

PROGRAM TECHNICAL DEEP DIVE

mRNA-Engineered MSC-EV — Intradiscal

IL-1RA-EV and GH1-EV for degenerative disc disease — technical deep dive

PI

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MAYO SITE

Jacksonville

ON NUMBER

Not yet in Salesforce

STAGE

Pre-IND — FDA INTERACT letter finalized 2026-08-11

MODALITY

REF_09 Modality 9, inheriting Modality 3 where the source cell is MSC

VERSION

V01

PREPARED

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Contents

1	Executive technical summary
2	The modality: biology and mechanism
3	This program's product, specifically
4	Process architecture, unit operation by unit operation
5	Analytical strategy
5a	Potency
5 (continued)	The analytical package — where this program is won
6	Comparability and technology transfer
6 (continued)	The engagement — four stages, sequenced against Qu's clock
7	Stability, storage and logistics
8	Supply chain and critical materials
9	Regulatory and CMC
10	Clinical and technical precedent
11	MADE capability against this modality
12	Open questions for Dr. Qu
13	Sources

1. Executive technical summary

This is the fastest-moving commercial opening in the Mayo relationship and it does not appear in any system of record. Dr. Qu invited MADE to scope the R&D-enabling studies unprompted, in the room, on 12 August: *"That would be the R&D enabling studies. It's going to cost money. If you guys are interested in working together, we can get that done"*^[S100].

There is a clock. His FDA INTERACT letter was finalized on 11 August — the day before the visit — with submission targeted for September and a 75-day agency response window ^{[S100][S101]}. Work started now lands before that feedback arrives; work started after it restarts.

Three things make this program technically unusual, and all three make it easier rather than harder.

The dosing basis is protein mass, not particle count. Qu was explicit and deliberate: *"people do like 10 billion particles, 20 billion particles. But we dose our stuff based on the amount of proteins... we have a clearer mechanism of action based on IL-1RA and GH1... probably it's gonna be a little bit more agreeable to FDA review"*^[S100]. The EV core identifies this as the position that converts an intractable potency problem into a tractable one: a defined over-expressed protein gives a quantifiable identity and a defensible dose basis where a whole-secretome product has neither. **Quantitative IL-1RA and GH1 immunoassays are therefore the release-defining methods, and they gate everything else.**

The producer cells are mRNA-engineered, not virally modified. Qu moved off AAV after COVID — *"after COVID, we just say use mRNA. Easy, simple, cheaper, scalable"*^[S100]. Non-integrating, transient modification puts the product in a materially different regulatory position from a vector-engineered one, and the EV core says that distinction should be established early rather than assumed.

MADE's platform fit is direct and was demonstrated in the same meeting. MADE presented its NFF → TFF → optional-chromatography downstream train to Mayo on 12 August ^[S101]. The RoosterBio partnership, publicly announced 5 May 2026, covers scalable MSC and EV manufacture including chemically defined EV collection media ^[S33] — and **Mayo's own EV process already runs on RoosterBio media**, established on 4 August ^[S101]. The producer platform and the collection media already match.

The strategic reading matters as much as the technical one. Syed proposed testing the relationship on a non-Jacksonville program and both Julie Allickson and Brian Hultgren endorsed the idea ^[S101]. Qu's EV program fits that description better than the cell bank does: it is time-boxed, it is his own program, it has a regulatory clock, and it does not depend on the lease or on any structural decision.

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 ^[S9]. 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 What Qu described

Attribute	As described	Manufacturing consequence
Platform	MSCs engineered by mRNA; moved off AAV post-COVID for scalability and cost	Transient transfection is a process step — mRNA sourcing, transfection efficiency and the timing of EV harvest all become process parameters
Product	EVs only; no whole-cell component	Downstream is the product. Cell removal, clearance and residual-cell testing are critical
Two products	IL-1RA-EV , with roughly a 9,000-fold increase in IL-1RA expression, and GH1-EV , a second gene in a separate vesicle. Provisional patent filed on GH1	Two drug substances, two release panels, possibly two INDs — must be confirmed
Indication and route	Degenerative disc disease, intradiscal injection — not IBD	Small volume at high concentration; local delivery raises specific biodistribution and safety questions
Dosing basis	Protein mass of IL-1RA and GH1, deliberately not particle count	This is the potency assay
Cell source	Placenta-derived master cell bank held; moved off iPSC-derived MSC to avoid FDA delay	Bank status, provenance and qualification need review — may be usable, may need re-banking under GMP

Source: investigator lunch, 12 August 2026 ^[S100].

3.2 The scientific claim

Qu's description of the effect: *"Once you poke it, it's going to collapse... your control doesn't improve, but you put IL-1RA, it improves. But you put IL-1RA + GH1, it improves a lot more. That is just protecting the disc like I've never seen before. Nobody targeted regeneration of the disc"*^[S100].

The combination effect is the program's central claim, and it is also a manufacturing question: two separately produced drug substances delivered together, whether co-formulated or co-administered, have different release, stability and regulatory consequences. **This is the single most important scope question and it is not yet answered.**

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) — The analytical package — where this program is won

5.1 Potency is already defined, which is rare

Because dose is expressed as protein mass of two named proteins, potency is not a search — it is a quantitation problem. Quantitative IL-1RA and GH1 assays by validated immunoassay become the release-defining methods.

