmission	Can the recipient be infected by due to contamination of the cord blood during procurement?
			Toxicity/Carcinogenicity	Unlikely that this factor could apply risk.
			Other	Consider other risks if applicable
Clinical application procedure	Complexity of the pre-implantation preparation and/or application method	Consider how complex the method of transplantation will be for this TCTP. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?	Unwanted immunogenicity	Does the preparation/application of the product involve handling that could cause critical change to the specifications of the final product?
			Engraftment failure	Does the preparation/application of the product involve handling that could cause engraftment failure?
			Disease transmission	Does the preparation/application of the product involve handling that could cause bacterial contamination of the product?
			Toxicity/Carcinogenicity	Does the preparation/application of the product involve handling that could cause introduction of chemical substances?
			Other	Consider other risks if applicable

Step 2C: Quantification of risks consequences

When the risk factors are selected and the potential risks are identified, the potential impact of this risk analysis needs to be determined according to the definitions present in section 3.4 and summarized in Annex IV.

5.3. INTERPRETATION OF THE OUTCOMES OF RISK ANALYSIS AND DEFINITION OF EXTENT OF STUDIES NEEDED BASED ON THE RISKS QUANTIFIED (STEP 3)

Using the EuroGTP II methodologies you will be able to perform a risk analysis, determine the risk profile and the level of risk associated with the novel product, process or procedure. As a result the tools (IAT / EuroGTP II algorithm) will provide the value of the individual risks and the *Final Risk Score* which is proportional to the number of risks evaluated (in the form of a level of risk).

It is important to state that HPC transplant centres should be prepared to invalidate treatment when proven problematic (in terms of safety and effectiveness) even when a novelty of negligible risk was implemented. HPC transplant centres should collect data and register of follow up in a systematic way and make them available to the scientific community regardless of the success of the treatment: not withholding results that point to a negative outcome or that turn out to be inconclusive. Therefore it is important in all processes, regardless of the level of risk, to monitor and register SARE / SAE.

The table below provides general guidance on the follow up studies needed in term of the level of risk determined (adjusted according to Provoost V. et al. 2014).

Table 5.3.– Generic review of Extend of Studies needed

Level of Risk	Extend of Studies needed
NEGLIGIBLE	**Step3A: Risk reduction strategies** A change in process could have a negligible level of risk because it is part of a therapy or procedure that is considered as established or standard. In this case multi-centred studies (ideally Randomized Controlled Trial (RCT)) are published in peer-reviewed journals and the procedures are performed according to a validated and/or standard protocol. **Minimal process validation** is needed. The technical performance of staff should be monitored and comparable with other TE or published studies, therefore standard Key Performance indicators (KPI) should be monitored on the technical quality of the staff performing the procedures. Dropping KPIs indicating protocol drift must lead to investigation of both the procedural steps and / or the possibility to re-train staff.
	Step 3B:Extent of clinical evaluation A **routine/safety follow up program** (e.g. EBMT Patient Registry[23]) is sufficient as the good practices states. Follow up procedures should be focused on assessing efficacy, comparing the clinical follow up with the results obtained before the implementation of the change in the process. Long-term (ideally trans-generational) health effects, including aspects such as fertility, oncology and mental health should be monitored.
LOW	**Step3A: Risk reduction strategies** Implementing a standard procedure or treatment in an HPC centre that has never performed this procedure exerts an **intensive validation**. Training of staff (as required by Joint Accreditation Committee ISCT-Europe & EBMT (JACIE)) is necessary in order to reach the outcomes published in scientific literature. A learning curve might be expected and should be part of the validation report. When implementing the procedure, additional quality controls must be performed to **monitor Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs)**. For example, when a TE is switching from T-cell depletion (TCD) to CD34+_selection (which they never performed before), engraftment rate, and graft versus host reactions should be carefully monitored.
	Step 3B:Extent of clinical evaluation A **safety follow up program** is necessary. Follow up procedures (conform EBMT Med-A, Med-B or Med-A cellular) should be focusing on assessing efficacy, comparing the clinical follow up with the results obtained before the implementation of the change in the process and in relation to the results published in scientific literature. The expected learning curve should be kept as short as possible and put in relation to the follow up program. Likewise, established techniques are prone to long-term (ideally trans-generational) follow up of the health effects, as established by EBMT.

Level of Risk	Extend of Studies needed
MODERATE	**Step3A: Risk reduction strategies** Novel procedures or treatments that exert a moderate risk and are considered innovative. The treatment has shown proof of principle and there is reassuring data in literature in terms of both safety and effectiveness at least in animal studies and pre-clinical data shows normal engraftment or response. The studies that have published this data should have a sound methodology and published in peer-reviewed journals. In order to implement an innovative treatment, an **enhanced validation** is necessary including and a range of **additional quality controls** performed to monitor Critical Process Parameters (CPPs), Critical Quality Attributes (CQAs), and the impact of the implemented TCTP should be carefully monitored. Since reassuring data of this innovative treatment is already available, a more specific monitoring of the published critical parameters can be performed instead of a registration of all critical parameters.
	Step 3B:Extent of clinical evaluation **Clinical evaluation and follow up programs**, conform the EBMT Patient Registry[23] should be implemented to assess reassuring mid-term safety (3 months up to life-long post transplantation including data on psychological wellbeing). These data collections should refer to patients undergoing the procedure as well as the donors where applicable.
HIGH	**Step3A: Risk reduction strategies** A new procedure can be offered to patients in an experimental design aiming at showing proof of principle, short-term safety and/or effectiveness. An **extensive validation** including and a range of **additional quality controls** performed to monitor Critical Process Parameters (CPPs), Critical Quality Attributes (CQAs), and the impact of the implemented changes is required. This extensive validation should include: **Non clinical studies**: preferably there should be studies showing the experimental procedure is safe in animals. **Pre-clinical Studies:** when experimental treatments encompass a laboratory phase, then at least the viability of cells should be looked at in detail, monitored and registered.
	Step 3B:Extent of clinical evaluation **Follow up program:** experimental treatments should only be offered to a selected and limited patient cohort and these patients should be clearly informed on the experimental status and should receive information about (the lack of knowledge about) possible risks, alternative treatments etc. ORHAs should only offer experimental treatments or treatments based on experimental procedures after approval by a commission of medical ethics.

A worked example demonstrating the whole process from novelty assessment to the definition of extent of studies is provided in the Annex VIII.

Step 3A: Risk reduction strategies – Use pre-clinical studies (*in vitro* and *in vivo*) to mitigate the identified risks

If the *Final Risk Score* is "low", "moderate" or "high" further studies may be performed, if not already done, to provide additional information to re-evaluate the level of risk (using step 2)

Additional guidance to facilitate the implementation of Step 3A (Risk reduction strategies) is provided in the form of matrices that can be used to select *in vitro* and *in vivo* tests appropriate to mitigate the risk previously identified in step 2.

The matrices suggest a number of different test criteria, which are again specific for different types of TCTP, each of which are also subdivided into specific tests. It then suggests which of these tests could be applied to address specific risk consequences (Table 5.4. and 5.5.).

Tests listed in the matrices are for guidance only and not intended to be an exhaustive list of mandatory tests.

Table 5.4. Pre-clinical evaluation – Examples of in vitro tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that might be used to address the respective risk consequences)

		Immuno-genicity		Engraft-ment failure	Toxicity/ Carcinogenicity			Disease transmission	
Criteria	**Specific test**	Systemic Im-mune response	Anaphylaxis	Engraftment failure	Cytotoxicity	Carcinogenicity	Teratogenicity	Blood borne infections	Infections acquired during procurement or processing
Sterility	Test for the presence of microbiological agents (According to JACIE Standards)			■				■	■
	Review environmental monitoring			■					■
	Stability (According to JACIE Standards)			■					
	Validation of test suitability (of all analytical methods applied)	■	■	■	■	■	■	■	■
Identity*	Confirmation of product specifications (e.g. HLA, Blood group, genetic markers. JACIE Standards)	■		■					
Purity**	Quantification of the target cells at various stages: Flow cytometry (e.g. CD34+ / CD 45+ cells; or CD 3) to monitor Graft versus host disease (GvHD)	■		■					
	Quantification of the target cells at various stages : Total Nucleated Cell (TNC) count	■							

* Characteristics of a product (HLA, blood group etc)

** Relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product

Criteria	Specific test	Immuno-genicity		Engraft-ment failure	Toxicity/ Carcinogenicity			Disease transmission	
		Systemic Im-mune response	Anaphylaxis	Engraftment failure	Cytotoxicity	Carcinogenicity	Teratogenicity	Blood borne infections	Infections acquired during procurement or processing
Potency***	Viability: apoptosis and/necrosis (e.g. Annexin 5/7 AAD staining or Terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labelling assay (TUNEL); Tripan blue)	■	■	■					
	Functionality: Cytological evaluation leukocytes (diff)	■		■					
	Functionality: CFU in clonogenic assays			■					
	Functionality: Long term culture initiating cell assay			■					
	Functionality: Lymphocytes subsets by flow cytometry			■					
Safety****	Stability Test Packaging: In case of novelty packaging			■					■
	Presence of viruses: To be tested before receipt of material; according to JACIE standards			■				■	
	Residual agents: mass spectrometry, chromatography	■	■	■	■	■	■		
	Residual cell/DNA: Fluorescence in situ hybridization (FISH), cytomorphological evaluations	■		■					

*** The therapeutic activity of a product as indicated by appropriate laboratory tests or adequately developed and controlled clinical data.

****Relative freedom from harmful effects to persons or products

Table 5.5. Pre-clinical evaluation – Examples of in vivo tests to assist in potentially reducing the risk consequences identified (Green cells represent the tests that might be used to address the respective risk consequences)

		Immunogenicity			Graft failure	Toxicity/ Carcinogenicity			Disease transmission	
Criteria	**Specific test**	Systemic Immune response	Localised immune response	Anaphylaxis	Graft failure	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Blood borne infections	Infections acquired during procurement or processing
Repopulation capacity	Immune deficient Mouse / small animal models e.g. cell labelling and imaging techniques									
Stem cell Functionality	Histology sections for immunohistochemistry-based assays (e.g. evaluation of the expression of specific proteins important for cellular function)									
	In vivo functional assessment									
Safety of compounds	Hematopoietic colony forming cell assays									

Step 3B: Extent of Clinical Evaluation

In order to determine safety and efficacy in the clinical application of novelties, evaluation and registration of follow up of outcome of the treatment in patients are necessary. When after preclinical evaluation of risk reducing steps a certain or not well defined risk is remaining, clinical follow up is indicated. Depending on the type of risk remaining to the novel aspects of the stem cell product, specific parameters in patients should be monitored to evaluate the safety and efficacy of the novelty. Currently, outcome of treatment of all stem cell recipients is monitored systematically by clinicians (EBMT members and non-members) by using the EBMT Minimal Essential Data (MED) forms. With this registration of all stem cell treatments via a centralized database, scientific research can be performed to evaluate best practices in the treatment of hematological disorders with HSC. The MED-AB forms cover all relevant items that are required to assess the clinical outcome in patients and to detect adverse reactions and complications ; the registration of variables collected by the MED-A form are essential for each hematopoietic stem cell transplantation. When more detailed aspects need to be monitored to evaluate patient outcome after stem cell transplantation, additional medical parameters can be found in the MED-B form Recently, a special MED-A form for Cell Therapy has come available.

To perform a clinical evaluation of stem cell novelties, the medical aspects mentioned in the MED A form are essential to collect. To guide the evaluation it is recommended that the outcome data are not only used to establish the safety and efficacy of the treatment within the one establishment, but that the data are uploaded in the EBMT Patient Registry[23] as well. The forms can be easily downloaded from the EBMT website. In the following table, an overview is given of the medical aspects that are considered essential for clinical follow up and that are covered in MED-AB forms.

After using the risk assessment tool to determine the level of risk of the application of the novelty, please decide whether a MED-A form would cover the evaluation of the risk, or if you need to complete the more extended MED-B form. Aspects that are not covered in the form can be collected using elements of the MED-A Cell Therapy form (see Clinical Evaluation and Follow up Cell Therapy tables), although the novelties that are covered by this guide are not cellular therapies.

Table 5.6 - Clinical evaluation and follow up - Hematopoietic Stem Cells: Bone Marrow, Peripheral Blood, Cord Blood, as stated in EBMT Minimal Essential Data forms

Test category	Detailed investigational options	EBMT – Form
Recovery & Graft performance	Absolute neutrophil recovery	MED **A** form 100 d
	Platelet reconstitution	MED **A** form 100 d
	Date of last platelet transfusion	MED B form 100 d
	Early graft loss	MED **A** form 100 d
	Hematopoietic chimaerism	MED B form 100 d
	Treatment for Early graft loss or non-recovery	MED B form 100 d
Acute GvHD	Maximum grade	MED **A** form 100 d
	Stage	MED **A** form 100 d
Treatment immediate post trx	Growth factors	MED B form 100 d
	Additional Cell infusions	MED **A** form 100 d
	Cell therapy (specified)	MED **A** form 100 d
Cell Therapy	Source of cells (auto/allo)	MED **A** form 100 d
	Type of cells	MED **A** form 100 d
	Chronological number of infusion	MED **A** form 100 d
	Indication	MED **A** form 100 d
	Number of infusions within 10 weeks	MED **A** form 100 d
Additional Disease Treatment	Yes/no	MED **A** form 100 d
	Reason (prophylaxis; relapse)	MED **A** form 100 d
	Chemo/drug administered	MED **A** form 100 d
	Radiotherapy	MED **A** form 100 d
Complications within the first 100 days	Infection related complications (bacterial, fungal, viral, parasites)	MED B form 100 d
	Systemic Symptoms of Infection	MED B form 100 d
	End-organ diseases	MED B form 100 d
	Documented pathogens	MED B form 100 d
	Non-infection related complications (specify)	MED B form 100 d
Best Response	Best disease status (response) after HSCT	MED **A** form 100 d
	Date of death (< 100d)	MED **A** form 100 d
Chronic GvHD at day 100	no/yes (date onset)	MED **A** form 100 d
	Maximum extent (during this period)	MED **A** form 100 d
	Maximum NIH score (during this period)	MED **A** form 100 d
First relapse / progression	First relapse or progression after HSCT	MED **A** form 100 d

Test category	Detailed investigational options	EBMT – Form
Relapse of leukaemias: method of detection	Clinical/hematological method: increase of blast cell count over 5% in the bone marrow	MED **A** form 100 d
	Cytogenetic method: reappearance of chromosome anomalies detected earlier in history of disease	MED **A** form 100 d
	Molecular method: reappearance of acute leukaemia specific molecular markers detected earlier in the history of the disease	MED **A** form 100 d
	Donor Cell Leukaemia?	MED **A** form 100 d
Disease status at 100 days	Clinical /hematological	MED B form 100 d
	Cytogenetic/FISH	MED **A** form 100 d
	Detection by molecular method	MED **A** form 100 d
Survival Status at 100 days	Alive/dead	MED **A** form 100 d
	Main cause of death	MED **A** form 100 d
	Contributory cause of death	MED **A** form 100 d

Table 5.7 – Clinical evaluation and follow up Cell Therapy, as stated in EBMT Cell Therapy Minimal Essential Data A form

Test category	Detailed investigational options	EBMT - Form
Indication for Cell therapy treatment	Treatment of a primary disease, including infections or infection prevention	MED **A** form 100 d
	Treatment or prevention of complications derived or expected from previous treatment including HSCT	MED **A** form 100 d
	Other:	MED **A** form 100 d
Therapy	Clinical trial	MED **A** form 100 d
	Institutional guidelines / standard treatment	MED **A** form 100 d
	Hospital exemption	MED **A** form 100 d
	Compassionate use	MED **A** form 100 d
	Performance score of patient an initiation of treatment	MED **A** form 100 d
	Cell origin	MED **A** form 100 d
Donor HLA match type	HLA identical sibling (including non-monozygotic twin)	MED **A** form 100 d
	Syngeneic (monozygotic twin)	MED **A** form 100 d
	HLA matched other relative	MED **A** form 100 d
	HLA mismatched relative (degree of mm 1HLA locus mm, ≥ 2 HLA locus mm)	MED **A** form 100 d
	Unrelated donor	MED **A** form 100 d
Cell therapy infusion unit – description & collection	Identification	MED **A** form 100 d
	Tissue Source	MED **A** form 100 d
	Collection procedure (incl. mobilizing agents)	MED **A** form 100 d

Table 5.8– Explanation and examples of the test categories

Test category	Explanation and examples
Cell Therapy Infusion unit - manipulation	Ex-vivo manipulation of the products contained in the cell therapy infusion unit (drugs, gene manipulation, recognition of specific target / antigen, selection, expansion, induced differentiation))
Therapy and cell infusions	Chronological number of cell therapy treatment for this patient
	Primary aim of the cell therapy treatment
	Patient preparative treatment (if yes, specify)
Cell Infusion Episodes	Were there more than once cell infusion episode during this treatment or procedure
	Cell type and number of cells infused
	Did the treatment that includes this cell therapy episode also include other type of treatment?
Response	Best clinical/biological response after the entire cell therapy treatment
	Complications & response
	First relapse or progression or significant worsening of organ function of the primary disease
	Last disease status
Toxicity during first 6 months after cell therapy was initiated	Acute Graft versus Host Disease (maximum grade)
	Chronic Graft versus Host Disease present (maximum extent & NIH score)
	Other complications or toxicities during this period (if yes, specify)
Secondary Malignancy	Did a secondary malignancy, lymphoproliferative or myeloproliferative disorder occur? If yes, donor cell leukemia or malignancy of the cellular product?
Graft assessment	Graft loss
Survival status	Alive / dead
	Main cause of death
	Contributory cause of death
Persistence of the infused cells	Were tests performed to detect the persistence of the cellular products during this period?

06

ART Specific Chapter – Specific guidance for the use of EuroGTP II methodologies and tools

There are 3 steps that need to be completed in order to determine the novelty, risks and extent of studies needed to perform before the process is implemented in the TE.

Define which type of TCTP you are evaluating

Firstly it is important to define for which TCTP you are going to use the tool, as this will generate specific risk factors. In case of ART, choose 'Assisted Reproductive Techniques' and subsequently which type of reproductive TCTP is the subject of the process under evaluation.

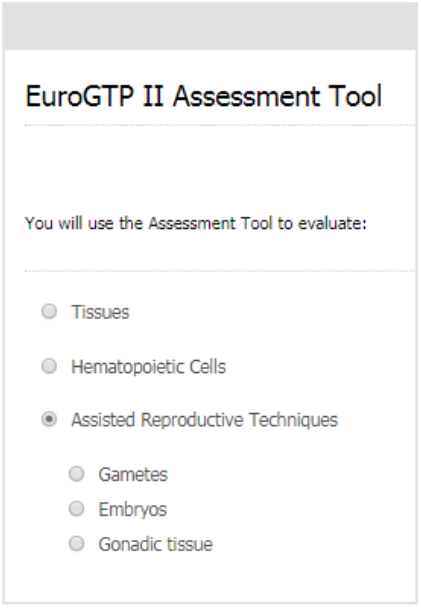

Figure 6.1.: Diagram of IAT: different options for ART

6.1. EVALUATION OF NOVELTY (STEP 1)

Before any risk analysis can be performed it has to be determined if the process change under evaluation consists of a novelty or not. If not, then no further action is needed in addition to the regular follow up of established protocols. If the change in process is indeed a novelty, the risk assessment needs to be performed (Step 2) and the tool will determine the specifics of the follow up needed.

The exercise in Step 1 consists on a set of questions, to determine if the users are facing a novelty. Novelty is present whenever the user answers "no" to at least one of the seven questions.

When facing all positive answers (yes), users are not dealing with any novelty. For this standard/established TCTP, the regular, internal validations and follow up procedures should be put in place/maintained.

One example is used to explain table 6.1. The example used is: vitrification of sperm procured via testicular extraction (TESE), where the standard protocol in your TE was slow freezing.

