

PROGRAM TECHNICAL DEEP DIVE

TSHR CAR-T — MC220711

Technology transfer, comparability and GMP manufacture — technical deep dive

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1. Executive technical summary

This is the best-documented program in the Mayo portfolio and the one where MADE can be most specific, because Mayo has already done the hard part. MC220711 TSHR CAR-T runs on the CliniMACS Prodigy T-Cell Transduction platform at the Center for Regenerative Biotherapeutics Advanced Biomanufacturing Facility, One Discovery Square, Rochester, with a complete batch-record set, an engineering-run tech transfer protocol already executed once, and a 61-page draft CMC ^{[S50][S51]}. A transfer here starts from documentation, not from a conversation.

Three observations shape MADE's technical position.

The process is Prodigy-native, and that is the first question to settle. Every batch record — initiation and T-cell enrichment, transduction, in-process sampling, harvest and final dose preparation — is written against the Prodigy TCT program ^{[S51][S52][S53][S54]}. A transfer either lands on a Prodigy at MADE, or it becomes a platform change with the full comparability burden the core describes. The CAR-T core is explicit that MADE's Prodigy status is not evidenced in the internal equipment record and must be confirmed against the BMRAM export before it is asserted. **This is the single highest-priority verification for this opportunity.**

The release panel has no functional potency assay. CAR-positive percentage at $\geq 20\%$ gated and CD3-positive at $\geq 80\%$ gated are both classified in the CMC as "Identity and Potency" ^[S50]. Those are identity attributes. Percent CAR-positive measures transgene expression, not function, and it is the first-listed FDA deficiency pattern for this modality — the agency challenges it at Phase II/III. The program is at Phase I/IND today, where a qualified biologically relevant assay is required and this position is defensible; it does not survive the move toward pivotal. Building a cytotoxicity or cytokine-release assay now, while the process is transferring anyway, is dramatically cheaper than building it under pivotal time pressure.

The vector position is unusually strong. The lentiviral vector is supplied by Vector BioMed on a third-generation HEK293T transient-transfection adherent platform, with a **drug master file on file with the FDA** and a full GMP release panel behind the certificate of analysis. The lot in hand, K340_TSHR_CAR LVV_0050 037.GMP.0050, reported a functional titer of 8.72×10^8 TU/mL and met all release specifications ^[S50]. Vector is the highest supply-chain risk in this modality and it is already resolved, with functional titer stated — which is the correct basis for MOI, and the one programs most often get wrong.

The right shape of engagement is a scoped technology transfer with comparability, run against Mayo's existing documentation, with an analytical workstream that closes the potency gap in parallel rather than afterwards.

2. The modality: biology and mechanism

A chimeric antigen receptor is a synthetic single-chain antibody fragment fused to T-cell signalling domains, so a T cell acquires MHC-independent recognition of a surface antigen. TCR-T differs in that it uses a native or affinity-matured T-cell receptor and therefore remains MHC-restricted, which constrains the eligible patient population by HLA type but opens intracellular targets that a CAR cannot reach.

The therapeutic entity is a living, dividing population, and that fact drives most of the CMC consequences. Potency is not a concentration; it is a functional capacity that can be lost without any change in cell count. Dose is expressed in CAR-positive cells rather than total cells, so transduction efficiency propagates directly into whether a dose can be met at all. And the product continues to expand in the patient, which is why persistence-linked attributes — memory subset distribution, exhaustion markers — are quality attributes rather than curiosities.

What the biology constrains in the process. Retroviral and lentiviral vectors require actively dividing cells, so transduction is downstream of successful activation. Suboptimal activation makes transduction fail regardless of how much vector is added, which is why a low transduction result is more often an activation problem than a vector problem ^[S11]. Large transgene inserts above roughly 6 kb transduce less efficiently, so dual-targeting and bispecific constructs may need a higher MOI and consequently carry more vector copy number risk ^[S11]. And patients who have had three or more prior lines of therapy present with low T-cell counts, poor viability and an exhausted phenotype — the starting material problem is a clinical fact about the population, not a collection-site defect.

3. This program's product, specifically

3.1 What it is

MC220711 is an autologous, lentivirally transduced CAR-T product targeting the **thyroid-stimulating hormone receptor**. TSHR is a target of real interest because its expression is largely restricted to thyroid tissue, which gives an unusually clean on-target/off-tumor profile compared with targets shared across normal lineages — and because thyroid ablation is clinically survivable in a way that B-cell aplasia or myeloid ablation is not. The clinical population is thyroid cancer.

Dose is expressed as **CAR-positive cells per kilogram of recipient weight**, calculated per patient from the collection order — the batch records carry the arithmetic on their face ^[S54].

