Cell and Gene Therapy Product Development Matrix – CMC

	Optimization (Research up to Pre-IND)	Development (Pre-IND to IND)
Manufacturing Process Risk Analysis References 1, 2, 3	• Optimization plan should include a basic process risk analysis. This should include: o Identification of process steps with potential for significant viable cell loss, contamination, variability, or other modes of failure. o Brief discussion of process development needed to mitigate process risks (significant viable cell loss, contamination, variability), enhance process control, and maximize cell yield and stability. • Failure Mode Effects Analysis (FMEA) is a useful risk analysis framework but is not required.	• Process risk analysis in the Development plan should be more detailed than the risk analysis performed in Optimization phase and should reflect understanding of the manufacturing process gained from process development studies already performed.
Manufacturing Process References 4 (section IIIB), 5, 6 (section IIIB), 7, 8, 9	• Manufacturing process description o Process Table Description of current version of manufacturing process in tabular form, as a series of steps, one step per row. Top row should be the beginning of the manufacturing process (usually collection of cells or tissue) proceeding to the final product or other endpoint. The table should include the four columns listed below. All four should be completed for each process step. ▪ Time (days or hours, as appropriate) ▪ Process Step Description ▪ Materials and Equipment ▪ Development Notes ▪ *Time column should indicate approximate timepoints in days or hours, as appropriate (i.e., what steps are performed on day 1, day 2, etc.). Timepoints may be expressed as ranges as needed.* ▪ *Process Step column should include brief descriptions of each process step, such as Cell Expansion Culture, Cryopreservation, CD34 Positive Selection, etc.* ▪ *Materials and Equipment column should list the raw materials (reagents and other consumables), and equipment (i.e., 37℃ CO_2 incubator, low-speed centrifuge, BSC, etc.) needed for each process step.*	• As in Optimization, with more detailed information defining the manufacturing process, and incorporating results of process development studies.

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	▪ *Development Notes for each step should include comments, issues, or plans regarding development. These typically address increasing yield and purity of the desired cell population, reducing risk of contamination, and simplifying manufacturing and facilitating scale-up/scale-out, often by changing from manual to semi-automated or automated processing, and adopting closed-systems. See slides on manufacturing process development and PAS 83 for further information about process development.* *Updates to the Development Notes column will document the development history of the product, one of the sections of the IND application.* ▪ Table should include sampling points*, process steps in which samples are taken for in-process testing or for final product release testing.* ▪ Table should include decision points*, when the manufacturing process may be modified or aborted based on results of in-process testing, or other factors. Describe basis for decision points – test results that would prompt a decision to continue/modify/discontinue process*. o Process Flow Diagram *Outline current version of the manufacturing process as a flow diagram, a series of steps from beginning of the manufacturing process to end.* Flow diagram should include: ▪ Approximate timepoints in days or hours, as appropriate. ▪ Sampling points. ▪ Decision points. • *Process table and flow diagram should be updated as needed to capture changes in manufacturing process with ongoing development.*	
Development Plan References 5, 7, 8, 9	• Process development plan should be provided, and should include the following: o Description of process development studies	• As in Optimization, with more detailed information. Should follow logically from manufacturing

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	o Specify manufacturing unit operations (process steps) to be improved. o Provide rationale for optimizing these process steps. o Specify equipment and disposables being tested/optimized. o Specify outcome measures for process optimization, i.e., viable cell yield for target population. o Brief description of comparability plan to bridge process changes.	process information, data, and conclusions in the previous section.
Manufacturing Components: Cells/Tissue		
Cell Source, Method of Collection References 4, 6 *These points apply to cell therapy products,* ex vivo *gene therapy products, and some cell-based devices. If developing an* in vivo *gene therapy product, this section should be skipped.*	• If product will be manufactured from primary cells, Optimization plan should address issues related to cell source, including: o Autologous, HLA matched-allogeneic, unmatched allogeneic "universal donor", xenogeneic, or other? o Source tissue (i.e., umbilical cord blood, bone marrow, skin, etc.). If source tissue has not yet been established, Optimization plan should specify candidate tissues and how source tissue will be selected. o Any relevant donor characteristics (i.e., HLA type, age, medical history, etc.). o Sourcing and availability of tissue/cells for R&D studies. This is particularly relevant to development of autologous products. Suitability of models such as healthy donor material should be discussed. • Optimization plan should address tissue/cell collection, including: o Detailed description of collection method (blood draw, surgical excision, bone marrow aspiration, apheresis, etc.). o Mobilization protocol, if any (any treatment donor receives to mobilize or activate cells *in vivo* prior to collection should be considered mobilization) o IRB approval status	• As in Optimization, with more detailed information and data supporting acceptance criteria for product of cell collection (volume, mass, dimensions of tissue, number of WBCs, etc.).

