

TECHNICAL EVALUATION & CDMO FRAME

# mRNA-Engineered MSC-EV — Intradiscal

MADE technical evaluation and CDMO frame

| PI                  | MAYO SITE    | ON NUMBER | MODALITY               | VERSION |
|---------------------|--------------|-----------|------------------------|---------|
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PREPARED

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## 1. At a glance

|                              |                                                                                                                                                                                                                                          |
|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Program                      | Two mRNA-engineered MSC-derived EV products — IL-1RA-EV and GH1-EV                                                                                                                                                                       |
| Modality                     | Extracellular vesicles, MSC-derived, with an engineered producer cell                                                                                                                                                                    |
| Phase                        | Pre-IND, heading to Phase 1                                                                                                                                                                                                              |
| Indication and route         | Degenerative disc disease, intradiscal injection                                                                                                                                                                                         |
| Regulatory status            | <b>INTERACT letter finalized 11 August 2026; submission targeted September, with a 75-day agency response window</b> — see §3                                                                                                            |
| Starting material            | Placenta-derived MSC master cell bank, held. iPSC-derived MSC was deliberately set aside to avoid regulatory delay                                                                                                                       |
| Drug substance               | Two separate EV drug substances from mRNA-engineered producer MSCs, harvested for vesicles only with no whole-cell component. IL-1RA-EV carries roughly a nine-thousand-fold increase in IL-1RA expression; GH1-EV is the second product |
| Drug product                 | <b>Not established.</b> Whether the two products are co-formulated or co-administered is unresolved, and it decides whether this is one drug product or two — see §15                                                                    |
| Dosing basis                 | <b>Protein mass of IL-1RA and GH1, not particle count</b>                                                                                                                                                                                |
| Where MADE would manufacture | Princeton, NJ — available now                                                                                                                                                                                                            |
| MADE reference               | Pre-IND; no MADE reference number assigned yet                                                                                                                                                                                           |
| MADE technical fit           | <b>Direct.</b> The producer platform, the collection media and the downstream train already align — see §5 and §13                                                                                                                       |

**This is a technical assessment, not a proposal.**

## 2. Program understanding

MSCs engineered by mRNA, harvested for extracellular vesicles only, with no whole-cell component. Two separate products in separate vesicles — IL-1RA-EV, with roughly a nine-thousand-fold increase in IL-1RA expression, and GH1-EV. A placenta-derived master cell bank is held, and iPSC-derived MSC was deliberately set aside to avoid regulatory delay. Dose is expressed as protein mass of IL-1RA and GH1 rather than as particle count.

**Three of those choices are unusually well judged, and they shape our whole read.**

**Dosing on protein mass rather than particle count.** This is the decision that makes the program fileable. Particle count says nothing about activity; a defined over-expressed protein gives a quantifiable identity, a defensible dose basis, and — critically — a potency assay that can actually be built. Most EV programs spend years on the potency problem. Yours has largely defined it away.

**mRNA rather than viral engineering.** Non-integrating, transient modification places the product in a materially different regulatory position from a vector-engineered one, and it is worth establishing that with the agency explicitly rather than leaving it to be inferred.

**Moving off iPSC-derived MSC.** A pragmatic call that removes a substantial characterization and review burden.

**What the public record adds.** There is no approved EV therapeutic and therefore no regulatory template to follow. In that setting, a program that has already fixed its dose basis on a measurable molecule starts several steps ahead — the potency argument the modality most often cannot make is already made.

Your priorities, and how MADE answers them

| Your priority                                      | How MADE answers it                                                                                                                 |
|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| A September submission supported, not delayed      | Stage 1 process definition is small, fixed and can start now, with outputs written to be usable in the submission's CMC narrative   |
| Dosing on protein mass carried through manufacture | The analytical plan is built around your protein-mass basis — the assay strategy quantifies exactly what you dose                   |
| Two products, one platform                         | IL-1RA-EV and GH1-EV share the producer platform and the downstream train, so development is scoped once and applied twice          |
| The mRNA position established, not inferred        | The non-integrating, transient distinction is raised deliberately in the regulatory framing rather than left for a reviewer to draw |

