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TECHNICAL WALKTHROUGH · PIVOTAL PROCESS WORKSHOP, 28 SEPTEMBER 2026

RTX-001 Ruby (CP-2) Pivotal Process Process Flow and Analytical Methods, Step by Step

The Resolution Therapeutics CP-2 process flow as the basis, the eight proposed changes marked where they land, the in-process controls and release panel, and where Made Scientific proposes to develop, qualify and control each unit operation

Program	RTX-001, autologous IL-10 and MMP-9 mRNA-engineered macrophages for decompensated cirrhosis; cryopreserved drug product; one leukapheresis processed to multiple doses for one patient
Prepared for	Resolution Therapeutics
Prepared by	Made Scientific, Inc. · Version 01 · 27 September 2026
Process basis	Resolution Therapeutics, US CDMO pivotal process: detailed process flow diagram and narrative for CP-2 (3 to 4 September 2026) and the workshop briefing pack (September 2026), both shared under the confidentiality agreement; all process figures are quoted as shared and remain subject to Resolution's final data review before process lock in December 2026
Analytical basis	The CP-2 in-process sampling points and release panel as shared; the MATCH GMP process publication (Fraser et al., Cytotherapy 2017) and the Resolution patent WO2024074376A1 for published precedent; Made Scientific's analytical transfer, comparability and QC positions prepared for the workshop
Companion to	Development Work Packages, DOE Plans, Version 01 (D-1 to D-8) · the workshop deck · the live capacity model
Scope	Technical content only. No commercial terms are stated in this document.

 Step references in the form "D4.3" point to the numbered steps of the CP-2 narrative as modeled by Made Scientific; "C1" to "C8" to the eight proposed changes in the briefing pack; "D-n" to the one-page plans of the Development Work Packages; "Q1" to "Q4" to the questions Resolution posed for the workshop.

How to read this document

The Ruby process is a single manufacturing attempt on one leukapheresis: a Day 0 selection and seeding session, four days of differentiation in the incubator, and a Day 4 session that now carries harvest, electroporation, a short recovery, formulation, fill and controlled-rate freezing into Day 5. This walkthrough follows the unit operations in order. For each one it gives, on one card, what the CP-2 narrative prescribes, the time Resolution has stated for the step and, where Made Scientific has modeled a different working figure, that estimate clearly labeled as an estimate, the grade, equipment and operators, the samples drawn and the tests they feed, the Made Scientific position on how the step is transferred, developed or controlled, and the questions we would like to settle on the day.

Part 4 then takes the analytical program on its own: the in-process controls by unit operation, the release panel with turnaround and the critical path, extended characterization and the potency candidates, the hold points and stability gaps, rapid safety methods, the reference-standard hierarchy, the QC-vial philosophy, the instrument bridges and the transfer sequence.

TEST ROLES USED THROUGHOUT IN-PROCESS RELEASE IN ARREARS CHARACTERIZATION C1 to C8 the eight proposed changes; amber cards are changed steps

Contents

What is fixed and what is open. The CP-2 narrative and briefing pack are the process basis; every duration labeled "RTx" is Resolution's stated figure. Every duration labeled "MADE estimate" is a working figure from Made Scientific's Day 4 scheduling model and is to be confirmed by the timed Day 4 rehearsal and the training runs; it is not a fact about the process. Every Made Scientific position is a proposal for discussion. No specification, acceptance limit, IPC action limit or sample volume has been shared, so none is stated as such; where a limit appears it is the published MATCH precedent or the patent target and is labeled.

1. The process in one page

One leukapheresis, one patient, one manufacturing attempt, multiple cryopreserved doses. Day 0 about 12 h (RTx); Days 1 to 4 in the incubator; Day 4 into 5 carries everything from harvest to freeze. Sampling points from the CP-2 narrative. Amber = a changed step; the tag names the change.

