

TECHNICAL EVALUATION & CDMO FRAME

TSHR CAR-T — MC220711

MADE technical evaluation and CDMO frame

PI	MAYO SITE	ON NUMBER	MODALITY	VERSION
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PREPARED

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1. At a glance

Program	MC220711 — autologous TSHR-directed CAR-T
Modality	Autologous, lentivirally transduced CAR-T
Phase	Phase 1 / IND
Regulatory status	IND stage. A draft CMC exists, batch records are executable documents, and critical process parameters are defined
Starting material	Autologous leukapheresis
Drug substance	TSHR-CAR-transduced autologous T cells, harvested from the Prodigy T-Cell Transduction run
Drug product	Cryopreserved final dose, expressed as CAR-positive cells per kilogram of recipient weight
Vector	Lentiviral, Vector BioMed, third-generation HEK293T transient transfection, FDA drug master file on file
Current platform	CliniMACS Prodigy, T-Cell Transduction process
Where MADE would manufacture	Princeton, NJ, with East Norriton, PA in the MADE network for scale
MADE reference	ON23725-2026
MADE technical fit	Strong. The process is well documented, the vector position is resolved, and the analytical gap is addressable

This is a technical assessment, not a proposal. It sets out how MADE reads the program, what we would recommend, and what we would need from your team to scope it properly.

2. Program understanding

From the materials your team shared. MC220711 is an autologous CAR-T targeting the thyroid-stimulating hormone receptor, manufactured on the CliniMACS Prodigy T-Cell Transduction platform at the Center for Regenerative Biotherapeutics Advanced Biomanufacturing Facility in One Discovery Square. Plasmid is sourced from Puresyn through the INDReady platform; lentiviral vector from Vector BioMed on a third-generation HEK293T transient transfection platform, with a drug master file on file with the FDA. Activation uses TransAct at a 1:25 ratio; transduction occurs on Day 2 at a titrated MOI. Dose is expressed as CAR-positive cells per kilogram of recipient weight.

Three things here are stronger than the modality norm, and they are worth naming because they materially reduce the risk of a transfer.

The **vector position is resolved**, with a functional titer of 8.72×10^8 TU/mL reported on the certificate of analysis and all release specifications met. Vector is normally the single largest supply-chain risk in this modality — long lead times, single sourcing, lot-to-lot variability in titer and impurity profile. Yours is in hand, from a supplier with an FDA drug master file, and quoted on *functional* titer, which is the correct basis for MOI and the one programs most often get wrong.

The **MOI titration study included diseased-donor material** — one healthy donor and two thyroid cancer donors — evaluating CAR expression, proliferation, viability, vector copy number and replication-competent lentivirus. Most programs characterize on healthy donors alone and then discover that heavily pre-treated patients yield low, exhausted T cells. Yours has already looked.

A tech transfer of this process has already been done once, from Process Development into the ODS facility under an engineering-run protocol. Your team knows what this transfer takes in effort and calendar, which makes scoping the next one a conversation rather than an estimate.

What the public record adds. TSHR is an attractive target precisely because its expression is largely thyroid-restricted, which gives a cleaner on-target/off-tumor profile than targets shared across normal lineages, and because thyroid ablation is clinically manageable in a way that other lineage ablations are not.

3. Regulatory position and pathway

Item	Position
Current filing status	Phase 1 / IND, with a draft CMC in preparation and a complete batch-record set behind it
Designations held	Not established — see §15
What the CMC package must support at this phase	The process described with major steps and critical parameters identified though not validated; identity by flow; viability typically above 70%; sterility, endotoxin and mycoplasma; vector copy number and transgene expression; a qualified, biologically relevant potency assay; and stability sufficient to support the proposed storage and shipping, with a real-time study initiated
What this phase does not require	Fully validated release methods, reference standards, or ICH-compliant long-term stability. Before Phase 2/3 manufacture, 21 CFR Part 211 compliance is not expected, IND specifications may be permissive, and there is no requirement for three PPQ lots
What a site change implies	A manufacturing site change on an active IND carries a comparability exercise and a site-change assessment. On the standard transfer-risk matrix, sponsor internal GMP facility to MADE GMP is HIGH-MEDIUM, with three to five bridging lots and equivalence margins on high-risk attributes. This program sits at the favorable end of that band — see §10
Long-term follow-up	Integrating vectors carry a fifteen-year follow-up expectation. It is a sponsor obligation, but the retain and sample-archive requirements land on the manufacturer, so they belong in the storage plan from the first GMP batch
Applicable guidance	FDA CBER, <i>CMC Information for Human Gene Therapy INDs</i> , final, January 2020; <i>Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products</i> , draft, July 2023; <i>Potency Assurance for Cell and Gene Therapy Products</i> , draft, December 2023; <i>Long-Term Follow-Up After Administration of Human Gene Therapy Products</i> , final, January 2020; and the CBER flexible-CMC announcement of 11 January 2026