3.2 The process as Mayo runs it today

Step	What Mayo does	Reference
Plasmid	Puresyn, Inc — INDReady plasmid DNA platform	[S50]
Vector	Vector BioMed, third-generation HEK293T transient transfection, adherent LVV expression platform; FDA DMF on file	[S50]
Collection	Leukapheresis, collected and transported to the Mayo GMP facility	[S50]
Initiation and enrichment	TCT process initiation and T-cell enrichment on the CliniMACS Prodigy	[S51]
Activation	TransAct at a 1:25 ratio (Miltenyi 200-076-204)	[S50]
Transduction	Day 2, on the Prodigy, at a titrated MOI	[S50][S52]
In-process sampling	Defined sampling procedure on the Prodigy	[S53]

Step	What Mayo does	Reference
Harvest and final dose	Harvest and preparation of the final dose from the TCT process	[S54]
Cryopreservation	Cryopreserved doses with a defined dosing schema	[S50]
Facility	CRB Advanced Biomanufacturing Facility, One Discovery Square, Suite 3, Room 4029	[S51]

3.3 The vector characterization study — a genuine strength

Mayo ran a bench-scale MOI titration study to confirm identity, functionality and potency of the vector lot, using cryopreserved purified CD4/CD8 cells from three donors — one healthy donor and **two thyroid cancer donors** — evaluating CAR expression by flow, proliferation, viability, VCN and RCL by VSV-G ^[S50].

Two things are worth naming. Including diseased-donor material rather than healthy donors alone is better practice than most programs manage, and it directly addresses the modality's known problem that heavily pre-treated patients yield low, exhausted T cells. And an MOI titration establishes the relationship between vector input and product attributes — which is exactly the dataset a comparability exercise needs if the transduction step moves.

3.4 The release panel, as written

Test	Method	CMC classification	Specification	Stage
Sterility	BACT/ALERT 3D, validated as an alternate to USP <71>; 14 days, one anaerobic and two aerobic bottles	Safety	No growth	FP
Endotoxin	Per CRB.RST.PRO.0043	Safety	≤5 EU/kg per dose	FP
Mycoplasma	USP <63>, BioFire	Safety	Not detected	IPC
CAR-T cells (%)	Flow cytometry	"Identity and Potency"	≥20% gated	IPC, FP
CD3-positive T cells (%)	Flow cytometry	"Identity and Potency"	≥80% gated	IPC, FP
Viability	NucleoCounter NC-200	Purity	≥70% of total cells	IPC, FP
Viable cell concentration	NucleoCounter NC-200	Characterization	Report result	IPM, FP
RCL	dPCR	Safety	Negative	FP
VCN	dPCR	"Identity"	0.2–5.0 copies per transduced cell	FP
Appearance	Visual inspection	Characterization	—	—

Source: draft CMC §1.15 ^[S50].

4. Process architecture, unit operation by unit operation

4.1 The chain, end to end

#	Unit operation	Purpose	Where it fails
1	Leukapheresis receipt and acceptance	Establish a defined, qualified starting population	Acceptance criteria not defined; material out of hold window; cryo thaw loss
2	Wash and volume reduction	Remove plasma, platelets, anticoagulant	Cell loss on an unoptimized closed system
3	Selection or enrichment	Define the input population (CD3, or CD4/CD8 ratio-controlled)	Purity and recovery vary with starting material quality
4	Activation	Drive cells into cycle so transduction can occur	Reagent choice changes phenotype; under-activation silently caps transduction
5	Transduction	Introduce the construct	MOI on the wrong titer basis; vector held after thaw
6	Expansion	Reach the CAR-positive dose	Platform ceiling; feeding strategy undefined
7	Activation reagent removal	Clear beads where used	Incomplete removal is a safety issue and a release spec
8	Harvest, wash and concentrate	Prepare for formulation	Yield loss; shear
9	Formulation and fill	Cryoprotectant, container, dose per unit	DMSO trade-off; fill configuration not specified
10	Controlled-rate freeze and storage	Preserve function	Profile unspecified; nucleation sensitivity

4.2 Starting material — the constraint everything else inherits

The single most useful thing an RFP can contain, and the one most often missing, is a minimum starting-material acceptance criterion: viable cell count, viability, and CD3-positive percentage, with a defined maximum age from collection to processing ^[S9]. Without it, neither party can say whether a failed batch was a process failure or an unmanufacturable input.

Three specific effects deserve naming. Heavily pre-treated patients yield low, exhausted T cells ^[S11]. High circulating tumor burden can contaminate the apheresis, which requires either a depletion strategy or a patient-selection criterion — a question that belongs to the protocol, not to manufacturing. And cryopreserving apheresis before shipping costs 10–20% viability on thaw, which must be carried into the dose-yield calculation rather than discovered at harvest ^[S11].

The chain-of-identity and chain-of-custody procedure is a design input here, not an afterthought. For a multi-site trial it determines whether the CDMO coordinates collection logistics or receives material against a sponsor-run network — a scope boundary worth settling in the first technical call.

4.3 Gene modification

Approach	Typical efficiency	What to watch
Lentiviral, T cells	40–70%	Construct size above ~6 kb depresses efficiency; MOI must be stated on functional titer (TU/mL), not physical ^[S11]
Retroviral, T cells	comparable	Requires actively dividing cells — activation quality is upstream of everything
mRNA electroporation	>80% initial	Transient: expression declines over 3–7 days. Insufficient where dosing needs persistent CAR expression ^[S11]

Vector copy number acceptance is typically 0.5–5 copies per cell, with values above 5 raising insertional-mutagenesis concern ^[S11]. A construct that needs a high MOI to hit its transduction target is therefore squeezed from both sides, and that tension should be resolved in development rather than discovered in release testing.

