

PROGRAM TECHNICAL DEEP DIVE

Allogeneic MSC-Derived EV / Carbomer

Neonatal MSC-EV drug substance and carbomer drug product — technical deep dive

PI	MAYO SITE	ON NUMBER	STAGE	
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MODALITY			VERSION	PREPARED
REF_09 Modality 9, inheriting Modality 3 where the source cell is MSC			V01	September 3, 2026

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1. Executive technical summary

This is the most manufacturing-ready EV program MADE is likely to see, and the RFP is unusually complete: a defined neonatal MSC master cell bank, a 3 L xeno-free bioreactor process, a TFF-based purification already in place, a 17-day drug substance cycle, a compounded carbomer drug product with a closed mixing method, ten months of accumulated drug substance stability, and full proposed release panels for both DS and DP [S60].

MADE's structural fit here is unusually strong and it is publicly documented. The RoosterBio partnership of 5 May 2026 covers exactly this shape of work — MSC producer cells, chemically defined EV collection media, and EV purification by tangential flow filtration and column chromatography [S33]. The program's existing process is already TFF-based, so this is alignment rather than adaptation.

Three technical observations shape the response.

The particle characterization method is MRPS, and MADE has no evidenced particle instrument. Mayo specifies microfluidic resistive pulse sensing for particle concentration and size distribution at both drug substance and drug product [S60]. The EV core identifies particle characterization as the modality's single most basic release attribute and notes it is absent from MADE's analytical inventory [S8]. **This is the gating capability question for this opportunity**, and it has two credible answers — qualify MRPS in house, or outsource to a qualified partner and say so. It does not have a third.

Nothing in either panel is classified as potency. The DS functional assay, a luminescent readout at $\geq 70\%$, is classified as *Characterization*. CD73 activity on the drug product is likewise *Characterization*. Tetraspanin identity on the drug product is marked "*Identity (TBD)*" — Mayo itself flags it as unsettled [S60]. This is a Phase I/II program where that is defensible, and it is precisely the position the EV core says will not survive the move toward pivotal.

The constructive reading is that Mayo has already built the assay it needs and has not yet given it the classification it deserves. CD73 is a defensible mechanism-linked attribute for an MSC-derived vesicle, it is already running at a quantitative $\geq 70\%$ specification, and it was used to qualify batch consistency of the compounding step [S60]. Converting it from characterization to a qualified potency assay is a smaller piece of work than building one from nothing — and it is the single most valuable thing a manufacturing partner could propose here.

The drug product is a compounded combination, not a fill. Drug substance is combined with Aquasonic 100 carbomer gel by passing material between two 60 mL syringes through a Luer-lock closed system, automated with a high-pressure syringe pump, then dispensed into cryovials [S60]. That is a distinct drug product manufacturing operation with its own excipient controls, its own consistency qualification and its own stability program. It should be scoped as such and not absorbed into fill-finish.

2. The modality: biology and mechanism

Extracellular vesicles are membrane-bound particles released by essentially all cell types. Therapeutic programs work with exosome-enriched preparations, typically 30–150 nm, alongside larger microvesicles at 100–1,000 nm; apoptotic bodies are a contaminant to be excluded rather than a product [59]. Most clinical EV programs use MSCs as the producer cell, with iPSC-derived cells and immortalized lines as the alternatives.

The therapeutic entity is cargo, not cell. EVs carry proteins, lipids and RNA — including miRNA — and deliver that cargo to recipient cells. For MSC-derived EVs this is increasingly understood as the actual mechanism behind MSC therapy itself: the paracrine effect, isolated and made into a product. That framing has a practical consequence. An MSC-EV program is not a simplification of an MSC program; it is the same biology with the cell removed, and it inherits the cell's variability while losing the cell's ability to respond to its environment after dosing.

Acellular composition changes the risk profile in both directions. There is no engraftment, no proliferation, no tumorigenicity concern in the way a cell product carries one, and no viability to maintain — EVs can be stored frozen and, in some programs, at less demanding temperatures. Against that, there is no living system to buffer manufacturing variability, so lot-to-lot cargo consistency is entirely a process control problem.