4. Starting material

Item	Position
Source	Autologous leukapheresis from the trial population
Collection	Collected and transported to the GMP facility

Item	Position
Acceptance criteria	Not established — viable cell count, viability and CD3-positive percentage are the three that matter. See §15
Fresh or cryopreserved	Not established — see §15. If apheresis is cryopreserved before processing, 10–20% viability is lost on thaw and has to be carried into the dose-yield calculation rather than discovered at harvest
Time from collection to processing	Not established — a defined maximum age is the companion to the acceptance criteria. See §15
Chain of identity and custody	MADE runs standard incoming handling with chain of identity and chain of custody. For a multi-site trial this determines whether the manufacturer coordinates collection logistics or receives material against a sponsor-run network — a scope boundary worth settling in the first technical call
On a failed collection	Not established — whether re-collection is protocol-permitted decides how a failed incoming material is handled. See §15
What the modality does to this input	Heavily pre-treated patients yield low, exhausted T cells, and high circulating tumor burden can contaminate the apheresis. Your MOI titration already looked at two thyroid cancer donors, which is more than most programs have when they set these criteria

5. Process flow

Unit operation	Your approach	MADE capability	Read
Leukapheresis receipt and acceptance	Collected and transported to the GMP facility	Standard incoming handling with chain of identity and custody	Fits
Enrichment and initiation	Prodigy TCT, closed and automated	Closed-system T-cell processing on the same platform	Fits
Activation	TransAct, 1:25 ratio	Same reagent; specify by specific activity and lot rather than mass	Fits
Transduction	Day 2, titrated MOI	Standard. MOI confirmation on a new vector lot; thawed vector is used immediately, since lentiviral titer falls by roughly half in two to four hours at room temperature	Fits
Expansion	Prodigy programmed sequence	Prodigy carries a standard-protocol ceiling near $2-3 \times 10^9$ cells, which accommodates an autologous CAR-positive dose	Fits
In-process sampling	Defined Prodigy sampling procedure	Transfers with the batch record. We would add a mid-expansion phenotype check, which is the control most often omitted and the one that explains a surprising release result	Fits
Harvest and final dose	Per-patient dose from CAR-positive cell count	Standard	Fits
Formulation, fill and cryopreservation	Controlled-rate freeze, cryopreserved doses	Controlled-rate freezing and LN2 storage in service — see §8	Fits

On the platform, we can be specific. A transfer of MC220711 lands on the platform your batch records are already written against, rather than becoming a platform change. It is an equipment transfer of a process your team has already moved once.

Two practical points, neither of them a constraint. Prodigy capacity is split evenly across our two sites, so site selection is a scheduling conversation we would want to have early rather than late. And we would confirm the tubing set your process uses against what we stock before committing to a run schedule.

6. Key equipment

Instrument	Your platform	MADE's installed position	Onboarding required
CliniMACS Prodigy	TCT process, end to end	14 in service of 16 held — 7 Princeton, 7 East Norriton in rooms P-182, L-140 and L-132	None
Controlled-rate freezer and LN2 storage	Controlled-rate freeze, cryopreserved doses	9 in service — 2 Princeton, 7 East Norriton	None
NucleoCounter NC-200	Viability and viable cell concentration	7 in service, Princeton — the same instrument your panel names	None
Beckman Coulter CytoFLEX, 13-color	Flow cytometry for CAR and CD3	4 in service, Princeton	Method bridge, not onboarding — see §7
Bio-Rad QX200 ddPCR	Vector copy number by dPCR	5 in service — 1 Princeton, 4 East Norriton in L-138 and L-144	None
BioFire	Mycoplasma by PCR	3 in service — 1 Princeton, 2 East Norriton	None
Endosafe	Endotoxin	7 in service — 4 Princeton, 3 East Norriton	None
CliniMACS Plus	Not used in your process	5 in service, Princeton	Available if a separate selection step is ever needed
LOVO	Not used in your process	4 in service — 3 Princeton, 1 East Norriton in L-140	Available if a separate wash step is ever needed

Nothing in this process requires an instrument MADE would have to acquire and qualify first.

