

PROGRAM TECHNICAL DEEP DIVE

Autologous Adipose MSC

Frozen drug substance and fresh drug product for Phase I/II — technical deep dive

PI	MAYO SITE	ON NUMBER	
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1. Executive technical summary

This program is technically straightforward and logistically demanding, and the logistics are where the response has to be strongest.

Mayo runs a two-tier autologous process: patient adipose tissue is collected and processed, adipose-derived MSCs are cultured, and drug substance is cryopreserved into 1 mL vials in liquid nitrogen. For administration a single DS vial is thawed and plated, cultured, harvested, formulated and loaded into a syringe — **and the product must be administered within 8 hours of loading** ^[S62]. The drug product is fresh, not frozen.

The RFI contains a location requirement MADE does not meet, stated plainly. Mayo asks that the manufacturer be "within a few hours of our clinical site(s) in Rochester, MN, Jacksonville, FL and/or Phoenix, AZ **or** have validated methods for transporting both starting adipose tissue and ready-to-administer MSC drug product", with shipping verification included in the comparability studies ^[S62]. Princeton, NJ and East Norriton, PA are not within a few hours of any of the three.

That is not a reason to decline; it is the thing to answer first and best. The RFI names the alternative explicitly, and validated transport is a solvable engineering problem with a defined verification path that Mayo has already agreed to fund inside comparability. A response that leads with a specific, credible logistics design — inbound tissue and outbound fresh product, with temperature and time data — is answering the question Mayo actually asked. A response that soft-pedals geography is not.

Two clinical constraints propagate into everything. The 8-hour post-loading administration window is the tightest in the Mayo portfolio. And release depends on **interim in-process sterility reads to support administration**, with final sterility resulting after treatment — a design the core describes for fresh products generally, and one that requires a defined physician-notification path when a sterility vial turns positive. Mayo's is 24 hours, with colony identification to species level ^[S62].

There is no potency assay at either stage. The MSC core is explicit that this is the modality's second-listed FDA deficiency and that identity by ISCT-style markers does not distinguish an MSC from a fibroblast, let alone establish function. Mayo's panel is a good identity panel — better than the ISCT minimum, with CD44 and HLA-ABC added — and it is not a potency panel ^[S62].

2. The modality: biology and mechanism

MSCs are plastic-adherent, self-renewing stromal cells isolated from bone marrow, adipose tissue, umbilical cord (Wharton's jelly), dental pulp and other sources. The 2006 ISCT position defined the minimum criteria that still anchor every release panel: plastic adherence, the surface marker profile above, and trilineage differentiation capacity into osteoblasts, adipocytes and chondroblasts ^[S20]. ISCT's 2019 position paper pushed the field toward naming the tissue of origin and the demonstrated function rather than relying on "mesenchymal stem cell" as a category, precisely because the category had become uninformative ^[S21].

The mechanism is paracrine, not structural. In the overwhelming majority of clinical applications MSCs do not engraft and do not differentiate into the target tissue. They act by secreting immunomodulatory, anti-inflammatory, angiogenic and trophic mediators — and increasingly by releasing extracellular vesicles carrying that cargo ^[S21]. This single fact drives most of what follows. If the therapeutic mechanism is secretion, then differentiation capacity is an identity attribute and secretory function is potency, and a program that tests the former as a proxy for the latter has no assurance of function.

Sources are not interchangeable. Bone marrow, adipose and cord-tissue MSCs have different expansion kinetics, different marker expression intensities and different functional profiles, despite all three meeting ISCT criteria ^[S11]. Bone marrow MSC proliferative capacity declines with donor age; cord-derived cells expand faster and further. A source change is a comparability exercise, not a materials substitution.

Donor variability is the defining property of the modality. Two donors meeting identical identity criteria can differ tenfold in IDO and PGE2 output ^[S11]. For an allogeneic product this is managed by qualifying donors on function before committing a bank; for an autologous product it cannot be managed at all, only characterized — which is why autologous MSC programs need wider specifications and a clear position on what happens when a patient's own cells underperform.

3. This program's product, specifically

3.1 The two-tier process

	Drug substance	Drug product
Input	Patient adipose tissue, collected and processed	One thawed DS vial
Process	Culture of adipose-derived MSCs, final collection, cryopreservation	Thaw and plate, culture, harvest, formulate, load into syringe
Form	Frozen, 1 mL vials	Fresh, in a syringe
Storage	Liquid nitrogen	None — administered within 8 hours of loading
Release	Endotoxin, mycoplasma, sterility, flow cytometry, karyotype	Cell dose, viability, in-process and final sterility, endotoxin, mycoplasma, Gram stain

Source: RFI, 18 August 2026 ^[S62]. Phase I/II clinical use.