3. Regulatory position and pathway

|                                   |                                                                                                                                                                                                                                                                                                                                                                                     |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Current filing status             | Pre-IND. <b>INTERACT letter finalized 11 August 2026, submission targeted September 2026, with a 75-day agency response window.</b> Work started now lands before that feedback arrives; work started after it restarts                                                                                                                                                             |
| What the letter asks              | <b>Not established</b> — and it is the cheapest scoping input available, because it names the CMC gaps already known. See §15                                                                                                                                                                                                                                                       |
| Designations held                 | <b>Not established</b> — see §15                                                                                                                                                                                                                                                                                                                                                    |
| Product classification            | EVs are typically regulated as biological products rather than as cell therapies in the US or ATMPs in the EU. Confirming it determines which guidance applies and whether gene-therapy long-term follow-up expectations attach at all                                                                                                                                              |
| Producer-cell modification        | mRNA engineering is non-integrating and transient, which is a materially different position from an integrating vector. Establish it with the agency explicitly rather than letting it be inferred — the INTERACT interaction is the right place                                                                                                                                    |
| One IND or two                    | <b>Unresolved, and it is the largest single scope question.</b> Two separately produced drug substances delivered together — co-formulated or co-administered — have different release, stability and regulatory consequences. See §15                                                                                                                                              |
| What the CMC package must support | Phase 1: the process described with critical parameters identified though not validated; identity, purity, safety and strength defined; a biologically relevant quantitative potency assay, qualified rather than validated; stability sufficient for the proposed storage and shipping. It does not need validated release methods, reference standards or ICH long-term stability |

|                            |                                                                                                                                                                                                                                                                                                         |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| What a site change implies | Not a site change — this is a laboratory-to-GMP transfer, which carries academic-origin transfer risk rather than site-change comparability. See §10                                                                                                                                                    |
| Applicable guidance        | FDA CBER's flexible-CMC position of 11 January 2026 applies to biological products generally. MISEV 2018 and 2023 are characterization standards, not regulatory requirements                                                                                                                           |
| Why the clock matters      | An INTERACT or pre-IND interaction is where classification, the potency strategy and the sterility assurance approach get settled, and the commitments made there become the CMC plan. Stages 1 and 2 running before the response arrives means the answers fold into the IND rather than restarting it |

## 4. Starting material

|                                    |                                                                                                                                                                                                           |
|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Source                             | Placenta-derived MSC master cell bank, held. iPSC-derived MSC was considered and deliberately set aside to avoid regulatory delay                                                                         |
| Which bank for the EV process      | <b>Not established.</b> Whether the placenta master cell bank or another line supplies the EV process is an open question, and it changes the work — see §15                                              |
| Collection                         | Not applicable — the process starts from an established bank rather than a fresh donor collection                                                                                                         |
| Acceptance criteria                | <b>Not established.</b> Bank provenance, qualification status and documentation have not been shared                                                                                                      |
| Fresh or cryopreserved             | Cryopreserved bank                                                                                                                                                                                        |
| Time from collection to processing | Not applicable for a banked allogeneic starting material                                                                                                                                                  |
| Chain of identity and custody      | No per-patient chain of identity. Bank inventory, vial reconciliation and passage tracking are the controls that matter                                                                                   |
| Passage budget                     | <b>Not established.</b> Senescent MSCs produce different EV cargo, so passage limits are a product-quality control rather than a housekeeping rule — and yield and cargo do not necessarily move together |
| On a failed collection             | Not applicable. The equivalent question is whether the existing bank is usable as held or needs re-banking under GMP, which is the difference between bank qualification and bank creation in Stage 4     |
| Second critical input              | <b>mRNA.</b> A transiently transfected process makes mRNA sourcing, quality and supply a starting-material question in its own right, not a consumable — see §5 and §15                                   |