Table 6.1: Exercise for assessing novelty

	YES	NO	NA
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?			
Explanation: Consider if your TE has previous experience working with the TCTP or not. **Example:** You want to implement vitrification of sperm in your TE, thus the answer to question A would be YES: you have previously prepared sperm and issued it for clinical use			

	YES	NO	NA
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?			
Explanation: Consider if the starting material is from the same donor population or not. **Example:** You want to implement vitrification of sperm in your TE, thus the answer to question B would be YES: the starting material is from the same donor population			

	YES	NO	NA
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?			
Explanation: Consider the starting material and how it is procured or collected and if this changes in the novel protocol or therapy. **Example:** You want to implement vitrification of sperm in your TE, thus the answer to question C would be YES: the starting material is procured using the same procedure. There is no change in TESE protocol, only the cryopreservation method is different.			

	YES	NO	NA
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?			

Explanation:
Consider the complete processing procedure of the product. If changes occur in the new protocol or therapy, answer the question accordingly.

Example:
You want to implement vitrification of sperm in your TE, thus the answer to question D would be NO: there are indeed changes in processing and preservation of the sperm when vitrification will be introduced in comparison to the standard slow freezing protocol currently used.

	YES	NO	NA
E. Will this TCTP be packaged and stored using a protocol and materials used previously in your establishment for this type of TCTP?			

Explanation:
Consider if changes occur in the packaging and storage and if you have experience with these items in your TE for the specific cell or tissue product where the novelty is introduced.

Example:
You want to implement vitrification of sperm in your TE, thus the answer to question E would be NO if there are changes in the type of packaging in the case that straws will be used for the vitrified sperm instead of vials.

	YES	NO	NA
F. Will this type of TCTP provided by your establishment be applied clinically using an implantation/application method used previously?			

Explanation:
Consider if product or therapy has been clinically applied previously and answer accordingly.

Example:
You want to implement vitrification of sperm in your TE, thus the answer to question F would be YES: there is no difference in clinical application for the sperm

	YES	NO	NA
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site before?			
Explanation: Consider the clinical application of the product and answer the question concerning the intended anatomical site of implantation or transplantation. **Example/Explanation:** The clinical application of vitrified sperm is the same as slow frozen sperm, so the answer would be YES in this example. In another example this question will be answered 'NO' if e.g. heterotopic transplantation of ovarian tissue strips is a new protocol, where previously only orthotopic transplantations were performed in your clinic.			

When having answered 'NO' to one of these questions, the level of risk must be determined in STEP 2.

6.2. LEVEL RISK ANALYSIS (STEP 2)

The 2nd step of the exercise aims to determine the risk associated with the novelties attenuated in the process being evaluated.

Every modification in the processes associated with the donation, procurement, testing, processing, storage and distribution of cells and tissues may have potential consequences for the quality of these products and safety of recipients and the corresponding offspring in ART.

Moreover, different levels of novelties represent different risks and distinct impact on the quality and safety of the tissue and cell products. The evaluation of such risks could be performed using the methodology proposed in the current rationale.

Step 2A: Identification of risk factors

At first select the risk factors associated with the changes in the process. There are 8 risk factors that could be applicable to changes in processes concerning gametes and embryos or ART treatments and 9 risk factors that could apply to ART when gonadic tissues are concerned. Definitions for the correct interpretation of risk factors and examples can be found in table 6.2.

Step 2B: Identification of risk consequences

Then, when a risk factor is applicable, potential risk consequences must be considered and the probability scored. The potential risk consequences must be considered in comparison with the TCTP prior to the implementation of novelty. When selecting the potential risk consequence, it is important to think of the potential harm that the novelty may cause to the recipients, the resulting child and/or the impact on the availability and accessibility of treatment. It is important to note that the risk consequences are **not** about the viability of the embryo. For example, if the viability of a blastocyst could be harmed because of a novel biopsy procedure, then the risk factor loss of viability and/or functionality should be chosen. However the quantification of the potential risks consequences should be assessed bearing the patient and thus the recipient in mind. So, the question to be asked is: would there be unexpected immunogenicity in the recipient when this damaged embryo would be transferred? Would there be implant failure or pregnancy loss? Would there be a risk of disease transmission in this patient? Some examples are given to explain the risks:

Potential risks associated with the clinical use of ART tissue and cell products are:

- **Unexpected immunogenicity:** This is only applicable for gonadic tissue and this option will also only appear if gonadic tissues are selected at the start of the risk assessment.
- **Implant failure and/or pregnancy loss:** for ART, this risk is self-explanatory. Additionally, also the loss of a batch of gametes or of embryos requiring an extra treatment for the patient should also be considered under this risk.
- **Disease transmission (including infection)**: Consider if the novelty in the TCTP has a potential risk of introducing disease transmission or infection in the recipient.
- **Toxicity / Carcinogenicity :** Consider if the novelty in the TCTP can introduce toxicity reactions in the recipient or even if there is a risk for carcinogenicity
- **Other:** Consider other risks associated with the changes in TCTP and score them accordingly. It is very important to make use of this category as many of the above stated risks might seem not attributable to ART recipients since gametes and embryos are clinically applied in a very specific way having other risks than tissues and cells being transplanted into recipients. As an example: the risk for complication in the recipient like pelvic inflammatory disease in the recipient could be a potential risk when certain novelties are introduced in ART.

In order to have a complete overview of the combination of risk factors and risk consequences, a complete table with examples is given (Table 6.2). It is important to note that not all risk factors apply to changes in protocols and procedures, likewise, not all risks consequences apply to a risk factor. For the ease of interpretation, the explanation of the risks is based on the example:

Table 6.2. Combined table of the Identification of the risk factors and the associated risks

	Risk factors	Examples and Explanation	Risks	Examples and Explanation
Donation	Donor Characteristics	Consider if the novelty in your process or procedures changes donor characteristics and if these changes could impart a risk to the recipient. *Examples:* • *Change in collecting sperm from peripubertal boys (12y-14y) to collecting sperm from pubertal boys (>14y)* • *Change from autologous to allogeneic donors: If the TCTP is sourced from an allogeneic donor, there may be risks that immunogenicity could impact on the clinical performance of the TCTP, and risks of disease transmission*	Unwanted immunogenicity	Not applicable for this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	Consider and quantify the risk that sperm collected from peripubertal boys might lead to pregnancy loss when used in assisted reproduction
			Disease transmission	Although highly unlikely consider and quantify the risk that sperm collected from peripubertal boys might lead to disease transmission in the recipient. In the example of the Southern European donors, if the donors would be from a specific area where the incidence of certain viral diseases would be known, then this might impact on the risk for disease transmission.
			Toxicity/ Carcinogenicity	It is highly unlikely that this change in donor characteristics would have a risk for toxicity in the recipient. In the case of gonadic tissue that came from a donor with oncological disease, this risk must be taken into account.
			Other	Consider other risks if applicable

	Risk factors	Examples and Explanation	Risks	Examples and Explanation
Procurement	Recovery/Procurement process and environment	Consider where and how the TCTP is collected, procured or recovered, and if this process could have an influence on the TCTP. How long does the process take, how complex is it, and what is quality of the environment *Examples:* • *Change from semen production in the clinic to collection of sperm at the home of the patient and transporting it to the TE.* • *Change to a new type of sterile semen container*	Unwanted immunogenicity	Not applicable for this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	It would be highly unlikely that the use of a new semen container during collection would impact on implant failure.
			Disease transmission	It could be possible that if this new container would be a non-sterile container, that this might influence disease transmission. Although the risk would be rare.
			Toxicity/ Carcinogenicity	Consider the risk that using a new semen container would have on the toxicity or carcinogenicity in the recipient.
			Other	Consider other risks if applicable.

	Risk factors	Examples and Explanation	Risks	Examples and Explanation
Processing/ storing /transport	Processing and environment	Consider where and how the TCTP is prepared. How long does processing take and how complex is it – this may impact on the risk of contamination, or that it may not be prepared to consistent specifications and quality. Also consider the quality of the processing environment, which may also affect the risk of contamination. *Examples:* • *Change from laser assisted hatching on day 3 to day 5 for trophectoderm biopsy.* • *Change from performing Intracytoplasmic sperm injection (ICSI) outside of the laminar flow hood to compared to doing ICSI enclosed in a hood.*	Unwanted immunogenicity	Not applicable for this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	Changing from ICSI in a laminar flow hood to outside of the hood will probably rarely effect pregnancy loss because of changes in the preparation.
			Disease transmission	If this procedure would take place in a different environment where the risk for environmental contamination would be higher, then a risk for disease transmission in the recipient might be impacted.
			Toxicity/Carcinogenicity	It is highly unlikely that the change from day 3 to day 5 laser assisted hatching would introduce toxic compounds in the recipient.
			Other	Consider other risks if applicable.
	Reagents	Consider any reagents used during recovery, processing, decontamination and storage of the TCTP. Could they damage the TCTP in any way, or could residual traces of reagent remain in the TCTP that could cause toxic or immunogenic effects in recipients? *Example:* • *Change to a new cryopreservation medium.* • *Change to a new anaesthetic during oocyte collection*	Unwanted immunogenicity	Not applicable it this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	The change in reagents will unlikely impact on the risk of pregnancy loss.
			Disease transmission	If this new reagent contains for example albumin from a source that is doubtful, then there is a risk for disease transmission to the recipient.
			Toxicity/Carcinogenicity	If this medium contains different types of antibiotics, than this might have an impact on toxicity reactions in the recipient.
			Other	Consider other risks if applicable

	Risk factors	Examples and Explanation	Risks	Examples and Explanation
Processing/ storing /transport	Storage Conditions	Consider any potential risks arising from how the starting material and TCTP are stored, not only after processing and before clinical application, but also in intermediate steps: e.g. between procurement and processing, during processing, and between processing steps. *Examples:* • *Change from storage of stimulation medication at room temperature to a refrigerated storage at 4°C.* • *Change from sperm being stored in liquid nitrogen to storage in the vapour phase.*	Unwanted immunogenicity	Not applicable in this example, only for gonadic tissue
			Implant failure/ pregnancy loss	The change is storage conditions might have a direct impact on implant failure when this preserved sperm would be used for insemination.
			Disease transmission	The change in storage might theoretically have an impact on disease transmission, even though the risk is very rare.
			Toxicity/Carcinogenicity	The impact of the novel storage conditions will, in this example, have very little even no impact on the introduction of toxic compounds.
			Other	Consider other risks in the patient if applicable
	Transport Conditions	Consider any potential risks arising from how the starting material and TCTP are transported, for example between the sites procurement and processing, and between the sites of storage and clinical application *Examples:* • *Change to a new type of dry-shipper for the distribution of frozen sperm to clinical sites* • *Change from only ART treatments from own patients to IVF for satellite patients where oocytes are collected in another clinic.*	Unwanted immunogenicity	Not applicable it this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	New transport conditions might have an impact on pregnancy loss if not adequately controlled.
			Disease transmission	Disease transmission is rarely impacted if only transport conditions are changed.
			Toxicity/Carcinogenicity	Toxicity could be impacted if not only transport conditions are changed, but maybe medium differences are also present between the satellite center and the current TE.
			Other	Consider other risks if applicable

	Risk factors	Examples and Explanation	Risks	Examples and Explanation
Processing/ storing /transport	Reliability of Microbiology Testing (in case of gonadic tissue)	Consider the risk that the testing methodology and / or presence of residual processing reagents such as antibiotics in the finished TCTP may impact the accuracy of any microbiology/mycology testing of the TCTP. This risk factor is not about blood tests on the donor. *Example:* • *Change to a new ovarian tissue processing medium that could mask the current microbiology testing because of the presence of antibiotics.*	Unwanted immunogenicity	This change could have an impact on unwanted immunogenicity when this tissue is transplanted in the recipient.
			Implant failure/ pregnancy loss	This change could lead to implant failure due to residual microbiological load that has an impact on the graft viability.
			Disease transmission	If this change in solely on processing medium, but it is still autologous use of the tissue, then the risk of disease transmission will probably not chance in comparison to the former procedure.
			Toxicity/ Carcinogenicity	There might be a risk for introducing toxic compounds.
			Other	Consider other risks if applicable
Product	Loss of viability and or functionality	Consider the risk that the changes in procedures of processes can have on the viability or functionality of the TCTP *Example:* • *Change from a 2-step cryopreservation protocol to a 5 step protocol.* • *Change from a blastomere biopsy program to a trophectoderm biopsy program. .*	Unwanted immunogenicity	Not applicable it this example, only for gonadic tissue
			Implant failure/ pregnancy loss	This novelty can have a direct impact on implant failure and pregnancy loss when the blastocysts are harmed because of inadequate technical expertise.
			Disease transmission	The impact on disease transmission due to harming of the embryo because of the new biopsy technique is highly unlikely.
			Toxicity/ Carcinogenicity	The loss of viability will probably only have a rare impact on the introduction of toxicity or carcinogenicity in the recipient.
			Other	Consider other risks if applicable

	Risk factors	Examples and Explanation	Risks	Examples and Explanation
Product	**Presence of unwanted cellular material and/or graft vascularity** (in case of gonadic tissue)	This risk must be considered from the perspective that for some TCTPs, the presence of intact vital cells is desirable, although it may also increase risks of, for example, immunogenicity or disease transmission. This presence might affect to tumour formation, immunogenicity and disease transmission risks. Vascular tissues may be more at risk to infiltration by pathogens or malignant cells than avascular tissues *Example:* • *When ovarian tissue autologous transplantation is performed in patients with a history of blood cancer at the moment of tissue procurement. The risk of transmission of malignant cells should be considered.*	Unwanted immunogenicity	Consider the risk that presence of cellular material/graft vascularity could have on unwanted immunogenicity in the recipient.
			Implant failure/ pregnancy loss	There could be a risk for implant failure when malignant cells are present in the graft.
			Disease transmission	If malignant cells are transplanted together with the graft, the there is a risk for the transmission of oncological disease.
			Toxicity/Carcinogenicity	Presence of cells might impact on the risk of carcinogenicity
			Other	Consider other risks if applicable
Clinical application procedure	**Complexity of the pre-implantation preparation and/or application method**	Consider how complex the method of clinical application will be for this TCTP. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be? Low feasibility of application standardization might have influence in the risks of implant failure and disease transmission at least. *Example:* • *Change to a new transfer catheter for clinical application.*	Unwanted immunogenicity	Not applicable it this example, only for gonadic tissue
			Implant failure/ pregnancy loss	There might be an impact on the risk of implant failure when a new transfer catheter is introduced.
			Disease transmission	It is highly unlikely that the risk for disease transmission would be impacted when only a new transfer catheter is implemented.
			Toxicity/Carcinogenicity	A new transfer catheter might, although unlikely, introduce toxicity to the recipient
			Other	Consider other risks if applicable

Step 2C: Quantification of risks consequences

When the risk factors are selected and the potential risk consequences are identified, the potential impact of this risk analysis needs to be determined according the definitions present in section 3.4 and summarized in Annex IV.

Step 2D: Assessment of risk reduction

Having calculated probability, severity and detectability, and thus an overall risk based on 'internal' knowledge and data, it may be possible to adjust this score by taking into account other external sources of information. This external data is not used to specifically reduce probability, severity or detectability, rather it is used to calculate a general reduction in the overall risk score.

Data that should be taken into account when calculating risk reduction may include:

- Published data in peer reviewed literature on the specific changes in the procedures or protocols could be helpful. Additionally guidelines from national and international scientific societies could be a source in information.
- Unpublished data from external sources: it might be interesting to gain information from other ART centres who have experience with the changes that you would like to implement in your processes or procedures.
- Advice and information from external experts: it might be interesting to get in contact with special interest groups of ESHRE to get expert opinions on certain novelties.
- Technical improvements from formal internal validation studies: it could be possible that you have own data from previous validation studies that can be used as retrospective validation data.
- Clinical outcome data from external sources (e.g. registries): national registries might be interesting to have a look at and for global European data, the European IVF Monitoring (EIM) consortium of ESHRE could be contacted (www.eshre.eu/eim).

When calculating the risk reduction factor, it is important that the quality and reliability of the data be considered. For systematic reviews and evidenced based guidelines or recommendations that are based on a solid methodology, the risk reduction can be considered high. For other information, it is important to consider a fair reduction factor and this could be prone to subjectivity.

6.3. INTERPRETATION OF THE OUTCOMES OF THE RISK ANALYSIS AND DEFINITION OF EXTENT OF STUDIES NEEDED BASED ON THE RISKS QUANTIFIED (STEP 3)

Using the tool you will be able to perform a risk analysis, determine the risk profile and the level of risk associated with the novel process or procedure. As a result the tool will provide the *Final Risk Score* and the respective classification as a level of risk. It is important to state that ART centres should be prepared to stop certain treatments when proven problematic (in terms of safety and effectiveness) even when a novelty of negligible risk was implemented. Therefore ART centres should always collect data and register follow up data in a systematic way. Data should be made available to the scientific community regardless of the success of the treatment: not withholding results that point to a negative outcome or that turn out to be inconclusive.[24] It is important in all processes, regardless of the level of risk, to monitor and register SARE / SAE.

The table below gives guidance on the relation of the level of risk in accordance to the clinical evaluation/follow up studies needed (Table 6.3 adjusted according to Provoost V. et al. 2014[24]).

Table 6.3.– Generic Review of Extend of Studies needed

Level of Risk	Extend of Studies needed
NEGLIGIBLE	**Step3A: Risk reduction strategies** A change in process could have a negligible level of risk because it is part of a therapy or procedure that is considered as established or standard. In this case multi-centred studies (ideally RCT) are published in peer-reviewed journal and the procedures are performed according to a validated and standard protocol. **Minimal process validation** is needed. The technical performance of staff should be monitored and comparable with other TE or published studies, therefore standard Key Performance Indicators (KPI) should be monitored on the technical quality of the staff performing the procedures. Dropping KPIs indicating protocol drift must lead to investigation of both the procedural steps and / or the possibility to re-train staff.
	Step 3B:Extent of clinical evaluation A **routine/safety follow up program** is enough as the good practices state. Follow up procedures should be focused on assessing efficacy, comparing the clinical follow up with the results obtained before the implementation of the change in the process. Long-term (ideally trans-generational) health effects, including aspects such as fertility, oncology and mental health should be monitored.

Level of Risk	Extend of Studies needed
LOW	**Step3A: Risk reduction strategies** Implementing a standard procedure or treatment in an ART centre that has never performed this procedure exerts an **intensive validation**. Training of staff is necessary in order to reach the outcomes published in scientific literature. Having a mentor/mentee relationship with an ART centre having experience is highly recommended. Specifications on performance should be determined and when these limits are met by training on spare tissues and cells, staff can be authorized for performing the procedure. A learning curve might be expected and should be part of the validation report. When implementing the procedure, additional quality controls must be performed to **monitor Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs)**. For example, when a TE is switching from IVF to ICSI (which they never performed before), fertilisation rated, and damage rates etc. of embryos should be carefully monitored in relation to the staff performing the procedure.
	Step 3B:Extent of clinical evaluation A **safety follow up program** is necessary. Follow up procedures should be focused on assessing efficacy, comparing the clinical follow up with the results obtained before the implementation of the change in the process and in relation to the results published in scientific literature. As the procedure or treatment encompasses an established or standard technique. The expected learning curve should be kept as short as possible and put in relation to the follow up program. Likewise, established techniques are prone to long-term (ideally trans-generational) follow up of the health effects. TE or ORHA implementing an established technique shall perform long-term follow up and could base their follow up items on the mentor facility. This way of working could lead to periodic evaluation of performance in the mentor/mentee relationship.
MODERATE	**Step3A: Risk reduction strategies** Novel procedures or treatments that exert a moderate risk and are considered innovative. The treatment has shown proof of principle and there is reassuring data in literature in terms of both safety and effectiveness at least in animal studies and pre-clinical data shows normal embryology development. The studies that have published this data should have a sound methodology and published in peer-reviewed journals. In order to implement an innovative treatment, an **enhanced validation** is necessary including and a range of **additional quality controls** performed to monitor Critical Process Parameters (CPPs), Critical Quality Attributes (CQAs), and the impact of the implemented changes on gametes, embryo's and gonadic tissue should be carefully monitored in the pre-clinical studies. Since reassuring non-clinical data of this innovative treatment should at least be already available, a more specific monitoring of the published critical parameters can be performed instead of a registration of all critical parameters.
	Step 3B:Extent of clinical evaluation **Clinical evaluation and follow up programs** should be implemented to assess reassuring mid-term safety (3 months up to 5 years post-delivery including data on psychological wellbeing) and these studies should refer to patients undergoing the procedure as well as the children born from it.