Lentiviral vector loses roughly half its titer in 2–4 hours at 25 °C. Thaw and use immediately; a thawed vector hold step in a batch record is a defect ^[S11].

4.4 Expansion — matching the platform to the dose

Platform	Practical ceiling	Fit for autologous CAR-T
Static flask	~2×10 ⁹ cells	Workable at small dose; not GMP-scalable, labor-intensive, inconsistent. A request for static flask above 5×10 ⁹ is a scale infeasibility ^[S11]
G-Rex (Wilson Wolf)	~2×10 ¹⁰ per G-Rex 100M	The natural fit for autologous doses of 1–5×10 ⁸ CAR-positive cells; gas exchange becomes limiting only at very high density ^[S11]
Rocking bioreactor (Xuri / WAVE)	1–10×10 ⁹ depending on bag	Supports high-density culture and perfusion feeding; less common for autologous and needs a perfusion strategy developed ^[S11]
CliniMACS Prodigy	~2–3×10 ⁹ standard protocol	Closed and automated end to end; a target above 5×10 ⁹ total cells may exceed it without custom programming ^[S11]

The choice is not only about ceiling. Prodigy collapses selection, activation, transduction, expansion and harvest into one closed automated run, which reduces operator variability and cleanroom occupancy but fixes the process into the instrument's programmed sequence. G-Rex is open-architecture and flexible but adds manipulations. A transfer that changes platform changes the shear environment, the feeding regime and the harvest mechanics simultaneously — three changes at once, which per the comparability framework raises the required bridging-lot count ^[S5].

4.5 Formulation, fill and cryopreservation

CryoStor CS10 at 10% DMSO is the standard, with some sponsors running proprietary or lower-DMSO formulations; DMSO concentration trades cryoprotection against post-thaw toxicity to the patient ^[S11]. Controlled-rate freezing at approximately –1 °C/min is typical, but some products are sensitive to nucleation temperature, and an RFP that does not specify the profile is signalling development work that has not been done ^[S11].

The specification-timing question matters more than it looks. Pre-freeze viability of 90% commonly drops to 60–70% post-thaw. If the release specification applies post-thaw, the pre-freeze target has to be set higher — and if that arithmetic was never done, the program discovers it on the first GMP batch ^[S11].

5. Analytical strategy

5.1 The release panel and where it is generated

Attribute	Method	Typically
Identity — T cell	Flow cytometry, CD3/CD4/CD8	In house
Identity — transgene	CAR expression by flow	In house
Purity	Flow cytometry, unwanted populations	In house
Strength	CAR-positive cell count, viability	In house
Safety — VCN	ddPCR	In house
Safety — RCL/RCR	qPCR or co-culture	Commonly outsourced
Safety — sterility	Automated culture	In house or outsourced by method
Safety — mycoplasma	PCR	In house
Safety — endotoxin	LAL	In house
Potency	See §5a	Mixed

5.2 The two methods that cause the most trouble

CAR expression by flow. The detection reagent is part of the method, not an implementation detail. Protein L, anti-scFv antibodies, tagged antibodies and target-antigen-Fc constructs give different numbers for the same product, and the reagent cannot be changed without bridging data ^[S11]. Tandem and bispecific CARs may not bind all reagents equally, so both binding domains need confirming. And CAR percentage measured immediately after transduction differs from CAR percentage at harvest because of internalization and shedding — the sampling timepoint has to be fixed in the method, or two sites running "the same" assay will disagree.

VCN by ddPCR. Primers must target integrated sequence, transgene or LTR, and not unintegrated vector DNA, or VCN is overestimated ^[S11]. These primers are distinct from the RCL/RCR primer set and are regularly confused. Given that VCN carries a safety cap, an overestimate is not a conservative error — it can fail a releasable batch.

5.3 In-process control

The useful in-process points are viability and count at each media exchange, transduction efficiency at a fixed post-transduction timepoint, and a mid-expansion phenotype check. The first tells you whether to continue; the second is the earliest signal that the dose will be met; the third is the one most often omitted

and the one that explains a surprising release result.

5a. Potency

Potency is the most common IND deficiency in cell and gene therapy, and the FDA's December 2023 draft guidance reframes it as a *potency assurance strategy* — process design, process control, material control, in-process testing and release assay together — rather than a single test ^[S13]. An assay matrix is acceptable, and for a complex product it remains acceptable through BLA ^[S1].