## 5. Process flow

| Unit operation               | Your approach                                                | MADE capability                                                                                                                                                                                                         | Read                                          |
|------------------------------|--------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|
| Master and working cell bank | Placenta-derived MSC bank, held                              | Cell bank qualification or GMP re-banking, depending on the bank's documented status                                                                                                                                    | Fits; the scope depends on the bank — see §15 |
| Producer expansion           | <b>Not established</b> — current laboratory scale not shared | PBS Biotech vertical-wheel, Sartorius Biostat STR, Eppendorf BioFlo 320, Corning CellSTACK and Terumo BCT Quantum Flex are publicly named platform options. At intradiscal dose volumes the scale requirement is modest | Fits                                          |

| Unit operation                       | Your approach                                                                  | MADE capability                                                                                                                                                                                                                                                               | Read                                                                                    |
|--------------------------------------|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| mRNA transfection                    | mRNA engineering, moved off viral vector                                       | Transfection method not yet described. Electroporation platforms are in service, and a reagent-based method transfers as a materials and process step rather than as equipment — see §6                                                                                       | Fits either way; method needs defining                                                  |
| Conditioning and EV production phase | <b>Not established</b> — duration, cell density and harvest trigger not shared | Chemically defined EV collection media come into MADE GMP operations through the RoosterBio partnership, and your process already runs on RoosterBio media                                                                                                                    | Fits, and the producer-media comparability question is largely answered before we start |
| Conditioned media harvest            | Not established                                                                | Standard. Producer viability tracked through conditioning, because dying cells release apoptotic bodies and debris that inflate particle counts and dilute the therapeutic fraction                                                                                           | Fits                                                                                    |
| Clarification                        | Normal-flow filtration                                                         | MADE presented its normal-flow filtration to tangential-flow filtration to optional-chromatography train to your team on 12 August                                                                                                                                            | Fits                                                                                    |
| Concentration and diafiltration      | TFF                                                                            | EV purification by tangential flow filtration is a publicly stated MADE capability                                                                                                                                                                                            | Fits                                                                                    |
| Polishing                            | Optional                                                                       | Column chromatography is a publicly stated MADE capability — SEC or anion exchange                                                                                                                                                                                            | Available if needed                                                                     |
| Sterile filtration                   | <b>Not established</b>                                                         | Standard. The constraint is your size distribution: a 0.22 µm filter excludes anything above 220 nm, so filtration and the sterility assurance strategy are designed together, and where filtration is not viable, sterility is achieved by closed aseptic processing instead | Design question, see §15                                                                |
| Fill and cryostorage                 | <b>Not established</b>                                                         | Standard. Cryoprotectant choice is part of the product, not a process aid                                                                                                                                                                                                     | Fits; formulation to define                                                             |

**On the downstream train.** Two things about this program are unusually clean. MADE presented the normal-flow to tangential-flow to optional-chromatography purification train in the same 12 August session, so the architecture has already been shown to your team. And MADE's strategic partnership with RoosterBio, announced 5 May 2026, brings RoosterBio MSCs, xeno-free expansion media and chemically defined EV collection media into our GMP operations, together with EV purification by TFF and column chromatography — while your process already runs on RoosterBio media. The producer-media question, normally a comparability item in any EV transfer, is largely answered before we start.

**On the engineered producer cell.** MADE holds a lentivirus-engineered MSC bank and Wharton's jelly umbilical-cord MSC EV programs. Engineering the producer cell to change the cargo is a problem we have worked before, and the mRNA route removes the vector supply chain from it entirely.