Level of Risk	Extend of Studies needed
HIGH	**Step3A: Risk reduction strategies** A new procedure can be offered to patients in an experimental design aiming at showing proof of principle, short-term safety and/or effectiveness. An **extensive validation** and a range of **additional quality controls** performed to monitor Critical Process Parameters (CPPs), Critical Quality Attributes (CQAs), and the impact of the implemented changes is required. This extensive validation should include: **Non clinical studies**: preferably there should be studies showing the experimental procedure is safe in animals. **Pre-clinical Studies:** when experimental treatments encompass a laboratory IVF phase, then at least the structural integrity of the gametes, embryos or gonadic tissue should be looked at in detail, monitored and registered. Clinical embryology data should indicate a normal cleavage embryo morphology and blastocyst formation.
	Step 3B:Extent of clinical evaluation **Follow up program:** experimental treatments should only be offered to a selected and limited patient cohort and these patients should be clearly informed on the experimental status and should receive information about (the lack of knowledge about) possible risks, alternative treatments etc. ORHAs should only offer experimental treatments or treatments based on experimental procedures after approval by a commission of medical ethics.

The purpose of step 3 is to provide users with guidance as to how to evaluate and mitigate the risks through an application of specific tests. This section is purely informative and far from complete.

Process validation

Process validation studies can be very helpful in tackling risks when novelties are addressed in procedures. Additional quality controls and monitoring of certain process indicators is critical. There are some reports in literature where ART process indicators can be found: The alpha consensus report on indicators concerning cryopreservation processes[25] and the Vienna consensus report on ART laboratory performance indicators[26].

When performing process validation studies, it is important to set out specific parameters that should be monitored and results that should be met. There is vast variety of test that can be carried when process validation studies are performed. The novelty being introduced in the process and the risk factors and risk consequences identified will determine which test to be used. As an example: fertilization rates, embryo cleavage patterns, blastocyst formation rates, packaging sealing tests when novel containers are introduced, cryopreservation survival rates when new steps in cryopreservation programs are introduced.

Pre-clinical In vitro studies

When novelties are introduced in ART, a variety of in vitro testing can be performed: Microscopic observations can be helpful in determining the morphological integrity of the gametes and embryos, the cell viability can be assessed by Live/Dead assays, DNA fragmentation assays, immunohistochemical testing of e.g. markers for apoptosis or proliferation can be informative in certain studies or analysing certain secreted factors in vitro cultures. Depending on the changes and novelties introduced, it is important to perform certain pre-clinical *in vitro* studies.

Pre-clinical in vivo studies

If possible, animal models should be used to verify safety of highly novel TCTP in ART. Although animal models can be helpful, it is known that the results cannot always be translated to the human. At least proof of principle should be shown in animal studies.

Clinical evaluation protocols

Clinical evaluation/ follow up should address clinical key performance indicators. Unfortunately at the moment there is no consensus on these parameters in ART. However, pregnancy rates, miscarriage rates, foetal abnormalities, delivery rates, health of the child born, complication in the patients after clinical application are possible parameters to take into account. Other tests can also be helpful in follow up the patients: checking the thrombotic response in patients, looking at local immunological responses upon transplantation of gonadic tissue e.g. in the latter case, resumption of regular menstruation and ovulation is of importance to verify a successful graft. In summary, the general wellbeing of the patients and the child born from novel ART treatments should be addressed.

Worked examples demonstrating the whole process from novelty assessment to the definition of extent of studies are provided in the Annex IX.

07

Tissue & Cell Database

The Tissue and Cells (T&C) database aims to be a compendium of tissues/cells products, preparation processes, applications and therapies.

7.1. PURPOSE OF THE T&C DATABASE

The purpose of the European *T&C database* is to promote the safe and effective use of TCTPs, by the provision of data related to the products and therapies available, and references relating to their efficacy.

The structure and contents of the database were defined in order to ensure its consistency, harmonize the characterization of TCTPs, and support the collection of efficacy and quality data associated with the clinical use of SoHO at European level.

The *T&C Database* was designed to be appropriate for the needs of:

- TEs and those engaged in the quality control and design of pre-clinical studies and clinical evaluation of TCTP;
- End users / ORHAs;
- CAs.

The distribution of TCTP between European Member States is a common practice, and the exchange of scientific and clinical information promotes the assessment of safety and efficacy of novel and traditional SoHO's therapies.

The aim of this tool is to provide structured and systematic information regarding TCTPs implemented by the TEs, and include an overview regarding new TCTPs, information on clinical application and references to available efficacy and safety data.

This information contained in the *T&C Database* intends to:

- Collate references and evidence relating to safety and efficacy data;
- Encourage stakeholders/CAs to accept the validity of data generated for products in other countries (harmonization of practices)
- Promote collaboration amongst TEs, encouraging multicenter collaborations for the development of novel TCTPs;
- Promote the accessibility for patients, by promoting knowledge amongst clinicians regarding the availability of TCTPs.

The data included in the *T&C Database* was voluntarily shared by TEs, with the intention of contributing to the knowledge base associated with novel and well established TCTPs within Europe.

This data should be periodically reviewed and updated by experts nominated by the European scientific associations that collaborated with the EuroGTP II Project: EBMT, EATB, EEBA and ESHRE. This review aims to ensure that the data is trustworthy and up to date, and to avoid redundant entries.

7.2. GENERAL PRINCIPLES

The *T&C Database* is a registry of TCTP consisting of information provided by European TEs.

TEs are encouraged to register information associated with clinical evaluation studies performed to determine the safety and efficacy of the TCTP distributed.

Furthermore, this information promotes dialogue between the European CAs and TEs seeking collaborations and sharing of expertise and information.

In order to assure consistency and scientific reliability, the EuroGTP II project has defined the principles and procedures required for the correct inclusion and interpretation of data submitted to *T&C Database*.

The technical guidelines (instructions and definitions) to correctly complete, submit and review data, are part of the current document and intend to assist users and contributors.

The principles applied should be periodically revised by experts in the future: strategies foreseeing this purpose will be defined in cooperation with the scientific associations.

TEs contributors should provide sufficient information to ensure that the data included is robust, comprehensive and evidence based. Data relating to authorisation status should also be provided.

Whilst the products entered by the TE are already listed in EU Coding Platform, the *T&C Database* provides additional information related with processing and clinical use and novel products / therapies, which were not part of the EU Platform. Each record includes a summary description and information about the current status of the TCTP with regard to clinical uses, risks assessed and authorizations status

Some TCTP entries may include the number of recipients already treated on an annual basis, the number of patients defined for the clinical evaluation studies, and cross references to the Notify Library (optional information).

7.3. ACCESSING THE T&C DATABASE:

The contents of the *T&C Database* are publicly accessible (http://db.goodtissuepractices.site/).

Three different levels of accesses were defined in order to achieve an appropriate security level, and allow the correct management of database contents (Table 7.1):

Table 7.1 – Users profiles of the T&C database

Level of Access:	Credentials holders	Functionalities:
Administrators	Hosts of *T&C Database*	Can view, add, edit and delete contents in the database
User	Members of the Experts' Committees defined by the Scientific Associations	Can view, add, and edit contents in the database
Guest	General public – free access	Can view contents in the database

7.4. INTRODUCTION OF DATA:

The introduction of data will be entered on a voluntary basis by the TEs and supervised/peer reviewed by experts nominated by the Scientific Associations (more details will be defined in the *GTP's Management Model*) that will promote the use of this database

7.5. DESCRIPTION OF CONTENTS

M – Mandatory field | **OP** – Optional field

Table 7.2. – Contents used to describe TE in the T&C Database

	Field Name	Description	Observations
M	EU TE Code + TE Name	(2 letters 6 Numbers) + Full Name of TE	Data iimported from *EU Coding Platform*
M	Country	Name of Country + ISO code (2 letters code of ME)	Data iimported from *EU Coding Platform*
M	City	Name of city	Data iimported from *EU Coding Platform*
OP	Website	Link	-

During the design and implementation, the information was directly imported from *EU Coding Platform*, this is an accredited source of information provided by the CAs of the different Member States. New TEs, or organisations authorized after the implementation of the *T&C Database*, will be added manually by the database's administrator, after confirming the authorization status in the *EU Coding Platform*.

Information related with TE's authorisation status require confirmation with *EU Coding Platform*, as there may be a delay with the information related with authorisations revoked.

Table 7.3. – Contents used to describe Products and Processes in the T&C Database

	Field Name	Description
M	**Product ID**	**EUTC Code and Name** **(Primary Key (PK))**
M	SoHO Class	Tissue/Cells/ART (Select Option)
M	Product Type	Amniotic Membrane/Cardiovascular/Ocular/Other Membranes/Mature cells/MSK/Progenitor Cells/ Skin/ Embryo /Oocyte /Ovarian Tissue/ Sperm /Testicular Tissue (Select Option)
OP	Product Sub classification	Adipose / Cardiovascular, Valves / Cardiovascular, Vessels / Mature Cell, Hepatocyte / Mature Cell, Keratinocyte / Mature Cell, Pancreatic Islet Cells/ Mature Cell, T Cell (DLI) / Mature Cells, MNC (DLI) / Membrane, Amniotic/ Membrane, Dura Mater/ Membrane, Fascia Lata / Membrane, Fascia Rectus / Membrane, Pericardium/ Musculoskeletal, Bone / Musculoskeletal, Cartilage / Musculoskeletal, Tendon & Ligament / Neuronal / Ocular / Other / Parathyroid / Progenitor Cell, Hematopoietic, Bone Marrow / Progenitor Cell, Hematopoietic, Cord Blood / Progenitor Cell, Hematopoietic, PBSC / Progenitor Cell, Hematopoietic, Unspecified / Reproductive, Embryos/Zygotes / Reproductive, Oocytes / Reproductive, Ovarian / Reproductive, Sperm / Reproductive, Testicular / Skin / Umbilical Cord (Tissue

	Field Name	Description
M	Product Name	Open text – (Product name given by the TE)
M	Product Characteristics	Open text – (Main characteristics/specifications of the product, defined by the TE)
OP	Donor/Recipient Relationship	Allogeneic (postmortal donors)/ Allogeneic (living donors)/ related / unrelated / Autologous
OP	Specific Donor Criteria	Open text (Optional) (Donor selection criteria applied, over and above EUCTD requirements)
OP	Collection/Recovery Method	Default Ejaculated Extracted (optional only for ART)
OP	Additive Solution	Describes additives introduced during the processing of the product. Text (optional)
OP	Pathogen Reduction	No pathogen reduction / Not specified / Antibiotics / Combined process / ETO / No pathogen reduction / Peracetic acid / Radiation sterilization/ Other (optional)
OP	Storage Solution	Open text (Optional)
OP	Preservation	Not specified/default / Cryopreserved / Dehydrated / Freeze dried / Frozen / Glycerol (high conc) / Refrigerated / Solvent dehydrated (optional)
OP	Other Info: (Storage Temperature; Storage requirements after issue and/or Shelf life from donation/ after issue)	Open text (Optional)
OP	Update (innovation and changes)	Open text (Optional)
M	Date of authorization of process and/or product	Date

Table 7.4. – Contents used to describe Clinical indications and associated information, in the T&C Database

	Indication	
M	Classification of Diseases	Code (1 letter + 2 digits) – Optional (http://apps.who.int/classifications/icd10/browse/2016/en)
OP	Supplementary information – Clinical Indications	Open text – details of clinical indication Users may choose to follow ICD10 detailed classification: Optional (http://apps.who.int/classifications/icd10/browse/2016/en)
M	Level of Risk – IAT Level	Result given by EuroGTP II IAT – Evaluation made by TEs Select from: Negligible/low; moderate; high; Not performed (authorised prior to EuroGTP II)
M	Risk Assessment Date	When was the risk assessment performed – DD/MM/YYYY
OP	Bibliographic References	Open text, allows to add links or/and references
OP	Notify references	Relevant Codes of Notify Library or Links

7.6. CODES USED:

- EU TE ID codes and Product ID Code –SEC Platform
- Classification of Diseases – http://apps.who.int/classifications/icd10/browse/2016/en
- Notify Library

7.7. STRUCTURE OF DATA

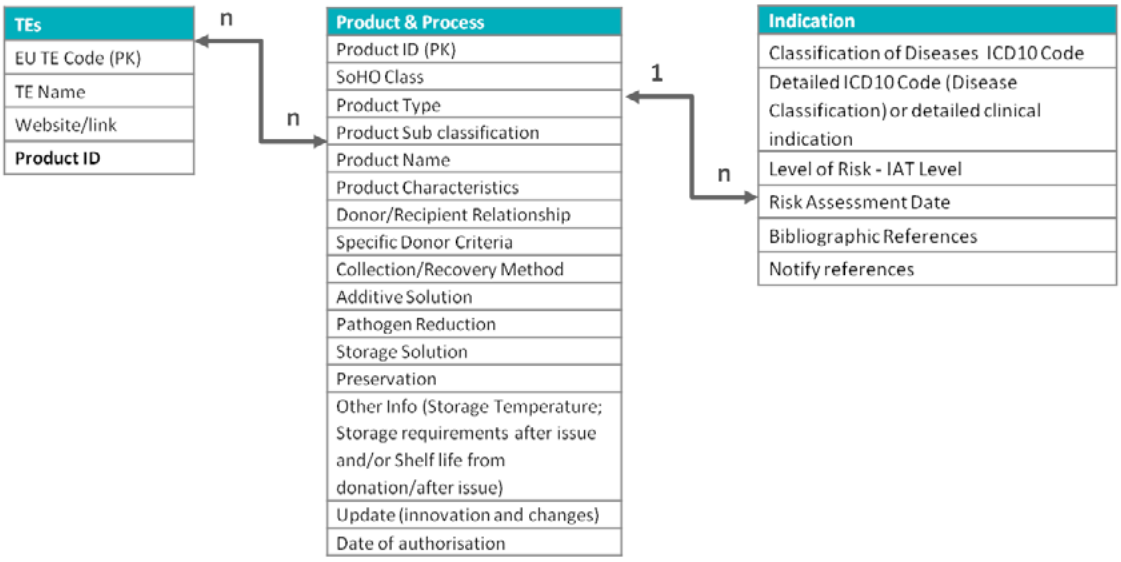

Figure 7.1.: Database Scheme / Entity Relationship Diagram

7.8. SEARCHES S AND PRACTICAL USE OF THE DATABASE:

As mentioned above, the database may be used to search for products and therapies made available by different TEs in Europe.

In principle, one TE can register several different TCTPs, and the same TCTPs can be prepared and distributed by several different TE in Europe.

Different users may find it useful to perform different searches depending on their interests and goals. Examples:

- TEs may want to know who in Europe is preparing a particular TCTP in order to establish a collaboration or gather scientific references;
- When CAs intent to search for references of TCTPS previously authorised in other Member States, but implemented for the first time by national TEs;
- Surgeons may search for new TCTP options to treat specific pathologies;

These examples were used to validate the functionality of the *T&C Database*.

08

Definitions*

*Unless stated otherwise, the definitions of this guide follow the definitions of EUTCD2,4,5,6,7 or proposed in 3rd Edition of the EDQM; Council of Europe. Guide to the Quality and Safety of Tissues and Cells for Human Application12, prior EU funded projects1,10,11 , or are new definitions proposed by EuroGTP II project.

Adverse event: Any untoward occurrence associated with the procurement, testing, processing, storage or distribution of tissues and cells. (See also: serious adverse event.)

Adverse reaction: Any unintended response, including a communicable disease, in the donor or the recipient that is associated with the procurement or human application of tissues and cells. (See also: serious adverse reaction.)

Allogeneic: Refers to cells and tissues donated by one person for clinical application to another person.

Allograft: Tissues or cells transplanted between two genetically different individuals of the same species.

Apheresis: Medical technique in which peripheral blood of a donor or patient is passed through an apparatus that separates out one particular constituent.

Assisted reproductive technologies (ART): All treatments or procedures that include the in vitro handling of human oocytes, spermatozoa or embryos for establishing a pregnancy.

Autologous: Cells or tissues removed from and applied in the same person. In ART, the terms 'autologous donors' and 'autologous use' apply to cases of preservation of fertility.

Best practice: A method or technique that has consistently shown results superior to those achieved with other means, and that is used as a benchmark.

Cells: Individual human cells or a collection of human cells when not bound by any form of connective tissue.

Clinical audit: *A process for monitoring standards of clinical care to see if it is being carried out in the best way possible (known as 'best practice'). Clinical audit can be described as a systematic 'cycle'. It involves measuring care against specific criteria, taking action to improve it if necessary, and monitoring the process to sustain improvement.*[27] (In the context of this guide clinical audit refers to retrospective or prospective evaluation of routinely collected clinical data.)

Clinical data: Information concerning safety or performance that is generated from the use of tissue or cells' (T&C) product and is sourced from the following: clinical investigation(s) of the T&C product concerned, clinical investigation(s) or other studies reported in scientific literature, of a T&C product for which equivalence to the T&C product in question can be demonstrated, reports published in peer reviewed scientific literature on other clinical experience of either the T&C product in question or a T&C product for which equivalence to the T&C product in question can be demonstrated , clinically

relevant information coming from post application surveillance, in particular the clinical follow up (definition adapted from Regulation (EU) 2017/745 [27]).

Clinical Evaluation/Follow up study: For the purposes of this document this term refers to *monitoring predefined clinical outcome indicators to evaluate quality, safety and efficacy/effectiveness of the blood, tissue or cell product for a predefined number of patients.*

Clinical evidence: *Clinical data and clinical evaluation results pertaining to a device of a sufficient amount and quality to allow a qualified assessment of whether the device is safe and achieves the intended clinical benefit(s), when used as intended by the manufacturer[27].*

Competent Authority (CA): Organisation(s) designated by an EU Member State as responsible for implementing the requirements of Directive 2004/23/EC.

Contamination: Accidental inclusion or growth of harmful micro-organisms, such as bacteria, yeast, mould, fungi, virus, prions, protozoa or their toxins and by-products. Contamination is different from colonisation, which is the natural, biological presence of micro-organisms.

Cord blood bank: Is a specific type of tissue establishment where hematopoietic progenitor cells collected from the placental and umbilical cord blood vessels are processed, cryopreserved and/or stored. It may also be responsible for procurement, testing or distribution.

Critical: Potentially having an effect on the quality and/or safety of or having direct contact with the cells and tissues.

Cross contamination: Transfer of micro-organisms from one material to another.

Cryopreservation: Preservation and storage of viable tissues and cells (including gametes and embryos) to preserve viability, either by freezing or vitrification, or alternatively (to extend their viable life) by low-temperature storage.

Cryoprotectant: A chemical compound that is able to protect cells and tissues against freezing injury. Also used as a compatible solute tolerated in high concentrations by cells and tissues for cryopreservation by vitrification.

Deceased donor: A person declared to be dead according to established medical criteria and from whom cells, tissues or organs have been recovered for the purpose of human application.

Decontamination: The process of removing or neutralising contaminants.

Distribution: Transportation and delivery of tissues or cells intended for human application.

Donation: Donating human tissues or cells intended for human applications.

Donor: Every human source, whether living or deceased, of human cells or tissues.

Efficacy/effectiveness: Presence of desired (clinical) effects depending on the mode of action of the product.

Embryo: Pre-implantation, reproductive tissue resulting from the combination of oocyte and sperm.

End user: A healthcare practitioner who undertakes human application procedures

Ethics committee: An independent body established in a Member State in accordance with the law of that Member State and empowered to give opinions for the purposes of this Regulation, taking into account the views of laypersons, in particular patients or patients' organisations[27].