Engineering the producer cell engineers the cargo. Where a program modifies the source cell — mRNA engineering to over-express a therapeutic protein, for instance — the vesicles carry that modification's product. This makes the potency question sharper and, usefully, more tractable: a defined over-expressed protein gives a quantifiable identity and potency handle that a whole-secretome product does not have.

3. This program's product, specifically

3.1 The two products

	Drug substance	Drug product
What	Neonatal MSC-derived extracellular vesicles	EV DS compounded with Aquasonic 100 carbomer gel
Source	Neonatal MSC master cell bank, existing	—
Process	3 L bioreactor, xeno-free; conditioned media filtered to remove cellular debris; TFF into 25 mM trehalose storage buffer	Thawed sterile DS plus sterile carbomer, mixed through a closed two-syringe Luer-lock system with a high-pressure syringe pump; dispensed to sterile cryovials
Duration	~17 days, WCB expansion through EV collection, TFF buffer exchange and vial freeze	Single day
Storage	–80 °C	–80 °C
Stability to date	Process development stability at –80 °C has reached 10 months, ongoing	10 months at –80 °C, ongoing
Administration	—	Thaw ~40 minutes at 4 °C; stable for application up to 4 hours at 4 °C, inclusive of the thaw

Source: RFP, 4 August 2026 ^[S60]. Indication is topical application.

3.2 What the process already gets right

Xeno-free from the start. A 3 L xeno-free bioreactor process avoids the bovine-EV contamination problem that the EV core identifies as the defining conditioning risk — serum-derived vesicles co-purify with product and are indistinguishable by particle counting. Mayo has designed that problem out rather than managing it downstream.

TFF-based purification. The core's primary go/no-go is isolation scalability: ultracentrifugation does not reach GMP commercial scale, TFF does. This process is already on the scalable architecture, which removes the largest single process risk most EV programs carry into transfer.

Trehalose storage buffer. Consistent with EV cryopreservation practice, and already part of the formulation rather than a late addition.

A ten-month stability record. Real accumulated data on both DS and DP, still running.

3.3 The release panels, as proposed

Drug substance

Parameter	Purpose as stated	Method	Specification
Sterility, aerobic/anaerobic/yeast and mold	Safety	USP <71>	No growth after 14 days
Endotoxin	Safety	USP <85>	<0.40 EU/mL
Mycoplasma	Safety	USP <63>	Not detected (in-process cells at harvest)
Pre-dilution particle concentration	Quantity	MRPS	≥1.5×10 ¹¹ particles/mL (in-process)
Post-dilution particle concentration and size distribution	Quantity	MRPS	Report results
Tetraspanins	Identity	Multiplex	CD9, CD63, CD81 present
Appearance	Characterization	Visual	Report results
Total protein	Purity	BCA	≥50 µg per 1×10 ¹¹ particles
Functional assay	Characterization	Luminescent output	≥70%
Residual DNA	Characterization	dPCR	Report results

Drug product

Parameter	Purpose as stated	Method	Specification
Sterility	Safety	USP <71>	No growth after 14 days
Endotoxin	Safety	USP <85>	<5 EU/kg per dose

Parameter	Purpose as stated	Method	Specification
Particle concentration and size distribution	Quantity	MRPS	Report results
Minimum fill	Quantity	USP <775>	Report results
Tetraspanins	Identity (TBD)	Multiplex	CD9, CD63, CD81 present
Appearance	Characterization	Visual	Report results
CD73 functional assay	Characterization	Luminescent output	≥70%

Source: RFP §Proposed release tests and specifications ^[S60].

4. Process architecture, unit operation by unit operation

4.1 The chain, end to end

#	Unit operation	Purpose	Where it fails
1	Source cell bank	Consistent producer	Donor variability; passage and senescence change cargo
2	Producer cell expansion	Reach production scale	Platform must be EV-harvest compatible
3	Conditioning	Switch to EV collection conditions	Serum-derived bovine EVs contaminate the prep if not removed
4	Conditioned media harvest	Collect the vesicles	Producer cell death releases non-EV particles that read as product
5	Clarification	Remove cells and debris	Carryover into downstream
6	Concentration and buffer exchange, TFF	Reduce volume, change buffer	Shear; membrane fouling; vesicle loss to the membrane
7	Polishing, SEC or anion exchange	Remove free protein and lipoproteins	Resolution versus yield trade-off
8	Sterile filtration	Sterility assurance	0.22 µm excludes vesicles above 220 nm
9	Formulation and fill	Final buffer, container	Cryoprotectant choice affects the product
10	Freeze and storage	Preserve	Aggregation and fragmentation across freeze-thaw