7. Analytical panel and release

Attribute	Your method	Specification	MADE position	In house or outsourced
Sterility	BACT/ALERT 3D, validated as an alternate to USP <71>; 14 days	No growth	Same approach	In house
Endotoxin	Per your procedure	≤5 EU/kg per dose	Endosafe	In house
Mycoplasma, USP <63>	BioFire PCR	Not detected	Same instrument	In house

Attribute	Your method	Specification	MADE position	In house or outsourced
CAR-T cells, percent	Flow cytometry	≥20% gated	CytoFLEX. The CAR detection reagent is part of the method and transfers exactly as written	In house
CD3-positive T cells, percent	Flow cytometry	≥80% gated	CytoFLEX	In house
Viability	NucleoCounter NC-200	≥70% of total cells	Same instrument	In house
Viable cell concentration	NucleoCounter NC-200	Report result	Same instrument	In house
Vector copy number	dPCR	0.2–5.0 copies per transduced cell	Bio-Rad QX200 ddPCR, with primers targeting integrated sequence rather than unintegrated vector DNA	In house
Replication-competent lentivirus	dPCR	Negative	Outsourced, as it is across the industry	Outsourced
Appearance	Visual inspection	—	Standard	In house

Every test in your panel sits within MADE's in-house analytical capability, with the standard exception of replication-competent lentivirus and adventitious agent testing.

A note on method transfer: we would be glad to take your methods as transferred, qualify MADE equivalents with a demonstrated bridge, or run a combination — whichever your team prefers. Where CAR expression by flow is concerned, the detection reagent is part of the method and cannot be changed without bridging data, and the sampling timepoint has to be fixed in the method, since CAR percentage measured immediately after transduction differs from CAR percentage at harvest.

7a. Potency

There is no functional potency assay in the panel today. CAR-positive percentage and CD3-positive percentage are both classified in the CMC as "Identity and Potency". Both are excellent identity attributes. Neither measures whether the product kills a TSHR-expressing target or releases cytokine when it meets one.

That position is defensible at Phase 1, where the agency expects a biologically relevant assay that is qualified rather than validated, and an assay matrix is acceptable. It does not survive the move toward pivotal, and inadequate potency assay is the most common IND deficiency across cell and gene therapy — with this exact pattern the first-listed one for CAR-T.

Our recommendation: develop a TSHR-antigen-specific cytotoxicity co-culture, with cytokine release as the confirmatory readout, during technology transfer rather than after it. Both run on instruments already in our laboratories — multi-mode plate reader for the readout, CytoFLEX for intracellular cytokine and phenotype. Your MOI titration dataset gives the dose-response relationship such an assay needs, so the work starts from something rather than nothing.

8. Fill, finish and presentation

Item	Position
Container closure	Not established — see §15
Fill volume and dose-per-container configuration	Not established. Doses are cryopreserved against a defined dosing schema; the per-container configuration is a planning input for both LN2 capacity and retains. See §15
Dose form	Cryopreserved cell suspension, dose calculated per patient as CAR-positive cells per kilogram of recipient weight, from the collection order
Labeling	Not established — see §15
Controlled-rate freeze	Controlled-rate freezing is part of your process; the profile itself is not stated in what we have seen. Around $-1^{\circ}\text{C}/\text{min}$ is typical, but some products are sensitive to nucleation temperature. Confirming the profile transfers is Stage 1 work — see §15
Storage	Cryopreserved, LN2 vapor phase
Specification timing	Worth settling explicitly: pre-freeze viability near 90% commonly reads 60–70% post-thaw. If the $\geq 70\%$ viability specification applies post-thaw, the pre-freeze target has to be set higher, and that arithmetic is better done now than on the first GMP batch

There is no aseptic fill-finish operation in this process in the small-molecule sense — the product is formulated and aliquoted into cryopreservation containers as the terminal step of the manufacture.