3.2 The release specifications

Pre-freeze drug substance

Test	Method	Specification
Sterility	BacT/Alert, USP <71>	No growth after 14 days
Endotoxin	Endosafe nexgen-PTS, USP <85>	Informational use only
Mycoplasma	PCR, BioFire, USP <63>	Not detected

Test	Method	Specification
Flow cytometry	—	CD90 ≥85%, CD73 ≥85%, CD105 ≥85%, CD44 ≥85%, HLA-ABC ≥60% of gated; CD14 ≤5%, CD45 ≤5%, HLA-DR ≤5%
Genetic stability	Karyotype	No clonal abnormalities

Final drug product

Test	Method	Specification
Cell dose	NucleoCounter NC-200	Consistent with order and trial group assignment
Viability	NucleoCounter NC-200	≥70%
In-process sterility, 48 h before harvest	BacT/Alert, USP <71>	No growth — pending at administration
Final product sterility	Gram stain	No organisms seen
Final product sterility	BacT/Alert, USP <71>	Sent; result follows treatment
Endotoxin	Endosafe nexgen-PTS, USP <85>	<0.2 EU/kg
Mycoplasma	BioFire, USP <63>	Not detected — rapid, available before treatment

Source: RFI ^[S62].

4. Process architecture, unit operation by unit operation

4.1 The chain, end to end

#	Unit operation	Purpose	Where it fails
1	Tissue procurement and receipt	Qualified starting tissue	Shipping window and temperature; donor screening incomplete
2	Isolation	Liberate and select MSCs	Enzyme residuals; fibroblast co-isolation
3	Primary culture and passage to P2–P4	Establish the population	Slow adherence from poor-quality tissue
4	Master cell bank	Single characterized origin for everything downstream	Under-sized bank; incomplete characterization
5	Working cell bank	Manufacturing-ready inventory	Passage budget consumed too early
6	Expansion to scale	Reach dose	Platform change alters phenotype and function
7	Harvest and detachment	Recover cells intact	Enzymatic damage to surface proteins; microcarrier carryover

#	Unit operation	Purpose	Where it fails
8	Wash and concentrate	Clear media and residuals	Yield loss
9	Formulation and fill	Cryoprotectant, container, dose	DMSO tolerance at the delivery route
10	Controlled-rate freeze and storage	Preserve function	Post-thaw potency loss, not just viability loss

4.2 Starting material and donor management

The questions that decide a program: is the tissue source defined; are donor qualification criteria defined; single-donor or pooled; what are the procurement logistics and the acceptable time and temperature from collection to processing; is isolation by enzymatic digestion or explant outgrowth; and — the one most often missing — what is the donor-to-donor variability management strategy ^[S9].

For bone marrow, mononuclear cells are separated on a density gradient, plated, and non-adherent hematopoietic cells washed away over the first three to seven days. For cord tissue, explant outgrowth yields a purer population with less fibroblast contamination but takes seven to fourteen days, while enzymatic digestion with collagenase liberates cells in hours and allows same-day plating at the cost of residual enzyme and matrix fragments that need clearing and controlling ^[S21]. Immunoselection on CD271 or CD146 enriches for clonogenic precursors and reduces variability but adds cost and regulatory surface ^[S21].

Passage and population-doubling limits must be defined and justified ^[S9]. This is not paperwork. It is the control that stands between the program and the senescence failure mode.

4.3 Expansion — the decision that carries the most comparability risk

Platform	Character	Typical yield	What it costs you
T-flask, cell factory (CellSTACK, Nunc)	2D, open, manual	Small cohorts, ≤ 10 doses	Labor and footprint — roughly 160 m ² and nine staff for ~100 doses a month; contamination risk from repeated vessel opening ^{[S21][S24]}
Hollow fiber (Terumo Quantum)	2D geometry, closed, automated feed and harvest	$10^7 \rightarrow >10^{10}$ cells in 14–21 days	Fixed vessel geometry; less process visibility ^{[S21][S25]}
Multi-tray and fixed-bed (HYPERStack, iCELLis)	2D geometry, scaled out	600–6,000 cm ² per vessel	Scale-out rather than scale-up
Stirred-tank with microcarriers	3D suspension	$5\text{--}10 \times 10^9$ per 50 L batch, 50–100 doses at 10^8 /dose	The largest phenotype risk — see below ^{[S21][S26]}

Moving from flasks to a stirred tank changes the mechanical environment, and MSCs respond to mechanics. Cells grown on microcarriers under agitation can differ from flask-grown cells in morphology, surface marker intensity and function ^[S11]. Harvest compounds it: enzymatic detachment damages surface proteins, incomplete detachment leaves cells on the carriers and cuts yield, and carrier fragments in the product are a safety concern requiring a clearance step and a test ^[S11]. A 2D-to-3D move is a full comparability exercise and should be planned and priced as one.