4.2 The producer cell — where MSC inheritance applies

When the source is MSC, apply the MSC checklist to the producer: donor variability is significant for MSC-EV programs; tissue source affects cargo, so bone marrow, adipose and cord-derived EVs are not interchangeable; and **senescent MSCs produce different EV cargo**, which makes passage limits a product-quality control rather than a housekeeping rule ^[S9].

Producer passage limits and senescence considerations affect both EV yield and EV composition, and those two do not necessarily move together — a passage that still yields particles may be yielding different particles. Any program without a passage-resolved cargo comparison has an untested assumption at its

center.

4.3 Conditioning — the step unique to this modality

Conditioning switches the culture into EV collection conditions, and the defining requirement is **serum-free conditioning**. Fetal bovine serum contains bovine extracellular vesicles that co-purify with the product and are indistinguishable from it by particle counting ^[S9]. A program that expands in serum must therefore have a defined washout and a serum-free collection phase, and must be able to demonstrate the bovine EV burden is cleared.

The questions that decide reproducibility: conditioning duration; cell density at conditioning; whether media is collected once or repeatedly from the same culture; and — the one most often omitted — **producer cell viability monitoring during conditioning**, because dying cells release apoptotic bodies and cellular debris that inflate particle counts and dilute the therapeutic fraction ^[S9].

RoosterBio's chemically defined EV collection media is directly relevant here: it removes the serum-EV problem at the source rather than managing it downstream ^[S33].

4.4 Isolation and purification — the scalability go/no-go

Method	Scales to GMP commercial	Notes
Ultracentrifugation	No	Standard in academic labs; does not scale. A program built on it needs a process change ^[S9]
Tangential flow filtration	Yes	The workhorse for concentration and buffer exchange; MADE capability ^[S33]
Size exclusion chromatography	Yes	Separates EVs from free protein; typically follows TFF
Anion exchange chromatography	Yes	Alternative polishing step; MADE has column chromatography capability ^[S33]
Immunoaffinity	Limited	High specificity, poor scalability and high cost

TFF plus SEC, or TFF plus anion exchange, are the two scalable architectures ^[S9]. Purification must remove serum and media components, free protein, lipoproteins and unwanted vesicle subpopulations — and each of those is a different separation problem, which is why single-step purification is rarely sufficient.

Sterile filtration is a design constraint, not a final step. A 0.22 µm filter excludes anything larger than 220 nm. If the therapeutic preparation includes microvesicles above that size, sterile filtration removes product. The EV size distribution and the sterility assurance strategy must be designed together, and where filtration is not viable the process must achieve sterility by closed aseptic processing instead ^[S9].

4.5 Formulation and storage

Storage is typically -80 °C, though some programs hold at -20 °C or 2–8 °C with supporting stability data ^[S9]. Trehalose is a common cryoprotectant for EVs and is part of the formulation rather than a process aid — it changes the product ^[S9].

Freeze-thaw is a real stress for this modality. EVs can aggregate, fragment or lose function across repeated freeze-thaw cycles ^[S9]. The number of permitted cycles has to be established, and the stability-indicating methods have to include particle count, size distribution, marker expression and potency — not viability, which does not exist here, and not particle count alone, which will not detect a functional loss.

5. Analytical strategy

5.1 Characterization against MISEV

The MISEV guidelines of 2018 and 2023 are the field's characterization standard. They are not regulatory requirements, and sponsors interpret them differently — which means "MISEV-compliant" is a claim to interrogate rather than accept ^[S9].

