CHEMISTRY, MANUFACTURING AND CONTROLS REQUIREMENTS FOR CELL, TISSUE AND GENE THERAPY PRODUCTS FOR CLINICAL TRIALS AND PRODUCT REGISTRATION

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Introduction

Chemistry, Manufacturing and Controls (CMC) dossier provides a detailed information of the cell, tissue and gene therapy products (CTGTP) manufacturing and quality controls. This includes the origin of the starting materials, quality of reagents, manufacturing process and process controls, analytical methods and stability studies (refer to Figure 1). CTGTP includes products that contain autologous or allogeneic human cells or tissues, viable animal cells or tissues or recombinant nucleic acids. The ultimate objective is to reflect manufacturer's ability to consistently produce, analyse and store the final product with sufficient quality that is suitable for administration to human subjects. The CMC information submitted is a commitment that the applicant performs manufacturing and testing of the CTGTP as stated in the dossier.

This document lays out Health Sciences Authority's expectations for the CMC information required in each section of the Quality module, as relevant and applicable, of CTGTP clinical trials and product registration applications. The purpose of this document is to provide clarity on the CMC requirements; from upstream cells/tissue procurement, testing to downstream product manufacturing, analytical methods and release to the clinical site so as to assure the product safety, quality and efficacy is maintained throughout its lifecycle. The structure of the quality dossier is aligned with the Module 3 of the ICH Common Technical Document for the Registration of Pharmaceuticals for Human Use or Part II of the ASEAN Common Technical Dossier.

The CMC document applies to clinical trials sponsor, individual investigator and commercial company developing a CTGTP. The amount of CMC information to be submitted in a clinical trials application would depend on the phase of trial. Changes to the manufacturing may be necessary as the product development proceeds and hence any change that could affect the safety, identity, quality, purity, potency, or stability of the product shall be submitted for review prior to implementation.

The CMC dossier should be compiled from establishment(s) involved in the manufacture, donor testing and analytical testing laboratories for clinical trials and/or product registration.