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	○ Characteristics of an adequate collection (volume, mass, dimensions of tissue, number of WBCs, etc.). ○ Other key characteristics of tissue/cell raw material, if any.	
Donor Screening and Eligibility Testing Reference 10	• If developing an allogeneic product, Optimization plan should specify the donor eligibility screening and testing performed. This should be in accordance with 21 CFR 1271. Unless the exceptions in 21 CFR 1271.90a apply, donor screening and testing should include: ○ Donors of all types of cells and tissues: ▪ Screening and testing for HIV-1, HIV-2, HBV (surface and core antigen), HCV, *T. pallidum* (syphilis). ▪ Screening for CJD. ○ Donors of viable WBC-rich cells or tissues: additional screening and testing for HTLV-1, HTLV-2, and CMV. ○ Any additional testing, such as HLA typing. • The Optimization plan should include a copy of the donor screening questionnaire.	• As in Optimization.
Vector (Gene Therapy Products) References 6, 7	• Gene Therapy Vector Construct Description of history and derivation of the gene therapy vector including: ○ The gene map, with relevant restriction sites, and any vector constructs used during generation of the final vector and their sources ○ Gene insert ○ Regulatory elements, such as promoter, enhancer, and poly-adenylation signal ○ Selection markers • Vector Diagram ○ Diagram of the vector identifying gene insert and regulatory regions, and any other relevant elements, such as pertinent restriction endonuclease sites. • Sequence Analysis Plan ○ Vectors 40 kilobases (kb) or less: ▪ Full sequencing of vector ▪ Sequence analysis	• As in Optimization, with more detailed information and data.

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	▪ Preparation of annotated sequence of the entire vector ▪ Summary of sequence analysis • Indicate origin and function of each component of the vector. • Should account for all nucleotides such as promoters, known coding sequences, polyadenylation signals, origins of replication and restriction sites used during construction of the vector or for diagnostic tests. • Evaluation of significance of all discrepancies between the expected sequence and the experimentally determined sequence. • Evaluation of significance of any unexpected sequence elements, including open reading frames. • Viral vector sequencing should be performed on the master viral bank. • Plasmid sequence should be obtained from the master cell bank. • Retroviral vector sequence should be obtained from the MVB/packaging cell line or from DNA obtained after transduction of a stable cell line. o Vectors greater than 40 kb: ▪ Summary of extent and results of any sequence analysis performed, including any testing performed by restriction endonuclease analysis. ▪ Sequence analysis of the gene insert, flanking regions, and any regions of the vector that are modified.	
Cell Bank System (if used) References 4, 6 (Section *III A 1 – Vector*)	• Description of cell banking system (Master Cell Bank, with or without an additional Working Cell Bank), if used. Should include: o History, source, derivation, characterization of each cell bank, and frequency at which testing is performed. • Specify characteristics, testing, and results of testing performed on the following cell banks: o Master Cell Bank (MCB)/Packaging Cell Line	• As in Optimization, with more detailed information and data.