Attribute	Method	Note
Particle concentration and size distribution	Nanoparticle tracking analysis (NTA) or tunable resistive pulse sensing (TRPS)	Dynamic light scattering is acceptable for QC only, not for characterization ^[S9]
EV-positive markers	CD9, CD63, CD81 tetraspanins; ALIX, TSG101, syntenin	Demonstrates the particles are EVs
EV-negative markers	Calnexin, GM130	Demonstrates absence of cellular contamination — the control most often skipped
Purity	Particle-to-protein ratio	Higher is purer; the single most useful purity number in this modality ^[S9]
Visualization	Cryo-EM preferred; TEM with negative staining acceptable	Typically outsourced
Cargo	Proteomics, miRNA-seq, lipidomics	Scope follows the claimed mechanism

5.2 Identity is a composition question

Identity here is not a surface phenotype, it is a cargo definition: which miRNAs, proteins or RNAs define this preparation, at what level, and what is the lot-to-lot consistency criterion ^[S9]. A program that has characterized cargo once, on one lot, has a description rather than a specification.

5.3 Safety testing, where the interferences live

Sterility. Test on the bulk EV preparation using a method compatible with the matrix — direct inoculation rather than membrane filtration, since the matrix and the vesicles themselves foul membranes ^[S9].

Endotoxin. EV preparations commonly interfere with the LAL assay, and method qualification requires extensive bridging ^[S9]. This is a known, budgetable piece of development work, and programs that discover it during release testing lose weeks.

Mycoplasma. Test the producer cells **and** the conditioned media ^[S9]. Testing only the cells misses contamination introduced during conditioning.

Residual host cell DNA and protein. More significant where the producer is an immortalized line; still a purity attribute where it is a primary cell ^[S9].

5a. Potency

5a.1 The stated position

REF_09 names potency assay development as the number one challenge for EV programs, and the reason plainly: cell-based functional assays correlate to mechanism poorly without significant development ^[S9]. There is no default assay in this modality. There is no equivalent of the CAR-T cytotoxicity co-culture or the MSC IDO readout that a program can adopt and qualify.

REF_04, MADE's potency-strategy reference, covers CAR-T, NK, TIL and MSC. **It has no EV section** ^[S4]. The guidance below is reasoned from REF_09's named assay families and the FDA potency framework, and is presented as such.

5a.2 Building an assay from the mechanism

Claimed mechanism	Candidate potency assay	MADE bench
Anti-inflammatory	TNF-α suppression in stimulated PBMCs ^[S9]	multi-mode plate reader ELISA ^[S4]
Pro-regenerative	Recipient cell proliferation or migration assay ^[S9]	multi-mode plate reader; imaging
Immunomodulatory	T-cell suppression or macrophage polarization ^[S9]	CytoFLEX + multi-mode plate reader ^[S4]
Defined over-expressed protein	Direct quantitation of the therapeutic protein per particle	ELISA; ddPCR for the transcript

The last row is worth dwelling on. A program that engineers its producer cells to over-express a specific therapeutic protein has a far more tractable potency problem than a whole-secretome program, because there is a defined molecule to measure and a defensible dose basis. Where a sponsor doses on **protein mass rather than particle count**, that is a deliberate and defensible regulatory choice — it ties the dose to a stated mechanism instead of to a physical count that says nothing about activity — and it defines the potency assay.

5a.3 Reference standards

REF_09 asks specifically whether each batch is tested against a qualified reference EV preparation ^[S9]. This is more important in EV than in most modalities: with no viability, no cell count and high assay variability, a reference standard is often the only way to make lots comparable over time. Establishing one early, from a well-characterized lot held back for the purpose, is cheap. Reconstructing one later is not.

5 (continued) – Reading this program's panels

5.1 What is strong

Total protein expressed as **µg per 1×10¹¹ particles** is a particle-to-protein ratio in disguise, and the EV core names that as the single most useful purity number in the modality. Setting it as a release specification rather than a characterization result is better practice than most programs manage.

Tetraspanin multiplex covering CD9, CD63 and CD81 is the correct positive identity panel. Residual DNA by dPCR is appropriate. Mycoplasma tested on in-process cells at harvest is right, and the core notes it should also cover conditioned media — worth confirming whether it does.