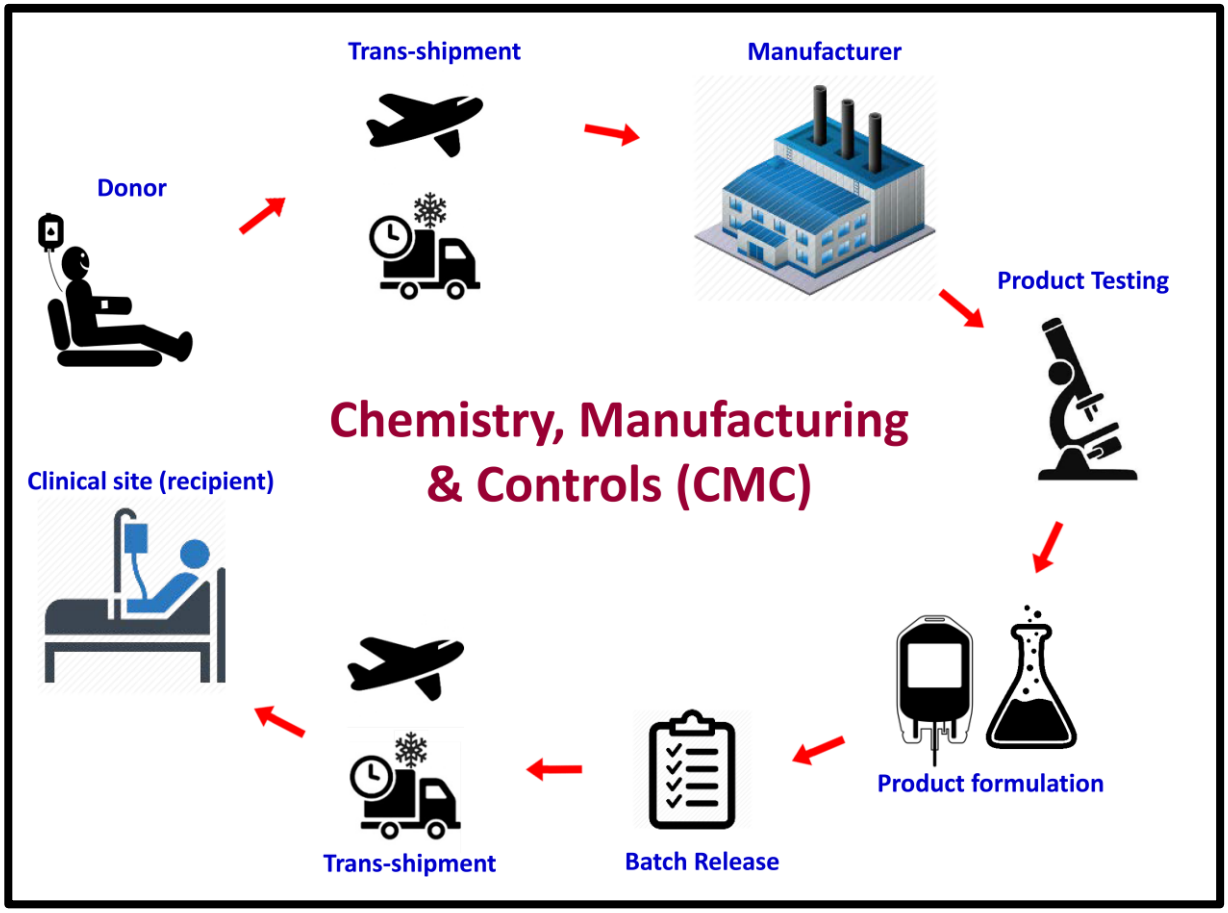

Figure 1: Overview of CTGTP manufacturing and quality controls

Dossier Sections	Description
Active Substance	
S1 General Information	
S1.1 Nomenclature	• International Non-proprietary Name (INN), The United States Adopted Names (USAN), proprietary name or common name of that is given to the product or an active substance.
S1.2 Structure	• Physical characteristics of cells (e.g. origin, phenotype, cell size, cell surface markers). • Schematic representation of structural component (e.g. scaffold, matrices, medical device) that is combined with a CTGTP. • Structural formula, molecular formula and relative molecular mass of chemical entity of therapeutic product that is combined with a CTGTP. • Schematic representation of amino acid sequence including post-translation modifications (e.g. glycosylation) and relative molecular mass of biologics entity of therapeutic product that is combined with a CTGTP. • For viral vectors, to provide schematic representation of regulatory and functional genetic elements (e.g. promoter, enhancer, restriction enzyme sites, transgene and selection markers). Information include but not limited to the composition of viral capsid, envelope structures, molecular weight, particle size, glycosylation sites, nature of the genome (single-stranded, double stranded, DNA or RNA, copy number of genomes per particles), tropism of viral vectors (e.g. specificity of viral vector for particular host tissue). • For plasmid vector, to provide schematic representation of regulatory and functional genetic elements (e.g. promoter, enhancer, restriction enzyme sites, transgene and selection markers). Information include but not limited to the physical properties, biochemical, growth characteristics, genetic markers and location (e.g. on plasmid, episomal or chromosomally) of inserted foreign genes. • For use of gene editing technology, provide description of the gene(s) edited and gene editing technology that was used.
S1.3 General properties	• Physicochemical and biological properties to achieve the defined biological effect. • Proposed mechanisms of action.
S2 Manufacture	