## 6. Key equipment

| Instrument                                   | Your platform                           | MADE's installed position                                                                                                                                                                                                                 | Onboarding required                                                                                                                                                                    |
|----------------------------------------------|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Producer bioreactor                          | Not established                         | PBS Biotech vertical-wheel, 6 in service at Princeton (3 MAG and 15 MAG); Sartorius Ambr 15 for small-scale screening, 1 Princeton; Terumo Quantum Flex hollow fiber, 1 Princeton; Sartorius Biostat STR 50 L single-use, 1 East Norriton | Scale selection once current yield per run is known                                                                                                                                    |
| Transfection                                 | mRNA delivery, method not yet described | Lonza 4D-Nucleofector, 1 in service at Princeton; Thermo Neon, 1 Princeton and 1 East Norriton. If your method is reagent-based rather than electroporation, it transfers as materials and process parameters instead                     | Define the method in Stage 1; it is a process parameter set either way                                                                                                                 |
| TFF                                          | TFF concentration and diafiltration     | EV purification by tangential flow filtration is a publicly stated MADE capability. Repligen KrosFlo KR2i units are held at East Norriton in warehouse storage, so the specific skid is to confirm before this program is committed to it | Membrane chemistry and cutoff qualified per process                                                                                                                                    |
| Chromatography, if polishing is used         | Optional                                | Column chromatography is a publicly stated MADE capability                                                                                                                                                                                | Resin selection and lot qualification                                                                                                                                                  |
| Particle concentration and size distribution | Not established                         | Particle Metrix ZetaView Evolution quatt, 1 at Princeton, in service — nanoparticle tracking analysis                                                                                                                                     | Method qualification for your matrix. If your laboratory uses a different measurement principle, an equivalency bridge is the route — settle it early rather than during comparability |
| IL-1RA and GH1 quantitation                  | Immunoassay                             | Molecular Devices SpectraMax M3, 3 East Norriton; Thermo plate reader, 2; Thermo Varioskan Lux, 1 Princeton                                                                                                                               | <b>The critical path.</b> Assay development, a qualification plan and a reference standard strategy — see §7a                                                                          |
| Tetraspanin identity and residual-cell flow  | Multiplex or bead-based flow            | Beckman Coulter CytoFLEX 13-color, 4 in service at Princeton; Miltenyi MACSQuant, 6 East Norriton; Agilent NovoCyte Penton, 1 East Norriton                                                                                               | Panel and gating strategy                                                                                                                                                              |
| Residual mRNA and host-cell DNA              | qPCR / dPCR                             | Bio-Rad QX200 ddPCR, 1 Princeton and 4 East Norriton; Thermo QuantStudio, 1 Princeton and 2 East Norriton                                                                                                                                 | <b>Method development, not method transfer</b> — see §7                                                                                                                                |
| Endotoxin                                    | LAL                                     | Endosafe, 4 Princeton and 3 East Norriton                                                                                                                                                                                                 | EV-matrix interference resolved before release testing                                                                                                                                 |
| Mycoplasma                                   | PCR or culture                          | BioFire, 1 Princeton and 2 East Norriton                                                                                                                                                                                                  | Test producer cells and conditioned media                                                                                                                                              |
| Nucleic acid and protein quantitation        | Standard                                | NanoDrop, 1 Princeton and 3 East Norriton                                                                                                                                                                                                 | Standard                                                                                                                                                                               |

| Instrument                   | Your platform   | MADE's installed position                                 | Onboarding required                         |
|------------------------------|-----------------|-----------------------------------------------------------|---------------------------------------------|
| Cryogenic freeze             | Not established | Controlled-rate freezers, 2 Princeton and 7 East Norriton | Freeze profile defined with the formulation |
| Cryo-EM or TEM visualization | Not established | Outsourced, as is normal in this modality                 | Vendor selection if required                |

## 7. Analytical panel and release

Because dose is protein mass of two named proteins, **quantitative IL-1RA and GH1 immunoassays become the release-defining methods**. They gate everything else, so they belong at the front of the analytical workflow rather than in its middle, and they need a reference standard strategy from the start.