9. Stability, storage and shipping

Item	Position
Stability status	Not established. Phase 1 expects stability sufficient to support the proposed storage and shipping with a real-time study initiated, and we would want to see what exists before proposing anything. See §15
Storage condition	LN2 vapor phase. The planning inputs are dose-per-container configuration, retain requirements, and the LN2 capacity a per-patient product consumes across a multi-year trial
Shipper	Not established — see §15
Qualification status of the shipping lane	Not established — see §15
Chain of custody, outbound leg	Not established. Whether the trial stays single-site at Rochester or adds collection and treatment sites decides how much of this MADE would own. See §15
The governing constraint	Vein-to-vein time is a quality attribute in this modality. The question is not whether manufacture is fast, but whether manufacture plus release testing plus shipping fits inside the clinically acceptable window with stability data covering it. Where programs fail this, the gap is usually release-test turnaround rather than culture time — which is why outsourced test turnaround belongs in the timeline conversation early
What cryopreservation buys	A cryopreserved product decouples manufacture from infusion scheduling, which is the main reason it is preferred, and it is what your process already does

10. Comparability

A transfer from a sponsor internal GMP facility to MADE GMP classifies as HIGH-MEDIUM on the standard transfer-risk matrix, with three to five bridging lots and equivalence margins on the high-risk attributes. **This program sits at the favorable end of that band, for concrete reasons:** the process is already in GMP rather than in a research laboratory, batch records exist as executable documents rather than protocols still to be written, critical process parameters are defined, and an engineering-run transfer protocol has already been written and executed once.

The analytical bridge is the part to settle first. Comparability is only as good as the assays that carry it, so the method question in §7 — your methods transferred, MADE equivalents qualified against a demonstrated bridge, or a combination — is answered before the engineering run, not after it. Where a functional potency assay is qualified in time, it joins the comparability set; where it is not, the set runs on the attributes below.

Attribute	Method	Priority
CAR expression, percent and MFI	Flow	HIGH
Target population, CD3/CD4/CD8	Flow	HIGH
Purity, absence of unwanted populations	Flow	HIGH
Cytotoxicity	Co-culture	CRITICAL where an assay exists — see §7a
Cytokine release	ELISA or flow	CRITICAL where an assay exists
Vector copy number	ddPCR	HIGH
Viability	NucleoCounter	HIGH
Memory subset distribution	Flow	MEDIUM
Exhaustion markers, PD-1 / LAG-3 / TIM-3	Flow	MEDIUM-HIGH — correlated with clinical efficacy
Replication-competent lentivirus clearance	dPCR	HIGH
Endotoxin, sterility, mycoplasma	Per method	Required every lot

The change inventory is short, which is the point. Site and facility, with a site-change assessment. Operator qualification against your batch records. TransAct lot, same reagent and same ratio, specified by activity. A new vector lot, which means an MOI confirmation — and your titration format is the right vehicle for it. Analytical methods, per the bridge above. And the cryopreservation profile, confirmed rather than assumed.

Ask for the Engineering Run #2 outcome data at the start. Yield, CAR percentage, vector copy number, viability and any deviations from that run are the single most valuable input to scoping this one — better than any estimate we could construct.

11. Technology transfer approach

Stage	What happens	Gate
Stage 0 — technical assessment	Read the process against the modality checklist, resolve the platform question, and produce a clarification list. This document is its first output	—

Stage	What happens	Gate
Stage 1 — paper transfer and gap closure	Batch records mapped to our document system; equipment fit confirmed; TransAct, media, cytokine and consumable sourcing qualified; vector supply confirmed for lead time and ship-to; analytical gap assessment across the panel. Potency assay development starts here, in parallel, not afterwards	—
Stage 2 — engineering run	Non-GMP, on our equipment with our materials and our operators, using healthy-donor apheresis. Measures the full yield chain and generates the post-change arm of the comparability dataset	Go / no-go sits here
Stage 3 — comparability	Head-to-head against your historical data on CAR percentage, CD3 percentage, vector copy number and viability, with a site-change assessment alongside	—
Stage 4 — GMP manufacture, release and clinical supply	Batch records finalized, environmental and aseptic qualification complete, first clinical batch, release and disposition	Readiness assessment before GMP activity

The go/no-go gate between Stages 2 and 3 is deliberate. Finding a difference at the engineering run is a good outcome if the program can adjust and re-run; it is an expensive one if GMP slots are already committed.