S2.1 Manufacturer(s)	• Name(s), address(es) and responsibilities of all site(s) involved in cell/tissue procurement site (e.g. apheresis site, tissue bank), product manufacturing, quality control testing (e.g. sterility testing laboratory) and storage. • Valid and relevant accreditation certificates or licences (e.g. AABB, AATB, JACIE, FACT, CAP, ISO 13485, ISO/IEC 17025, GMP, GTP).
S2.2 Description of manufacturing process and in-process controls	• A flow diagram of the manufacturing process with in-process controls and acceptance criteria (e.g. control limits). • A detailed description of the manufacturing steps from: ➢ Human cell/tissue collection (e.g. leukapheresis, biopsy, cord blood bank, tissue bank), ➢ vector production (e.g. viral vectors, bacterial plasmids and mRNA production, purification procedures, vector quality testing and storage conditions), ➢ enzymatic digestion, ➢ cell selection (e.g. method, devices and reagents used), ➢ cell expansion (e.g. culture media components, culture flasks/bags/bioreactor, open or closed culture system, culture temperature, duration), ➢ gene modifications (e.g. transfection, infection, electroporation or gene editing components), ➢ purification, irradiation, process operating parameters (e.g. duration, temperature, centrifugation speed), ➢ in-process control tests (e.g. viability, pH, cell number, dissolved oxygen, titre, bioburden, impurities), ➢ hold times, transfer steps, production duration, harvesting, formulation, filling and storage. • Transport procedures for starting material and finished product (e.g. transport temperature, duration and packing information) and hold times of materials to the manufacturing facility and during manufacturing process should be provided. • Information on batch size, scale of production, quantity of starting materials (e.g. volume apheresis materials) and expected yield. • Define the batch (or lot) numbering system, as applicable.
S2.3 Control of Materials	• Document the source, quality and control of all materials and components used in the manufacturing.
	<u>Human cells</u> • Sources of cells and tissues (e.g. autologous or allogeneic source).

	• Collection procedures (e.g. leukapheresis, biopsy, mobilization of stem cells, operating parameters, volume or number of cells) and collection container. Where relevant provide the Singapore Medical Devices Registration Number for the container. • Description on donor screening (medical history and physical examination); donor testing (e.g. HIV-1; HIV-2, HBV, HCV, Syphilis, emerging relevant communicable disease agents as applicable and relevant to the local context); and donor qualification criteria. • Quality parameters with acceptance criteria of the donor cells and tissues. • Preparation of Master Cell Bank and Working Cell Bank (source, history, derivation, characterization tests, cryopreservation procedure, storage condition and stability data, size of the cell banks). • Identity of cell lines verified by genotypic and/or phenotypic markers, cell number, viability, purity, sterility (bacterial and fungal), mycoplasma, adventitious viruses, growth characteristics and genetic stability, *where applicable*).
	<u>Plasmid and Viral vectors</u> • Detailed description on the cloning steps of the recombinant construct. • Recombinant construct map identifying the plasmid or virus backbone, gene insert, regulatory elements and selection markers. • Therapeutic gene insert sequences and sequence analysis. • Origin, identity and biological characteristics of the packaging cell line or helper virus. • Description of procedures used for preparation of the final vector construct including host cells and helper virus used, propagation of recombinant clone, establishment and size of bacterial or viral seed banks. • Characterisation tests (e.g. genomic/phenotypic characterisation, molecular size, restriction endonucleases analysis, annotated sequencing data, transcription/expression of therapeutic sequences, adventitious agent virus, residual replication competent virus, viral titre, plasmid concentration) on the seed banks and vector construct. • Storage condition, shelf life and stability data.
	<u>Bacterial Cell banks</u> • Bacterial host strain name. • Preparation of Master Cell Bank and Working Cell Bank (source, history, derivation, characterization