RECORD · CONTROL	UNIT OPERATION · CLOCK · GRADE	SAMPLES AND TESTS AT THIS STEP
Process buffer, DS formulation buffer, electroporation buffer, base culture medium, recovery medium; mRNA vial thawed and transferred to a weldable storage pouch with tubing left for welding (new in CP-2)	**1 Buffer and reagent preparation** C9 Day -15 to -1 · RTx about 3 h · Grade A (ISO 5) · Class A MSC, peristaltic pump, balance, welder, sealer	mRNA pouch integrity and concentration (proposed) IN-PROCESS
Receipt within 48 h of collection; apheresis Tuesday and Wednesday for the pivotal, arrival next afternoon; Day 0 start within 24 h of receipt; Prodigy input envelope (MATCH precedent) at or below 20 × 10^9 WBC, below 400 × 10^6 WBC/mL, at or below 3.5 × 10^9 CD14, 50 to 300 mL	**2 Day 0 set-up and starting-material assessment** D0.1 to D0.2 · RTx 0.5 h + 0.5 h · Grade C · Prodigy, NC-202, flow	CD45+ count (NC-202); %CD14+ (BD FACSLyric) IN-PROCESS
Loading and priming (approximate split); CD14 microbead selection and wash on the Prodigy; cirrhotic donors commonly above 30% monocytes, so the target-cell load is checked against the column capacity	**3 CD14+ enrichment** D0.3 to D0.4 · RTx 3.33 h prep + 6.5 h run · Grade C · Prodigy	CD45+ count post-enrichment; %CD14+ purity IN-PROCESS · MATCH precedent: yield at least 40% of input CD14+
Custom application (CAP) from Miltenyi; limited bag number; filtered air required; replaces up to 18 to 20 MACS500 bags filled by CellConnect or Cue; bags to the CO_2 incubator	**4 Seeding into up to 10 MACS1000 bags via the Prodigy formulation bar** C1 C2 C3 D0.5 · RTx about 1 h (TBC) · Grade C · Prodigy formulation bar, welder, balance	Cell count end of Day 0 (NC-202) IN-PROCESS
Serum-free medium plus M-CSF (MATCH precedent TexMACS + 100 ng/mL M-CSF, 50% feeds on days 2 and 4); feed schedule for the 4-day culture not stated in CP-2; incubators per batch 1 to 1.5, measured on the day	**5 Monocyte-to-macrophage differentiation** C4 Days 1 to 4 · 96 h · Grade C incubator	None stated by RTx · proposed D2 to D3 conversion-efficiency read (count, 25F9/CD206) IN-PROCESS
Set-up 0.33 h; harvest and pooling of up to 10 bags by gravity and welder or peristaltic pump; the 7 h harvest-and-wash figure was MACS500 time, expected less with MACS1000 (S. Howe)	**6 Day 4 harvest and pooling** C3 D4.1 to D4.2 · RTx 0.33 h + 2.75 h · MADE estimate 1.75 h · Grade C · BSC, welder · 2 operators	Cell count start of Day 4 (NC-202) IN-PROCESS
Automated, continuous wash and buffer exchange into electroporation buffer; the wide range likely reflects variable cell loads (RTx); model hold before the step at most 0.5 h	**7 Rotea wash and buffer exchange** D4.3 · RTx 4.25 h · MADE estimate 1.25 h · Grade C · Rotea · 1 operator	Cell count after buffer exchange (NC-202) IN-PROCESS
Adjust to target concentration (patent: 50 to 100 × 10^6/mL small scale, 200 × 10^6/mL large-scale fluidics); load cartridge; calculate mRNA volume for the mRNA:cell ratio; one RTx table says Grade B, the other Grade C	**8 Transfection set-up** D4.4 · RTx 1.5 h · Grade C, BSC steps in Grade B · balance, welder, NC-202 · 2 operators	Cell count before transfection (NC-202) IN-PROCESS
Continuous-flow closed electroporation; about 5 min run plus about 5 min close-out inside a 1.75 h transfection block; replaces the Lonza 4D-Nucleofector LV; program and operating range to be finalized by RTx with Kytopen before transfer	**9 Electroporation** C5 D4.5 · RTx 0.17 h · MADE estimate 0.75 h · Grade C · Kytopen Flowfect · 2 operators	Post-transfection cell count (NC-202) IN-PROCESS
Recovery medium added, transfer to up to 4 MACS bags (RTx PFD shows about 3 MACS500), bags to the incubator; window 2 to 6 h, target about 4 h (TBC); T0 = final Flowfect step complete	**10 Recovery medium addition and recovery culture** C6 D4.6 to D4.7 · RTx 1.17 h + about 4 h (TBC) · Grade C incubator · 2 then 0.5 operators	Proposed end-of-recovery viability and stress read (NC-202; Annexin V/DRAQ7) IN-PROCESS CHARACTERIZATION
Set-up 0.5 h; harvest and pooling into the harvest bag 1 h	**11 Second harvest and pooling** D4.8 to D4.9 · RTx 0.5 h + 1 h · Grade C · BSC · 2 operators	Post-harvest count (NC-202) IN-PROCESS