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	○ Master Viral Bank (MVB) ○ Working Cell Bank (WCB)/Working Viral Bank (WVB)	
Manufacturing Components: Raw Material/Reagents References 4 (section IIIA), 6 (section IIIA), 11, 12, 13, 14	• Optimization plan should describe: ○ Testing to define optimal or preferred reagents. • Raw Materials Table The Optimization plan should include a table of raw materials which includes the following information for each raw material item: ○ Manufacturing step(s) at which it is used ○ Vendor(s)/supplier(s) ○ Source (i.e., chemical, recombinant, human, bovine, porcine) ○ Quality (USP, pharmaceutical, clinical-grade, GMP, research-use-only) ○ Ancillary material or excipient (see USP <1043>) *Ancillary material = used in manufacturing process, not intended to be in final product (i.e., culture medium, cytokines, immunomagnetic particles, etc.)* *Excipient – intended to be part of final product formulation.* • For any animal-derived materials, materials manufactured in-house, or materials labeled For Research Use Only, the Optimization plan should include: ○ Justification for use ○ Description of risk mitigation • For any bovine-derived raw materials, including serum, the Optimization plan should specify: ○ Country of origin (Australia, New Zealand, or other negligible BSE risk country). ○ Quality (should be GMP if possible) ○ Further qualification testing, if any • Beta-lactam antibiotics If beta-lactam antibiotics are used, this should be addressed in the Optimization plan, which should include: ○ Justification for use of beta-lactam antibiotic(s).	• Development plan should include the information needed for Optimization plan, with more detailed information and data as indicated below. • Raw materials qualification plan, and results of any qualification testing already performed. *Qualification may be limited to review of the Certificate of Analysis (CoA), but more extensive testing may be required depending on quality of the material and intended use – see the description of risk-based materials qualification in USP <1043>.* *The terms raw materials and reagents are used interchangeably. FDA says reagents, USP says raw materials.* *Research-grade or otherwise non-GMP raw materials may be used at Phase I if there is no higher-quality alternative available, provided the material is qualified by additional testing, to mitigate risk. USP <1043> has further information on the risk-based approach to qualification of raw materials.* • Batch-to-batch raw materials testing, if relevant (plans and if available, results). Batch-to-batch testing is particularly important for serum and other variable biological material. • Raw Materials Table The Development plan should include a table of raw materials which includes the following

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	o Description of precautions to prevent hypersensitivity reactions	information for each raw material item: o Manufacturing step(s) at which it is used o Final concentration at use o Vendor(s)/supplier(s) o Source (i.e., chemical, recombinant, human, bovine, porcine) o Quality (USP, pharmaceutical, clinical-grade, GMP, research-use-only) o Ancillary material or excipient o Proposed qualification (C of A, any additional testing, intention to cross-reference manufacturer's DMF). • Determination of residual ancillary materials. Should describe: o Determination of residual ancillary materials in the final product by testing or calculation. o Specify which materials will be tested, and why these have been chosen *Testing is preferable for materials with known or potential toxicities.* o Test procedures used to detect residual ancillary materials, including limits of detection or limits of quantitation. • Certificates of Analysis for all raw materials should be obtained prior to IND preparation
Testing References 4, 5, 6, 7, 8, 9, 15, 16		
In-Process Testing	• In-Process Testing Table The Optimization plan should include a table of in process testing, specifying the following:	In-Process testing information as in Optimization. In-process testing should be defined and in place prior to IND submission.

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	o Tests used, process step, and intermediate acceptance criteria, if any (i.e., process continuation criteria). o Draft version of in-process testing should be defined prior to Pre-IND meeting *In-process testing is typically a subset of the release testing panel, focused to provide useful information about the process intermediate tested and the manufacturing process.*	
Release Testing	The Optimization plan should address product release testing, and should include the following: o Descriptions of analytical methods chosen or being evaluated, and potential acceptance criteria. Acceptance criteria should be based on data from lots used in preclinical studies. o Product Release Testing Table ▪ Release testing table should list safety, purity, identity, and potency tests, analytical methods, and acceptance criteria. o The Optimization plan should result in analytical methods having been selected, with the Phase I version of acceptance criteria defined, in time for the Pre-IND meeting. *Specifications are the quality standards (i.e., the tests and analytical procedures, and the acceptance criteria) that confirm the quality of products and other materials used in manufacturing. Acceptance criteria are the acceptable numerical limits or ranges for the tests described.* Descriptions of release testing should include the following test categories and tests: o Safety testing ▪ Sterility cultures (aerobic, anaerobic, yeast/fungal). 21 CFR 610 or USP <71> methods, or a qualified alternative, such as an automated sterility culture system, bioMérieux BacT/ALERT or BD Bactec, for example. ▪ Mycoplasma PCR or other rapid method ▪ Adventitious agent (viral infectious disease) testing, if necessary for release *Testing performed as part of donor eligibility is sufficient unless testing cell banking*	In-Process testing information as in Optimization. In addition: o Proposed, rather than potential, acceptance criteria for each test. Acceptance criteria should be based on data from lots used in preclinical studies. o Product Release Testing Table ▪ Release testing table should list safety, purity, identity, and potency tests, analytical methods, acceptance criteria, and test sensitivity and specificity. o The Development plan should result in analytical methods having been qualified in time for IND submission. • Data supporting specifications for minimum cell dose, minimum viable cell dose. *Phase I version of acceptance criteria should be in place prior to IND submission.* *For-Information-Only specifications may be used for potency testing at Development stage and are permissible though not necessarily desirable as late as Phase II.* *Analytical methods should be qualified by end of Development phase.*