Final Product: Any tissue or cell preparation intended to be transplanted or administered after the final release step.

Follow up: Subsequent examinations of a patient, living donor or recipient, for the purpose of monitoring the results of the donation or transplantation, care maintenance and initiating post-donation or post-transplantation interventions.

Gamete: Mature human germ cell, whether oocyte or sperm.

Good practice: A method or technique that has consistently shown results superior to those achieved by other means and which is currently used as a benchmark.

Graft: Part of the human body that is transplanted in the same or another person to replace a damaged part or to compensate for a defect.

Hematopoietic Stem Cells (HSC): Primitive hematopoietic cells capable of self-renewal as well as maturation into any of the hematopoietic lineages, including committed and lineage-restricted progenitor cells, unless otherwise specified and regardless of tissue source. Also referred to as 'hematopoietic progenitor cells'.

Human application: The use of tissues or cells on or in a human recipient and extracorporeal applications.

Implantation/grafting: The process of inserting a piece of tissue or cells into a recipient.

Informed consent: A person's voluntary agreement, based upon adequate knowledge and understanding of relevant information, to donate, to partici-

pate in research or to undergo a diagnostic, therapeutic or preventive procedure.

Non-partner donation: Means that the donor is another person apart from the couple.

Novelty: Any change that could significantly affect the quality and/or safety of the TCTP and/or the safety of recipients.

Organisations responsible for human application (OHRA): A healthcare establishment or unit of a hospital or another body that carries out human application of human tissues or cells.

Packaging: Packaging, including primary and secondary packaging, aims to protect tissues and cells and to present them to the operator (starting or in-process packaging) or to the clinical user (final packaging) in a suitable manner.

Partner donation: Means the donation of reproductive cells between a man and a woman who declare that they have an intimate physical relationship.

Patient: In ART, relates to individuals or couples seeking treatment.

Preservation: The use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of cells or tissues.

Process: A series of related actions to achieve a defined outcome.

Processing: All operations involved in the preparation, manipulation, preservation and packaging of tissues or cells intended for human applications.

Procurement Organisation (PO): Means a health care establishment or unit of a hospital or another body that undertakes the procurement of human tissues and cells and that may not be accredited, designated, authorised or licensed as a tissue establishment.

Procurement: A process by which tissue or cells are made available.

Qualification: According to EU GMP, the action of proving that any equipment works correctly and actually leads to the expected results. More generally, qualification is applied to the inputs to a process, i.e. equipment, facilities, materials and software (and their suppliers), as well as operators and the relevant written procedures.

Quality: Totality of characteristics of an entity that bear on its ability to satisfy stated and implied needs. Consistent and reliable performance of services or products in conformity with specified standards.

Randomised control trial (RCT): A study in which samples or subjects are allocated at random into groups, called the 'study' and 'control' groups, to receive or not receive an experimental therapeutic intervention.

Recipient: Person to whom human tissues, cells or embryos are applied.

Recovery or Retrieval: The procedure of removing cells, tissues or organs from a donor for the purpose of transplantation or assisted reproduction.

Reproductive cells: Means all tissues and cells intended to be used for the purpose of assisted reproduction.

Risk assessment: Identification of potential hazards with an estimation of the likelihood that they will cause harm and of the severity of the harm should it occur.

Safety: Relative risk: proportional difference from a suggested baseline value.

Serious adverse event (SAE): Any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for patient or which might result in, or prolong, hospitalisation or morbidity. In addition, the definition of SAE includes the total loss of germinal tissues, gametes or embryos for one cycle and any mix-up of gametes or embryos.

Serious Adverse Reaction (SAR): An unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity. The definition of SAR should be extended to the offspring in the case of non-partner donation, only for cases of transmission of genetic diseases.

Severity: Directive 2006/86/EC defines serious as: fatal, life-threatening, disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity. EuroGTP II project follows the grading system for severity has been agreed and is presented in the SoHO V&S project[10].

Single arm study/trial: Sample of individuals with the targeted medical condition is given the experimental therapy and then followed over time to observe their response.[28]"

Storage: Maintaining the tissues and cells under appropriate controlled conditions until distribution.

Surveillance: Systematic collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for

assessment and public health responses, as necessary.

T&C Supply chain: *The sequence of processes and activities involved in the donation, procurement/retrieval, processing, testing, transport, preservation, storage, distribution and application of T&C*

Tissue Establishment (TE): A tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or distribution of human tissues and cells are undertaken. It may also be responsible for procurement or testing of tissues and cells. In the field of ART, TE applies to establishments performing ART activities: ART centres, ART laboratories, sperm banks, etc.

Tissue: All constituent parts of the human body formed by cells; An aggregate of cells joined together by, for example, connective structures which perform the same particular function, e.g. ovarian tissue.

Toxicity: Degree to which a substance can damage an organism.

Transplantation: The transfer (engraftment) of human cells, tissues or organs from a donor to a recipient with the aim of restoring function(s) in the body.

Transport: To transfer or convey tissues and cells from one place to another.

Validation: Establishing documented evidence that provides a high degree of assurance that a specific process, piece of equipment or environment will consistently produce a product meeting its predetermined specifications and quality attributes; a process is validated to evaluate the performance of a system with regard to its effectiveness based on intended use.

Vigilance: An alertness or awareness of serious adverse events, serious adverse reactions or complications related to donation and clinical application of cells, tissues and organs involving an established process at a local, regional, national or international level for reporting.

09

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Annex I

Partners and Experts of EuroGTP II Project

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— Annex II —

Template Form: Characterization of TCTP

Name/of TCTP	
Brief summary	Highlighting the significant proprieties of the TCTP and/or clinical applications under study main differences with related SoHO products Justification for the implementation of change, including the key benefits of the innovation..
TCTP Characterization	Main characteristics of the TCTP/Critical attributes of TCTP. Example: (How is the TCTP processed; what, if any, changes have been made to the established preparation or treatment protocol?; number of cells, structural characteristics), origin of TCTP (autologous/allogeneic), excipients or other reagents or residues could be transplanted with the TCTP (such as carriers or preservants), and description of novelty (if applicable)
Clinical application	In what format is it presented for clinical application? (e.g. need to add solutions, cut, etc) What, if any, excipients or other reagents or residues could be transferred through the clinical application with the TCTP (such as carriers or preservatives)?
Identification of risks	Description of the possible risks and adverse reactions anticipated based on prior experiences and risk assessment
Clinical indication(s) of the TCTP	
Minimal Follow up data required to assess safety and efficacy	
SARE monitoring and report	Communication procedures with the TEs
Information on prior pre-clinical evaluations	Brief description of essays and results obtained in validation studies and quality controls, performed before issuing the novel TCTP for clinical application.
Date	

—Annex III—

Template forms: Methodologies for Assessing the Risks associated to novel TCTP.

Methodologies for Assessing the Risks
associated to novel Tissue and/or Cellular Therapies/Products (TCTPs).
-Tissues Template-

Please follow the EuroGTP II Guide in order to correctly evaluate your TCTPs

TCTP characterization

The evaluation of the level of novelty and the risks associated, should start with a characterization of the novel process or TCTP.

Tissues

- ☐ Musculoskeletal
- ☐ Cardiovascular
- ☐ Amniotic Membrane
- ☐ Ocular Tissues
- ☐ Skin
- ☐ Other

Name of the product, therapy or process under evaluation:

Description of TCTP under evaluation:
(Describe the relevant aspects of the TCTP, detailing the modifications/novelties associated with **donation**, **processing** and **clinical application** under evaluation)

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-Tissues Template-

Step 1

The purpose of this exercise is to evaluate our proposed methodology for determining if a TCTP, therapy or process is novel or not.

Please answer the following questions in order to determine if the product or process is novel. This process represents the first stage of the overall procedure for evaluating novelty and risk.

	Yes	No	Not Applicable/ Not Relevant
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?			
Justify:			
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?			
Justify:			
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?			
Justify:			
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?			
Justify:			
E. Will this TCTP be packaged and stored using a protocol and materials used previously in your establishment for this type of TCTP?			
Justify:			
F. Will this type of TCTP provided by your establishment be applied clinically using an implantation method used previously?			
Justify:			
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site before?			
Justify:			

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Donor Characteristics

Consider whether the donor population you intend to obtain the TCTP from could impart any risk, for example if the TCTP is sourced from an allogeneic donor, there may be risks that immunogenicity could impact on the clinical performance of the TCTP, and risks of disease transmission'

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Procurement process and environment

Consider where and how the TCTP is collected, procured or recovered, and if this process could have an influence on the TCTP. How long does the process take, how complex is it, and what is quality of the environment - for example, these factors may impact on the probability that the TCTP becomes contaminated, or damaged during recovery

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Processing and environment

Consider where and how the TCTP is processed, namely how long does the processing take and how complex is it(including all physical and chemical treatments applied to the product) – this may impact on the risk of contamination, or that it may not be prepared to consistent specifications and quality. Also consider the quality of the processing environment, which may also affect the risk of contamination. (Please notice that risks associated to reagents are considered in the following specific risk factor 'Reagent').

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reagents

Consider any reagents used during processing, decontamination, preservation, storage and transport of the TCTP. Could they damage the TCTP in any way, or could residual traces of reagent remain in the TCTP that could cause toxic or immunogenic effects in recipients?

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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Good Tissue & cell Practices

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reliability of Microbiology Testing

Consider the risk that the nature of the TCTP, the testing methodology and/or the presence of residual processing reagents such as antibiotics in the finished TCTP may impact the accuracy of any microbiology tests. Note, this refers specifically to bacteriology/mycology testing of the TCTP, not any blood tests performed on the donor.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Storage Conditions

Consider any potential risks arising from how the starting material and TCTP are stored, between procurement and processing, during processing, and between processing and clinical application.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Transport Conditions

Consider any potential risks arising from how the starting material and TCTP are transported, for example between the sites procurement and processing, and between the sites of storage and clinical application.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Presence of unwanted cellular material and/or graft vascularity

This risk must be considered from the perspective that for some TCTPs, the presence of intact vital cells is desirable, although it may also increase risks of, for example, immunogenicity or disease transmission
This presence might affect to tumour formation, immunogenicity and disease transmission risks.
Vascular tissues may be more at risk to infiltration by pathogens or malignant cells than avascular tissues

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Complexity of the immediate pre-implantation preparation and/or application method

Consider how complex the method of clinical application will be for this TCTP. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?
Highly complex methods of application could influence the risks of implant failure and/or disease transmission.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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Methodologies for Assessing the Risks associated to novel Tissue and/or Cellular Therapies/Products (TCTPs).

-HSC Template-

Please follow the EuroGTP II Guide in order to correctly evaluate your TCTPs.

TCTP characterization

The evaluation of the level of novelty and the risks associated, should start with a characterization of the novel process or TCTPs.

Hematopoietic Cells

- ☐ Bone Marrow
- ☐ Peripheral blood
- ☐ Cord Blood
- ☐ Other

Name of the product, therapy or process under evaluation:

Description of TCTP under evaluation:
(Describe the relevant aspects of the TCTP, detailing the modifications/novelties associated with **donation**, **processing** and **clinical application** under evaluation)

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- HSC Template-

Step 1

The evaluation of the level of novelty and the risks associated, should start with a characterization of the novel process or Tissue and Cellular Therapies/Products (TCTPs).

Please answer the following questions in order to determine if the product or process is novel. This process represents the first stage of the overall procedure for evaluating novelty and risk.

	Yes	No	Not Applicable/ Not Relevant
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?			
Justify:			
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCP?			
Justify:			
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCP?			
Justify:			
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCP?			
Justify:			
E. Will this TCTP be packaged and stored using a protocol and materials used previously in your establishment for this type of TCP?			
Justify:			
F. Will this type of TCTP provided by your establishment be applied clinically using an implantation method used previously?			
Justify:			
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site before?			
Justify:			

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- HSC Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Donor Characteristics

Consider whether the novelty in your process has an impact at the moment of the donation.
This factor requires that you consider whether the donor population you intend to obtain the TCTP from, could cause any risk for the recipient

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Procurement process and environment

Consider where and how the TCTP is recovered currently and whether the changes proposed with the novel method changes recovery time, complexity, quality of the environment?
For example, how long does the process take, how complex is it, and what is how does the procurement devices affect the quality of the HPC?

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Processing and environment

Consider the current processing method for the TCTP how the novelty in processing can affect the product. How long does the novel preparation process take and how complex is it – this may have an impact on the risk of contamination, or cell characteristics that may not be consistent with product specifications. Also consider the quality of the processing environment, which may also affect the risk of contamination.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reagents

Consider any reagents used during recovery, processing, decontamination, and storage of the TCTP. Could they damage the TCTP in any way, or could residual traces of reagent remain in the TCTP that could cause toxic or immunogenic effects in recipients.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reliability of Microbiology Testing

Consider the risk that the testing methodology and / or presence of residual processing reagents such as antibiotics in the finished TCTP may impact the accuracy of any microbiology/mycology testing of the TCTP. This risk factor is not about blood tests on the donor.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Storage Conditions

Consider any potential risks arising from how the starting material and TCTP are stored, between procurement and processing, during processing, and between processing and implantation.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Transport Conditions

Consider any potential risks arising from how the starting material and TCTP are transported, for example between the sites of procurement and processing, and between the sites of storage and implantation.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Presence of unwanted cellular material

Consider the risk of s the presence of inactivated cells, debris or cell components which may cause, immunogenicity or disease transmission.

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Complexity of the pre-implantation preparation and/or application method

Consider how complex the method of transplantation will be for this TCTP. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?

Applicable Yes ☐ No ☐

Justify:

Risks					
Unwanted immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Extensive ☐	Substantial ☐

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Methodologies for Assessing the Risks
associated to novel Tissue and/or Cellular Therapies/Products (TCTPs).
-ART Template-

Please follow the EuroGTP II Guide in order to correctly evaluate your TCTPs.

TCTP characterization

The evaluation of the level of novelty and the risks associated, should start with a characterization of the novel process or TCTP.

ART

- ☐ Gametes
- ☐ Embryos
- ☐ Gonadic tissue*

Name of the product, therapy or process under evaluation:

Description of TCTP under evaluation:
(Describe the relevant aspects of the TCTP, detailing the modifications/novelties associated with **donation**, **processing** and **clinical application** under evaluation)

*When assessing Gonadic tissue products/therapies, please consider also the risk factors and risk consequences identified in the tissue section.

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-ART Template-

Step 1

The purpose of this exercise is to evaluate our proposed methodology for determining if a TCTP, therapy or process is novel or not.

Please answer the following questions in order to determine if the product or process is novel. This process represents the first stage of the overall procedure for evaluating novelty and risk.

	Yes	No	Not Applicable/ Not Relevant
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?			
Justify:			
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?			
Justify:			
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?			
Justify:			
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?			
Justify:			
E. Will this TCTP be packaged and stored using a protocol and materials used previously in your establishment for this type of TCTP?			
Justify:			
F. Will this type of TCTP provided by your establishment be applied clinically using an implantation method used previously?			
Justify:			
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site before?			
Justify:			

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-ART Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Donor Characteristics

Consider if the novelty in your process or procedures changes donor characteristics and if these changes could impart a risk to the recipient.

Applicable Yes ☐ No ☐

Justify:

Risks					
Implant failure / Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-ART Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Recovery/Procurement process and environment

Consider where and how the TCTP is collected, procured or recovered, and if this process could have an influence on the TCTP. How long does the process take, how complex is it, and what is quality of the environment

Applicable Yes ☐ No ☐

Justify:

Risks					
Implant failure / Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-ART Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Processing and environment

Consider where and how the TCTP is prepared. How long does processing take and how complex is it – this may impact on the risk of contamination, or that it may not be prepared to consistent specifications and quality. Also consider the quality of the processing environment, which may also affect the risk of contamination.

Applicable Yes ☐ No ☐

Justify:

Risks					
Implant failure / Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-ART Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reagents

Consider any reagents used during recovery, processing, decontamination and storage of the TCTP. Could they damage the TCTP in any way, or could residual traces of reagent remain in the TCTP that could cause toxic or immunogenic effects in recipients?

Applicable Yes ☐ No ☐

Justify:

Risks					
Implant failure / Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-ART Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Storage Conditions

Consider any potential risks arising from how the starting material and TCTP are stored, not only after processing and before clinical application, but also in intermediate steps: e.g. between procurement and processing, during processing, and between processing steps.

Applicable Yes ☐ No ☐

Justify:

Risks					
Implant failure / Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-ART Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Transport Conditions

Consider any potential risks arising from how the starting material and TCTP are transported, for example between the sites procurement and processing, and between the sites of storage and clinical application

Applicable Yes ☐ No ☐

Justify:

Risks					
Implant failure / Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
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Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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-ART Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Loss of viability and or functionality

Consider the risk that the changes in procedures of processes can have on the viability or functionality of the TCTP

Applicable Yes ☐ No ☐

Justify:

Risks					
Implant failure / Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

-ART Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Complexity of the pre-implantation preparation and/or application method

Consider how complex the method of clinical application will be for this TCTP. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?
Low feasibility of application standardization might have influence in the risks of implant failure and disease transmission at least.

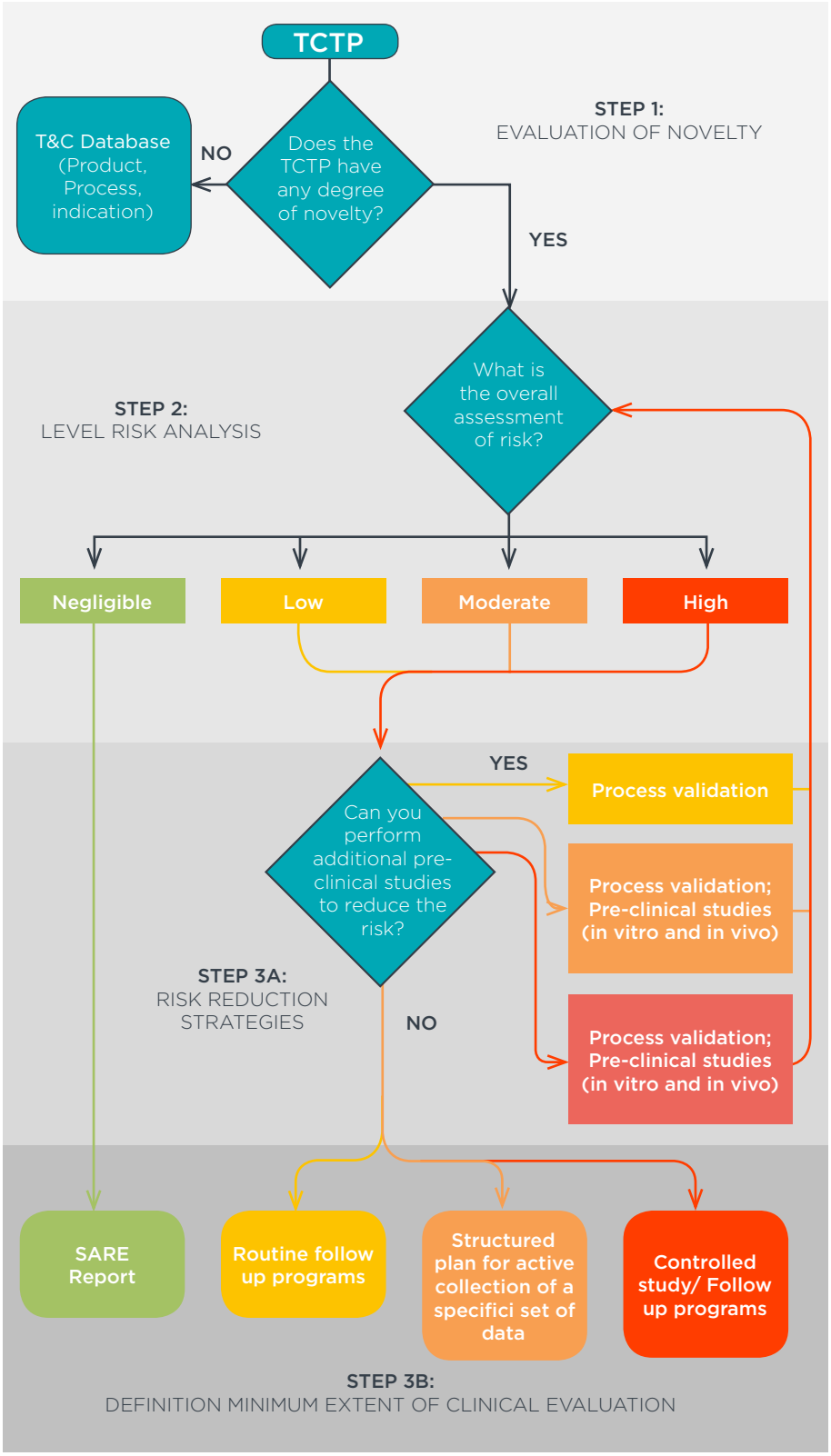
Applicable Yes ☐ No ☐

Justify:

Risks					
Implant failure / Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None ☐	Limited ☐	Moderate ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatning ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

—Annex IV—

Methodologies Wall Chart


TCTP
Does the TCTP have any degree of novelty?
NO
T&C Database (Product, Process, indication)
YES
STEP 1:
EVALUATION OF NOVELTY
What is the overall assessment of risk?
STEP 2:
LEVEL RISK ANALYSIS
Negligible
Low
Moderate
High
Can you perform additional pre-clinical studies to reduce the risk?
YES
Process validation
Process validation; Pre-clinical studies (in vitro and in vivo)
Process validation; Pre-clinical studies (in vitro and in vivo)
STEP 3A:
RISK REDUCTION STRATEGIES
NO
SARE Report
Routine follow up programs
Structured plan for active collection of a specifici set of data
Controlled study/ Follow up programs
STEP 3B:
DEFINITION MINIMUM EXTENT OF CLINICAL EVALUATION

Probability levels (Definitions from V&S SoHO Project)

LEVEL OF PROBABILITY	DEFINITION
1 - Rare	Difficult to believe it could happen
2 - Unlikely	Not expected to happen but possible
3 - Possible	May occur occasionally
4 - Likely	Probable but not persistent
5 - Almost certain	Likely to occur on many occasions

Severity levels (Definitions from V&S SoHO Project)

LEVEL OF SEVERITY	DEFINITION
1- Non-serious	Mild clinical or psychological consequences for the recipient, however with no hospitalisation, or anticipated long term consequences/disability
2- Serious	Hospitalisation and/or: Persistent/significant disability or incapacity Intervention to preclude permanent damage Evidence of a serious transmitted infection Significant decrease in the expected treatment success Birth of a child with an infectious or genetic disease following ART with donor gametes or embryos
3- Life-threatening	Major intervention necessary to prevent death Evidence of a life threatening transmissible infection Birth of a child with life threatening genetic disease following ART with donor gametes of embryos
4 - Fatal	Death of the patient

Detectability levels

LEVEL OF DETECTABILITY	DEFINITION
1 - Very high	The potential defect will almost certainly be detected before clinical application in the recipient
2 - Moderately high	There is a reasonable chance that the potential defect will be detected before clinical application in the recipient
3- Low	There is a low chance that the potential defect will be detected before clinical application in the recipient
4 – Very low	It is unlikely that the potential defect will be detected before clinical application in the recipient
5 - Cannot be detected	The potential defect will be detected only after clinical application in the recipient

Percentage risk reduction definitions

PERCENTAGE RISK REDUCTION		DEFINITION
0	**None**	There is no relevant data available to support reducing the calculated risk score
25	**Limited**	There is a moderate relevant data available to support reducing the calculated risk score, based predominantly on unpublished data
50	**Moderate**	There is moderate amount of good quality relevant data available to support reducing the calculated risk score, including published and unpublished data from external sources, and some data which has been through an independent peer review process
75	**Substantial**	There is high quality relevant data to support reducing the calculated risk score, including data that has been peer reviewed and published
95	**Extensive**	There is an extensive amount of high quality relevant data, including multiple peer reviewed publications, that demonstrates that the probability of the risk occurring, having a significant impact, and/or being undetected is negligible

Annex V

EuroGTP II Algorithm for the calculation of Final Risk Score

EuroGTP II Algorithm for the calculation of Final Risk Score

1. Estimate the Preliminary Score associated with the TCTP:

$$\textbf{Preliminary Score} = \Sigma \textbf{ risks} =$$

$$= \Sigma \left(\left(S \times P \times D\right) - \left(\left(S \times P \times D\right) \times (\%\text{risk reduction})\right)\right)$$

P = *Probability*
S = *Severity*
D = *Detectability*

The combined risk is determined following the described steps:

$$\textbf{Combined Risk Value} = \frac{\textbf{Preliminary score} \times \textbf{Highest Possible score}}{\textbf{(Max S} \times \textbf{Max P} \times \textbf{Max D} \times \textbf{Number of Applicable Risks Consequences)}}$$

Max P = 5
Max S = 4
Max D =5

Applicable Number of Risks Consequences = Range from: 1 to 45 for tissues (including gonadic tissues) and HSC; 1 to 32 for ART (See details in the specific chapters: 4 – Tissues, 5 – HSC and 6 – ART

Highest Possible Risk Score = (Max S × Max P ×Max D ×Number of Risks) x Risk Factors = 4500 for Tissues and HSC, and 3200 for ART

$$\textbf{Final Risk Score} = \frac{\textbf{Combined Risk Value} \times 100}{\textbf{Highest Possible score}}$$

Two ancillary rules have been implemented in the algorithm to ensure that individual highly scored risks are not masked by adding various low risk scores. Thus, independently of the determined *Final Risk Score*, individual risks with scores higher than 30, result in "moderate risks" and, individual risks with scores higher than 50, result in "high risks".

(Demonstration of the algorithm with practical examples – Annex VII, Annex VIII and Annex IX)

The Preliminary and *Combined Risk Scores* resulting from the risk assessment doesn't have a direct correspondence with the *Final Risk Score.*

The calculation of the *Final Risk Score* must be proportional to the number of risk consequences evaluated in the assessment of the TCTP.

Table 2.1. Levels of risk based in the Final Risk Value determined by the algorithm

0 – 2	Negligible Risk
>2 – 6	Low Risk
>6 – 22*	Moderate Risk
>22*	High Risk

* Lower values may result in moderate and high risk scores due to the application of the ancillary rules (described in the algorithm).

Annex VI

Risk reduction strategies and definition of clinical evaluation for Tissues

Table of contents of Annex VI

Acronyms

AFM – Atomic Force Microscopy

AM – Acetoxymethyl

ATP – Adenosine Triphosphate

BOP – Bovine corneal opacity permeability

CT – Computed Tomography

DAPI – 4',6-diamidino-2-phenylindole

DMMB – Dimethylmethylene Blue Assay

ECD – Endothelial Cell Density (cornea)

ECM – Extracellular matrix

ELISA – Enzyme-linked immunosorbent assay

EM – Electron Microscopy

EthD-1 – Ethidium Homodimer-1

GAGs – Glycosaminoglycans

GC-MS – Gas chromatography - Mass spectrometry

GuCl – Guanidine hydrochloride

H&E – Haemotoxylin and Eosin

HPLC – High Performance Liquid Chromatography

ICC – Immunocytochemistry

ICE – Isolated chicken eye

ICRS – International Cartilage Repair Society

IHC – Immunohistochemistry (antigen detection)

IKDC – International Knee Documentation Committee Subjective Knee Form

KOOS – Knee Injury and Osteoarthritis Outcome Score

LDI – Laser Doppler imaging

MALDI – Matrix-assisted laser desorption/ionization

MRI – Magnetic resonance imaging

MTT – t3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

MVP – Moisture Vapour Permeability

NRS – Numeric Rating Scale

OCT – Optical coherence tomography

PAS – Periodic acid–Schiff

PCR – Polymerase chain reaction

pERG – Pattern electroretinography

PFA – Paraformaldehyde

PGs – proteoglycans

PROM – Patient Reported Outcome Measures

QIRC – Quality of Life Impact of Refractive Correction

QoL – Quality of life

RCM – reflectance confocal microscopy

RNA – Ribonucleic acid

RT-PCR – Reverse transcription polymerase chain reaction

SAGE – Serial analysis of gene expression

SARE – Serious Adverse Reactions and Events

TEM – Transmission Electron Microscopy

TER – Transepithelial resistance

TEWL – Trans Epidermal Water Loss

TOF – Time of Flight

TUNEL – Terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labelling assay

VAS – visual analogue scale

VEP – Visual Evoked Potentials

WOMAC – Western Ontario and McMaster Universities Osteoarthritis Index

WOMET – Western Ontario Meniscal Evaluation Tool

WVTR – Water Vapour Transmission Rate

Definitions:

Donor Cell Functionality – The ability of donor cells to perform their required function; assays of donor cell functionality may address for example manufacture of specific ECM components, or secretion of specific growth factors

Donor Cell Viability – The ability of donor cells to survive; assays of donor cell viability measure generalized aspects of the health of cells, such as membrane integrity or mitochondrial activity

Tests listed in the matrices are for guidance only and **not intended to be an exhaustive list of mandatory tests**.

The references provided in this document aim to describe the generic assays/tests suggested as pre-clinical and clinical evaluations. These references do not describe the specific tests applicable to the different type of tissues.

Corneas

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Corneas

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
In vitro cytotoxicity	Transepithelial resistance (TER)	■	■		■	■	■	■	■	■	■		
	Staining with Trypan blue	■	■		■	■	■	■	■	■	■		
	Cell apoptosis by detection of specific markers (e.g. caspase 3).	■	■		■	■	■	■	■	■	■		
	Microculture viability assays (e.g. Mitochondrial dehydrogenase performance (MTT*) test[1])	■	■		■	■	■	■	■	■	■		

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Donor cell viability	Glucose uptake				■	■	■						
	Endothelial Cell Density (ECD)[2,3] using Trypan Blue				■	■	■						
	Measurement of Adenosine Triphosphate (ATP) levels				■	■	■						
	Hoechst/Ethidium/Calcein (HEC)[3,4] staining - endothelial cell triple staining viability assay (Hoechst, EthD-1 and Calcein Acetoxymethyl (AM))				■	■	■						
	Mitochondrial activity (e.g. MTT)				■	■	■						
Donor cell functionality	Immunostaining to determine the expression of different proteins and /or markers (e.g. ZO-1; Na+/K+ ATPase, p63, K12, αSMA etc.)				■	■	■						
	Transparency				■	■	■						
	Central Corneal Thickness				■	■	■						
	Tomography and Microscopy				■	■	■						
	Measurement of expression of specific markers/proteins through molecular assays, IHC and/or ELISA				■	■	■	■	■	■	■		

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Histological evaluation of the extracellular matrix (ECM)	Histological analysis to determine the presence of each layer (Paraformaldehyde (PFA)[4] fixing and Periodic acid–Schiff (PAS)[5] staining)				■	■	■						
	Biophysical investigations of ECM structure, collagen fibril orientation and distribution of GAGs in the collagen matrix: x-ray diffraction and Transmission Electron Microscopy (TEM)	■	■		■	■	■						
	PAS staining[5]	■	■		■	■	■						
	Haemotoxylin and Eosin (H&E)[1] staining	■	■		■	■	■						
	Immunohistochemistry (IHC)	■	■		■	■	■						
	Tomography and Microscopy				■	■	■						
	Enzyme-linked immunosorbent assay (ELISA)				■	■	■						
Biochemical evaluation of the extracellular matrix (ECM)	Quantification of ECM contents: collagen, Glycosaminoglycans (GAGs), mucopolysaccharides, etc.	■	■	■	■	■	■						
Histological evaluation of cell content	Morphology: intercellular borders, polymorphism, dystrophy, degeneration	■	■	■	■	■	■						
	Staining with Alizarin red S[6]	■	■	■	■	■	■						
	Presence of tight junctions, hemidesmosomes, etc.	■	■	■	■	■	■						
	H&E staining[1]	■	■	■	■	■	■						
	PAS staining[5]	■	■	■	■	■	■						
	Scanning/Transmission microscopy	■	■	■	■	■	■						
	Staining with alizarin red S[6]	■	■	■	■	■	■						
Residual DNA content	DAPI** and Hoechst staining[7]	■	■	■	■	■	■						
	In situ hybridization	■	■	■	■	■	■						
	Polymerase chain reaction (PCR)	■	■	■	■	■	■						

***** 4', 6-diamidino-2-phenylindole***

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biomechanical properties	Use of Atomic Force Microscopy (AFM)[8]												
In vitro functionality	Cell-biology and metabolic assays.												
	Physiological measures of EC function (e.g. perfusion and modulation of bicarbonate concentrations to turn off the endothelial pump and switch back on – measure rates of swelling and thinning).												
Residual processing & preservation reagents	Chemical and biochemical tests												
	Immuno-based assays (IHC, immunocytochemistry (ICC), ELISA, etc.)												
	Direct detection and quantification methodologies, (e.g. (High Performance Liquid Chromatography - Mass spectrometry (HPLC-MS); Gas chromatography - Mass spectrometry (GC-MS); Reagent specific assays)												

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Corneas

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility	Ocular staining assays to evaluate defects: fluorescein test, rose bengal test, lissamine green test[9]												
	Presence of palpebral signs (meibomitis), conjunctivitis, corneal perforation, corneal ulceration, blood in the anterior chamber, neovascolarization												
Immunological response	Histology sections to investigate signs of inflammation (e.g., vessels, neovascularization, etc.), the presence of proinflammatory agents, such as cytokines, or the presence of infiltrates (monocytes, macrophages, etc.).												
	Gross examination of eye and corneal; transparency												
	Use of specific (transgenic, knockout, etc.) animal models. Careful consideration should be given to the choice of strain												
Ocular Functionality	Imaging (e.g. OCT)												
	Histology sections for IHC-based assays (e.g. evaluation of the expression of specific proteins important for cellular function)												
	In vivo functional assessment: a) pERG; b) VEP[10]; c) Evaluation of the light reflex (Iridal response)												
	Morphological assessment (histology, IHC, Electron Microscopy (EM), etc.)												

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Irritation / Corrosion/ Toxic effect	Isolated rabbit eye test (ex vivo)							■	■	■			
	Isolated chicken eye (ICE) test (ex vivo)							■	■	■			
	Bovine corneal opacity permeability (BOP) test (ex vivo)							■	■	■			
Health	General condition/well-being after implantation (alive and well, sick, dead)	■	■	■	■	■	■	■	■	■	■	■	■
	Presence of ocular infections	■	■	■	■	■	■	■	■	■	■	■	■
	Growth/weight increase	■	■	■	■	■	■	■	■	■	■		
	Unexplained fever (due to immune induced reaction and/or toxicity)	■		■					■			■	■
	Visual acuity evaluation: use of animal maze				■	■	■						

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: Corneas

Test category	Detailed investigational options
Physical investigation (functional)	1. Assessment of visual acuity 2. Eye movements 3. Visual field 4. Measurement of intraocular pressure
Physical investigation (Anatomy)*	1. Observation of external structures (cornea, eye lid, sclera, conjunctiva, pupil and iris, etc.) 2. Assessment of pupils 3. Analysis of the fundus 4. Presence of defects, pathologies, inflammation, etc. 5. Topography 6. Pachymetry 7. Endothelial cell density 8. Optical Coherence Tomography for cornea/retina
Overall Clinical outcome measures*	1. Graft transparency 2. Endothelial cell density and loss 3. Severe Adverse Reactions and Events 4. Best corrected visual acuity 5. Topography 6. Graft rejection 7. Infection 8. Optical Coherence Tomography 9. Angio Optical Coherence Tomography 10. Fluoro angiography 11. Schirmer test 12. Measurement of mechanical sensation (esthesiometry - Cochet Bonnet anaesthesiometer)

**** Depends on the type of patient and the procedure; Select the appropriate combination and schedule of tests according to the risk category of the patients (low/medium/high)***
***** These tests will be done pre and post operatively so that improvement can be evaluated***

Test category	Detailed investigational options
Patient Reported outcome measures*	**Note:** It is important to use only Quality of Life (QoL) and visual disability instruments that have been validated by Rasch analysis, which takes into account both difficulty of task and an individual's ability. Users should consider if the Patient Reported Outcome Measure's (PROMs) they propose to use meet this criteria. 1. EQ-5D QoL - https://euroqol.org/) 2. Proceedings of PROMs which are more specific for Ophthalmology treatments and that are available in the UK at https://onlineproms.co.uk/ , such as: Patient-reported outcomes are measured using questionnaires (CatQuest) Quality of Life Impact of Refractive Correction (QIRC) Visual analogue scale (VAS) satisfaction Numeric Rating Scale (NRS) to assess pain 12-Item Short Form Health Survey (SF-12) or 36-Item Short Form Health Survey (SF-36) 3. Ocular surface disease index
Procedure or graft failure	1. Graft failure. Slit lamp examination can reveal clinical signs of graft rejection including: • corneal edema • keratic precipitates on the corneal graft, but not on the peripheral recipient cornea • corneal vascularisation • stromal infiltrates • Khodadoust line • an epithelial rejection line • subepithelial infiltrates 2. Corneal endothelial cell density (where possible) 3. Confocal microscopy 4. High intra ocular pressure 5. Infection 6. Optical Coherence Tomography 7. Angio Optical Coherence Tomography 8. Fluoro angiography 9. Examination of the fundus

***** Other PROMS are available***

Test category	Detailed investigational options
Post operative complications**	1. Slit lamp and fundus examination to evaluate • Post-op infection - (corneal scraping) • Suture problems • Corneal vascularisation • Epithelial defects • Haemorrhage • Graft detachment • Graft rejection • Inflammation • Eyelid disorders (blepharitis, ptosis, trichiasis) • Symblepharon and conjunctival disorder • Corneal melting/perforation • Cataract • Retinal detachment 2. Ocular hypertension (after tonometry) 3. Pain/photophobia/burning (patient reported symptoms) 4. Re-bubbling rate 5. Re-grafting rate 6. Systemic disease transmission

***** The clinician will determine which examinations are relevant; Important to distinguish failure due to non-graft related reasons from graft related failure; Routine follow up for systemic infection/disease is not needed, however if a recipient develops a post-operative systemic infection investigation is needed and reported as an Serious Adverse Reactions and Events (SARE).***

Sclera

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Sclera

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
Histological evaluation of the extracellular matrix (ECM)	Histological analysis to determine the presence of each layer (etc PFA fixing and PAS staining)				■	■	■						
	H&E staining[1]	■	■		■	■	■						
	Assessment of Morphology (Microscopy)				■	■	■						
Biochemical evaluation of the ECM	Quantification of collagen, GAGs, mucopolysaccharides, etc.				■	■	■						
Residual processing & preservation reagents	Chemical and biochemical tests appropriate to the specific reagent	■	■	■	■	■	■	■	■	■	■	■	■
	Direct detection and quantification methodologies (e.g. HPLC-MS; GC-MS; . Reagent specific assays)	■	■	■	■	■	■	■	■	■	■	■	■

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Sclera

		Immuno-genicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility	Ocular staining assays to evaluate defects: fluorescein test, rose bengal test, lissamine green test[9]				■	■	■						
	Presence of palpebral signs (meibomitis), conjunctivitis, corneal perforation, corneal ulceration, blood in the anterior chamber, neovascolarization				■	■	■						
Ocular Functionality	Imaging (e.g. OCT)				■	■	■						
	Morphological assessment (histology, IHC, EM, etc.)				■	■	■						
Health	General condition/wellbeing after implantation (alive and well, sick, dead)	■	■	■	■	■	■	■	■	■	■	■	■
	Presence of ocular infections	■	■	■	■	■	■	■	■	■	■	■	■
	Growth/weight increase	■	■	■	■	■	■	■	■	■	■		
	Unexplained fever (due to immune induced reaction and/ or toxicity)	■		■					■			■	■