Hollow fiber occupies a useful middle position for programs at clinical scale: it is closed and automated, which addresses the contamination and labor problems of flasks, while preserving the 2D growth geometry, which materially reduces the comparability burden of the move ^{[S21][S25]}.

4.4 Media — the choice with the longest regulatory shadow

Fetal bovine serum works, is well characterized, and is where most academic processes start. It also carries xenogeneic contamination risk, real lot-to-lot variability in growth factor content, TSE and BSE considerations, and increasing regulatory pushback, particularly in the EU ^{[S11][S21]}. Human platelet lysate at 5–10% supports equivalent or better expansion and removes the xeno concern, but carries its own lot-to-lot variability; pooling reduces variability at the cost of increased donor exposure ^{[S11][S21]}. Fully defined xeno-free media give the most consistent and most defensible position, historically at the cost of slower growth, though second-generation formulations have narrowed that gap ^[S21].

Treat an FBS-to-xeno-free conversion as a major change, not a materials swap. It shifts growth rate and cell behavior and requires comparability ^[S11]. The right time to do it is before the pivotal process is locked. A program that plans to convert "later" is planning a comparability exercise at the worst possible moment, and REF_09 asks specifically whether a conversion plan exists ^[S9].

4.5 Cell banking

Allogeneic MSC products run a two-tier bank. The master cell bank is established at passage 2–4, typically 50–200 vials at $1\text{--}5\times 10^6$ cells each, and carries the full characterization burden — identity, purity, viability, sterility, mycoplasma, endotoxin, viral screening, karyotype, tumorigenicity and potency. The working cell bank derives from a single MCB vial expanded to passage 5–7, typically 200–1,000 vials, with a lighter but still substantial panel ^[S21].

The arithmetic matters more than it looks. At 10^8 cells per dose and roughly 15-fold expansion per passage, one WCB vial at 2×10^6 cells yields on the order of 45 doses after two passages ^[S21]. Bank sizing, passage budget and dose count are one calculation, not three, and getting it wrong is discovered late. Clinical passage limits are typically set at P9–P12 to bound senescence and karyotypic drift, and agencies expect demonstration that end-of-production cells retain potency, safety and identity equivalent to early passage ^[S21].

4.6 Formulation, fill and cryopreservation

The route of administration constrains the formulation more than in most modalities. Intrathecal and intra-articular delivery tolerate less DMSO and less residual than intravenous infusion, and some programs require a wash-and-resuspend at the clinical site — which pushes complexity onto the pharmacy and needs an instruction-for-use written with that in mind. Post-thaw viability specification of $\geq 80\%$ is the common position; the more important question, and the less often asked, is whether post-thaw *potency* has been measured at all ^[S21].

5. Analytical strategy

5.1 The release panel

Attribute	Method	Typical specification
Identity	Flow: CD73, CD90, CD105, CD34, CD45, CD14, HLA-DR	CD73/CD90/CD105 $\geq 95\%$; CD34/CD45 $\leq 2\%$ [S20] [S21]
Viability	7-AAD, trypan blue or AO/PI	$\geq 80\%$ viable
Sterility	USP <71> direct inoculation, or automated culture	No growth
Mycoplasma	PCR or culture	Negative
Endotoxin	LAL	<2.5 EU/mL for IV; <5 EU/dose
Adventitious viruses	Donor serology; in vitro and in vivo assays at MCB	Negative or non-reactive
Karyotype	G-banding, 20 metaphases, or FISH	Normal diploid
Process residuals	ELISA or LC-MS/MS	FBS protein <50 ng/10 ⁶ cells, or hPL limit
Host cell DNA	qPCR	<10 ng/dose where enzymatic digestion is used
Potency	See §5a	Program-specific

Compiled from the MADE MSC modality report's release-panel table, which cites ISCT 2006, 21 CFR 1271 and 610, USP <71>/<63>/<85>, Ph. Eur. and ICH Q5A [S21].

5.2 Where identity testing is weaker than it looks

The ISCT panel is necessary and not sufficient. It does not separate an MSC from a fibroblast, and for an immunomodulatory product tested on identity alone there is no assurance of intended function [S11]. CD146, CD271 and SSEA-4 offer better specificity but none is universally accepted, so the practical answer is not a better identity panel — it is a functional potency assay carrying the weight that identity cannot.