Assay	Measures	Priority	MADE bench
Cytotoxicity, co-culture with antigen-positive targets	Direct killing	Primary — most MOA-relevant	multi-mode plate reader — luminescence or fluorescence readout ^[S4]
Cytokine release on target stimulation, IFN- γ / TNF- α / IL-2	Effector activation	Primary	multi-mode plate reader ELISA; intracellular by flow ^[S4]
CAR expression, percent positive and MFI	Transgene expression	Secondary, identity-linked	CytoFLEX ^[S4]
VCN	Integration level	Secondary, safety and potency surrogate	QX200 ddPCR ^[S4]
Proliferation on activation	Functional capacity	Supportive	NucleoCounter, Vi-CELL BLU ^[S4]
Memory phenotype, Tcm / Tem / Tscm	Product quality attribute	Supportive	CytoFLEX multi-color ^[S4]

Phase-appropriate build. Phase 1 needs a biologically relevant, quantitative assay that is qualified rather than validated — cytokine release plus CAR expression is a defensible Phase 1 matrix. Phase 2 adds cytotoxicity and begins qualification with acceptance criteria. BLA expects a validated cytotoxicity assay as primary release, with cytokine release confirmatory ^[S4].

The pitfall, stated plainly. Percent CAR-positive alone is not a potency assay. It is an identity attribute. Programs that carry it as potency into Phase 2 get challenged, and the remediation — developing and qualifying a cytotoxicity assay under pivotal time pressure — is the expensive version of work that costs little if started at transfer ^[S4].

5 (continued) — Reading this program's release panel

The panel is competently built and phase-appropriate for Phase 1. Two classifications deserve discussion, and both are the kind of thing better raised by a manufacturing partner early than by a reviewer late.

CAR-positive percentage is classified as potency. It is not. It measures how many cells carry and display the transgene; it says nothing about whether those cells kill a TSHR-expressing target or release cytokine when they meet one. The core's §5a sets out the phase-appropriate build and the FDA's position ^{[S4][S1]}. At Phase 1, an assay matrix that is qualified rather than validated is acceptable and this panel is inside that. At Phase 2 the agency expects a functional readout with qualification underway.

MADE's specific recommendation: develop a TSHR-antigen-specific cytotoxicity co-culture with cytokine release as the confirmatory readout, starting during transfer. Both are within MADE's evidenced analytical bench — multi-mode plate reader for the readout, CytoFLEX for intracellular cytokine and phenotype ^{[S4][S8]}. The MOI titration dataset already generated gives a head start on establishing the dose-response relationship such an assay needs.

VCN is classified as identity. The 0.2–5.0 copies per transduced cell range is squarely the industry safety range, where above five raises insertional-mutagenesis concern ^[S11]. Classifying it as identity rather than safety is defensible on the argument that it evidences the modification, but the acceptance criterion is doing safety work. Worth aligning the classification with the criterion before an IND reviewer asks why they differ.

The rest of the panel is sound. BACT/ALERT 3D as a validated alternate to USP <71> is standard and accepted. Endotoxin on a per-kilogram basis is correct for an infused autologous product. Running CAR and CD3 at both in-process and final product is good practice — it means the program can distinguish a transduction failure from a harvest loss.

6. Comparability and technology transfer

6.1 Transfer risk depends on where the process is coming from

Source	Risk	Bridging lots	Statistical approach
Academic or research lab	HIGH	3–5, minimum 3	Equivalence margins for CQAs
Sponsor internal lab	HIGH-MEDIUM	3–5	Equivalence for high-risk CQAs
Another CDMO	MEDIUM	2–3	Quality range, equivalence for high-risk CQAs
MADE development to MADE GMP	LOW-MEDIUM	1–2	Quality range

Source: transfer risk matrix ^[S5].

An academic-origin process is high risk not because the science is weaker but because the artifacts a transfer runs on — validated methods, batch records, defined critical process parameters, equipment history — do not exist yet and have to be created. That work is real and should be scoped, not assumed away.

6.2 What a CAR-T comparability protocol has to demonstrate

CQA	Method	Priority
CAR expression, percent and MFI	Flow	HIGH
Target population, CD3/CD4/CD8	Flow	HIGH

CQA	Method	Priority
Purity, absence of unwanted populations	Flow	HIGH
Cytotoxicity	Co-culture	CRITICAL
Cytokine release	ELISA or flow	CRITICAL
VCN	ddPCR	HIGH
Viability	NucleoCounter, Vi-CELL BLU	HIGH
Memory subset distribution	Flow	MEDIUM
Exhaustion markers, PD-1 / LAG-3 / TIM-3	Flow	MEDIUM-HIGH — correlated with clinical efficacy
Residual activation beads	Visual or magnetic	MEDIUM
RCL/RCR clearance	qPCR or co-culture	HIGH
Endotoxin, sterility, mycoplasma	Per method	Required every lot

Source: CQA comparability matrix ^[S5].

6.3 The staged plan MADE runs

Stage 0 — technical assessment. Read the process against the modality checklist, identify what is defined and what is assumed, and produce the sponsor clarification list. Weeks, not months, and it is what this document supports.

Stage 1 — paper transfer and gap closure. Process documentation review, equipment fit mapping, materials sourcing and qualification, analytical method gap assessment. Analytical runs in parallel from day one rather than following process, because method development is the longer pole.