| Attribute                                               | Your method                              | MADE position                                                                                                                                                                                                               | In house or outsourced      |
|---------------------------------------------------------|------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| <b>Potency</b> — quantitative IL-1RA; quantitative GH1  | Immunoassay against a reference standard | Plate readers in service. Development and qualification is the critical path, and the reference standard strategy is part of it                                                                                             | In house                    |
| Identity — tetraspanins CD9, CD63, CD81                 | Multiplex or bead-based flow             | CytoFLEX and MACSQuant in service                                                                                                                                                                                           | In house                    |
| Identity — negative markers, calnexin and GM130         | Recommended addition                     | Demonstrates the absence of cellular contamination, which particle counting cannot detect. The control most often omitted                                                                                                   | In house                    |
| Quantity — particle concentration and size distribution | Nanoparticle tracking or equivalent      | ZetaView in service. The modality's most basic quantity attribute                                                                                                                                                           | In house                    |
| Purity — particle-to-protein ratio                      | Derived from the two above               | The most informative purity number in this modality                                                                                                                                                                         | In house, derived           |
| Residuals — <b>residual mRNA</b>                        | To be developed                          | <b>Specific to your program; a generic EV panel would miss it.</b> A transiently transfected producer cell carries mRNA that has to be shown cleared from the vesicle preparation. This is development work, not a transfer | In house, after development |
| Residuals — host-cell DNA and protein, residual cells   | qPCR, ELISA, flow                        | ddPCR, QuantStudio, plate readers and CytoFLEX in service                                                                                                                                                                   | In house                    |
| Safety — sterility                                      | USP <71>, direct inoculation             | The matrix and the vesicles foul membranes, so direct inoculation is the compatible method                                                                                                                                  | Outsourced, as standard     |
| Safety — endotoxin                                      | LAL                                      | Endosafe in service. EV matrices commonly interfere with LAL and the method needs bridging work — resolve it in Stage 2, not at release                                                                                     | In house, after method work |
| Safety — mycoplasma                                     | PCR or culture                           | BioFire in service. Test producer cells <b>and</b> conditioned media                                                                                                                                                        | In house                    |
| Safety — adventitious agents                            | Standard panel at the bank               | Standard                                                                                                                                                                                                                    | Outsourced, as standard     |

| Attribute                                                         | Your method            | MADE position                                                                                                                                                                                                           | In house or outsourced |
|-------------------------------------------------------------------|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| Compendial — appearance, pH, osmolality, sub-visible particulates | Standard               | Standard                                                                                                                                                                                                                | In house               |
| Reference EV preparation                                          | <b>Not established</b> | Recommended. With no viability and no cell count in this modality, a qualified reference preparation is often the only way to make lots comparable across time — and it is what the potency assay is calibrated against | In house               |

**One method needs a decision with your team.** Particle concentration and size distribution is the modality's most fundamental quantity attribute. We would want to agree whether to qualify it in our laboratory against your material or run it with a named partner laboratory at a stated turnaround — and we would rather settle that explicitly than leave it implied.

## 7a. Potency

**Potency here is not a search, it is a quantitation problem — and that is rare in this modality.** Because dose is expressed as protein mass of two named proteins, the potency assay is defined by the dose basis rather than discovered after it. Quantitative IL-1RA and GH1 immunoassays are the release-defining methods.

Three consequences follow. They gate everything, so they sit at the front of the analytical workstream. They need a reference standard strategy from the start, because there is no viability and no cell count in this modality to anchor lots against each other. And they make the whole program more fileable — which is precisely the judgment made when the dosing basis was chosen.

What remains open is not the choice of assay but its qualification: assay format, reference material, acceptance ranges, and whether an orthogonal bioactivity readout is added to support the mechanism claim alongside the quantitation. That is Stage 2 work and it is the reason Stage 2 starts early.

## 8. Fill, finish and presentation

Fill and finish is **not established** for this program, and at pre-IND that is the expected position rather than a gap. The entries below record what is decided, what is constrained, and what Stage 1 has to settle.

|                   |                                                                                                                                                                         |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Container closure | <b>Not established</b> — see §15                                                                                                                                        |
| Fill volume       | <b>Not established.</b> Intradiscal delivery is small volume at high concentration, so the fill is constrained by dose and by what the injection can accept             |
| Dose form         | <b>Not established.</b> Whether IL-1RA-EV and GH1-EV are co-formulated in one container or co-administered from two is the largest open scope question — see §3 and §15 |
| Dose basis        | Protein mass of IL-1RA and GH1. Protein mass per injection, injections per patient and Phase 1 cohort size are open, and together they set batch size — see §15         |
| Formulation       | <b>Not established.</b> Cryoprotectant choice is part of the product rather than a process aid, and it is a comparability item once fixed                               |

|                        |                                                                                                                                          |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Labeling               | <b>Not established</b> — Phase 1 clinical labeling defined in Stage 4                                                                    |
| Controlled-rate freeze | <b>Not established.</b> Controlled-rate freezers are in service at both MADE sites if the formulation needs one                          |
| Storage                | <b>Not established.</b> -80 °C is the common position in this modality; -20 °C and 2–8 °C are used by some programs with supporting data |