	tests, cryopreservation procedure, storage condition and stability data, size of the cell banks). • Characterisation tests (e.g. viability, genotypic/phenotypic characterisation, adventitious agents, transgene expression and/or activity).
	Xenogeneic Cells • Source, origin and health status of the animals. • Screening and detection of known infectious agents. • Description on sacrifice of animals, extraction of organs/ tissues or cells. • Shipping condition, duration and validation of transportation of the animal material to the manufacturing facility.
	Reagents • Tabulation of all reagents used in the manufacturing process indicating the identity, supplier, source (human or animal origin), the manufacturing stage in which the reagent is used, concentration used during manufacturing, reagent quality (e.g. clinical or research grade). • Certificate of Analysis (CoAs) or specifications of all reagents used in the manufacturing process. • If research grade reagent is used, a qualification program should be established that includes safety testing (sterility, endotoxin, mycoplasma, and adventitious agents), functional analysis, purity testing, and assays (e.g., residual solvent testing). • Transmissible Spongiform Encephalopathies (TSE) and adventitious agent risk assessment for reagents from animal origin, *where applicable.* Please refer to Checklist for the Registration of Class 2 CTGTP containing materials of animal origin. • If beta-lactam antibiotics (e.g. penicillin) are used, it is recommended to provide a rationale for their use and describe precautions to prevent hypersensitivity reactions.
	Scaffold, matrices, devices, biomaterials, biologics components • Description of the chemical, biological, physical and mechanical properties. • Device or therapeutic product name, product owner, registration status, manufacturing site details. Where relevant provide the Singapore Medical Devices or Therapeutic Product Registration Number. • Instruction For Use (IFU), package insert or CoAs. • Biocompatibility and structural characteristics study data to support the use of the material with cells/tissues.

	• Assess the stability (e.g. degradation profile, impurities level, aggregation) and safety concerns of the scaffold, matrices, devices, biomaterials and biologics components throughout the expected lifetime of the product.
	<u>Equipment</u> • Equipment such as apheresis machine, electroporator, irradiator, cell selection system, culture bags, chromatography matrices, tubing that come into contact with the product. Where relevant provide the Singapore Medical Devices Registration Number. • Instruction For Use (IFU) or CoAs. • Supporting data to demonstrate the performance of the device, *where applicable*.
S2.4 Control of Critical Steps and Intermediates	• The critical steps and intermediates in the manufacturing process need to be identified. • Description of in-process quality control tests and acceptance criteria. • Justifications on acceptance criteria. • Batch data of in-process control test results. • Describe the traceability system from human cell/tissue starting materials, critical raw materials, in-process intermediate product, final product and to the recipient.
S2.5 Process Validation and/or Evaluation	• Description of process validation studies, especially for pivotal trials and product registration. • Validation data to assure reproducibility of the manufacturing process and quality of the product. • Description of media fill test procedure and submit media fill test results to assess aseptic processing. • Procedures in place to investigate lot failures, out-of-specifications results and ways to implement corrective actions.
S2.6 Manufacturing Process Development	• Description of the manufacturing process development history. • Comparability studies to determine the impact of manufacturing changes on quality of active substance(s).
S3 Characterisation	
S3.1 Elucidation of Structure and other Characteristics	• Characterisation studies to demonstrate the biological activity, phenotypic, genotypic, purity and immunochemical properties of the active substance. • Complete sequence of the therapeutic and genetic elements for selectivity/regulation/control of the therapeutic sequences should be provided. Restriction endonuclease mapping data to provide.

	• Tissue tropism, infectivity, virulence, replication capacity, ratio of infectious to non-infectious particles and immunological characteristics information. • Potential insertional mutagenesis and associated risks to be evaluated. • Viral vector shedding, replication competence and possible reactivation of endogenous virus should be provided.
S3.2 Impurities	• Description of product and process-related impurities (e.g. residual bovine serum, growth factors, antibodies, antibiotics, selection beads, feeder cells, unwanted cell populations, non-viable cells, undifferentiated cells, residual replication competent virus, helper viruses, residual DNA associated with vector) introduced during manufacturing which may raise safety concerns such as potential toxicities, tumorigenicity or immunogenicity. • The manufacturing process aims to remove the product and process-related impurities, and the detection of the level of residual impurities to ensure product safety. • Describe the test procedures to detect the impurity level. • To provide the impurities acceptance level and with justifications.
S4 Control of Active Substance	
S4.1 Active Substance Specifications	List of characterisation tests and defined acceptance criteria: ➢ Identity, e.g. cell surface markers, genotypic markers, biochemical assays ➢ Purity, e.g. cell population of interest, vector content ➢ Impurities, e.g. endotoxin, unwanted cell populations, residual undifferentiated cells, residual replication competent virus ➢ Microbiological, e.g. bacterial, fungal, mycoplasma, adventitious agent tests ➢ Potency which quantitatively measure the biological activity of the product and related to the clinical response. ➢ Cell viability and total viable cell number ➢ Others such as appearance
S4.2 Analytical Procedures	• Detailed description of the analytical test procedures including the principle of the method, reagents, assay controls and test procedures.
S4.3 Validation of Analytical Procedures	• For compendial methods, to provide appropriate references. • For non-compendial methods, to provide the validation data to demonstrate specificity, sensitivity,