Stage 2 — engineering run. Non-GMP, on the destination equipment, with the destination materials. The purpose is to find the differences while they are cheap, and to generate the pre-change arm of the comparability dataset if the sponsor has no historical data in a comparable format.

Stage 3 — comparability. Head-to-head against the risk-weighted CQA set, with the bridging-lot count set by transfer risk rather than by convention.

Stage 4 — GMP manufacture and release. Batch records finalized, environmental and aseptic qualification complete, first clinical batch, release and disposition.

A go/no-go gate sits between Stage 2 and Stage 3. Discovering at the engineering run that the platform change moved the memory phenotype is a good outcome if the program can stop, adjust and re-run — and an expensive one if GMP slots are already committed.

6 (continued) — The transfer plan for this program

6.1 Transfer risk classification

Sponsor internal GMP facility to MADE GMP: **HIGH-MEDIUM** on the standard matrix, with three to five bridging lots and equivalence margins on high-risk CQAs ^[S5].

But this program sits at the *favorable* end of that band, and the reasons are concrete. The process is already in GMP, not in a research lab. Batch records exist and are executable documents, not protocols to be written. Critical process parameters are defined. An engineering-run transfer protocol has already been written and run once, from Process Development into the ODS facility ^[S51] — which means Mayo has already been through a transfer of this process and knows what it costs.

Ask for the Engineering Run #2 outcome data. It is the single most valuable input to scoping this transfer, and Mayo has it.

6.2 The change inventory

Change	Risk	Note
Prodigy availability at MADE	Determines everything else	If yes, this is an equipment-transfer exercise. If no, it is a platform change: shear, feeding, harvest mechanics and the closed-system architecture all move at once, and bridging-lot count rises accordingly ^{[S5][S11]}
Site and facility	Medium	Suite classification, environmental monitoring, and a site-change assessment
Operators	Medium	Prodigy TCT operator qualification and training to Mayo's batch records
TransAct lot	Low-Medium	Same reagent, same ratio; specify by lot and confirm activity ^[S11]
Vector lot	Medium	New lot means an MOI confirmation, per the modality's known lot-to-lot variability ^[S11] . Mayo's titration format is the right vehicle
Analytical methods	Medium	Either transfer Mayo's methods, or qualify MADE equivalents and demonstrate equivalency
Cryopreservation and storage	Low-Medium	Confirm the controlled-rate profile transfers and post-thaw specification arithmetic holds

6.3 Staged plan

Stage 0 — assessment. This document, plus the Prodigy confirmation and the Engineering Run #2 data request. Weeks.

Stage 1 — paper transfer and gap closure. Batch records mapped to MADE's document system; equipment fit confirmed; TransAct, media, cytokine and consumable sourcing qualified; vector supply agreement with Vector BioMed confirmed for lead time and minimum order; analytical gap assessment against the ten-test panel, with the potency development scoped and started here rather than later.

Stage 2 — engineering run. Non-GMP, on MADE equipment, with MADE materials and MADE operators, using healthy-donor apheresis. Measure the full yield chain and produce the post-change arm of the comparability dataset.

Stage 3 — comparability. Head-to-head against Mayo's historical data on CAR percentage, CD3 percentage, VCN, viability, and — if the potency assay is by then qualified — function. A site-change assessment accompanies it.

Stage 4 — GMP manufacture, release and clinical supply.

7. Stability, storage and logistics

Vein-to-vein time is the governing constraint and it is a quality attribute in this modality. The question is not whether the manufacturing process is fast but whether manufacturing duration plus release testing plus shipping fits inside the clinically acceptable window, with product stability data that actually covers that window ^[S9]. Where it does not, the gap is usually release testing rather than culture time — which is why outsourced test turnaround belongs in the timeline conversation early ^[S11].

Bridging therapy during the manufacturing wait is a protocol question with a manufacturing consequence: it sets how much schedule slip a program can absorb before a patient is harmed.

Cryopreserved product decouples manufacture from infusion scheduling and is strongly preferred for that reason alone. Storage is LN2 vapor phase; the practical planning inputs are dose-per-container configuration, retain requirements, and the LN2 capacity a per-patient product consumes over a multi-year trial.

8. Supply chain and critical materials

Category	Material	Typical source	Risk
Vector	Lentiviral or retroviral	Sponsor-supplied or vector CDMO	HIGH — often single-source, 3–6 month lead time, lot-to-lot variability ^[S6]
Selection	CliniMACS MicroBeads; Prodigy tubing sets	Miltenyi Biotec	MEDIUM — proprietary single-source ^[S6]
Activation	TransAct	Miltenyi Biotec	MEDIUM — alternative Dynabeads, but not interchangeable without comparability ^[S6]
Activation	Dynabeads CD3/CD28	Thermo Fisher	MEDIUM — same ^[S6]
Media	TexMACS, X-VIVO 15, CTS OpTmizer	Miltenyi, Lonza, Thermo	LOW-MEDIUM — multiple options, but lot-to-lot performance differs; incoming growth-performance QC recommended ^[S11]
Cytokines	IL-2	Multiple	LOW — but specify by specific activity (IU/mg), which differs between vendors and lots ^[S11]
Cytokines	IL-7, IL-15	CellGenix, Miltenyi	LOW-MEDIUM to MEDIUM — fewer GMP sources, 6–8 week lead times ^[S11]
Cryo	CryoStor CS10	BioLife Solutions	LOW-MEDIUM ^[S6]