Two flow-cytometry practicalities that cause disagreement between sites: a viability dye is essential, because dead cells bind antibodies non-specifically and inflate marker positivity [S11]; and the gating strategy — debris exclusion, doublet discrimination, viability gate — is part of the method and must transfer with it.

5a. Potency

5a.1 The trap, stated first

Trilineage differentiation is not a potency assay for an immunomodulatory indication. It confirms stem-cell character. It does not measure the secretory mechanism that is the therapeutic mode of action in most MSC clinical programs, and the FDA has raised this deficiency repeatedly [S4][S1]. If an RFP names trilineage

differentiation as potency, that is a regulatory risk to flag and an alternative to recommend, not a preference to accommodate ^[S9].

5a.2 The assays that do measure mechanism

Assay	Measures	Priority	MADE bench	The pitfall
IDO activity, kynurenine readout	Immunomodulatory potency	Primary for immunomodulation	multi-mode plate reader ^[S4]	Requires IFN- γ stimulation; concentration, duration and cell density change the result dramatically. Standardize or it will not transfer ^[S11]
MLR suppression	Functional immunosuppression	Primary alternative	CytoFLEX + multi-mode plate reader ^[S4]	Responder PBMC donor variability becomes assay variability. Use cryopreserved PBMCs from a single qualified lot ^[S11]
PGE2 secretion	Immunomodulatory mediator	Primary alternative	multi-mode plate reader ELISA ^[S4]	PGE2 is unstable — sample time and temperature change the answer ^[S11]
TSG-6 secretion	Anti-inflammatory mediator	Secondary	ELISA ^[S4]	
VEGF, HGF, IGF-1 secretion	Angiogenic potency	Primary where the MOA is angiogenic	multi-mode plate reader ^[S21]	Mechanism-match required — do not use for an immunomodulatory claim
ISCT identity panel	Identity	Required	CytoFLEX ^[S4]	Necessary, not sufficient
Trilineage differentiation	Stem-cell character	Supportive only	Histology ^[S4]	Not potency for immunomodulation

Match the assay to the claimed clinical mechanism. An anti-fibrotic program in liver disease is not well served by an IDO assay; a hepatic stellate cell co-culture is closer to its mechanism ^[S21]. An angiogenic program needs a tube-formation or secretion readout. The right question at first contact is not "what is your potency assay" but "what do you claim these cells do in this indication" — the assay follows from the answer.

IFN- γ licensing is worth understanding as a process lever, not only an assay condition. MSC immunomodulatory output is inducible; priming changes it. A program that primes in manufacture and a program that does not are making different products, and the potency assay has to reflect which one is being dosed.

5 (continued) — Reading this program's panels

The identity panel is better than the ISCT minimum, and that is worth acknowledging. CD90, CD73 and CD105 positive with CD14, CD45 and HLA-DR negative is the ISCT core; adding CD44 and HLA-ABC extends it. The MSC core's caution still applies — this identifies an MSC and does not distinguish it from a fibroblast, and

for an autologous product it says nothing about whether this patient's cells are functional ^[S11].

Karyotype on drug substance is a strong inclusion. Genetic stability testing on an expanded autologous product addresses the passage-dependent quality question directly, and many programs omit it.

Endotoxin on drug substance is informational only, and specified at <0.2 EU/kg on drug product. That is a coherent design — the DS is an intermediate — but it means the endotoxin control point is entirely at DP, on a fresh product with an 8-hour clock. Worth confirming the assay turnaround supports it comfortably rather than marginally.

The potency gap. For an autologous MSC program the core's guidance is uncomfortable but honest: donor variability cannot be managed, only characterized, because the donor is the patient. Specifications therefore need to be wider, and the program needs a stated position on what happens when a patient's own cells underperform. A functional assay would not gate release in an autologous setting the way it does for an allogeneic bank — but it would tell the investigator what was delivered, and it would build the dataset that makes a pivotal-stage specification defensible.

MADE's specific recommendation: an IDO or PGE2 readout run as characterization on every batch, not as a release gate. It costs little, uses the evidenced multi-mode plate reader bench ^{[S4][S8]}, accumulates the data the program will need at Phase 2, and does not put a patient's dose at risk of failing on an assay that has no established acceptance range yet. Population doubling time per passage should be tracked alongside it as the cheapest senescence signal available ^[S11].

6. Comparability and technology transfer

6.1 Transfer risk

The risk matrix is modality-independent: academic or research-lab origin is HIGH risk with three to five bridging lots and equivalence margins on CQAs; another CDMO is MEDIUM with two to three lots; MADE development to MADE GMP is LOW-MEDIUM with one to two ^[S5]. Most Mayo MSC programs are academic-origin, which sets the expectation honestly at the start.