	accuracy, repeatability, detection limit, linearity, range and suitability (*where applicable*).
S4.4 Batch Analyses	• To submit batch data to demonstrate batch consistency using proposed manufacturing process. • Data to provide in tabular format include batch number, batch size, manufacturing site, manufacturing date, control test methods, acceptance criteria and test results.
S4.5 Justification of Specification(s)	• Justifications for the active substance specifications.
S5 Reference standards or materials	• Reference materials used for testing of active substance (*where applicable*). • Reference standards used for any assay should provide characterisation data or CoA.
S6 Container Closure System	• Description and identity of materials used for construction of container closure system. • Information on sterilisation of container and the suitability for the intended use. • To submit the CoA, specifications and schematic drawing.
S7 Stability data	• If active substance not immediately processed into final product, to provide the storage condition and shelf-life. • Stability protocol describing the test parameters, acceptance criteria, testing frequency and test temperature. • Stability data to support the proposed storage condition and shelf-life.
Final Product	
P1 Description and composition of CTGTP	• Qualitative and quantitative composition of all components in the CTGTP, diluent and placebo. • Description of the dosage form. • Type of container closure used.
P2 Pharmaceutical and Manufacturing Process Development	• Description on components of final product: ➢ Active substance ➢ Viral vectors ➢ Non-cellular components (e.g. scaffold, matrices, biomaterials, biologics components ➢ Excipients • Manufacturing process development • Product formulation development • Microbiological attributes • Container closure system • Compatibility of final product with diluent and with the delivery device.

	• Comparability studies to determine the impact of manufacturing changes during product development and among different manufacturing sites.
P3 Manufacture	
P3.1 Manufacturer(s)	• Name(s), address(es) and responsibilities of all site(s) involved in development and proposed production, include manufacturing, quality control testing (e.g. sterility testing laboratory) and batch release. • Valid and relevant accreditation certificates and licences (e.g. CAP, ISO 13485, ISO/IEC 17025, GMP).
P3.2 Batch formula	Describe the batch formula of the CTGTP including batch size, list of all components (e.g. active substance, excipients, diluents) of the product.
P3.3 Description of manufacturing process and in-process controls	• Flow diagram of the manufacturing process with in-process control criteria. • Detailed description of all the manufacturing steps from final step of active substance manufacture, formulation, filtration, filling, packaging, freezing and storage. • Description on the shipping condition (e.g. temperature and packaging) and duration of finished product from manufacturer site(s) to product administration site(s). • Description on the preparation of CTGTP (e.g. thawing, addition of diluent, loading into delivery device) for administration or implantation.
P3.4 Control of Critical Steps and Intermediates	• Description of critical in-process tests and acceptance criteria. • Describe the traceability system from human cell/tissue starting materials, critical raw materials, in-process product, final product and to the recipient.
P3.5 Process Validation	• Description of process validation, especially for pivotal trial and product registration. • Validation data to assure reproducibility of the manufacturing process. • Description of media fill test procedure and media fill test results to assess aseptic processing • Procedures in place to investigate lot failures, out-of-specifications results and ways to implement corrective actions.
P4 Control of Excipients	• List of excipients, source, specifications and CoA. • The interaction between the excipient(s) and active substance should be discussed. • List of materials of human or animal origin used in manufacturing process. • A risk assessment of potential contamination with adventitious agents.