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	system, or there is a potential source of adventitious agents downstream of primary cell collection. o Identity testing Analytical methods capable of identifying the desired product. Cell surface markers, gene expression, other methods. ▪ Description of analytical methods being considered. ▪ Data supporting choice of analytical methods for identity testing, potential specifications for acceptance. o Purity *Relative freedom from extraneous material (contaminants) in the finished product. Unintended cellular phenotypes, residual reagents used in manufacturing process.* ▪ Endotoxin • Description of analytical method -- LAL, chromogenic or kinetic ELISA ▪ Cellular and non-cellular contaminants • Description of analytical methods being evaluated. • Data supporting detection of undesired cell types and non-cellular contaminants, potential specifications for acceptance. o Viability ▪ Measurement of % viable cells. *There is no requirement to use the Trypan Blue exclusion method, and fluorescence-based methods such as acridine orange/propidium iodide or DAPI are more analytically robust.* ▪ Description of analytical method and specifications. Minimum acceptable viability specification is typically 70%. o Cell number/dose ▪ Description of analytical methods being considered, such as automated cell counting.	

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	▪ Data supporting potential specifications for minimum cell dose, minimum viable cell dose. o Potency ▪ Description of candidate analytical methods being considered for potency testing. *For-Information-Only specifications permissible.* *Draft version of acceptance criteria should be in place prior to Pre-IND meeting*	
Stability Testing References 4, 5, 6, 7, 8	• Stability testing plans, in-process stability and final product stability – draft versions. Should include: o Test parameters – sterility, cell number, viability, selected identity, candidate potency tests, other. o Stability testing timepoints, specify test battery for each timepoint.	• In-process and final product stability testing plans - finalized. Should include information as in Optimization. • In-process and final product stability testing should be initiated prior to IND submission. *Stability studies may be performed using product manufactured in process qualification runs.*
Container/Closure References 4, 5, 6, 7, 8	• Container testing plan, should include: o Candidate product containers. Closed-system containers (bags, closed-system vials) strongly preferred. o Test parameters – cell number, viability, selected identity, candidate potency tests. • Execute late in Optimization phase	• Container testing and qualification plan. Should include: o Candidate product containers. Closed-system containers (bags, closed-system vials) strongly preferred. o Test parameters – sterility, cell number, viability, selected identity, candidate potency tests. • Test potential containers early in Development phase if not done in Optimization. • Container(s) should be selected and qualified by late Development.
Shipping References 4, 5, 6, 7, 8	• Define shipping steps required (i.e., cells from collection site to manufacturing facility, manufacturing facility to clinical site, etc.)	• Shipping and shipping qualification plan, should include, for each shipping step: o Shipping conditions, based on stability study data. o Temperature specifications.

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		o Equipment, including shipping containers and temperature monitor(s)/logger(s). o Transport company selection and qualification. • Shipping qualification to be completed by end of Development phase. *Shipping qualification may be performed using product manufactured in process qualification runs.* *Product shipping conditions, equipment, and materials should be defined in close collaboration with the GMP manufacturing facility staff, who should perform shipping qualification studies.*

References

In addition to the documents referenced, the FDA web pages for Cellular & Gene Therapy Guidances and Tissue Guidances are invaluable sources of information.