6.2 The changes that carry real risk in this modality

Change	Risk	Why
2D flask to 3D microcarrier	Highest	Mechanical environment, morphology, marker intensity, function, plus a new harvest mechanism and a carrier-clearance requirement ^[S11]
FBS to hPL or xeno-free	High	Growth rate and cell behavior shift; regulatory position improves but comparability is required ^[S11]
Tissue source change	High	Sources are not interchangeable despite meeting the same identity criteria ^[S11]
Donor or bank change	High	Tenfold potency range between qualifying donors ^[S11]

Change	Risk	Why
Flask to hollow fiber	Medium	Closed and automated, but 2D geometry preserved — the lowest-risk route to closed manufacturing [S21]
Passage extension	Medium	Senescence and karyotypic drift; needs end-of-production equivalence data [S21]

6.3 The CQA set a comparability protocol must cover

Identity by flow and purity at HIGH priority; **immunomodulatory activity by IDO, PGE2 or MLR at CRITICAL**; viability HIGH; proliferative capacity HIGH; karyotype MEDIUM and rising with passage; endotoxin HIGH; sterility and mycoplasma required every lot [S5]. Add population doubling time, which is the cheapest early senescence signal available and the one most often not tracked [S11].

6.4 The staged plan MADE runs

Stage 0 — technical assessment. Read the process against the modality checklist, name what is defined and what is assumed, produce the sponsor clarification list.

Stage 1 — paper transfer and gap closure. Documentation review, equipment fit, materials sourcing and qualification, analytical gap assessment. In this modality the analytical gap is usually larger than the process gap, so it starts first, not second.

Stage 2 — engineering run. Non-GMP on destination equipment with destination materials, and — specific to MSC — with population doubling time tracked per passage, so the senescence question has data behind it before GMP.

Stage 3 — comparability. Head-to-head on the risk-weighted CQA set, with functional potency carrying the weight.

Stage 4 — GMP manufacture and release.

6 (continued) — The transfer plan for this program

6.1 The logistics design — answer this first

Mayo's requirement is explicit and offers a route: validated transport of **both** starting adipose tissue inbound and ready-to-administer drug product outbound, with shipping verification performed as part of comparability [S62].

Inbound — adipose tissue. The controlling variables are time from collection to processing start, transport temperature, and container. This is a well-understood shipment and the acceptance criteria should be defined jointly with Mayo before the first run.

Outbound — fresh product in a syringe, inside 8 hours of loading. This is the hard one. The window covers transport, receipt, and administration. Any design has to establish what portion of the 8 hours transport may consume and what remains for the site, and demonstrate viability and dose at the far end of it, not at the near end.

Two structural options are worth putting to Mayo rather than assuming:

- **Ship the drug product.** Requires a qualified courier lane and a validated shipper, with data

supporting viability across the full window under worst-case conditions.

- **Ship drug substance and perform the final culture-and-formulate step near the clinical site.**

This preserves the 8-hour window at the site rather than spending it in transit. It implies a presence or partner near the clinical sites and is a different commercial conversation — but it is the design that actually solves the constraint rather than working around it, and Mayo's three-site footprint suggests they have thought about this.

Say which MADE recommends, and why. This is the section of the response Mayo will read most closely.

6.2 The change inventory

Change	Risk	Note
Transport, inbound tissue and outbound fresh DP	Highest — and specified as a comparability item by Mayo	See §6.1
Culture platform and vessel	High	If Mayo runs flasks and MADE proposes a closed platform, that is a comparability exercise; the MSC core notes hollow fiber is the lowest-risk route out of flask work
Media and supplement — FBS, hPL or xeno-free	High	Not stated in the RFI. Ask. If FBS, a conversion plan and its timing is a live question ^[S9]
Passage budget	Medium-High	Autologous, so the budget is per patient; confirm limits and their justification
Analytical methods	Medium	Mayo will accept transferred methods, MADE equivalents, or a combination, with equivalency demonstrated
Cryopreservation of DS	Medium	Confirm controlled-rate profile and post-thaw plating performance transfer

6.3 What Mayo has specified

Technology transfer supported with batch records and training; methods may be Mayo's, MADE's equivalents, or a combination, validated or verified phase-appropriately; **comparability of at least three runs using the intended in-house analytical methods**, with method validation permitted concurrently; and shipping verification inside comparability ^[S62].

7. Stability, storage and logistics

Master and working cell bank stability are assessed at 6, 12, 24 and 36 months under storage at $\leq -150^{\circ}\text{C}$ in vapor-phase liquid nitrogen, testing post-thaw viability, marker expression and potency; a loss of more than 10% in viability or potency triggers investigation and possible disqualification of affected vials ^[S21].