## 9. Stability, storage and shipping

|                                |                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Stability status               | <b>Not established.</b> No stability data has been shared, and at pre-IND a real-time study started at the intended storage condition is what Phase 1 requires rather than a completed program                                                                                                                                  |
| Storage condition              | <b>Not established</b> — see §8                                                                                                                                                                                                                                                                                                 |
| Stability-indicating methods   | Must cover particle count, size distribution, marker expression and potency. There is no viability in this modality, and a program tracking particle count alone will report a stable product that has lost function. Here potency means quantitative IL-1RA and GH1, which makes the stability program unusually interpretable |
| Freeze-thaw tolerance          | <b>Not established.</b> The number of permitted cycles has to be fixed explicitly and written into the instruction-for-use in a form a clinical site will follow — EVs can aggregate, fragment or lose function across cycles                                                                                                   |
| Shipper                        | <b>Not established</b> — see §15                                                                                                                                                                                                                                                                                                |
| Shipper qualification status   | <b>Not established.</b> Shipping qualification belongs in Stage 4, ahead of first clinical supply                                                                                                                                                                                                                               |
| Chain of custody, outbound leg | <b>Not established.</b> Intradiscal administration is a procedural setting, and site-side handling needs an instruction-for-use written for it                                                                                                                                                                                  |

## 10. Comparability

This is a laboratory-to-GMP transfer rather than a site change, so the applicable expectation is academic-origin transfer risk — the higher end of the standard matrix, with bridging lots and equivalence margins agreed on the critical quality attributes rather than a light-touch two-lot comparison. EV programs carry an additional burden that is worth naming early: **the producer cell process and the vesicle process both transfer, and both need comparability.**

**Two products, one platform, one comparability design.** IL-1RA-EV and GH1-EV share the producer platform and the downstream train, so the comparability protocol is written once and executed twice. That is the practical benefit of the two products being separate vesicles from a common process.

| Change to be demonstrated                        | Risk        | Against what, and how                                                                                                                   |
|--------------------------------------------------|-------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Producer expansion platform, flask to bioreactor | <b>High</b> | Inherits the full MSC 2D-to-3D question — the cells' mechanical environment affects what they secrete, and the product is the secretion |
| Producer donor, tissue source or passage         | <b>High</b> | Cargo composition changes with all three. Passage-resolved cargo comparison is the evidence                                             |

| Change to be demonstrated                         | Risk        | Against what, and how                                                                                                                      |
|---------------------------------------------------|-------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| Transfection step, laboratory to GMP scale        | High        | Transfection efficiency and the timing of EV harvest are process parameters that set expression, and expression is the dose                |
| Isolation and purification train                  | Medium      | Your process moves onto the NFF → TFF → optional-polishing architecture; impurity profile and subpopulation recovery compared head to head |
| Conditioning schedule, single or repeated harvest | Medium      | Later collections may differ in cargo from earlier ones                                                                                    |
| Formulation and cryoprotectant                    | Medium-High | Part of the product once fixed                                                                                                             |

**The CQA set the protocol has to cover.** Quantitative IL-1RA and GH1 as the critical attributes, because they are the dose; particle concentration and size distribution; tetraspanin marker profile; calnexin and GM130 absence; particle-to-protein ratio; residual mRNA; endotoxin; and sterility and mycoplasma on every lot. A retained reference preparation makes the comparison tractable, which is the second reason to establish one early.