5.2 The gaps worth raising

No negative marker control. The MISEV framework pairs the positive tetraspanin panel with negative markers — calnexin and GM130 — to demonstrate the absence of cellular contamination. Neither appears in either panel ^[S60]. Cellular debris carryover is invisible to particle counting and to tetraspanin presence; the negative markers are what detect it. This is a small addition with real assurance value.

Nothing is classified as potency. Discussed in §1. The recommendation is specific: qualify the CD73 luminescent assay as the potency assay for this product, and reclassify it accordingly. It is already quantitative, already specified at ≥70%, already mechanism-adjacent for an MSC-derived vesicle, and already trusted enough that Mayo used it to qualify compounding consistency. A reference EV preparation, retained from a well-characterized lot, would complete it — the core notes a reference standard matters more in this modality than in most, because there is no viability and no cell count to anchor lots against each other.

Endotoxin interference has not been addressed in the document. The core flags LAL interference as a common and budgetable surprise in EV matrices requiring extensive bridging. Mayo may already have resolved it; if not, it belongs in the transfer plan rather than in release testing.

Drug product identity is marked TBD by Mayo. That is an invitation. A manufacturing partner with a view on it is more useful than one who accepts the panel as written.

6. Comparability and technology transfer

6.1 Transfer risk

The standard matrix applies — academic origin HIGH with three to five bridging lots, another CDMO MEDIUM with two to three ^[S5]. EV programs skew toward the academic end, and they carry an extra burden: the producer cell process and the vesicle process both transfer, and both need comparability.

6.2 The changes that carry real risk

Change	Risk	Why
Ultracentrifugation to TFF/SEC	Highest	Different impurity profile, different vesicle subpopulation recovery. Unavoidable for any program heading to commercial scale ^[S9]

Change	Risk	Why
Producer donor, tissue source or passage	Highest	Cargo composition changes; the product is the cargo ^[S9]
Serum-containing to serum-free conditioning	High	Removes bovine EV contamination — an improvement that still changes the measured product
Producer expansion platform, 2D to bioreactor	High	Inherits the full MSC 2D-to-3D comparability question, and the cells' mechanical environment affects what they secrete ^[S11]
Formulation or cryoprotectant	Medium-High	Trehalose and buffer are part of the product
Collection schedule, single to repeated harvest	Medium	Later collections may differ in cargo from earlier ones

6.3 The CQA set

Particle concentration and size distribution HIGH; tetraspanin and ALIX/TSG101 marker profile HIGH; **calnexin and GM130 absence HIGH** — cellular contamination is a purity failure that particle counting cannot see; particle-to-protein ratio HIGH; cargo identity CRITICAL where cargo is the claimed mechanism; potency CRITICAL; endotoxin HIGH; sterility and mycoplasma required every lot ^{[S5][S9]}.

6.4 The staged plan MADE runs

Stage 0 — technical assessment. Read the process against the checklist. In this modality expect the isolation-scalability question, the potency position and the endotoxin method to be the three largest open items.

Stage 1 — paper transfer and gap closure. Documentation review, platform fit, media and producer-cell sourcing, analytical gap assessment. Endotoxin and sterility method development for the specific matrix starts here, not at release.

Stage 2 — engineering run. Non-GMP on destination equipment, with producer viability tracked through conditioning and a particle-to-protein ratio established for the destination process.

Stage 3 — comparability. Head-to-head on the risk-weighted CQA set, against a retained reference preparation.

Stage 4 — GMP manufacture and release.

6 (continued) — The transfer plan for this program

6.1 What Mayo has specified

Mayo will support technology transfer with batch records and training, and will accept either its own methods transferred, MADE in-house equivalents, or a combination — provided equivalency is demonstrated and all methods are validated or verified phase-appropriately. **Comparability will be at least three runs using the**

intended in-house analytical methods, with method validation permitted concurrently. Stability studies on DS and compounded DP are expected annually unless supported by manufacturing data within the preceding four months ^[S60].

Three runs against defined methods, with concurrent validation permitted, is a workable and well-calibrated ask. It matches the core's MEDIUM-risk transfer expectation.