The point worth pressing: **potency stability, not just viability stability**. A bank that holds 80% viability at 36 months but has lost immunomodulatory output is a bank that will produce conforming, ineffective product. Programs that only monitor viability have not tested the thing that matters.

Dose ranges across the modality span four orders of magnitude, roughly 10^5 to 10^9 cells, driven by indication, route and source — $1\text{--}2 \times 10^6$ cells/kg intravenously for acute GVHD, $1\text{--}10 \times 10^7$ per dose intra-articularly for knee osteoarthritis, 120×10^6 locally for Crohn's perianal fistula, $1\text{--}2 \times 10^7$ intrathecally for spinal cord injury ^{[S21][S27]}. Weight-normalized dosing predominates where organ mass scales with body weight; fixed doses are usual for local injection. Only about 39% of published MSC trials compared more than one dose cohort, so dose-response is generally uncharacterized across the field ^[S27] — which means a program's chosen dose is often empirical, and that is worth knowing before designing a process around hitting it exactly.

8. Supply chain and critical materials

Category	Material	Typical source	Risk
Starting material	Bone marrow, adipose or cord tissue	Donor collection or tissue bank	MEDIUM — donor variability is inherent, not a supply failure ^[S6]
Isolation	Collagenase, hyaluronidase	Worthington, Nordmark and others	LOW — GMP grade from multiple sources ^[S6]
Media supplement	Human platelet lysate	Compass Biomedical, STEMCELL, Macopharma	MEDIUM — lot-to-lot variability; pooling trades variability for donor exposure ^[S6]
Media supplement	FBS	Multiple	LOW on supply, HIGH on regulatory — the trend is away from it ^[S6]
Media	Xeno-free defined formulations	Multiple commercial suppliers	LOW-MEDIUM — qualification per formulation
Growth factors	FGF-2, EGF, PDGF	Multiple	LOW-MEDIUM
Bioreactor	Microcarriers	Cytiva, Corning, SoloHill	MEDIUM — the specific carrier type requires qualification and is not substitutable ^[S6]
Cryo	DMSO-based cryoprotectant	BioLife and others	LOW-MEDIUM

Unlike CAR-T, this modality has no single dominant supply risk on the scale of viral vector. The risk is distributed and biological: the tissue donor, the platelet lysate lot, the microcarrier surface chemistry. Each is individually manageable and collectively the reason incoming material performance testing earns its place.

9. Regulatory and CMC

9.1 The four deficiencies that recur

1. Donor variability not adequately characterized
2. Potency assay not correlated to the clinical mechanism — commonly trilineage differentiation
3. Senescence characterization insufficient
4. Passage-dependent quality attribute changes not controlled

Source: FDA deficiency patterns by modality ^[S1]. All four are the same underlying problem: the product is defined by what it is rather than by what it does. A CDMO can materially close all four, and doing so early is far cheaper than doing so under pivotal pressure.

9.2 Phase-appropriate expectations

Phase 1 needs the process described with critical parameters identified though not validated; identity by flow; viability typically above 70%; sterility, endotoxin and mycoplasma; a biologically relevant, quantitative potency assay that is qualified rather than validated; and stability sufficient for proposed storage and shipping with a real-time study started. It does not need validated release methods, reference standards or ICH-compliant long-term stability ^[S1].

The January 2026 flexible-CMC position applies here as it does everywhere: before Phase 2/3 manufacture, 21 CFR Part 211 compliance is not expected, specifications may be permissive, and there is no requirement for three PPQ lots ^[S12]. MSC RFPs from academic sponsors are frequently over-specified against the sponsor's actual phase, and right-sizing that is real value ^[S1].

9.3 The xeno-free direction

Regulatory expectation is moving away from FBS, most firmly in the EU ^{[S9][S21]}. A program planning a pivotal trial on FBS-supplemented media should have a conversion plan with a comparability strategy attached, and should run the conversion before the process is locked rather than after.

10. Clinical and technical precedent

10.1 The modality is approvable

On 18 December 2024 the FDA approved Ryoncil (remestemcel-L-rknd), an allogeneic bone-marrow MSC therapy, for steroid-refractory acute graft-versus-host disease in patients from two months of age — the first FDA-approved MSC therapy. Efficacy rested on MSB-GVHD001 (NCT02336230), a single-arm, multicenter study in 54 pediatric patients ^[S22]. After a long drought of approved MSC products in the United States, this is the existence proof that the regulatory path closes.