## 11. Technology transfer approach

| Stage                                                       | What happens                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Gate                                                                                                   |
|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| <b>Stage 0</b> — technical assessment                       | This document, and the four questions at the head of §15 answered                                                                                                                                                                                                                                                                                                                                                                                                                             | Scope fixed on the answers to the four questions — particularly one IND or two                         |
| <b>Stage 1</b> — process definition and technology transfer | Process description workshop and gap assessment against your current laboratory process; raw material and mRNA supply strategy; single-use and closed-system design; upstream definition covering MSC expansion, transfection, the EV production phase and harvest trigger; downstream definition covering clarification, TFF concentration and diafiltration, optional polishing, sterile filtration, fill and cryostorage; draft process flow, bill of materials and an IND risk assessment | Process flow, bill of materials and risk assessment issued — written to be usable in the CMC narrative |
| <b>Stage 2</b> — analytical development                     | The IL-1RA and GH1 potency assays first, with a qualification plan and reference standard strategy, then identity, purity, residuals, safety and compendial. Endotoxin and sterility method work for the EV matrix starts here, not at release                                                                                                                                                                                                                                                | Potency assays qualified against a reference standard                                                  |
| <b>Stage 3</b> — 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 we have used elsewhere and would offer here explicitly                                                                                                                                                                                                              | Yield and potency reproducibility confirmed; convert-to-GMP decision taken                             |
| <b>Stage 4</b> — 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                                                                                                                                                                                                                                                                                                                              | Formal readiness assessment before GMP activity begins                                                 |

**On sequencing.** Your INTERACT submission goes in during September with a seventy-five-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. That is the reason to start small and start soon.

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

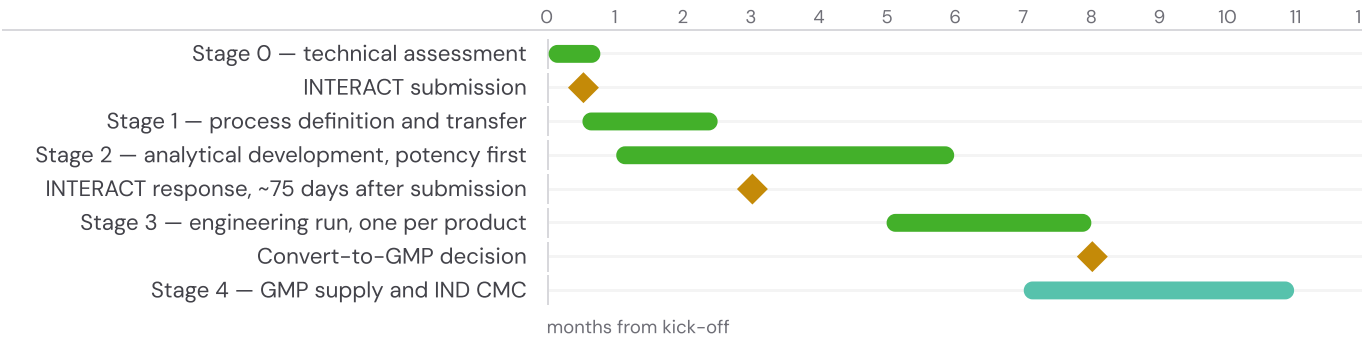
## 12. Indicative timeline

Stage durations below are indicative and are firmed up in Stage 1, once current scale, yield per run and cohort size are known. The totals are industry planning ranges, not a MADE commitment.

| Stage                                                | Duration                                      |
|------------------------------------------------------|-----------------------------------------------|
| Stage 0 — technical assessment                       | 2–4 weeks                                     |
| Stage 1 — process definition and technology transfer | 1–2 months                                    |
| Stage 2 — analytical development, potency first      | 3–5 months, starting in parallel with Stage 1 |
| Stage 3 — engineering run, one per product           | 2–3 months                                    |
| Stage 4 — GMP supply and IND CMC                     | 3–4 months                                    |
| <b>Total, platform-adapted</b>                       | <b>4–6 months</b> [industry range]            |
| <b>Add for academic-origin knowledge transfer</b>    | <b>2–4 months</b> [industry range]            |
| <b>Total, custom process with no platform</b>        | <b>12–18 months</b> [industry range]          |

The dosing basis is what removes the usual potency-development modifier from the critical path. In most EV programs a potency assay has to be invented before it can be qualified, and that is where the extra months go; here the assay is defined by the dose and only needs building and qualifying.

**Against your regulatory clock:**



The two gates that matter are placed where they actually fall. Stages 1 and 2 straddle the INTERACT response deliberately, so the agency's feedback lands on work in progress rather than on work not yet begun.