Wash and concentrate into saline (intermediate bag), much shorter than the first Rotea (RTx); then wait for the stained TruCount; dose setting is performed on drug substance	**12 Rotea wash and concentrate; TruCount** D4.10 to D4.11 · RTx 0.33 h + 1 h · MADE estimate 1.25 h · Grade C · Rotea, FACSLyric · 1 operator	Post-Rotea count (NC-202); TruCount viable macrophage enumeration (FACSLyric) gates the fill **IN-PROCESS**
DS formulation (dilute in DS formulation buffer), then FDP formulation (CryoStor CS10 addition); "TBC for Ares" (RTx); QC samples for release and characterization are taken here	**13 DS then FDP formulation** **C1 C7** D4.12 · RTx 2.5 h (TBC) · MADE estimate 1.25 h with fill · Grade C · Ares X20 · 2 operators	Release and characterization samples **RELEASE** **CHARACTERIZATION** · cell count results
Container prep and Ares set-up 0.25 h; bag fill, bags 1 to 20, 0.33 h; QC vial fill 0.08 h; DP 10 mL in CryoMACS 50 at 1 × 10^7 cells/mL, 4 to 9 bags, about 20 × 1 mL vials; "opportunity for concurrent VI?"	**14 Fill: dose bags and QC vials** **C7** D4.13 · RTx 0.67 h · Grade C · Ares X20, heat sealer · 2 operators	Fill weight and count first vs last (proposed) **IN-PROCESS**
Post-fill visual inspection, label, overwrap, vacuum seal, metal cassette, temporary fridge	**15 Inspection, label, overwrap and cassette** **C8** D4.14 · RTx 0.25 h · MADE estimate 0.75 h · Grade C · VI light box, vacuum sealer · 2 operators	Visual inspection USP <790> **RELEASE**
MSC close; transfer to the controlled-rate freezer; CRF run; transfer to vapor-phase LN_2; T4 = CRF cycle initiated; mapping and cassette work not yet started by RTx	**16 Controlled-rate freeze and storage** **C8** D4.15 · RTx 0.33 h · MADE estimate 1.0 h · Grade C · CryoMed LN_2 CRF, metal cassette · 1 operator	Chamber and product-simulating probe profile **IN-PROCESS** · release testing on QC vials from step 13: identity, dose, transgene, IL-10/MMP-9, MMP-9 activity, appearance, sterility (7 d), mycoplasma, endotoxin **RELEASE**

C9 (mRNA supply MS-1 to MS-2) is a starting-material change, not a process change; it lands at step 1. APS = the aseptic process simulation covers the open manipulations of steps 2, 6, 8, 11, 13 and 14.

2. The clocks that govern the process

The clocks that govern the Ruby process. Two are set by the starting material and the collection network, one by the culture, one is the new window CP-2 introduces, and one is the reason release takes a week after the freeze. The Day 4 elapsed time is the open question and is treated below as a range, not a figure.

Clock	Starts	Ends	Limit and where it is written
Collection to receipt	End of leukapheresis at the collection site	Receipt at Made Scientific	**Within 48 h** of collection (RTx); apheresis Tuesday and Wednesday for the pivotal, arrival next afternoon; the published MATCH product used 2.5 blood volumes on a Spectra Optia MNC program. The collection network is contracted separately by Resolution (Blood Centers of America, September 2026).
Receipt to Day 0 start	Receipt	Prodigy set-up	**Within 24 h** of receipt (RTx); starting-material assessment (CD45+, %CD14+) and volume adjustment into the Prodigy input envelope precede the run.
Culture	Bags into the incubator at the end of Day 0	Day 4 harvest set-up	**96 h** (4 days, was 5; change C4). Feed schedule for the 4-day culture is not stated in CP-2; MATCH fed at days 2 and 4 in a 7-day culture.
Post-electroporation window (new)	T0, final Flowfect step complete	T4, CRF cycle initiated	Recovery **about 4 h** (RTx target, TBC), window **2 to 6 h**; inside it sit recovery, the second harvest, Rotea, formulation, fill, inspection and cassette. Made Scientific's proposed definition of T0 to T4; Resolution to confirm the actual start and end points (change C6; D-3).
Release	QC samples at DS/FDP formulation (step 13)	Sterility result	**7 days** for BacT/ALERT sterility, the longest release test; identity, dose, transgene and ELLA results in 1 to 3 days. Because the product is cryopreserved, release gates shipment to the site, not infusion timing.
Process lock	Now	**December 2026**	All eight changes are subject to Resolution's final data review before process lock; analytical transfer is planned from January 2027 and process transfer from April 2027, with transfer complete by the end of 2027.