Reference	Title	Description	File Name
1	FDA Guidance for Industry: ICH Q9 Quality Risk Management – 2006.	Describes considerations for risk management/mitigation in development of biologic products	FDA Guidance – ICH Q9 Quality Risk Management – 2006.pdf
2	Lopez, *et al*. A quality risk management model approach for cell therapy manufacturing.	Discusses application of quality risk management to cell therapy manufacturing specifically. Risk Anal. 2010 Dec;30(12):1857-71	Lopez et al., 2010 - Quality risk management model for cell therapy manufacturing
3	Risk Analysis and Management	Slides outlining FMEA risk analysis in cell and gene therapy development.	Risk analysis and risk management slides.pdf
4	FDA Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs) - 2008	Overview of regulatory considerations and requirements for the CMC section of cell therapy IND applications	FDA Guidance - Content and Review of CMC Information for Human Somatic Cell Therapy INDs – 2008.pdf

Reference	Title	Description	File Name
5	USP <1046> Cellular and Tissue-based Products	USP chapter on development of cell therapy and tissue-engineered products, emphasis on CMC aspects but addresses clinical as well.	USP 1046 - Cellular and Tissue-based Products (NF, Supplement).pdf
6	FDA Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) - 2008	Overview of regulatory considerations and requirements for the CMC section of gene therapy IND applications	FDA Guidance - Content and Review of CMC Information for Human Gene Therapy INDs – 2008.pdf
7	USP <1047> Gene Therapy Products	USP chapter on development of gene therapy products, emphasis on CMC aspects but addresses clinical as well.	USP 1047 - Gene Therapy Products (NF, supplement).pdf
8	Successful Development of Quality Cell and Gene Therapy Products	Overview of manufacturing-related tasks and regulatory expectations in development of cell and gene therapy products.	Successful Development of Quality Cell and Gene Therapy Products - Denise Gavin, FDA OCTGT.pdf
9	PAS 83: Developing human cells for clinical applications in the EU and USA - 2012	Overview of cell therapy product development, including practical aspects and US FDA regulatory considerations.	PAS 83 - Developing human cells for clinical applications in the EU and USA – 2012.pdf
10	FDA Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products - 2007	FDA regulatory requirements for screening and eligibility testing of donors of cells and tissues for use in manufacturing cell therapy and gene therapy products	FDA Guidance - Eligibility Determination for Donors of HCT-Ps – 2007.pdf
11	USP <1043> Ancillary Materials for Cell, Gene, and Tissue-Engineered Products	Selection, qualification, and use of raw materials/reagents in manufacturing cell therapy, gene therapy, and tissue-engineered products	USP 1043 - Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.pdf
12	FDA Proposed Rule: Use of Materials Derived From Cattle in Medical Products Intended for Use in Humans and Drugs Intended for Use in Ruminants - 2007	Regulatory considerations and requirements for use of bovine-derived material (including fetal bovine serum) in manufacturing medical products, including cell therapy and gene therapy products.	FDA Proposed Rule - Use of Materials Derived From Cattle in Medical Products Intended for Use in Humans and Drugs Intended for Use in Ruminants – 2007.pdf
13	9 CFR 113.53 - Requirements for ingredients of animal origin	Regulatory requirements for use of bovine-derived material (including fetal	9 CFR 113.53 - Requirements for ingredients of animal origin

Reference	Title	Description	File Name
	used for production of biologics	bovine serum) in manufacturing biologic products, including cell therapy and gene therapy products.	used for production of biologics.pdf
14	EMEA Guideline on the use of bovine serum in the manufacture of human biological medicinal products - 2013	EU guideline and considerations for use of bovine serum, including FBS, in manufacturing biologi products, including cell therapy and gene therapy products. Applies to development of products for EU market, but has information useful for US cell and gene therapy product development.	EMEA Guideline on the use of bovine serum in the manufacture of human biological medicinal products – 2013.pdf
15	USP <1027> Flow Cytometry (NF, supplement)	Discusses methodology and applications of flow cytometry in cell therapy and gene therapy product development.	USP 1027 - Flow Cytometry (NF, supplement).pdf
16	FDA Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products - 2011	Reviews progressive development and implementation of potency testing for cell therapy and gene therapy products.	FDA Guidance - Potency Tests for Cellular and Gene Therapy Products – 2011.pdf