6.2 The change inventory

Change	Risk	Note
Particle characterization instrument	Gating	MRPS specified. Qualify in house or outsource — see §11
Producer cell bank handling	Medium	Existing neonatal MSC MCB transfers; confirm provenance, passage budget and remaining inventory
Bioreactor platform	Medium	3 L today. Confirm whether the intended MADE platform matches or scales, and whether cargo has been compared across scale
Collection media	Medium	If moving to a chemically defined EV collection medium, that is an improvement and a comparability item ^[S33]
TFF membrane and cassette	Medium	Membrane chemistry and cutoff qualify per process
Compounding step	Medium-High	The two-syringe closed system and syringe-pump automation must transfer as a validated operation, not as a technique
Analytical methods	Medium	Ten tests across two panels; equivalency demonstration per method

6.3 Staged plan

Stage 0 — assessment. This document, plus the MRPS decision and the questions in §12.

Stage 1 — paper transfer and gap closure. Batch records mapped; MCB provenance and inventory confirmed; bioreactor and TFF fit established; carbomer and excipient sourcing qualified; the ten analytical methods gap-assessed with the endotoxin interference question resolved first; the potency reclassification proposed to Mayo.

Stage 2 — engineering run. Non-GMP through both DS and compounded DP, with producer viability tracked through conditioning and a particle-to-protein ratio established for the destination process.

Stage 3 — comparability. Three runs minimum against Mayo's specified methods, with concurrent method validation and shipping verification included.

Stage 4 — GMP manufacture, release and supply.

7. Stability, storage and logistics

Storage at -80 °C is the common position; -20 °C and 2–8 °C are used by some programs with supporting data ^[S9]. Stability-indicating methods must cover particle count, size distribution, marker expression and potency over time — a stability program that tracks only particle count will report a stable product that has

lost function.

Freeze-thaw tolerance must be established explicitly, including the number of permitted cycles, and the instruction-for-use has to reflect it: if a vial cannot survive a second freeze, the clinical site needs to know that in a form they will actually follow.

For a topical or locally delivered EV product the final formulation may be a gel or other vehicle rather than a simple buffer, which adds a compounding step, its own excipient controls, its own stability program and — depending on the vehicle — a possible combination-product regulatory question. That step should be scoped as drug product manufacture in its own right, not as a fill operation.

8. Supply chain and critical materials

Category	Material	Typical source	Risk
Producer cells	MSC working cell bank	Donor-derived, or RoosterBio off-the-shelf allogeneic bioprocess-ready banks ^[S33]	MEDIUM donor-derived; LOW via the partnership , which is the point of it
Expansion media	Xeno-free MSC expansion media	RoosterBio ^[S33] ; alternatives exist	LOW-MEDIUM
Collection media	Chemically defined EV collection media	RoosterBio ^[S33]	LOW-MEDIUM — and it removes the serum-EV problem at source
TFF	Membranes and cassettes	Repligen, Cytiva, Sartorius	MEDIUM — membrane chemistry and cutoff are qualified per process
Chromatography	SEC or AEX resin, columns	Cytiva and others	MEDIUM — resin lot qualification
Formulation	Trehalose, buffer components	Multiple	LOW
Vehicle, where applicable	Gel or carrier excipients	Depends on formulation	MEDIUM — may carry its own compendial and stability requirements

The distinctive supply position in this modality is that **MADE's partnership addresses the two highest-variability inputs at once** — the producer cell bank and the collection media ^[S33]. For a sponsor with a proprietary source cell the partnership is less directly relevant, and MADE's EV infrastructure applies on its own ^[S9].

9. Regulatory and CMC

9.1 Classification comes first

EVs are typically regulated as biological products rather than as cell therapies in the US or ATMPs in the EU, and the pathway resembles recombinant proteins or vaccines more than it resembles cell therapy in some respects ^[S9]. **Confirm the classification with the sponsor and flag any ambiguity**, because it determines which guidance applies, what the CMC package looks like, and whether the long-term follow-up expectations of gene therapy apply at all.

Where the producer cell has been genetically modified, check whether the regulator views the product as gene-therapy-derived. A program engineering its MSCs with mRNA — non-integrating and transient — is in a materially different position from one using an integrating vector, and that distinction is worth establishing early rather than assuming.