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: Sclera

Test category	Detailed investigational options
Physical investigation (functional)	1. Eye movements 2. Measurement of intraocular pressure
Physical investigation (Anatomy)	1. Observation of external structures (cornea, eye lid, sclera, conjunctiva, pupil and iris, etc.) 2. Presence of defects, pathologies, inflammation, etc. 3. Topography 4. Pachymetry 5. Optical Coherence Tomography for cornea/retina
Overall Clinical outcome measures	1. Severe Adverse Reactions and Events 2. Topography 3. Infection 4. Optical Coherence Tomography
Patient Reported outcome measures	1. EQ-5D (QoL - https://euroqol.org/) 2. Proceedings of Patient Reported Outcome Measure's (PROMs) which are more specific for Ophthalmology treatments and that are available in the UK at https://onlineproms.co.uk/ , such as: • Patient-reported outcomes are measured using questionnaires (CatQuest) • QIRC • VAS satisfaction • Numeric Rating Scale (NRS) to assess pain • 12-Item Short Form Health Survey (SF-12) or 36-Item Short Form Health Survey (SF-36) 3. Ocular surface disease index

Test category	Detailed investigational options
Procedure or graft failure	1. Graft failure. Slit lamp examination 2. Confocal microscopy 3. High intra ocular pressure 4. Infection 5. Optical Coherence Tomography
Post operative complications	1. Slit lamp • Post-op infection • Suture problems • Hemorrhage • Graft detachment • Inflammation • Eyelid disorders (blepharitis, ptosis, trichiasis) • Symblepharon and conjunctival disorder • Corneal melting/perforation • Cataract • Retinal detachment 2. Ocular hypertension (after tonometry) 3. Pain/photophobia/burning (patient reported symptoms) 4. Re-grafting rate 5. Systemic disease transmission

Amniotic Membrane

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Amniotic Membrane

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
	Thermal gravimetric analysis				■	■	■						
In vitro cytotoxicity	Cell proliferation	■	■		■	■	■	■	■	■	■		
	Microculture viability assays (e.g. MTT).	■	■		■	■	■	■	■	■	■		
	Direct contact method[11]	■	■		■	■	■	■	■				
Donor cell viability	MTT test (mitochondrial activity of cells)	■	■		■	■	■	■					
	Fluorescence microscopy (Live/dead staining)				■	■	■						
	Proliferation test				■	■	■						

Criteria	Specific test	Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
		Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Donor cell functionality	Differentiation potential				■	■	■						
	RT-PCR, Real time PCR (expression levels of molecules related to the properties of the amniotic membrane e.g. cytokines)				■	■	■						
	ELISA, Western Blotting (content of specific protein)				■	■	■						
	Water absorption				■	■	■						
	Trypan blue staining				■	■	■	■					
	Assessment of the membrane architecture (e.g. IHC analysis, Immunophenotipical characterization)	■	■		■	■	■	■	■	■	■		■
	Flow cytometry				■	■	■	■					
Histological evaluation of the extracellular matrix (ECM)	H&E Staining[1], Mallory's trichrome[12]				■	■	■						
	PAS staining[5]				■	■	■						
	Scanning electron microscopy				■	■	■						
	Transmission electron microscopy				■	■	■						
Histological evaluation of cell content	Light microscopy (e.g. Hematoxylin and eosin staining)				■	■	■						
	Scanning electron microscopy				■	■	■						
	Transmission electron microscopy				■	■	■						
Absence of donor cells, cell remnants & nucleic acids	DAPI staining	■	■	■	■	■	■						
	Spectrophotometric analysis	■	■	■	■	■	■						
Biochemical evaluation of ECM quality	IHC				■	■	■						
	Infrared spectrometry analysis (degradation of the tissue)				■	■	■						

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biomechanical properties	Tensile testing				■	■	■						
In vitro functionality	Cell count and proliferation assay	■	■		■	■	■						
	Microbial permeability	■	■	■				■	■			■	■
	Oxygen permeability				■	■	■	■					
	Water vapour transmission rate (WVTR)[13]				■	■	■	■					
	Moisture Vapour Permeability (MVP)[14]				■	■	■	■					
	Flow cytometry for cells viability (e.g. Propidium iodide) and apoptosis (e.g. Annexin V, Caspase 3/7)	■	■		■	■	■						
	Differentiation of mesenchymal stem cells isolated from the tissue and cultured under specific condition	■	■		■	■	■						
	Immunofluorescence detection of intracellular molecules	■	■		■	■	■						
	Flow cytometry for antigen expression pattern analysis	■	■		■	■	■						
	ICC	■	■		■	■	■						
	RT-PCR, Real time PCR (e.g. expression of regulatory proteins related to the undifferentiated state)	■	■		■	■	■						
	ELISA, Western Blotting (content of specific protein)	■	■		■	■	■						
	Serial analysis of gene expression (SAGE), microarray (gene expression analysis)*	■	■		■	■	■						
Residual processing & preservation reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS Reagent specific assays)	■	■	■				■	■	■	■		

**** Relevant for clinical applications where the intended effect is to actively promote healing***

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Amniotic Membrane

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility	Histology and staining of cellular infiltrates												
	Measurement of serum/ wound fluid - Cytokines, chemokines (e.g. ELISA, flowcytometry, etc)												
	Blood testing – HLA (donor antigens)												
Functionality	number of adhesions												
	mean wound size reduction												
	Barrier												
	scar reduction												
Health	General condition/wellbeing after implantation (alive and well, sick, dead)												
	Local infections												
	Growth/weight increase												
	Unexplained fever (due to immune induced reaction and/ or toxicity)												

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: Amniotic Membrane

	Clinical Indication*		
	Tissue patch, barrier or wrap	Surface wound healing	Ocular surface healing
Test category	Detailed investigational options		
Physical investigation (functional)	1. Mechanical performance		1. Assessment of visual acuity 2. Eye movements 3. Visual field 4. Measurement of intraocular pressure
Physical investigation (Anatomical)	1. Absence of calcification 2. Magnetic resonance imaging 4. Cerebrospinal fluid leaks 5. Integration with native tissue (biopsy)	1. Bleeding/ seroma formation (visual assessment) 2. Size of wound 3. Revascularisation 4. Scar retraction	1. Observation of external structures (cornea, eye lid, sclera, conjunctiva, pupil and iris, etc.) 2. Assessment of pupils 3. Analysis of the fundus 4. Presence of defects, pathologies, inflammation, etc. 5. Topography 6. Pachymetry 7. Endothelial cell count 8. Optical Coherence Tomography for cornea/retina
Overall Clinical outcome measures	1. Alloimunisation 2.Prevention of adhesions 2. Urodynamics		1. Graft transparency 2. Endothelial cell density and loss 3. Severe Adverse Reactions and Events 4. Best corrected visual acuity 5. Topography 6. Graft rejection 7. Infection 8. Optical Coherence Tomography 9. Angio Optical Coherence Tomography 10. Fluoro angiography 11. Schirmer test 12. Measurement of mechanical sensation (esthesiometry - Cochet Bonnet anaesthesiometer)

**** In situations where the amniotic membrane is used for induction of tissue regeneration (e.g. Maxillofacial surgery - Osteonecrosis of the jaw; Orthopaedic surgery - tendinopathy treatment; Orthopaedics - treatment of osteoarthritis) please consider tests appropriate to the tissue being treated***

	Clinical Indication		
	Tissue patch, barrier or wrap	Surface wound healing	Ocular surface healing
Test category	Detailed investigational options		
Patient Reported outcome measures		1. Pain score	1. EQ-5D (QoL - https://euroqol.org/) 2. Proceedings of Patient Reported Outcome Measure's (PROMs) which are more specific for Ophthalmology treatments and that are available in the UK at https://onlineproms.co.uk/ , such as: • Patient-reported outcomes are measured using questionnaires (CatQuest) • QIRC • VAS satisfaction • Numeric Rating Scale (NRS) to assess pain • 12-Item Short Form Health Survey (SF-12) or 36-Item Short Form Health Survey (SF-36) 3. Ocular surface disease index

	Clinical Indication		
	Tissue patch, barrier or wrap	Surface wound healing	Ocular surface healing
Test category	Detailed investigational options		
Procedure or graft failure			1. Graft failure. Slit lamp examination can reveal clinical signs of graft rejection including: • corneal edema • keratic precipitates on the corneal graft, but not on the peripheral recipient cornea • corneal vascularisation • stromal infiltrates • a Khodadoust line • an epithelial rejection line • subepithelial infiltrates 2. Corneal endothelial cell count (where possible) 3. Confocal microscopy 4. High intra ocular pressure 5. Infection 6. Optical Coherence Tomography 7. Angio Optical Coherence Tomography 8. Fluoro angiography 9. Examination of the fundus

	Clinical Indication		
	Tissue patch, barrier or wrap	Surface wound healing	Ocular surface healing
Test category	Detailed investigational options		
Post operative complications	1. Infection 2. Haemorrhage	1. infection 2. inflammation	1. Slit lamp and fundus examination to evaluate: • Post-op infection - (corneal scraping) • Suture problems • Corneal vascularisation • Epithelial defects. • Haemorrhage • Graft detachment • Graft rejection • Inflammation • Eyelid disorders (blepharitis, ptosis, trichiasis) • Symblepharon and conjunctival disorders • Corneal melting/perforation • Cataract • Retinal detachment 2. Ocular hypertension (after tonometry) 3. Pain/photophobia/burning (patient reported symptoms) 4. Re-bubbling rate 5. Re-grafting rate 6. Systemic disease transmission
Examples (of Clinical Applications)	1. Cardiac surgery - device wrapping to prevent adhesions 2. Neurosurgery - malformation of the newborn spinal cord 3. Neurosurgery - Dural reconstruction	1. Plastic surgery - wound healing 2. Plastic surgery - bioregeneration 3. Plastic surgery - skin graft donor site healing 4. Burn surgery - treatment of burn wounds 5. Burn surgery - post stomal ulcer	1. Ophthalmology - promote healing of the ocular surface

Skin

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Skin as a biological dressing on (burn) wounds

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procure-ment or processing
Process Validation	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	■
	Validation of the reliability of microbi-ology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport method-ologies				■	■	■						
In vitro Cytotox-icity	Microculture cytotoxicity assays (co-culture with keratinocytes or fibroblasts)						■	■					
Donor cell viability	trypan blue exclusion of cells (in suspension)						■						
	Microculture viability assays (e.g. MTT).						■						
Donor cell function-ality	Growth factor production (e.g. ELISA)						■						
Histological evaluation of the ECM	H&E staining				■								
	Collagen (Mason Trichrome)				■								
	Thickness				■								
Histological evaluation of cell content	H&E staining				■								

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biomechanical properties	pliability, stiffness				■								
	Epidermal-dermal attachment				■								
In vitro Functionality	Tears upon handling (preparation after storage)				■								
Residual processing & preservation reagents	concentration measurement in wash out fluids							■					
	cytotoxicity test of wash out fluid							■					
	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; Reagent specific assays)							■					
	pH of washing fluid							■					

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Skin

Note: since the use of skin is a temporary biological dressing , the risk may never be so high that the results of in vitro tests are not sufficient to decide the new method for this type of skin is suitable or not for clinical use

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test***	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Immunological response	biopsies during healing time		■										
	staining for inflammatory cells		■										
	adherence to wound				■		■						
	wound healing time (closure)						■						
	wound contraction, scar quality						■						
	granulation tissue formation						■						
Health	General condition/wellbeing after implantation (alive and well, sick, dead)	■	■	■	■	■	■	■	■	■	■	■	■
	Wound infection	■	■	■	■	■	■	■	■	■	■	■	■
	Growth/weight increase	■	■	■	■	■	■	■	■	■	■		
	Unexplained fever (due to immune induced reaction and/or toxicity)	■		■					■			■	■

****** Specifc tests using porcine wound model; comparative***

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: Skin

Test category	Detailed investigational options
Physical investigation (functional)	1. Elasticity, using a cutometer 2. Adherence of graft to wound bed
Physical investigation (Anatomical)	1. Non-invasive imaging (for example Laser Doppler Imaging) 2. Histological evaluation of tissue biopsies (H&E staining) 3. Stimulation of granulation tissue
Overall Clinical outcome measures	1. Wound closure. Evaluate by: • Visual assessment • Quantitative evaluation using a grid system • Computerised image analysis of wound photographs • By inference from treatment records, e.g. stopping use of ointments or dressings 2. Quality of healing. Objective assessment, e.g. Vancouver Scar Scale
Patient Reported outcome measures	1. QoL evaluated by using a questionnaire for the patient (pain, itching, scaring, pigmentation/vascularity, surface texture, surface area, scar height, sensitivity, psychological aspects, etc.)
Procedure or graft failure	1. Detachment of graft during dressing change (e.g. due to poor fixation/adherence to the wound bed)
Post operative complications ***(Causing difficulties in moving the graft material)***	1. Infection 2. Formation of seroma or haematoma between the graft and wound bed 3. Adherence of donor skin to the wound bed
Clinical indications	1. Applied following excision of necrotic tissue to: • Prepare the wound for autografting • Protect the wound from infection • Reduce fluid/heat loss 2. Coverage of meshed autografts
General notes	1. The type of wound will determine the appropriate tests 2. Burn wounds should be followed up for a minimum of two years. Longer follow up is advised. 3. Consider resource requirements 4. The quality of the wound bed preparation prior to graft application is critical to success of the graft.

Acellular Dermis

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Acellular Dermis

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
In vitro biocompatibility	Cell adhesion (histological analysis)				■			■					
	Cell proliferation				■			■					
	Non-invasive analysis (e.g. OCT)				■	■	■						
In vitro Cytotoxicity	Microculture cytotoxicity assay (e.g. MTT, trypan blue)				■			■	■				
Presence of donor cells, cell remnants & nucleic acids	Histological analysis (H&E staining)	■	■		■								
	Quantitative analysis of DNA		■		■								
	Qualitative analysis of DNA (e.g. DAPI stain)		■		■								

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Histological evaluation of the ECM	H&E staining	■	■		■	■	■						
	Elastin (Verhoeff-Van Gieson[15], Orcein[16])					■	■						
	Collagen IV Immunostain*				■								
	Non-invasive imaging techniques to evaluate 3D structure and vasculature of the ECM (e.g. OCT, reflectance confocal microscopy (RCM))				■	■	■						
	Space in the ECM interfibres (e.g. OCT)				■	■	■						
Biochemical evaluation of ECM quality	Resistance to collagenase digestion				■	■	■						
	Assessment of collagen nativity (chymotrypsin assay[17])				■	■	■						
	Quantification of ECM contents (e.g., collagen and elastin)				■	■	■						
Biomechanical properties	Mechanical tensile testing (ultimate tensile stress, ultimate tensile strain, stiffness)				■	■	■						
	Suture pullout resistance**				■	■	■						
In vitro Functionality	Tears upon handling (preparation after storage)						■						
Residual processing & preservation reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; Reagent specific assays)	■	■	■	■			■	■	■	■		
	pH of washout fluid				■			■					

**** Only relevant if basement membrane is important.***

***** As an indicator of ease of suturing.***

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Acellular Dermis

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility	implantation subcutaneous model, in growth of host cells		■		■			■					
Immunological response	porcine wound model; comparative		■										
	biopsies during healing time		■										
	staining for inflammatory cells		■										
Functionality	porcine full thickness wound model				■	■	■						
	incorporation in wound bed				■	■	■						
	take of autograft on product, wound healing time (closure)				■	■	■						
	wound contraction, scar quality				■	■	■						
Health	General condition/wellbeing after implantation (alive and well, sick, dead)	■	■	■	■	■	■	■	■	■	■	■	■
	Wound infection	■	■	■	■	■	■	■	■	■	■	■	■
	Growth/weight increase	■	■	■	■	■	■	■	■	■	■		
	Unexplained fever (due to immune induced reaction and/or toxicity)	■		■					■			■	■
Other, functional test	implantation in abdominal wall (rat/ porcine), adhesions*				■	■	■						
	occurrence of calcification or early breakdown (bulging)				■	■	■						

**** Specific for hernia repair indication***

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: Acellular Dermis used (for treating burns*)

Test Category**	Detailed investigational options
Physical investigation (functional)	1. Elasticity, using cutometer 2. Range of motion during articulation (can be assessed by physiotherapy) 3. Permeability of wound (Trans Epidermal Water Loss (TEWL) evaluation, e.g.by using a TEWAmeter) 4. Skin hydration/surface evaporation, using corneometer 5. Pigmentation and colouration (Mexameter) 6. pH (compared to healthy skin from the same patient (Normal range is 5.5 - 6.0) 7. Dermal scan, compared to healthy skin from same anatomical area using commercially available apparatus (e.g. OCT, Laser Doppler imaging (LDI), etc.)
Physical investigation (Anatomical)	1. Wound contraction (e.g. evaluated by using planimetry)
Overall Clinical outcome measures	1. Wound closure. Evaluate by: 1.1 - Visual assessment 1.2 - Quantitative evaluation using a grid system 1.3 - Computerised image analysis of wound photographs 1.4 - By inference from treatment records (e.g. stopping use of ointments or dressings) 2. Quality of healing. Objective assessment (e.g. Vancouver Scar Scale)
Patient Reported outcome measures	1. QoL evaluated by using a questionnaire for the patient (pain, itching, scaring, pigmentation/vascularity, surface texture, surface area, scar height, psychological aspects, etc.) 2. Sensitivity (touch)
Procedure or graft failure	1. Non integration with wound bed*** 2. Seroma/haematoma formation
Post operative complications	1. Infection
Examples	1. To regain mechanical function of damaged skin

**** Other clinical indications exist but were not consider in the this guide: plastics (e.g. Hypospadia correction and Oculoplasty); wound healing (e.g. Chronic vascular/diabetic ulcers, Following excision of dermal malignancies); Tendon/ligament repair (e. g. re-enforcement of tendon/ligament repair & improvement of tissue regeneration); Biological patch/barrier material (e.g. Breast reconstruction, Abdominal wall repair)***
*****General remark: as technologies evolve, the suggested apparatus should be adapted to the new available technologies, accordingly***
******May be due to either infection, poor wound bed preparation, or patient factors(e.g use of drugs that reduce peripheral blood flow - the key measure is lack of vascularisation)***

Cardiovascular Tissues – Heart Valves and Vascular Grafts

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Cardiovascular Tissues

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation tests	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
In vitro cytotoxicity	Extract cytotoxicity[11]				■	■			■				
	Contact cytotoxicity[11]				■	■		■					
Donor cell viability	Microculture viability assays (e.g. MTT, fibroblast culture)	■	■							■	■		
	Expression of cell surface markers	■	■										
Physical / morphological properties	Evaluation of the morphology/anatomy of processed tissue (leaflet morphology, fenestrations, coaptation of leaflets, calcification, atheromatosis.)	■	■	■									■
	Hydrodynamic properties: competency test under pressure and pulsatile flow testing						■						

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biomechanical properties	Uniaxial/biaxial tensile strength testing assays						■						
	Cyclic testing						■						
	Suture pullout						■						
Histological evaluation of the ECM	Safranin O[18] (proteoglycans (PGs) & GAGs)					■	■						
	Alizarin Red S or Von Kossa[19] (Calcium)					■	■						
	Van Gieson[15] (Collagen)					■	■						
	Masson´s Trichrome staining[20]					■	■						
	Protein quantification (e.g., collagen and elastin)				■	■	■						
Histological evaluation of cell content	H&E stain	■	■		■								
	DAPI staining	■	■		■								
Presence of donor cells, cell remnants & nucleic acids	DNA quantification				■	■							
	Qualitative testing (DAPI)				■	■							
Biochemical evaluation of ECM quality	Quantification of ECM contents, e.g. collagen and elastin					■							
	Collagenase resistance					■							
	Collagen nativity					■							
Residual processing reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; Reagent specific assays)	■	■	■	■			■	■	■	■		
	IHC	■	■	■	■			■	■	■	■		

