Checklist for Preclinical Studies

CATALYZE
Accelerating Translation

Cell and Gene Therapy

Notes

This CGT-focused checklist consolidates early-phase assays for viral gene therapies and cell therapies.
Note: Completion of all studies is not required; please indicate which studies have been completed.

Key references: FDA Cellular & Gene Therapy Guidances (e.g., CMC for Human Gene Therapy INDs, potency assurance), ICH Q5A(R2) viral safety (2023/2024), USP general chapters (<71>, <85>, <63>) and USP resources on rapid methods, plus peer/industry sources for AAV analytics and handling. Use phase-appropriate rigor: fit-for-purpose at Pre-IND, qualification in Phase I/II, full validation by BLA where applicable; align with FDA via INTERACT/Pre-IND.

Modality	Category	Test/Technique	Purpose	Phase Applicability	Sample Requirements	Key Output/ Acceptance Criteria	Reference/ Guideline	Notes
Gene Therapy (AAV/AdV/mRNA/ DNA)	☐Strength/ Dose	Vector Genome Titer (ddPCR/qPCR)	Dose setting and comparability across lots; robust across in-process and purified samples	Pre-IND, Phase I	DNase pretreatment; capsid lysis (viral vectors); validated primers for GOI/ITR or common elements	vg/mL (or copies/mL) within target; method precision meeting internal criteria	FDA CGT CMC IND (2020); USP AAV PCR control; ICH Q5A(R2) risk-based testing	Use particle controls for AAV; for non-viral vectors, ensure plasmid integrity control
Gene Therapy (AAV/AdV)	☐Capsid Content	Full/Empty/Partial Ratio (SV-AUC / CD-MS / AEX / SEC-MALS)	Quantify empty, partial, full capsids; set process controls and limits	Pre-IND, Phase I	High particle load (AUC); buffer exchange for CD-MS; chromatography setup	% full, % partial, % empty; trending and phase-appropriate limits	USP AAV8 empty/full app note; Waters CDMS; FDA/CBER discussions on empty capsids	Orthogonal methods recommended; consider platform limits by serotype
Gene Therapy (viral vectors)	☐Potency	Cell-based Transduction + Expression/Function (Assay Matrix)	Demonstrate MoA: gene transfer and biological effect	Phase I (qualification), Phase II (tightening)	Relevant cell model; quantitative readouts (mRNA/protein/ activity)	EC50/IC50, expression fold-change, functional activity within defined ranges	FDA Potency Assurance (2023 Draft); Phase-appropriate potency development	Use assay matrix if single assay cannot capture both transfer and function
Gene Therapy (viral vectors)	☐Safety/ Impurities	Replication-Competent Virus (e.g., rcAAV, RCA for AdV)	Demonstrate absence of replication-competent contaminants	Pre-IND, Phase I	Amplification on permissive cells + qPCR/readout	Not detected / below LOD	FDA CGT CMC IND; ICH Q5A(R2)	Critical for helper-dependent systems; include controls
Gene Therapy (viral vectors)	☐Safety/ Adventitious Agents	Adventitious Virus Testing Strategy	Risk-based testing of banks/harvests and viral clearance (if applicable)	Pre-IND, Phase I	MCB/WCB/LIVCA testing; bulk harvest panels	Panel negative/controlled; documented clearance steps	ICH Q5A(R2); EMA/CHMP adoption; FDA CBER guidances	Plan INTERACT/Pre-IND to align testing panels and clearance rationale
Gene Therapy (viral vectors)	☐Residuals	Residual Host Cell DNA & Size Distribution	Quantify residual DNA and demonstrate fragmentation control	Pre-IND, Phase I	qPCR/ddPCR; size analysis (capillary electrophoresis)	ng/dose within limits; majority <200 bp	ICH Q5A(R2); FDA/EMA updates	Include benzonase verification and acceptance criteria
Gene Therapy (viral vectors)	☐Residuals	Helper Plasmid DNA / Helper Virus Markers	Demonstrate clearance of process-related genetic impurities	Pre-IND	Targeted qPCR/ddPCR (rep/cap/helper)	Below action limits; consistent removal	FDA CGT CMC IND; ICH Q5A(R2)	Trend across unit operations
Gene Therapy (all)	☐Sterility & Pyrogen	Sterility (USP <71>) & Endotoxin (USP <85>)	Ensure sterility and acceptable endotoxin burden for parenteral use	Phase I release	DP lots; validated sampling	Sterile; endotoxin per dose within USP K-based limits	USP <71>; FDA endotoxin points-to-consider	Run product-specific suitability for LAL method
Gene Therapy (all)	☐Mycoplasma	Mycoplasma (USP <63> or validated rapid PCR)	Detect mycoplasma contamination	Phase I release	Culture-based (28-day) or validated rapid PCR	Not detected; validated LOD	USP <63>; JCM 2023 rapid assay comparison	Rapid methods acceptable with validation and suitability
Gene Therapy (all)	☐Off-target effects	Next-gen sequencing	Detect off-target effects in other tissues			Report, show any effect on downstream gene expression	FDA recommendation	FDA wants detailed report
Cell Therapy (autologous/ allogeneic)	☐Identity	Cell Identity/Phenotype (Flow cytometry, immunophenotyping)	Confirm product identity and expected phenotype (e.g., CAR expression, CD markers)	Pre-IND, Phase I	Qualified antibodies/panels; reference controls	% positive cells; MFI ranges; identity pass/fail	FDA CGT guidances; industry practice	Include viability gating and isotype/ compensation controls