9.2 Phase-appropriate CMC is a judgment call here

FDA and EMA expectations for EVs are less well defined than for cell therapies, so phase-appropriate CMC is a judgment call rather than a checklist ^[S9]. The general framework still governs: a biologically relevant quantitative potency assay at Phase 1, qualified rather than validated; identity, purity, safety and strength defined; stability sufficient for proposed storage and shipping ^[S1]. The January 2026 flexible-CMC position applies to biological products generally ^[S12].

MISEV compliance is not regulatory compliance. A sponsor asserting MISEV alignment has said something about characterization completeness, not about regulatory sufficiency, and the two should not be conflated in either direction ^[S9].

9.3 The value a CDMO adds early

An FDA pre-IND or INTERACT interaction is the right place to settle classification, the potency strategy and the sterility assurance approach. A program approaching one of those meetings benefits disproportionately from having a manufacturer's view of what is achievable at scale, because the commitments made in that meeting become the CMC plan. Being in the room, or at least in the document, before the meeting is worth more than any amount of process optimization afterwards.

10. Clinical and technical precedent

There is no approved EV therapeutic. That is the single most important piece of context for any EV program, and it cuts both ways: there is no established regulatory template to follow, and there is no incumbent to displace.

The FDA has acted against unapproved exosome products. The agency has issued public safety notifications about products marketed for regenerative and cosmetic uses without approval, following adverse events. The practical consequence for a legitimate program is that the modality carries reputational baggage

the sponsor did not create, and that claims language matters more here than elsewhere — a clinical EV program should be visibly distinguishable from the wellness market in everything it publishes.

The MSC precedent applies by inheritance. The December 2024 pairing described in the MSC core — Ryoncil approved on the 18th, Alofisel withdrawn on the 13th after a 568-patient confirmatory trial returned 2.4% against placebo where 212 patients had shown 15.8% — is directly relevant, because MSC-EV programs inherit MSC's product-consistency problem without inheriting the cell's capacity to respond in vivo. If inconsistent MSC potency is a plausible explanation for that replication failure, then EV cargo consistency deserves at least as much analytical attention, not less.

Route matters more than in most modalities. Locally delivered EV programs — intradiscal, intra-articular, topical — face a fundamentally different biodistribution problem from systemically delivered ones, and dose comparisons across routes are not meaningful. Programs dosing on protein mass rather than particle count are, in effect, taking a position on this.

11. MADE capability against this modality

11.1 The partnership is the capability, and it is public

The RoosterBio strategic technology and supply partnership, announced 5 May 2026, integrates RoosterBio's off-the-shelf MSCs, proprietary MSC expansion media and chemically defined EV collection media into MADE's GMP operations, and explicitly covers **extracellular vesicle purification via tangential flow filtration and column chromatography** ^[S33].

Because it is published, it can be described to a sponsor without qualification. Against REF_09's EV fit-check:

Fit-check item	Position
Bioreactor compatible with EV production	Platform options publicly named: Corning CellSTACK, Eppendorf BioFlo 320, Sartorius Biostat STR, Terumo BCT Quantum Flex, PBS Biotech vertical-wheel ^[S33]
TFF system for EV isolation at scale	Publicly named capability ^[S33]
Column chromatography for polishing	Publicly named capability ^[S33]
NTA or equivalent particle characterization	Not evidenced — see §11.2
Cryo-EM	Outsourced, as REF_09 expects ^[S9]
Sterile filtration, EV-compatible	Standard capability; the constraint is the product's size distribution, not the equipment

MADE has manufactured GMP MSC lots for global customers since technology transfer began in Q3 2025, with recent campaigns yielding approximately 0.5 billion viable cells per CS10 ^[S33]. For an EV program the producer-cell track record is the relevant one, and it is real and quotable.

11.2 Particle characterization — in service, and it is NTA

MADE holds a Particle Metrix ZetaView Evolution quatt (PMX-440-Quatt-C) at Princeton, in service — verified 23 August 2026 with qualification attested by MADE operations ^[S8]. Particle concentration and size distribution, the modality's most basic quantity attribute, is covered.

One distinction survives qualification and should not be blurred by it. ZetaView is nanoparticle tracking analysis. Where a sponsor's panel specifies microfluidic resistive pulse sensing, the two are different measurement principles — NTA tracks Brownian motion, MRPS measures a resistive pulse through a constriction — and they do not report identical size distributions on the same preparation. An equivalency bridge is required, and it is a real piece of analytical work rather than a formality.