How this becomes a proposal. A MADE proposal presents this plan as numbered work orders grouped into milestones — process establishment and feasibility, process and analytical development, GMP readiness, and GMP manufacture — with each work order carrying its own deliverables, assumptions and exclusions, and Mayo's deliverables stated alongside ours, so both teams always know what the critical path needs next. The first milestone concludes in a joint review of the data with a penalty-free go/no-go decision, and the move from development into GMP activity is a formal stage-gate readiness assessment. The plan above is written to drop into that structure without translation.

12. Indicative timeline

Stage	Duration
Stage 0 — assessment	2–4 weeks
Stage 1 — paper transfer and gap closure	1–3 months
Analytical development, in parallel from Stage 1	2–4 months [industry range]
Stage 2 — engineering run	1–2 months
Stage 3 — comparability	2–3 months
Stage 4 — GMP readiness and first clinical batch	1–2 months
Total, equivalent closed platform	4–6 months [industry range]
Total, platform change required	12–18 months [industry range]

Autologous scheduling complexity adds approximately one month to GMP readiness in either case [industry range].

On an equivalent closed platform, the path looks like this:



13. MADE experience that supports this

On this modality specifically. MADE's quality leadership carries collective experience from prior organizations directly on gene-modified T cells: CAR-T QC laboratory build-out and commercial approvals at Bristol Myers Squibb, QC leadership through a BLA and FDA pre-approval inspection at Novartis, and BLA and pre-license inspection work at Adaptimmune. This is experience held by individuals now at MADE, and we describe it that way deliberately — MADE as a company has not yet completed a client audit.

On the analytical bench. Beckman Coulter CytoFLEX 13-color flow cytometry, multi-mode plate readers for cytotoxicity and cytokine readouts, Bio-Rad QX200 ddPCR, Thermo QuantStudio, NucleoCounter NC-200 and Beckman Vi-CELL for count and viability.

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

14. Guidance and suggestions

Three observations offered as strengthening, not correction.

Percent CAR-positive is doing potency work that it cannot do, as set out in §7a. Building the functional assay now, while the process is moving anyway, is far less disruptive than building it under pivotal timeline pressure.

Vector copy number is classified as identity and specified as safety. The 0.2–5.0 copies per transduced cell range is the industry safety range, where values above five raise insertional-mutagenesis concern. The classification and the acceptance criterion are pulling in different directions, and a reviewer is likely to ask why. Aligning them before the question arrives is a small edit with a real benefit.

Set the starting-material acceptance criteria explicitly, per §4. Without a minimum viable count, viability and CD3 percentage with a defined maximum age, neither party can say whether a failed batch was a process failure or an unmanufacturable input — and in a modality where out-of-specification release is a real rate rather than a tail risk, that distinction decides who owns the outcome.

15. Open questions

Regulatory. Does the program hold any FDA designations we should account for in the CMC plan? Where does Mayo expect Phase 2 and commercial supply to be manufactured, and on what timeline does that decision need to be made?

Platform and transfer. Is Prodigy TCT the required platform, or would an equivalent closed system with comparability be acceptable? What were the Engineering Run #2 outcomes? Which Prodigy tubing set is in use?

Vector. What is the remaining inventory of lot O37.GMP.0050, and the reorder lead time? Does the supply agreement cover a contract manufacturer as ship-to? What MOI was selected from the titration, and on what basis?

Starting material. Leukapheresis acceptance criteria — viable count, viability, CD3 percentage? Maximum age from collection to processing? Is apheresis cryopreserved before processing? What is the protocol position if incoming material fails acceptance, and is re-collection permitted?

Fill, storage and shipping. What is the container closure and fill volume, and how many containers per patient dose? What is the controlled-rate freeze profile? What labeling applies? Does the $\geq 70\%$ viability specification apply pre-freeze or post-thaw? What stability data exists for the cryopreserved product, and what shipper and shipping lane are in use today?

Analytics. Would you prefer your methods transferred, or MADE equivalents qualified with a demonstrated bridge? Which CAR detection reagent is in use, and at what timepoint is CAR percentage measured? Would you share the MOI titration dataset to support potency development?

Clinical. 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, or will collection sites be added?

16. Suggested next step

A ninety-minute technical call with your process development and quality teams, covering the platform question, the Engineering Run #2 data, and the potency assay proposal. We would come to it having read your batch records and your draft CMC, so the time can be spent on decisions rather than on background.