## 13. MADE experience that supports this

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**Modality.** The RoosterBio strategic technology and supply partnership, announced 5 May 2026, covers scalable MSC and extracellular vesicle manufacturing — off-the-shelf MSCs, xeno-free expansion media, chemically defined EV collection media, and EV purification by tangential flow filtration and column chromatography. 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. On the engineered producer cell specifically, MADE holds a lentivirus-engineered MSC bank and Wharton's jelly umbilical-cord MSC EV programs — changing the producer cell to change the cargo is not a new problem here.

**Analytical bench.** Plate readers, NanoDrop, flow cytometry, ddPCR, dPCR, endotoxin, mycoplasma and particle sizing, all in service across the two sites as set out in §6, with QA-audited external laboratories for what is better placed outside.

**Sites.** Princeton, NJ, available now. Grade B background with Grade A biological safety cabinets for open manipulations; EV downstream processing is largely closed and runs as Grade B / Closed System.

**Quality and regulatory.** Collective experience from prior organizations across cell therapy quality systems, method qualification, comparability and regulatory CMC, including forty-plus products through FDA INDs. This is experience held by individuals now at MADE; the company itself has not yet completed a client audit, and we say so wherever this is described.

## 14. Guidance and suggestions

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**Start Stage 1 small and start it before the INTERACT response.** The submission goes in during September with a seventy-five-day window. A process flow, a bill of materials and an IND risk assessment in your hands before the agency responds is worth more than the same documents afterwards, because the response can then be answered rather than absorbed.

**Settle one IND or two before anything else is scoped.** Co-formulated and co-administered are different products with different release panels, different stability programs and possibly different filings. Every downstream estimate moves with that answer.

**Establish a reference standard strategy alongside the potency assays, not after them.** The assays are quantitative against a reference; without a qualified reference preparation the numbers are not comparable across time, and this modality has no viability or cell count to fall back on.

**Plan residual mRNA as development work.** It is specific to a transiently transfected producer cell, a generic EV panel would miss it, and there is no method to transfer — only one to build.

**Raise the mRNA position explicitly at INTERACT.** Non-integrating and transient is a materially better regulatory position than a vector-engineered product, and it is worth stating rather than leaving a reviewer to infer it.

**Add negative markers to the panel.** Calnexin and GM130 detect cellular contamination that particle counting and tetraspanin presence cannot — a small addition with real assurance value.

## 15. Open questions

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**The four that set the scope, in this order.**

1. **What does the INTERACT letter ask the agency?** It names the CMC gaps already known, and it is the cheapest possible scoping input.

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

1. **Which cell source for the EV process** — the placenta master cell bank, or another line? What is its qualification status and documentation? This decides whether Stage 4 is bank qualification or re-banking, which are materially different pieces of work.

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

**Process.** What is the current scale and yield per run in your laboratory today? What transfection method and mRNA source are used, and what is the mRNA supply route at GMP scale? What is the conditioning duration, cell density and harvest trigger? Single or repeated media collection? Is producer viability monitored through conditioning? Is there a sterile filtration step, and how does it sit against your EV size distribution?

**Analytics.** Which assays already exist, and in whose hands? Is there a qualified reference preparation for IL-1RA and GH1 quantitation, or does one need creating? What particle-sizing method does your laboratory use today? Has endotoxin interference in the EV matrix been assessed?

**Formulation and presentation.** What formulation and cryoprotectant are intended? What container closure and fill volume? What storage condition, and is there any stability data at it yet? How many freeze-thaw cycles are permitted?

**Regulatory.** Has the product's classification been confirmed with the agency? Are any designations held? Has the non-integrating, transient position of mRNA engineering been raised explicitly?

**Program.** What is the target IND date, and what funds the work?

## 16. Suggested next step

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A short scoping call — an hour would do it — on the four questions at the head of §15. If they resolve as we expect, a small fixed-scope Stage 1 could be defined inside a week and started on a purchase order rather than waiting on a master agreement, which puts the work in motion before your INTERACT response lands rather than after.