That is a method-comparability question, not a capability gap, and it is the kind of thing better raised in the first technical conversation than discovered during comparability.

The remaining fit-check items are settled: TFF and column chromatography are publicly named MADE capability ^[S33], sterile filtration is standard, and cryo-EM outsources as REF_09 expects.

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 Penton; 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
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. EV downstream processing is largely closed, which suits the environment well; the open steps are formulation and fill.

11.5 Team experience

MADE's quality leadership carries collective prior-organization experience across biologics and cell therapy quality systems, method qualification, comparability and inspection readiness — including commercial cell therapy quality leadership at Bristol Myers Squibb and Novartis, quality risk management at Adaptimmune and GSK, and regulatory CMC covering forty-plus products through FDA INDs. 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

REF_O2 carries no EV per-batch band ^[S2]. The nearest structural analogue is allogeneic MSC at a published industry band, since the producer-cell campaign dominates the cost base — but EV adds a full downstream purification train, a characterization panel with no cell count in it, and method development for endotoxin and sterility that most modalities do not need. **Build EV estimates bottom-up rather than borrowing the MSC band.**

Duration follows the standard model: 12–18 months for a custom process with no platform, 4–6 months where a platform applies — and the RoosterBio platform is precisely what can make the accelerated case real for an MSC-EV program ^{[S2][S33]}. Add two to four months for academic origin, and two to four months for potency assay development, which in this modality should be assumed present unless the sponsor demonstrates a qualified assay already exists ^[S2].

12. Open questions for Dr. Wyles's team

Producer cells. What is the provenance and passage history of the neonatal MSC master cell bank, and what remains in inventory? What passage budget applies, and has EV cargo been compared across passage? Is the bank single-donor?

Process. Is 3 L the intended clinical scale, or is scale-up planned — and if so, has cargo been compared across scale? What is the conditioning duration and cell density? Single or repeated media collection? Is producer viability monitored through conditioning? Is mycoplasma tested on conditioned media as well as on cells at harvest?

Analytics. Would Mayo prefer MRPS transferred, or an equivalent qualified with a bridging dataset? Has endotoxin interference in the EV matrix been assessed? **Would Mayo consider reclassifying the CD73 luminescent assay as the potency assay?** Is there a retained reference EV preparation, and is every lot tested against it? Are negative markers — calnexin, GM130 — assessed anywhere in development, even if not on release?

Drug product. What defines drug product identity, given the panel marks it TBD? What are the carbomer's own specifications and supplier? Is the 4-hour post-thaw application window supported by data at the intended clinical site conditions?

Regulatory. Has the product's classification been confirmed with the FDA — biological product, and is the carbomer combination treated as a formulation or as a device component? Has a pre-IND or INTERACT meeting occurred or been scheduled?

Program. What dose and application regimen is planned, and how many patients in Phase I/II? What is the target IND 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). Note: carries no EV per-batch band
[S4]	REF_04 — Potency Assay Strategy Advisor (MADE internal). Note: has no EV section. The §5a guidance is reasoned from REF_09's named assay families and the FDA potency framework
[S5]	REF_05 — Comparability and Tech Transfer Planning Framework (MADE internal)
[S8]	MADE internal capability inventories, as specified in proposal-generator design.md §4.8. The <code>xlsx</code> 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 9, and its inheritance rule from 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
[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
[S60]	Request for Proposal — Mayo Clinic Allogeneic Mesenchymal Stem Cell Derived Extracellular Vesicle / Carbomer Clinical Manufacturing, 4 August 2026, 6 pp. Mayo Clinic Center for Regenerative Biotherapeutics
[S61]	Salesforce opportunity 23785-2026, Allogeneic MSC-derived extracellular vesicle (EV); stage Engaged; primary contact Saranya Wyles; retrieved 2026-08-22
—	MISEV 2018 and MISEV 2023 — Minimal Information for Studies of Extracellular Vesicles, International Society for Extracellular Vesicles. Characterization standards, not regulatory requirements