P5 Control of CTGTP	
P5.1 CTGTP Specification(s)	• List of characterisation tests and defined acceptance criteria: ➢ Identity, e.g. cell surface markers, genotypic markers, biochemical assays. ➢ Purity, e.g. cell population of interest, vector content ➢ Impurities, e.g. endotoxin, unwanted cell populations, residual undifferentiated cells, residual replication competent virus. ➢ Microbiological, e.g. bacterial, fungal, mycoplasma, adventitious agent tests. ➢ Potency tests which quantitatively measure the biological activity of the product and related to the clinical response. ➢ Cell viability and total viable cell number per dose or implant. ➢ Others such as appearance. • To provide justifications when the test results are not available prior to release of the final product and action plans should test results fail to meet the release specifications (e.g. product sterility failure).
P5.2 Analytical Procedures	• Detailed description of the analytical test procedures including the principle of the method, reagents, assay controls and test procedures.
P5.3 Validation of Analytical Procedures	• For compendial methods, to provide appropriate references. • For non-compendial methods, to provide the validation data to demonstrate specificity, sensitivity, accuracy, repeatability, detection limit, linearity, range and suitability (*where applicable*). • If antibiotics used in the manufacturing process, you should provide documentation that the antibiotics were removed prior to sterility testing. If the antibiotics cannot be removed from the final product, we recommend that you assess the validity of the sterility assay using the bacteriostasis and fungistasis testing that is designed to ensure that any residual antibiotic present in the product does not interfere with the results of sterility testing.
P5.4 Batch Analyses	• To submit batch data (or CoA for final product) to demonstrate batch consistency using proposed manufacturing process. • Data to provide in tabular format include batch number, batch size, manufacturing site, manufacturing date, control test methods, acceptance criteria and test results.
P5.5 Characteristics of Impurities	• To provide characterisation of impurities if not provided in S.3.2 Impurities.

P5.6 Justification of Specification(s)	• Justification for the product specifications.
P6 Reference standards or materials	• Reference materials used for testing of CTGTP (*where applicable*). • Reference standard used for any assay should provide characterisation data or CoA.
P7 Container Closure System	• Description and identity of materials used for construction of container closure system. • Information on sterilization of container and the suitability for the intended use. • To submit the CoA, specifications and schematic drawing. Where relevant provide the Singapore Medical Devices Registration Number.
P8 Stability data	• Stability protocol describing the test parameters, acceptance criteria, testing frequency and test temperature. • Tabular stability data to support the proposed storage condition and shelf-life. Tabular stability data to support the proposed in-use (e.g. after thawing, addition of diluent, after opening from the transport container) and in-device storage condition and shelf-life.
Appendices	
A.1 Facilities and Equipment	• Summary of information on manufacturing flow in the manufacturing facility and equipment come in contact with the product.
A.2 Adventitious Agents Safety Evaluation	• List of materials of human or animal origin used in manufacturing process. • Please refer to Checklist for the Registration of Class 2 CTGTP containing materials of animal origin. • Risk assessment of potential contamination with adventitious agents and TSE agents.
A.3 Excipients	
A.4 Environmental Risk Assessment (ERA)	• ERA evaluates the potential environmental risks when the CTGTP containing genetically modified organisms (e.g. viral vectors) are released into the environment and adverse effects to the ➢ the staff administering the CTGTP. ➢ Care-givers and family members who may have direct contact with the patient. ➢ animals, plants and micro-organisms ➢ environment-at-large • Specific mitigation measures (e.g. limit exposure, monitor release of the product) if the CTGTP significantly affect the quality of the environment. • Please contact the Genetic Modification Advisory Committee (www.gmac.sg) for ERA.

	• To submit GMAC's recommendations with the application for CTGTP Clinical Trials or Product Registration.
Literature References	List of key literature references should be provided.

REVISION HISTORY

Version	Date of publication	Amendment Summary
1	Oct-2024	This document GN-ATPB-1-4 Version 1 replaces the previous document ATPB-GN-008-000 dated March 2021.