Category	Material	Typical source	Risk
Formulation	PlasmaLyte A, HSA	Baxter, B. Braun, CSL, Grifols	LOW ^[S6]

The vector line is the one that moves programs. Where the sponsor supplies vector, the questions are supply agreement, lead time, minimum order and COA specification. Where MADE would source it, add four to eight months, or run it as a parallel workstream from day one ^[S2]. Either way it should be on the critical path diagram explicitly rather than assumed available.

9. Regulatory and CMC

9.1 What the phase actually requires

The January 2026 FDA flexible-CMC announcement matters commercially as well as scientifically. Before Phase 2/3 manufacturing, compliance with 21 CFR Part 211 is not expected; IND specifications may be permissive; there is **no requirement for three PPQ lots**, and PPQ lots may be designed for concurrent release ^[S12]. A meaningful fraction of RFP requirements are stricter than the agency requires at the sponsor's phase, and right-sizing that is genuine value a CDMO can add rather than a corner cut ^[S1].

Phase 1 needs: the process described with major steps and critical parameters identified though not validated; identity by flow; viability typically above 70%; sterility, endotoxin and mycoplasma; VCN and transgene expression; a qualified, biologically relevant potency assay; and stability sufficient to support proposed storage and shipping with a real-time study initiated. It does not need fully validated release methods, reference standards, or ICH-compliant long-term stability ^[S1].

9.2 The six deficiencies that recur in CAR-T

1. Potency assay not MOA-correlated — CAR expression without a functional readout
2. Insufficient VCN characterization and acceptance criteria
3. Missing or inadequate RCL/RCR testing strategy
4. Inadequate comparability data for vector lot changes
5. Vein-to-vein timeline versus product stability
6. Missing long-term follow-up plan for integrating vectors

Source: FDA deficiency patterns by modality ^[S1]. Items 1, 4 and 6 are the ones a CDMO can materially help with at transfer; items 2, 3 and 5 are shared with the sponsor.

Integrating vectors carry a 15-year long-term follow-up expectation ^[S16]. It is a sponsor obligation, but the retain and sample-archive requirements land on the manufacturer, so it belongs in the storage plan from the first GMP batch.

10. Clinical and technical precedent

Four approved autologous CAR-T products — tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel, idecabtagene vicleucel, lisocabtagene maraleucel — established that the modality is manufacturable at commercial scale and, just as usefully, established where it strains. The public record of that strain is the most valuable precedent for a program entering transfer.

Manufacturing failure and out-of-specification release are real rates, not tail risks. Early commercial experience saw out-of-specification product for viability and dose in a non-trivial minority of manufactures, most often traced to starting-material quality in heavily pre-treated patients. Programs that set acceptance criteria on the apheresis, rather than discovering the problem at release, absorb this better.

The point-of-care model is being tested inside Mayo itself. Mayo's own CD19 CAR-T program manufactures in-house under a point-of-care model, which is the enterprise's own demonstration that proximity to the patient has manufacturing value. Any transfer conversation should be explicit that moving to a CDMO trades that proximity for capacity, quality-system depth and a path to late-phase supply — and should say what is given up as well as what is gained.

Vector supply has been the sector's recurring bottleneck, not cell processing. Programs that treated vector as a procurement line rather than a critical-path workstream are the ones that slipped.

11. MADE capability against this modality

11.1 Analytical bench — evidenced against the registers

Verified 23 August 2026 against the Princeton register (464 assets) and the East Norriton register (1,530 assets), with qualification status attested by MADE operations on the same date ^[s8].

Need	Instrument	In service
Identity, purity, phenotype, exhaustion panel	Beckman Coulter CytoFLEX, 13-color	4 Princeton
Additional flow capacity	Miltenyi MACSQuant Analyzer; Agilent NovoCyte Penteon; Nexcelom image cytometer	6, 1 and 2 respectively, East Norriton, in L-135 and L-141
Rapid in-process flow	Accellix cytometer	1 East Norriton, in L-141
Cytotoxicity, cytokine and ELISA readouts	Thermo Varioskan Lux; Thermo plate reader; Molecular Devices SpectraMax M3	1, 2 and 3 respectively — Princeton and East Norriton L-135/L-141
Vector copy number, editing efficiency	Bio-Rad QX200 ddPCR	1 Princeton, 4 East Norriton in L-138 and L-144
Orthogonal dPCR	Thermo QuantStudio	1 Princeton, 2 East Norriton
Count and viability	NucleoCounter NC-200	7 Princeton
Count and viability	Beckman Vi-CELL	2 East Norriton, in V-018 and V-019

Need	Instrument	In service
Particle concentration and size	Particle Metrix ZetaView Evolution quatt	1 Princeton
Mycoplasma	BioFire	1 Princeton, 2 East Norriton
Endotoxin	Endosafe	4 Princeton, 3 East Norriton
Nucleic acid quantitation	NanoDrop	1 Princeton, 3 East Norriton
Colony counting (CFU)	Reichert colony counter	1 East Norriton, in V-042
Hematology differential	Sysmex XN-330	held at East Norriton, in storage

11.2 Process equipment — evidenced against the registers

Verified 23 August 2026 against both site registers, with qualification status attested by MADE operations on the same date ^[S8].