10.2 And a promising small trial is not a product

On 13 December 2024, five days earlier, the European Commission withdrew the marketing authorization for Alofisel (darvadstrocel) — allogeneic expanded adipose MSCs for complex perianal fistulas in Crohn's disease — at Takeda's request ^[S23]. The original ADMIRE-CD study in 212 patients had shown a 15.8% difference over placebo in combined remission at 24 weeks ^[S28]. The confirmatory ADMIRE-CD II study in 568 patients showed **2.4%**, missing its primary endpoint and every secondary endpoint ^[S23].

No new safety signal was identified. This was not a toxicity failure; it was an efficacy failure on replication at scale.

The manufacturing reading of that outcome is the one worth carrying into any MSC program. Product consistency — donor-to-donor functional variability, potency drift across passage and bank, mechanism-mismatched potency assays that cannot detect any of it — is a leading hypothesis for why an effect that looked real in 212 patients did not hold in 568. It does not prove the mechanism is wrong. It does mean that a program which cannot measure its own product's function is running a risk it cannot see, and that the analytical work described in §5a is not a compliance exercise but the thing that makes a pivotal result interpretable.

10.3 The field's own pattern

Dose-response is largely uncharacterized: about 39% of MSC trials compare more than one dose cohort ^[S27]. Route-dependent bioavailability differs by orders of magnitude — intravenously administered MSCs are substantially trapped in the pulmonary capillary bed, which is why locally delivered programs and systemically delivered programs are not comparable on dose. And the modality's approvals to date are in indications where the immunomodulatory mechanism is closest to the clinical endpoint.

11. MADE capability against this modality

11.1 Process equipment — evidenced

Two sources agree here, and one of them is public. The internal equipment inventory evidences **Terumo Quantum Cell Expansion**, Sepax cell processing, Xuri W25 rocking bioreactor, KrosFlo TFF, BIOSTAT B-DCU II, controlled-rate freezing and LN2 storage ^[S8]. The RoosterBio partnership announcement of 5 May 2026 — public, and therefore already sayable — names the manufacturing platform options as **Corning CellSTACK**, **Eppendorf BioFlo 320**, **Sartorius Biostat STR**, **Terumo BCT Quantum Flex** and **PBS Biotech vertical-wheel bioreactors**, with flask production supported to 40×CS10 scale and single-use bioreactor tech transfer advancing toward client readiness ^[S33].

Terumo Quantum is the directly relevant one. Hollow-fiber expansion is the lowest-comparability-risk route from an academic flask process to closed, automated clinical manufacture — it removes the contamination and labor exposure of open flask work while preserving 2D growth geometry, so the cells are not simultaneously being asked to tolerate a new mechanical environment ^{[S21][S25]}. For a Mayo program currently running flasks, this is a real and specific answer rather than a generic capability claim.

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
Terumo Quantum Flex — hollow fiber	1	Princeton. The lowest-comparability-risk route out of an academic flask process
Sartorius Biostat STR — stirred tank	1	East Norriton, 50 L single-use, in V-018
Sartorius Ambr 15 — small-scale screening	1	Princeton
PBS Biotech vertical-wheel	6	Princeton — 3 MAG and 15 MAG
Eppendorf BioFlo 320, to 40 L	attested	Held per MADE operations, 23 August 2026; absent from both registers
LOVO — harvest and wash	4	3 Princeton; 1 East Norriton in L-140
CTS Rotea — harvest and wash	5	3 Princeton; 2 East Norriton
Xuri / WAVE W25 rocking	21	East Norriton, in L-140 and P-182
Sartorius Biostat B-DCU II	—	4 Princeton, Inactive
Terumo Quantum, original	—	1 Princeton, Inactive; superseded by the Quantum Flex
iCELLis — fixed bed	—	Not held. The only REF_09 MSC fit-check item MADE does not have

The expansion fleet spans screening (Ambr 15), hollow fiber (Quantum Flex), vertical-wheel (3 L and 15 L), rocking bag (Xuri) and stirred tank to 50 L (Biostat STR), with BioFlo 320 to 40 L alongside. iCELLis is the single genuine absence, and there is no MSC expansion requirement across that range that needs it.

The public track record sits alongside it. MADE has manufactured GMP MSC lots for global customers since technology transfer began in Q3 2025, at approximately 0.5 billion viable cells per CS10 ^[S33].

11.3 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

Need	Instrument	In service
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
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

11.4 Manufacturing environment

Princeton, NJ and East Norriton, PA. Grade B background with Grade A BSC for open manipulations; closed-system steps described as Grade B / Closed System. Open flask work in an MSC process is precisely the manipulation that drives the case for moving to a closed platform.