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Cardiovascular Tissues

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility	IHC staining (Post explantation: cell infiltration)		■		■			■					
	HLA matching	■							■				
	Calcification		■		■	■							
	EchoDoppler / Echocardiography / computed tomography (CT) Scan / Magnetic resonance imaging (MRI)	■				■	■						
Functionality in vivo (stenosis or regurgitation)	Echocardiography for regurgitation and stenosis evaluation; bleeding, thrombosis, infection					■	■						
	Regurgitation grade					■	■						
	Tissue regeneration	■	■		■	■	■	■	■				
	Bleeding events						■						
	Rupture of the graft						■						
	Thrombosis / Thromboembolic event				■								
Health	General condition/wellbeing after implantation (alive and well, sick, dead)	■	■	■	■	■	■	■	■	■	■	■	■
	Infection/endocarditis	■	■	■	■	■	■	■	■	■	■	■	■
	Growth/weight increase	■	■	■	■	■	■	■	■	■	■		
	Unexplained fever (due to immune induced reaction and/or toxicity)	■		■					■			■	■
Valves functionality/ integrity	Post explantation histological analysis	■	■	■	■	■	■	■	■	■		■	■
	Thrombogenicity					■	■						
	Morphological evaluation post explantation structural integrity, fibrosis, calcification					■							
	Radiograph analysis					■	■						

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: Heart Valves and Vascular Grafts

	Clinical Indication	
	Heart Valves	Vascular Grafts
Test category	**Detailed investigational options**	
Graft failure (during procedure / immediately after implantation)	1. Perioperative (surgical) graft Failure (transoesofageal echocardiography)	1. Perioperative (surgical) graft Failure(Doppler echo)
Post operative complications	1. Unexplained fever (due to immune induced reaction and/or toxicity) 2. Bleeding events 3. Rupture of the graft; 4. Thrombosis / Thromboembolic event 5. Infection/endocarditis	1. Unexplained fever (due to immune induced reaction and/or toxicity) 2. Bleeding events 3. Rupture of the graft; 4. Thrombosis / Thromboembolic event 5. Infection/endocarditis
Patient Reported symptoms and outcome	1. Fatigue 2. Loss of physical capacity 3. Dyspnoea	1. Pain in the operated limb 2. Colour and temperature changes in the skin distal of the graft 3. Decreased functional capacity of the operated limb
Physical investigation (discrete outcome measures with quantifiable results) **And** **Overall Clinical outcome measures**	1. Graft related mortality 2. Graft normal function (Auscultation / echocardiogram/ MRI) 3. Abnormal function (increase peak pressure gradient) due to mismatch, calcific degeneration with/ without stenosis – (Auscultation / Echocardiogram and CT scan) 4. Abnormal function - Annular dilation (by echocardiogram or CT scan) 5. Regurgitation (by echocardiogram or MRI) 6. Graft related re-operation (due to graft survival)	1. Lack of pulsation 2. Graft related mortality 3. Graft normal function (pulse palpation / Auscultation / Doppler echo) 4. Abnormal function (increase pressure gradient) due to mismatch, calcific degeneration with/without stenosis – (Auscultation / Doppler echo / CT Scanner) 5. Abnormal function - graft dilation (Aneurism formation) by Doppler echo, angiography or CT scan) 6. Graft related re-operation (due to graft survival)

Bone

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Bone

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation tests	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the efficacy of the demineralization process				■								
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
In Vitro Immunogenecity	Mixed lymphocyte reaction				■			■	■				
In vitro cytotoxicity	Co-culture of cells with graft (toxicity/ proliferation)				■			■					
In vitro biocompatibility	Microculture toxicity assays				■			■	■				
	Contact toxicity testing				■			■	■				
Presence of donor cells, cell remnants & nucleic acids	DAPI staining	■	■		■								
	Safranin O (lipids)				■			■					
	Lipid content (solvent extraction)				■			■					
	Cell specific markers	■	■		■								

Criteria	Specific test	Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
		Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biomechanical properties	Ultimate tensile stress (load at failure)						■						
	Ultimate compressive stress (load at failure)						■						
	Presence of microfractures after stress					■	■						
	Elastic modulus						■						
	Shear testing						■						
	Three point pending						■						
Residual processing reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; Reagent specific assays)			■	■			■	■	■	■		
Histological evaluation of the ECM	Von Kossa staining					■	■						
	Van Gieson[15] staining					■	■						
	H&E staining	■	■		■								
In Vitro functionality	in vitro osteoinduction				■	■							
	BMP content				■	■							
Biochemical evaluation of the ECM	Collagen denaturation					■	■						

Pre-clinical evaluation –Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Bone

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility	Histology and staining of cellular infiltrates												
Immunological response	Analysis of HLA (alloimunisation)												
Functionality	Osteogenesis in extraskeletal sites												
	Bone induction chamber												
	Healing of a critical size defect												
	Fusion												
Health	General condition/ wellbeing after implantation (alive and well, sick, dead)												
	Infection												
	Growth/weight increase												
	Unexplained fever (due to immune induced reaction and/or toxicity)												

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: **Bone**

	Clinical Indication			
	Joint revision	Spinal surgery	Fracture repair	Replacement of lost bone mass
Test category	Detailed investigational options			
Physical investigation (functional)	1. Prosthesis survival rate	1. Spinal curve correction 2. Length of hospital stay	1. Full weight bearing	
Physical investigation (Anatomical)	1. Stem subsidence 2. Cortical repair (radiography) 3. Graft incorporation (radiography, CT scan) 4. Trabecular remodelling (radiography, CT scan)	1. Bone graft mass (radiography) 2. Graft incorporation (union with host bone) 3. Bone bridging (fusion) between vertebral bodies - arthrodesis	1. Radiographic assessment of union, callus formation	1. Radiographic assessment of bone fill 2. Bone biopsy
Patient Reported outcome measures	1. Harris Hip Score (pain and function) 2. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[21] test	1. Pain scores 2. MacNab score 3. Oswestry Disability Index 4. SF-36 score 5. Neck disability index	1. Numeric pain scale (0-10) 2. Numeric satisfaction score (0-5)	
Procedure or graft failure	1. Dislocation (e.g. Prosthesis dislocation) 2. Periprosthetic fracture 3. Need for revision 4. Aseptic loosening	1. Pseudarthrosis (non-union) rate 2. Loss of correction	1. Non-union or delayed union	

	Clinical Indication			
	Joint revision	Spinal surgery	Fracture repair	Replacement of lost bone mass
Test category	Detailed investigational options			
Post operative complications	1. Infection 2. Alloimmunisation 3. Pain	1. Dural tear 2. Neurologic Injury 3. Haematoma 4. Infection 5. Adjacent segment degeneration 6. Dysphagia 7. Alloimmunisation 8. Pain	1. Infection 2. Alloimmunisation 3. Pain	
Examples	1. Hip replacement/revision	1. Scoliosis surgery 2. Spinal fusion		

Tendons

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Tendons

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation tests	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
In Vitro Immuno-genecity	Mixed lymphocyte reaction	■	■										
In vitro cytotoxicity	Microculture toxicity assays				■			■	■				
	Contact toxicity testing				■			■	■				
	Co-culture of cells with graft (toxicity, proliferation)				■			■	■				
In vitro Biocom-patibility	Pro-inflammatory response	■	■		■	■							
	Co-culture of cells with graft (maintenance of phenotype)	■	■		■	■							
Presence of donor cells, cell remnants & nucleic acids	DAPI staining	■	■		■								
	Quantitative DNA analysis (total DNA content)				■								
	H&E staining	■	■		■							■	
	Cell specific markers	■	■		■								

Criteria	Specific test	Immuno-genicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
		Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procure-ment or processing
Biomechanical properties	Ultimate tensile stress (load at failure)					■	■						
	Ultimate tensile strain (extension at failure)					■	■						
	Displacement under constant load (creep)					■							
	Elastic modulus/ stiffness					■	■						
Residual processing reagents	Direct detection and quantification method-ologies,(e.g. HPLC-MS; GC-MS; Reagent specific assays)			■	■			■	■	■	■		
Histological evaluation of the ECM	Van Gieson[22] stain					■	■						
	Inter-fibre space					■	■						
Biochemical evalua-tion of the ECM	Collagen denaturation					■	■						
	Collagenase resistance					■	■						

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Tendons

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Test criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility	Histology and staining of cellular infiltrates	■	■		■			■					
	Macrophage type identification[23]	■	■										
	Histological assessment of graft/donor tissue interface				■	■	■	■					
Immunological response	Analysis of HLA (alloimu-nisation)	■											
Functionality	Radiography/CTScan/MRI to establish bony fusion				■	■	■	■					
	Force plate analysis				■	■							
	Tetracycline labelling for new bone formation				■								
	Joint stability				■								
Health	General condition/well-being after implantation (alive and well, sick, dead)	■	■	■	■	■	■	■	■	■	■	■	■
	Infection	■	■	■	■	■	■	■	■	■	■	■	■
	Growth/weight increase	■	■	■	■	■	■	■	■	■	■		
	Unexplained fever (due to immune induced reaction and/or toxicity)	■		■					■			■	■
	Quality of gait				■				■				

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: Tendons

Test category	Detailed investigational options
Physical investigation (functional)	1. Laxity (KT-1000, Lachman test) 2. Range or motion (ROM) assessment
Physical investigation (Anatomical)	1. Graft biopsy to evaluate cellularity, collagen structure, necrosis, inflammatory cell infiltrate 2. Bone resorption 3. Tunnel enlargement 4. MRI 5. Graft (bone) incorporation
Overall Clinical outcome measures	1. Patellofemoral crepitus 2. Hop and jump tests
Patient Reported outcome measures	1. International Knee Documentation Committee Subjective Knee Form (IKDC score)[24] 2. Lysholm score[25] 3. Cincinatti score 4. Knee Injury and Osteoarthritis Outcome Score (KOOS)[24] 5. Tegner score[25,26] 6. Frequency/level of sporting participation
Procedure or graft failure	*Note some of the clinical outcome measures (e.g. excess laxity) may denote graft failure.* 1. Graft rupture 2. Requirement for revision
Post operative complications	1. Effusion 2. Cyst formation 3. Post-op infection 4. Pain 5. Elevated temperature
Examples	1. ACL Revision/Repair

Meniscus

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Meniscus

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation tests	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
In vitro cytotoxicity	Extract cytotoxicity assays[1]				■			■	■				
	Contact cytotoxicity assays[1]				■			■					
	Co-culture of cells with allograft (toxicity/proliferation)				■			■					
In Vitro Immunogenecity	Mixed lymphocyte reaction	■	■										

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Donor cell functionality	Evaluation of donor cell phenotype - quantification of secreted/ produced biomolecules (PGs, GAGs, proteins)				■	■	■						
	Donor cell viability (e.g. trypan blue, live dead staining, flow cytometry, confocal microscopy)				■	■	■						
Histological evaluation of the ECM	Haemotoxylin & Eosin staining				■	■	■						
	Safranin O, Alcian Blue - PGs				■	■	■						
	IHC to evaluate type II collagen				■	■	■						
Presence of donor cells, cell remnants & nucleic acids	DAPI staining	■	■		■								
	Residual nucleic acid quantification	■	■		■								
	Haemotoxylin & Eosin staining	■	■		■								
Residual processing reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; Reagent specific assays)	■	■	■	■			■	■	■	■		
Biochemical properties of the ECM	Collagen denaturation					■	■						
	Collagenase susceptibility					■	■						
	Evaluation of proteoglycan quality – GuCl extraction					■	■						
	Composition of the ECM - Water (gravimetic/aW assessment) - Collagen (hydroxyproline) - GAGs (Dimethylmethylene Blue Assay (DMMB))				■	■	■						

		Immunogenicity			Graft failure			Toxicity/Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biomechanical properties	Static tensile modulus												
	Dynamic tensile modulus												
	Indentation test												
Morphological/physical properties	Microscopic surface examination												

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Meniscus

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Test criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility/Integration with recipient tissue	Characterisation of recipient cell infiltrates												
	Evaluation of donor cell content												
	Histological assessment of graft/donor tissue interface												
	Evaluation of graft vascularity												
	Biomechanical evaluation of graft insertion												
Immunological response	Analysis of HLA (alloimunisation)												
Graft quality/ remodelling	Composition of the ECM - Water (gravimetic assessment) - Collagen (hydroxyproline) - GAGs (DMMB)												
Functionality	Radiography/CT/MRI to establish bony fusion												
	Tetracycline labelling for new bone formation												
	Evaluation of recipient knee articular cartilage quality												
Health	General condition/well-being after implantation (alive and well, sick, dead)												
	Infection												
	Growth/weight increase												
	Unexplained fever (due to immune induced reaction and/or toxicity)												
	Quality of gait												

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: **Meniscus**

Test category	Detailed investigational options
Physical investigation (functional)	*May intersect with Patient Reported Outcome Measures below*
Physical investigation (Anatomical)	1. Post-operative MRI/Xray or Arthroscopy, to investigate position, integration and degeneration of graft 2. Graft degeneration investigated by arthroscopy/arthrotomy (International Cartilage Repair Society (ICRS) grading score[27] can be used to grade cartilage degeneration) 3. Biopsy to investigate ECM structure, donor cell phenotype and IHC, ,matrix remodelling, localized immunogenicity 4. Alloimmunisation
Overall Clinical outcome measures	1. Standard knee functionality scales
Patient Reported outcome measures	1. Lysholm Knee Score[26] 2. Activity level 3. IKDC Score[24] 4. SF-36 5. Functional Knee score 6. Tegner score 7. Cincinnati Knee Rating[28]
Procedure or graft failure	1. Graft survival
Post operative complications	1. Swelling 2. Pain 3. Effusion 4. Synovitis
Examples	Meniscal transplantation

Fresh Cartilage

STEP 3A: RISK REDUCTION STRATEGIES

Pre-clinical evaluation – Examples of ***in vitro*** tests to assist in potentially reducing the risk consequences identified (**blue** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Fresh Cartilage

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Test criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Process Validation tests	Validation of the efficacy of the decontamination process	■	■	■	■	■	■					■	■
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)	■	■	■	■	■	■					■	
	Validation of the reliability of microbiology analytical methods	■	■	■	■	■	■						■
	Aseptic handling (Media fill) validation	■	■	■	■	■	■						■
	Validation of packaging integrity following simulated use (including sealing tests)	■	■	■	■	■	■						■
	Validation of the transport methodologies	■	■	■	■	■	■						■
	Validation of the stability of the TCTP during storage ('shelf life')				■	■	■						
In vitro cytotoxicity	Extract cytotoxicity assays[1]				■			■	■				
	Contact cytotoxicity assays[1]				■			■					
	Co-culture of cells with allograft (toxicity/proliferation)				■			■					
In Vitro Immunogenecity	Mixed lymphocyte reaction	■	■										
Evaluation of donor cell functionality	Evaluation of donor cell phenotype - quantification of secreted/produced biomolecules (PGs, GAGs, proteins)				■	■	■						
	Donor cell viability (trypan blue, live dead staining)				■	■	■						

		Immunogenicity			Graft failure			Toxicity/Carcino-genicity			Disease transmission		
Test criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Histological evaluation of the ECM	H&E staining				■	■	■						
	Safranin O, Alcian Blue - PGs				■	■	■						
	IHC to evaluate type II collagen				■	■	■						
Residual processing reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; Reagent specific assays)	■	■	■	■			■	■	■	■		
Biochemical properties of the ECM	Collagen denaturation					■	■						
	Collagenase susceptibility					■	■						
	Evaluation of proteoglycan quality – guanidine hydrochloride (GuCl) extraction[29]					■	■						
	Composition of the ECM - Water (gravimetic/aW assessment) - Collagen (hydroxy-proline) - GAGs (DMMB)				■	■	■						
Physical/morphological properties	Macroscopic surface examination					■	■						

Pre-clinical evaluation – Examples of ***in vivo*** tests to assist in potentially reducing the risk consequences identified (**Green** cells represent the tests that might be used to address the respective risk consequences) - Tissues: Fresh Cartilage

		Immunogenicity			Graft failure			Toxicity/Carcino-genicity			Disease transmission		
Test criteria	Specific test	Systemic Immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of Donor Derived Infectious Agents	Infections acquired during procurement or processing
Biocompatibility/Integration with recipient tissue	Characterization of recipient cell infiltrates	■	■		■			■					
	Evaluation of donor cell viability	■	■		■	■	■						
	Histological assessment of graft/donor tissue interface				■	■	■	■					
	Evaluation of graft vascularity				■	■	■						
	Biomechanical evaluation of graft insertion				■		■						
Immunological response	Analysis of HLA (alloimunisation)	■	■										
Graft quality/ remodelling	Composition of the ECM - Water (gravimetic assessment) - Collagen (hydroxyproline) - GAGs (DMMB)				■	■							
Functionality	Radiography/CT/MRI to establish bony fusion				■	■	■						
	Tetracycline labelling for new bone formation				■	■	■						
	Evaluation of recipient knee articular cartilage quality					■							
Health	General condition/wellbeing after implantation (alive and well, sick, dead)	■	■	■	■	■	■	■	■	■	■	■	■
	Infection	■	■	■	■	■	■	■	■	■	■	■	■
	Growth/weight increase	■	■	■	■	■	■	■	■	■	■		
	Unexplained fever (due to immune induced reaction and/or toxicity)	■		■					■			■	■
	Quality of gait				■	■			■				

STEP 3B: DEFINITION OF CLINICAL STUDIES

Clinical evaluation and follow up plans - Tissues: Fresh Cartilage

Test category	Detailed investigational options
Physical investigation (functional)	1. Range of Motion 2. Daily living activities functionality
Physical investigation (Anatomical)	1. Post-operative MRI/CT scan 2. Arthroscopy, to investigate position, integration and degeneration of graft 3. Radiography to evaluate mechanical axis 4. Alloimmunisation
Overall Clinical outcome measures	1. Standard knee functionality scales
Patient Reported outcome measures	1. Lysholm Knee Score[26] 2. Activity level 3. IKDC Score[24] 4. SF-36 5. Western Ontario Meniscal Evaluation Tool (WOMET)[30] 6. Tegner score 7. Cincinnati Knee Rating[28] 8. Kujala score[31,32] 9. KOOS[24] 10. WOMAC[21] 11. VAS for pain
Procedure or graft failure	1. Graft rupture/Resorption 2. Requirement for revision
Post operative complications	1. Infection 2. Immune reaction 3. Repetitive effusion
Examples of Application	Large focal osteochondral injury of the patella

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– Annex VII –

Worked Example of risk assessment Tissues

EuroGTP II Interactive Assessment Tool

TCTP: *Tissues - Cardiovascular*

The following information refers to TCTP: *Decontamination of Heart Valves*

Evaluation performed on: *2019-01-16 14:42:38*

Description of TCTP under evaluation:

Heart Valves Allografts are currently decontaminated with an antibiotic solution prior to being cryopreserved. TE is proposing to change the formulation of our antibiotic solution

	Yes	*No*	*NA*
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?	X		
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?	X		
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?	X		
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?		X	
E. Will this TCTP be packaged , stored , and distributed using a protocol and materials used previously in your establishment for this type of TCTP?	X		
F. Will this type of TCTP provided by your establishment be applied clinically using an application method used previously?	X		
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site and/or same clinical indication before?	X		

	Justification provided for Evaluation of Novelty questions
A.	TE already provides Heart Valves
B.	Donor selection criteria are not changing
C.	Procurement procedure is not changing
D.	The composition of a critical processing reagent is changing
E.	Storage and packaging are not changing
F.	Clinical application is not changing
G.	Clinical application is not changing

Risk Factor	Risk	Probability	Severity	Detectability	Potential Risk	Risk Reduction	Risk
Reagents	Unwanted immunogenicity	3	2	4	**24**	0%	**24**
Reagents	Implant failure	2	3	4	**24**	50%	**12**
Reagents	Disease transmission	3	3	2	**18**	50%	**9**
Reagents	Toxicity / Carcinogenicity	1	2	4	**8**	75%	**2**
Reliability of Microbiology Testing	Disease transmission	3	3	4	**36**	0%	**36**