REF_09 fit-check item	In service	Detail
CliniMACS Prodigy	14	7 Princeton; 7 East Norriton in P-182, L-140, L-132
CliniMACS Plus	5	Princeton
LOVO	4	3 Princeton; 1 East Norriton in L-140
CTS Rotea	5	3 Princeton; 2 East Norriton in L-132 and P-184
Xuri / WAVE W25	21	East Norriton, in L-140 and P-182
G-Rex ecosystem — Wilson Wolf GatheRex	3	2 Princeton; 1 East Norriton in L-140
Controlled-rate freezing and LN2	9	2 Princeton; 7 East Norriton
Sepax	2	1 Princeton; 1 East Norriton
Electroporation — Thermo Neon	2	1 Princeton; 1 East Norriton in L-132
Electroporation — Lonza 4D-Nucleofector	1	Princeton
Thermo CTS Xenon	—	1 Princeton, Inactive
MaxCyte	—	Not held. Neon and 4D-Nucleofector cover the need

Fourteen qualified CliniMACS Prodigy units across two sites is the headline for this modality, and it is a substantial capacity position rather than a bare capability. It also means a transfer of a Prodigy-native process is an equipment transfer rather than a platform change — the distinction that separates a four-to-six month engagement from a twelve-to-eighteen month one.

The practical planning input is distribution rather than availability: capacity is split evenly across Princeton and East Norriton, which is a site-selection conversation to have early.

11.3 Manufacturing environment

Princeton, NJ and East Norriton, PA. Suites operate at Grade B background with Grade A BSC for open manipulations; closed-system steps are described as Grade B / Closed System. Autologous CAR-T at Phase 1/2 dose scale sits comfortably inside this envelope.

11.4 Team experience

MADE's quality leadership carries collective prior-organization experience directly on this modality — CAR-T QC laboratory build-out and commercial approvals at Bristol Myers Squibb (ABECMA, BREYANZI), QC leadership through the KYMRIAH BLA and FDA pre-approval inspection at Novartis, the TECELRA BLA and pre-license inspections at Adaptimmune, and BLA filing experience on CTL-019. This is prior-organization experience held by named individuals now at MADE; MADE itself has not yet completed a client audit, and that distinction is stated wherever this is described to a client.

11.5 Indicative economics and duration

Custom process, no platform	12–18 months, kick-off to first released GMP batch ^[S2]
Platform-adapted process	4–6 months ^[S2]
Academic-origin process	add 2–4 months to knowledge transfer ^[S2]
Autologous scheduling complexity	add 1 month to GMP readiness ^[S2]
Vector to be manufactured, not supplied	add 4–8 months, or run in parallel ^[S2]

These are industry-standard scoping ranges for directional use. They are not MADE pricing and do not become MADE pricing until finance has scoped the specific program ^[S2].

11 (continued) — MADE fit for this program

11.7 The platform question — answered

MADE holds sixteen CliniMACS Prodigy units and fourteen are in service — seven at Princeton and seven at East Norriton in rooms P-182, L-140 and L-132. Verified 23 August 2026 against both site registers, with qualification attested by MADE operations on the same date ^[S8].

This was the gating question for the opportunity, and the answer is strongly favorable. A transfer of MC220711 lands on the platform the batch records are written against, which makes this an equipment transfer of a process Mayo has already transferred once — the 4–6 month case rather than the 12–18 month one.

CliniMACS Plus (5, Princeton) and LOVO (4 across both sites) are also in service, which matters if the process ever needs a separate selection or wash step.

Two practical points remain, and neither is a capability question. Capacity is split evenly across the two sites, so site selection is a scheduling conversation to have early. And the tubing set Mayo's process uses should be confirmed against what MADE stocks before a run schedule is committed.

11.8 What MADE brings that Mayo does not have today

Capacity and scheduling depth. A single Mayo suite running a per-patient autologous process bounds enrollment by suite time. A CDMO with multiple qualified suites removes that bound.

A path to late phase and commercial supply. Mayo's facility is built for early translation. The question this program will face on a positive Phase 1 is where Phase 2/3 and commercial supply happen, and answering it before it is urgent is worth more than answering it after.

Analytical development capability for the potency gap, as set out in §5 above.

Quality-system depth for the CMC. MADE's quality leadership carries collective prior-organization experience taking gene-modified T-cell products through BLA and FDA pre-approval inspection — ABECMA and BREYANZI at Bristol Myers Squibb, the KYMRIA BLA and PAI at Novartis, TECELRA at Adaptimmune. This is prior-organization experience held by named individuals now at MADE; MADE itself has not yet completed a client audit.