11.5 Team experience

MADE's quality leadership carries collective prior-organization experience across cell therapy quality systems, comparability and method transfer — including IND approval work on an MSC-adjacent program (FCR001) at Bristol Myers Squibb, quality risk management and inspection-readiness programs at Adaptimmune and GSK, and regulatory CMC leadership covering forty-plus products through FDA INDs at Halloran and Minaris. 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.

11.6 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]
Complex potency assay development	add 2–4 months to the analytical timeline ^[S2]

Industry-standard scoping ranges for directional use. Not MADE pricing until finance has scoped the program ^[S2]. Note that the potency-development modifier applies to most MSC programs coming out of an academic lab, and should be assumed present unless the sponsor demonstrates otherwise.

12. Open questions for Dr. Heimbach and Dr. Huebert's team

Logistics — ask these first. What is the acceptable time and temperature window for adipose tissue from collection to processing start? Of the 8-hour post-loading window, how much may transport consume? Would Mayo consider a drug-substance shipment with final drug product preparation near the clinical site? Which of the three sites will enroll, and in what proportion?

Process. What culture vessel and format is used today? What media and supplement — FBS, human platelet lysate, or xeno-free — and if FBS, is a conversion planned? What passage and population-doubling limits apply, and what justifies them? What is the target cell dose, and does it vary by trial group? Adipose tissue collection method and typical quantity?

Analytics. Would Mayo prefer its methods transferred or MADE equivalents qualified? Is population doubling time tracked per passage? **Would Mayo support running an IDO or PGE2 characterization assay on every batch to build the potency dataset ahead of Phase 2?** What is the endotoxin assay turnaround against the 8-hour window?

Clinical. Indication and route of administration? Expected patients per month? What happens if a patient's cells fail to expand to dose — is re-collection protocol-permitted? What is the current physician-notification path on a positive sterility, and does MADE need to replicate it exactly?

Program. Is this Phase I/II starting fresh, or continuing an existing trial? Target first-patient date?

13. Sources

Tag	Source
[S1]	REF_01 — Regulatory Intelligence Knowledge Base (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 specified in proposal-generator design.md §4.8. The xlsx files were not available to this machine; instrument names come from that specification and must be confirmed against the BMRAM export before client use.
[S9]	REF_09 — Modality-Specific Evaluation Depth Checklists, Modality 3 (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
[S20]	Dominici M et al. Minimal criteria for defining multipotent mesenchymal stromal cells. Cytotherapy 2006. PMID 16923606

Tag	Source
[S21]	MADE MSC modality research report, PROJ-047 bibliographer — chapters 1 (biology), 7 (potency and mechanism assays), 8 (dosing, routes and stability), 9 (CMC, manufacturing and quality control), 10 (regulatory). Compiled with primary citations; DOIs and PMIDs are carried in the source chapters
[S22]	FDA, approval of remestemcel-L-rknd (Ryoncil) for steroid-refractory acute GVHD in pediatric patients, 18 December 2024; Summary Basis for Regulatory Action
[S23]	European Medicines Agency, Alofisel withdrawn from the EU market, 13 December 2024; ADMIRE-CD II outcome
[S24]	Schirmaier C et al. Scale-up of MSC expansion. Stem Cells Translational Medicine 2014. DOI 10.5966/sctm.2013-0146
[S25]	Lock LT et al. Quantum hollow-fiber MSC expansion. Cytotherapy 2017. DOI 10.1016/j.jcyt.2017.09.004
[S26]	Mizukami A, Swiech K. Mesenchymal stromal cells: from discovery to manufacturing. Frontiers in Bioengineering and Biotechnology 2018. DOI 10.3389/fbioe.2018.00121
[S27]	Renesme L et al. Establishment of a consensus definition and reporting of MSC dosing. Stem Cells Translational Medicine 2022. DOI 10.1002/sctm.21-0289
[S28]	Panés J et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: ADMIRE-CD. Lancet 2016. PMID 27477896
[S33]	Made Scientific and RoosterBio announce strategic technology and supply partnership to expand scalable MSC and extracellular vesicle manufacturing. madescientific.com/news, published 5 May 2026. Publicly disclosed and therefore quotable
[S62]	Request for Information — Mayo Clinic Autologous Mesenchymal Stem Cells Clinical Manufacturing, 18 August 2026, 6 pp. Mayo Clinic Center for Regenerative Biotherapeutics
[S63]	Salesforce opportunity 23735-2026, Autologous Adipose-MSC; stage Technical Appraisal; primary contact recorded in SFDC as a single merged record for Dr. Heimbach and Dr. Huebert; retrieved 2026-08-22