Risk Factor	Applicable	Comment
Reagents	Y	Our current antibiotic solution contains 5 antibiotic. The manufacturing of one antibiotic has been discontinued. We are replacing this with another antibiotic. Risk of immunogenicity: we know that traces of antibiotic can be retained in the tissue. There is a risk that the new antibiotic may cause allergies. Manufacturer guidance suggest that 1/10 000 patients may have allergic reactions. (Probability: we considered this is possible because there is evidence this antibiotic can cause an allergic responses in a small number of patients, therefore we selected a score of 3; Severity: despite the nature of this graft, it is unlikely to be life threatening; Detectability: there is no way to implement a routine quality control test to ensure the absence of traces in the graft. We have no evidence to suggest whether or not the antibiotic remains in the graft after treatment, which justifies a high score for detectability; Risk Reduction: there is no evidence of risk reduction at this stage because we have no data viable or literature regarding this issue) Implant failure: Because is a new chemical that has not been applied to cardiac tissue previously the risk that it may damage the tissue needs to be considered. Following a literature search we identified evidence suggesting that this antibiotic does not damage heart valves allografts. (Probability: is unlikely because we have some evidences that show the antibiotic does not damage the graft in any detectable way; Severity: a mechanical/sudden failure of the graft would have severe consequences for the recipient; Detectability: we cannot routinely test the properties of the graft during routine quality control; Risk Reduction: we do have some documented evidences to suggest the interaction of the antibiotic with the graft is safe) Disease Transmission: The purpose of the antibiotic solution is to reduce or eliminate bio burden in the heart valve, if does not do this the valve could transmit disease, which in the valve recipient could be very serious. We have received advise from a microbiologist expert that our new antibiotic is highly active and is an effective substitute for the former one. However we do not know if the efficacy of the antibiotic in combination with our solution would be compromised. (Probability: we have not done any validation test with this antibiotic; Severity: an infection could have severe consequences for the recipient; Detectability: we routinely evaluate individual grafts, however we know there are limitations in the reliability of the testing process; Risk Reduction: we have evidence provided by a microbiologist adviser of the efficacy of this antibiotic) Toxicity/carcinogenicity: we know that traces of antibiotic can be retained in the tissue. This may cause toxic / carcinogenic effects in the recipient due to the new chemical itself or the interaction with other reagents in the antibiotic solution. The information from the manufacturer suggests there is no evidence of toxic or carcinogenic effects (Probability: Difficult to believe that an interaction between the antibiotic could happen; Severity: the potential quantities that would be transferred into the recipient are very low ; Detectability: there is no routine test; Risk Reduction: The information from the manufacturer suggests there is no evidence of toxic or carcinogenic effect)
Reliability of Microbiology Testing	Y	It is possible due to residuals of quantities of the antibiotic compromising pos decontamination. Disease Transmission: (Probability: there is evidence that it could potential lead to a false negative result; Severity: an infection could have severe consequences for the recipient Detectability: there is no routine test in place which considers the presence of this antibiotic; Risk Reduction: currently we have no evidence)

Preliminary Score: ***83***
Number of Applicable Risks Consequences: ***5***
Number of Risks Consequences: ***5***
Max individual Risk value = ***36***

Highest Possible Risk Score = *5 * 4 * 5 * 5 * 9 =* ***4500***
Applicable Risk Score = *5 * 4 * 5 * 5 =* ***500***

Combined Risk Value = *(Risk Value * Highest Possible Risk Score) / Number of Applicable Risks = (83 * 4500) / 500* ***747***
Final Risk Score = *(Final Risk Score * 100) / Highest Possible Risk Score = (747 * 100) / 4500* ***17***

Your assessment has Final Risk Score of: **17**

This suggests that your TCTP falls into the Level of Risk:

Level of Risk	*Extent of Studies needed*
Moderate	**Step3A: Risk reduction strategies** The assessment indicates that more evidence is needed to support safe and effective use of this TCTP and mitigate risk. Process validation should be performed, however if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. Pre-clinical *in vitro* evaluation, specific to the identified risks, should be performed if not already done Pre-clinical *in vivo* evaluation, specific to the identified risks, using an animal model should be done if applicable (and if not already completed).
	Step 3B: Extent of clinical evaluation A structured plan for active collection of a specific set of data relating to the safety and efficacy of the TCTP should be put in place, in addition to routine clinical follow up. Ethical approval may be required and the principles of Good Clinical Practices (GCP) adhered to. Consideration should be given to restricting provision of the TCTP to a limited number of patients and/or centres until the risks have been adequately mitigated.

You should first consider if **there are any pre-clinical studies you can undertake to address the specific risks identified**, especially those risks that form a large part of the risk score. Please refer to the EuroGTP II manual for suggestions as to appropriate studies.
If, having completed all pre-clinical studies you consider feasible, your risk is still moderate or high, and you feel that the benefit of using the TCTP clinically justifies this level of risk, you should consider what type(s) of clinical assessment could be implemented to address this risk.

Please refer to the EuroGTP II Guide for suggestions as to appropriate types of assessment.

Annex VIII

Worked Example of risk assessment HSC

TCTP: *Hematopoietic Cells - Peripheral blood*

The following information refers to TCTP: *Plerixafor*

Evaluation performed on: *2019-01-16 14:51:17*

Description of TCTP under evaluation: *Mobilization of healthy haplo identical donors with plerixafor*

	Yes	No	NA
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?		X	
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?		X	
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?	X		
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?	X		
E. Will this TCTP be packaged , stored , and distributed using a protocol and materials used previously in your establishment for this type of TCTP?	X		
F. Will this type of TCTP provided by your establishment be applied clinically using an application method used previously?	X		
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site and/or same clinical indication before?		X	

	Justification provided for Evaluation of Novelty questions
No justification has been provided	

Risk Factor	*Risk*	*Probability*	*Severity*	*Detectability*	*Potential Risk*	*Risk Reduction*	*Risk*
Donor Characteristics	Unwanted immunogenicity	2	1	2	**4**	25%	**3**
Donor Characteristics	Engraftment failure	2	1	3	**6**	25%	**4.5**
Reagents	Unwanted immunogenicity	2	1	1	**2**	0%	**2**
Reagents	Engraftment failure	2	1	1	**2**	0%	**2**
Complexity of the pre-implantation preparation and/or application method	Unwanted immunogenicity	2	1	1	**2**	0%	**2**

Risk Factor	*Applicable*	*Comment*
Donor Characteristics	Y	Use of a new mobilisation agent
Reagents	Y	pro-inflammatory 6-sulfo-LacNac+ detected

Preliminary Score: ***13.5***
Number of Applicable Risks Consequences: ***5***
Number of Risks Consequences: ***5***
Max individual Risk value = ***4.5***

Highest Possible Risk Score = *5 * 4 * 5 * 5 * 9 =* ***4500***
Applicable Risk Score = *5 * 4 * 5 * 5 =* ***500***

Combined Risk Value = *(Risk Value * Highest Possible Risk Score) / Number of Applicable Risks = (13.5 * 4500) / 500* ***121.5***
Final Risk Score = *(Final Risk Score * 100) / Highest Possible Risk Score = (121.5 * 100) / 4500* ***3***

Your assessment has Final Risk Score of: **3**

This suggests that your TCTP falls into the Level of Risk:

Level of Risk	*Extent of Studies needed*
Low	**Step3A: Risk reduction strategies** Implementing a standard procedure or treatment in an HPC centre that has never performed this procedure exerts an **intensive validation**. Training of staff (as required by Joint Accreditation Committee ISCT-Europe & EBMT (JACIE)) is necessary in order to reach the outcomes published in scientific literature. A learning curve might be expected and should be part of the validation report. When implementing the procedure, additional quality controls must be performed to **monitor Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs)**. For example, when a TE is switching from T-cell depletion (TCD) to CD34+_selection (which they never performed before), engraftment rate, and graft versus host reactions should be carefully monitored.
	Step 3B: Extent of clinical evaluation A **safety follow up program** is necessary. Follow up procedures (conform EBMT Med-A, Med-B or Med-A cellular) should be focusing on assessing efficacy, comparing the clinical follow up with the results obtained before the implementation of the change in the process and in relation to the results published in scientific literature. The expected learning curve should be kept as short as possible and put in relation to the follow up program. Likewise, established techniques are prone to long-term (ideally trans-generational) follow up of the health effects, as established by EBMT.

Please refer to EuroGTP II Guide for additional details.

Annex IX

Worked Examples of risk assessment ART

EuroGTP II Interactive Assessment Tool

TCTP: *Assisted Reproductive Techniques - Gametes*

The following information refers to TCTP: *oocyte*

Evaluation performed on: *2019-01-16 15:09:05*

Description of TCTP under evaluation:

Usage of a new aspiration pump for oocyte recovery: to change the manual aspiration into aspiration with pump

	Yes	No	NA
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?	X		
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?	X		
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?		X	
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?	X		
E. Will this TCTP be packaged , stored , and distributed using a protocol and materials used previously in your establishment for this type of TCTP?	X		
F. Will this type of TCTP provided by your establishment be applied clinically using an application method used previously?	X		
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site and/or same clinical indication before?	X		

	Justification provided for Evaluation of Novelty questions
C.	Since previously only aspiration pump or hand aspiration has been used, but not an aspiration/follicle irrigation system used

Risk Factor	Risk	Probability	Severity	Detectability	Potential Risk	Risk Reduction	Risk
Procurement process and environment	Implant failure / Pregnancy loss	1	2	2	**4**	75%	**1**
Procurement process and environment	Toxicity / Carcinogenicity	1	1	1	**1**	75%	**0.25**
Loss of viability and or functionality	Implant failure / Pregnancy loss	1	2	2	**4**	75%	**1**

Risk Factor	*Applicable*	*Comment*
Donor Characteristics	N	In this case the donor population is the same.
Procurement process and environment	Y	In the case with a new aspiration/irrigation system the process of oocyte retrieval is different and might affect the quality of the oocytes/embryos (e.g. affect the incidence of aneuploidy or oocyte/embryo degeneration), however this pump has been used by other centers and therefore a substancial risk reduction can be applied.
Processing and environment	N	In this case the processing and environment is the same; however, might be different if the system requires special containers that might change the environment surrounding the eggs (like temperature, pH, etc.)
Reagents	N	The processing in this case should be the same and with the same medium
Storage Conditions	N	Should be the same
Transport Conditions	N	Should be the same
Complexity of the pre-implantation preparation and/or application method	N	Clinical Application has not changed in this example
Loss of viability and or functionality	Y	In this case there might be loss of viability due to pressure, temperature, pH, etc. factors that might result in a higher aneuploidy rate or higher degeneration rate. If there would be data from literature of from other centers using this pump and having good results with this pump, then there could be a substancial risk reduction.

Preliminary Score: ***2.25***
Number of Applicable Risks Consequences: ***3***
Number of Risks Consequences: ***4***
Max individual Risk value = ***1***

Highest Possible Risk Score = *5 * 4 * 5 * 4 * 8 =* ***3200***
Applicable Risk Score = *5 * 4 * 5 * 3 =* ***300***

Combined Risk Value = *(Risk Value * Highest Possible Risk Score) / Number of Applicable Risks = (2.25 * 3200) / 300* ***24***
Final Risk Score = *(Final Risk Score * 100) / Highest Possible Risk Score = (24 * 100) / 3200* ***1***

Your assessment has Final Risk Score of: **1**

This suggests that your TCTP falls into the Level of Risk:

Level of Risk	*Extent of Studies needed*
Negligible	**Step3A: Risk reduction strategies** A change in process could have a negligible level of risk because it is part of a therapy or procedure that is considered as established or standard. In this case multi-centred studies (ideally RCT) are published in peer-reviewed journal and the procedures are performed according to a validated and standard protocol. **Minimal process validation** is needed. The technical performance of staff should be monitored and comparable with other TE or published studies, therefore standard Key Performance indicators (KPI) should be monitored on the technical quality of the staff performing the procedures. Dropping KPIs indicating protocol drift must lead to investigation of both the procedural steps and / or the possibility to re-train staff.
	Step 3B: Extent of clinical evaluation A **routine/safety follow up program** is enough as the good practices state. Follow-up procedures should be focused on assessing efficacy, comparing the clinical follow-up with the results obtained before the implementation of the change in the process. Long-term (ideally trans-generational) health effects, including aspects such as fertility, oncology and mental health should be monitored.

Please refer to EuroGTP II Guide for additional details.

EuroGTP II Interactive Assessment Tool

TCTP: *Assisted Reproductive Techniques - Gametes*

The following information refers to TCTP: *Sperm*

Evaluation performed on: *2019-01-16 16:03:14*

Description of TCTP under evaluation: *Change from slow ejaculated sperm to lyophilisation of ejaculated sperm*

	Yes	*No*	*NA*
A. Has this type of TCTP previously been prepared and issued for clinical use by your establishment?	X		
B. Will the starting material used to prepare this TCTP be obtained from the same donor population previously used by your establishment for this type of TCTP?	X		
C. Will the starting material for this TCTP be procured using a procedure used previously by your establishment for this type of TCTP?	X		
D. Will this TCTP be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of TCTP?		X	
E. Will this TCTP be packaged , stored , and distributed using a protocol and materials used previously in your establishment for this type of TCTP?	X		
F. Will this type of TCTP provided by your establishment be applied clinically using an application method used previously?	X		
G. Has your establishment provided this type of TCTP for implantation or transplantation into the intended anatomical site and/or same clinical indication before?	X		

	Justification provided for Evaluation of Novelty questions
B.	There is no change in donor population
C.	collection is the same in the slow protocol as the lyophilisation protocol
D.	the procedure is completely different and in the exercise your TE has no experience with lyophilisation of sperm
E.	if the same containers can be used for the cryopreserved sperm, them the answer is yes. if the exercise would have an additional change: from vial to straw e.g., then this answer should also be 'no'

Risk Factor	*Risk*	*Probability*	*Severity*	*Detectability*	*Potential Risk*	*Risk Reduction*	*Risk*
Processing and environment	Implant failure / Pregnancy loss	5	2	1	**10**	0%	**10**
Processing and environment	Disease transmission	2	2	1	**4**	75%	**1**
Reagents	Implant failure / Pregnancy loss	1	2	1	**2**	25%	**1.5**
Storage Conditions	Implant failure / Pregnancy loss	4	2	1	**8**	0%	**8**
Loss of viability and or functionality	Implant failure / Pregnancy loss	5	2	1	**10**	0%	**10**

Risk Factor	*Applicable*	*Comment*
Donor Characteristics	N	Since there are no changes in the donor characteristics
Procurement process and environment	N	Changes in the cryopreservation protocol have no effect on the procurement. There are no extra risk need to be evaluated during this procurement
Processing and environment	Y	Changing to lyophilisation has definetely an effect and risk need to be considered: For this example, it is likely that due to the lyophilisation of the sperm that there is no implantation when using this sperm because of loss of functionality or viability of the sperm after thawing. The severity is serious in this example: if the sperm is no viable after lyophilisation and thawing then there is a significant decrease in the expected treatment success – thus score 2. It could be that you want to use 'life threatening' here because there will not be a pregnancy or the gametes might be destroyed. We would like to point out that 'fatal' is only used if there is a risk of death of the patient and not the embryo or the foetus. This assessment is on the risks for the recipient, not the embryo. If the sperm would not be vital after using this novel cryopreservation method, we would most certainly detect this. In this example, you might consider risk reduction based on animal studies, however there are not data in a human setting. So at this stage a risk reduction is not possible. It is important to only take into account the processing steps when evaluating this risk: during the processing steps, DNA fragmentation can be introduced in the sperm. Literature shows that the preparation for lyophilisation is quite easy and quick, so to shift from slow freezing to lyophilisation might not increase the complexity of the method, so there is no risk of introducing contaminants because of a very complex procedure. So the risk of disease transmission would be unlikely. In this case, it would occur, it could be serious as hospitalization could be necessary. The presence of virus could be detected: for several viruses, PCR can be performed. In the case a sperm that a sperm bank would be interested in using lyophilisation, a validation could be performed by which PCR testing after thawing and hydration of the sample is performed. A risk reduction can be applied when for example a validated post-thawing wash is performed of which it is known that HIV and HCV can be removed. Moreover, this post-thaw wash is referenced in many peer-reviewed data. As an example you might end up with. There is no evidence that the preparation process would introduce toxic substances or carcinogenicity into the sperm. This risk is not applicable and therefore should not be addressed. It is possible to always include other risk factors than those cited in the tool.
Reagents	Y	When different types of reagents are used in the lyophilisation protocol and thus, a potential risk needs to be considered. When considering the reagents needed in the lyophilisation procedure it is important to not take the processing steps into account, otherwise you might end up with the same result as before. Only consider the new reagents. For example: the reagents used for lyophilisation would be TE buffer (1mM tris, 1 mM EDTA, pH 8.0). Would they have an impact on the implant failure, including pregnancy loss? Probably not, because most of the reagents are not toxic for gametes or sperm. However if they turn out to have an impact, the result would be fatal. Would it be possible to detect this: yes, it is possible to look at the morphological changes of the sperm and / or perform vitality staining. There is data that suggests that this TE buffer has no effect on sperm, however these data might be not in combination with a lyophilisation procedure.
Storage Conditions	Y	It could be possible that because of the fact that lyophilisation is going to be performed, that additional care has to be taken when considering the storage condition during the preparation steps, for example: say that the sample would need to be put on ice after procurement and before lyophilisation. Cryostorage after lyophilisation is at 4ºC, so no liquid nitrogen would be necessary. Consider the risks with this change in the protocol.
Transport Conditions	N	In this example we expect no differences in transport conditions
Complexity of the pre-implantation preparation and/or application method	N	We expect no changes in the method of application in this example. However, in this example could be the case, when the manipulation after storage is very different and would have an impact on the outcome. Hypothetically, say that lyophilized sperm would need to be put in a very different insemination catheter and this would for example take much more time to load. In this case, you will have to consider this risk. So it is only the complexity of the application method or the preparation for clinical application.
Loss of viability and or functionality	Y	Risks need to be considered. It is known from the literature that sperm (from animals) becomes immotile after lyophilisation. This could impact in the success of the treatment

Preliminary Score: ***30.5***
Number of Applicable Risks Consequences: ***5***
Number of Risks Consequences: ***4***
Max individual Risk value = ***10***

Highest Possible Risk Score = *5 * 4 * 5 * 4 * 8 =* ***3200***
Applicable Risk Score = *5 * 4 * 5 * 5 =* ***500***

Combined Risk Value = *(Risk Value * Highest Possible Risk Score) / Number of Applicable Risks = (30.5 * 3200) / 500* ***195.2***
Final Risk Score = *(Final Risk Score * 100) / Highest Possible Risk Score = (195.2 * 100) / 3200* ***6***

Your assessment has Final Risk Score of: **6**

This suggests that your TCTP falls into the Level of Risk:

Level of Risk	*Extent of Studies needed*
Low	**Step3A: Risk reduction strategies** Implementing a standard procedure or treatment in an ART centre that has never performed this procedure exerts an **intensive validation**. Training of staff is necessary in order to reach the outcomes published in scientific literature. Having a mentor/mentee relationship with an ART centre having experience is highly recommended. Specifications on performance should be determined and when these limits are met by training on spare tissues and cells, staff can be authorized for performing the procedure. A learning curve might be expected and should be part of the validation report. When implementing the procedure, additional quality controls must be performed to **monitor Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs)**. For example, when a TE is switching from IVF to ICSI (which they never performed before), fertilisation rated, and damage rates etc. of embryos should be carefully monitored in relation to the staff performing the procedure.
	Step 3B: Extent of clinical evaluation A **safety follow up program** is necessary. Follow-up procedures should be focused on assessing efficacy, comparing the clinical follow-up with the results obtained before the implementation of the change in the process and in relation to the results published in scientific literature. As the procedure or treatment encompasses an established or standard technique. The expected learning curve should be kept as short as possible and put in relation to the follow up program. Likewise, established techniques are prone to long-term (ideally trans-generational) follow-up of the health effects. TE or ORHA implementing an established technique shall perform long-term follow-up and could base their follow-up items on the mentor facility. This way of working could lead to periodic evaluation of performance in the mentor/mentee relationship.

Please refer to EuroGTP II Guide for additional details.

EURO GTP II

Good Tissue & cell Practices

Co-funded by the Health Programme of the European Union