That has three consequences. **They gate everything**, so they belong at the front of the analytical workstream, not in its middle. **They need a reference standard strategy** — the EV core notes a qualified reference preparation matters more in this modality than in most, because there is no viability and no cell count to anchor lots against each other. And **they make the whole program more fileable**, which is precisely the judgment Qu made when he chose the dosing basis.

5.2 The rest of the panel

Layer	Attribute	Method
Potency	Quantitative IL-1RA; quantitative GH1	Validated immunoassay against a reference standard
Identity	Tetraspanins CD9, CD63, CD81	Multiplex or bead-based flow
Identity — negative	Calnexin, GM130	Demonstrates absence of cellular contamination — the control most often omitted
Quantity	Particle concentration and size distribution	NTA or equivalent
Purity	Particle-to-protein ratio	Derived — the most useful purity number in the modality
Residuals	Host-cell DNA and protein; residual mRNA ; residual cells	qPCR, ELISA, flow
Safety	Sterility, endotoxin, mycoplasma, adventitious agents	Standard, with EV-matrix method development for endotoxin and sterility
Compendial	Appearance, pH, osmolality, sub-visible particulates	Standard

Residual mRNA is specific to this program and is the item a generic EV panel would miss. A transiently transfected producer cell carries mRNA that must be shown to be cleared from the vesicle preparation, and the method for it is a development item rather than a transfer item.

5.3 The capability gap, stated plainly

Particle concentration and size distribution is the modality's most basic quantity attribute, and **MADE has no evidenced particle characterization instrument** ^[s8]. The same gap applies to the Wyles program. Resolving it once serves both. Two credible answers: qualify in house, or outsource to a named partner with stated turnaround.

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]
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.

6 (continued) — The engagement — four stages, sequenced against Qu's clock

The outline below was drafted from the 12 August meeting and is MADE's read, not a priced proposal ^[S101].

Stage 1 — process definition and technology transfer. Process description workshop and gap assessment against the current lab process; raw material and mRNA supply strategy; single-use and closed-system design; define upstream (MSC expansion, transfection, EV production phase, harvest trigger) and downstream (clarification, TFF concentration and diafiltration, optional polishing, sterile filtration, fill and cryostorage); draft process flow, bill of materials and an IND risk assessment.

Stage 2 — analytical development, the critical path. The IL-1RA and GH1 potency assays first, with a qualification plan and reference standard strategy; then identity, purity, residuals, safety and compendial as above.

Stage 3 — engineering run. One run per EV product at intended clinical scale, confirming yield, potency reproducibility and the analytical package end to end — **structured so the run can convert to GMP if results support it**, which is an approach MADE has used elsewhere and should offer explicitly.

Stage 4 — GMP supply and IND CMC. Cell bank qualification or re-banking as required; GMP drug substance and drug product for the Phase 1 cohort with stability; CMC authoring support for Module 3.

6.1 Sequence against his clock, not MADE's

INTERACT goes in during September with a 75-day agency response window. **Stages 1 and 2 should be running before that feedback arrives**, so the answers fold into the IND rather than restarting it.

The commercial implication is specific: offer Stage 1 as a small, fast, fixed-scope engagement that starts on a purchase order rather than on a master agreement ^[S101]. A scoped Stage 1 delivered in days rather than weeks is what makes this the proof point.

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_O9'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_O9 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. Qu

Ask these first, in this order.

1. **What does the INTERACT letter actually ask FDA?** It names the CMC gaps he already knows he has, and it is the cheapest possible scoping input.

1. **One IND or two?** Are the two EVs co-formulated or co-administered? This changes the entire analytical and regulatory package.

1. **Which cell source for the EV process** — the placenta master cell bank, or the adipose line? What is its qualification status and documentation?

1. **Dose and cohort size** — protein mass per injection, injections per patient, patients in Phase 1. This sets batch size and therefore everything downstream.

Then: current scale and yield per run in the lab today. Which assays already exist, and in whose hands — his lab, Mayo QC, or nowhere. Funding source and timing, given both his clinical programs run on benefactor dollars. And where it runs: Princeton is available now, and is also the natural proof-point site Syed proposed to Julie ^[S101].

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 .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 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
[S100]	Fellow recording, MADE × Mayo Jacksonville investigator lunch, 12 August 2026 — Dr. Qu on the mRNA-engineered EV platform, the two products, the intradiscal indication, the protein-mass dosing basis, the placenta master cell bank, and the direct invitation to scope the R&D-enabling studies. Transcribed and attributed by content in website/business-docs/pi-notes.html; Fellow diarization on this recording is unreliable
[S101]	website/business-docs/qu-ev-proposal-outline.html — MADE working outline prepared for Simona Jusyte from the 12 August meeting. Records the INTERACT timing and 75-day response window, the four-stage scope, MADE's NFF → TFF → chromatography presentation in that session, the RoosterBio media alignment established 4 August, and the pilot positioning endorsed by Julie Allickson and Brian Hultgren. A working outline, not a proposal; nothing in it is priced
[S102]	Salesforce — no opportunity exists for this program as of 2026-08-22. The nearest record, 23625-2026 "EV MFG" with Julie Allickson as primary contact, was deleted upstream on 2026-08-22 and appears to have been consolidated into opportunity 23785-2026 (Wyles)
—	MISEV 2018 and MISEV 2023 — Minimal Information for Studies of Extracellular Vesicles, International Society for Extracellular Vesicles. Characterization standards, not regulatory requirements