11.9 Indicative investment and duration

Duration, if the process lands on an equivalent Prodigy platform	4–6 months on the accelerated model, since the process, batch records and CPPs already exist [REF_O2 band]
Duration, if a platform change is required	12–18 months on the custom model, plus comparability [REF_O2 band]
Potency assay development	add 2–4 months to the analytical timeline, running in parallel [REF_O2 band]
Autologous scheduling complexity	add 1 month to GMP readiness [REF_O2 band]

The spread between the two duration rows is the commercial reason the Prodigy question is worth answering this week rather than next month.

12. Open questions for Dr. Kenderian's team

Platform and transfer. Is Prodigy TCT the required platform, or is Mayo open to an equivalent closed system with comparability? **What were the Engineering Run #2 outcomes** — yield, CAR percentage, VCN, viability, and any deviations? Which Prodigy tubing set is in use?

Vector. What is the remaining inventory of lot O37.GMP.0050, and what is the reorder lead time from Vector BioMed? Is there a supply agreement, and does it cover a CDMO as the ship-to? What MOI was selected from the titration study, and on what basis?

Starting material. What are the leukapheresis acceptance criteria — viable count, viability, CD3 percentage? What is the maximum age from collection to processing? Is apheresis cryopreserved before processing? What is the protocol position if incoming material fails acceptance?

Analytics. Would Mayo prefer its methods transferred, or MADE equivalents qualified with demonstrated equivalency? Is there appetite to develop a functional potency assay during transfer, and would Mayo share the MOI titration dataset to support it? What is the current thinking on moving CAR percentage out of the potency classification before the pivotal filing?

Clinical. What is the maximum acceptable vein-to-vein time? Expected patients per month at steady state? What happens on a manufacturing failure — is re-collection protocol-permitted? Is the trial single-site at Rochester, or will collection sites be added?

Program. Where does Mayo expect Phase 2 and commercial supply to be manufactured, and on what timeline does that decision need to be made?

13. Sources

Tag	Source
[S1]	REF_01 — Regulatory Intelligence Knowledge Base, CGT CMC regulatory framework (MADE internal, proposal-generator appraisal engine)
[S2]	REF_02 — COGS and Timeline Modeling Framework (MADE internal)
[S4]	REF_04 — Potency Assay Strategy Advisor (MADE internal)
[S5]	REF_05 — Comparability and Tech Transfer Planning Framework (MADE internal)
[S6]	REF_06 — Supply Chain and BOM Risk Analysis Framework (MADE internal)
[S8]	MADE internal capability inventories — Equipment_List_from_BMRAM.xlsx and List_of_Analytical_Instruments_at_Made_Scientific_Feb_2026.xlsx, as described in proposal-generator design.md §4.8. The xlsx files themselves were not available to this machine; instrument names are taken from that specification and must be confirmed against the BMRAM export before client use.
[S9]	REF_09 — Modality-Specific Evaluation Depth Checklists, Modality 1 (MADE internal)
[S11]	REF_11 — Cell Therapy Manufacturing and Analytical Deep Knowledge v2.0 (MADE internal)
[S12]	FDA CBER, Flexible CMC Requirements announcement, 11 January 2026
[S13]	FDA CBER, Potency Assurance for Cell and Gene Therapy Products, draft guidance, December 2023, Docket FDA-2023-D-4299
[S14]	FDA CBER, Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products, draft guidance, July 2023
[S15]	FDA CBER, CMC Information for Human Gene Therapy INDs, final guidance, January 2020
[S16]	FDA CBER, Long-Term Follow-Up After Administration of Human Gene Therapy Products, final guidance, January 2020
[S50]	DRAFT TSHR CMC, 12 August 2026, 61 pp. Mayo Clinic Center for Regenerative Biotherapeutics. §1.3 plasmid, §1.4 lentiviral vector, §1.15 quality control and release testing
[S51]	CRB.RST.PROT.0022 rev 1 — TSHR CAR-T Tech Transfer Protocol: Engineering Run #2, transferring MC220711 from Process Development to the One Discovery Square Advanced Biomanufacturing Facility, Suite 3, Room 4029
[S52]	CRB.RST.MBR.0064 — Transduction of T Cells During the TCT Process, TSHR
[S53]	CRB.RST.MBR.0065 — Taking a Sample During the TCT Process on the Miltenyi Prodigy, TSHR
[S54]	CRB.RST.MBR.0066 rev 1 — Harvest and Preparation of the Final TSHR CAR-T Dose from the TCT Process on the Miltenyi Prodigy

Tag	Source
[S55]	CRB.RST.MBR.OO63 rev 1 — Initiation of the TCT Process on the Miltenyi Prodigy, TSHR CAR; and MBR.OO12 Manufacturing Schema for the TCT Process (MC220711 TSHR CAR)
[S56]	Salesforce opportunity 23725-2026, TT Comparability and GMP Manufacture of TSHR CAR-T; stage Technical Appraisal; retrieved 2026-08-22