Cell Therapy (autologous/ allogeneic)	☐Potency	Functional Potency (e.g., cytotoxicity, cytokine release, proliferation)	Demonstrate biological activity aligned to MoA (e.g., CAR-T killing)	Phase I (qualification)	Relevant target cells/assays; quantitative endpoints	EC50/response window; system suitability	FDA Potency Assurance (2023 Draft)	Assay matrix may be required for complex MoA
Cell Therapy (autologous/ allogeneic)	☐Safety/ Impurities	Residuals (e.g., beads, cytokines, serum, DMSO)	Quantify process-related residuals and ensure patient safety	Pre-IND, Phase I	Appropriate ELISA/HPLC/GC methods	Below phase-appropriate limits	FDA CGT CMC; USP micro QC resources	Include leachables from disposables if applicable
Cell Therapy (autologous/ allogeneic)	☐Safety/ Microbial	Sterility (USP <71>) & Endotoxin (USP <85>) & Mycoplasma	Ensure sterility and low endotoxin for short shelf-life products	Phase I release	Rapid sterility/endotoxin methods may be needed	Sterile; endotoxin within dose limits; mycoplasma ND	USP <71>, <85>, <63>; USP evolving rapid methods	Justify rapid methods and demonstrate suitability per USP <1223> if used
Cell Therapy (genetically modified cells)	☐Genetic Safety	Vector Copy Number (VCN) & Integration Site Analysis	Assess genetic modification burden and insertional risk	Pre-IND, Phase I	qPCR/ddPCR for VCN; NGS for integration sites	VCN within defined range; no high-risk integrations identified	FDA CGT guidances; industry practice	Long-term follow-up plans per FDA guidance
CGT (all)	☐Stability & Handling	In-use/Shipping Stability (temperature excursions, hold times)	Define handling windows for clinical sites and supply chain	Pre-IND, Phase I	Stress studies (freeze–thaw, agitation, light) and real-world shipping	Potency retention; aggregation/viability within limits	Frontiers AAV 2019; USP micro QC resources	Document chain-of-identity/chain-of-custody controls
CGT (all)	☐Documentation	Reference Standard Qualification & Method Lifecycle	Establish internal reference materials and phase-appropriate method status	Pre-IND→BLA	Well-characterized lots; traceable controls	COA covering identity/purity/potency; method fit-for-purpose → qualification → validation	FDA CGT CMC; ICH Q5A(R2); FDA early-phase CMC talks	Plan comparability for process changes and platform methods