Day 4 elapsed time: four figures, one rehearsal

The single-batch Day 4 (harvest set-up to CRF start) is the number the staffing model turns on, and it is not yet measured. The figures in circulation are listed with their basis so that none is mistaken for a fact. The Made Scientific model uses shorter step times than the CP-2 narrative for harvest, the first Rotea and formulation with fill, on the expectation that the MACS1000 format and the Ares X20 shorten them; that expectation is what the rehearsal and the training runs will test.

Source	Single-batch Day 4	Basis
Resolution CP-2 process flow diagram labels	**about 20 h**	Day 4 about 14 h to end of recovery plus Day 4-into-5 about 6 h, as labeled on the RTx diagram
Resolution CP-2 narrative, step times added sequentially	**about 21 h**	About 10 h to the end of recovery transfer, up to 4 h recovery, about 6.9 h final harvest to freeze
Made Scientific hour-by-hour occupancy map	**17 h**	Rotea, Flowfect, recovery, Rotea, Ares drawn hour by hour; a planning map, no formulas
Made Scientific Day 4 scheduling model	**12.0 h** (14.5 operator-hours)	Compressed step times (harvest 1.75 h, first Rotea 1.25 h, formulation and fill 1.25 h) with a 0.5 h maximum hold between steps; **a working estimate**
Made Scientific rehearsal target	**11.5 to 14 h**	To be confirmed by the timed Day 4 rehearsal on 28 September 2026 and the training runs

> **What every figure agrees on.** Day 4 exceeds one 12-hour shift under every estimate, so the Day 4 session is staffed as a standing second and third shift. The two levers that shorten the equipment day without shortening the biology are staggering the harvest starts by 4 to 6 h (the recovery hold is the natural offset, so one Rotea, one electroporator and one Ares can serve two batches) and defining a hold window at every hand-off so a queue is a recorded window rather than a deviation.

Where the suite time goes: Day 0 about 12 h (RTx), Prodigy-bound, one leukapheresis per instrument per day; Days 1 to 4 are incubator time with no stated sampling; Day 4 into 5 is 11.5 to 21 h depending on the source above; QC runs the TruCount before the fill and the release panel from the step-13 samples. Operator loading per step is given on each step card; the scheduling model puts a single batch at 14.5 operator-hours on Day 4 (estimate).

3. Unit operations, step by step

1 Buffer and reagent preparation, mRNA pouch transfer

Day -15 to -1 · RTx about 3 h · Grade A (ISO 5) · Class A MSC C9

What happens

1. Process buffer, DS formulation buffer, electroporation buffer, base culture medium and recovery medium are prepared with the peristaltic pump, balance, tube welder and heat sealer in a Class A safety cabinet.
2. New in CP-2: the RTX001_01 mRNA vial is thawed and its contents transferred to a weldable storage pouch, leaving enough tubing for future welding, so the Day 4 transfection set-up is a closed weld rather than an open transfer.
3. The mRNA is Resolution-supplied; the pivotal supply moves from the MS-1 (EU) provider to the MS-2 provider under Resolution's comparability Studies 1 and 2.

Controls and records

Item	Requirement
Time	RTx about 3 h; no MADE estimate differs
Grade	A (ISO 5), Class A MSC
Materials	Critical consumables and mRNA per the key terms; medium must be antibiotic-free for growth-based sterility to be valid (MATCH precedent)
Hold	mRNA pouch hold to a maximum pre-use time, to be set (hold-point table, 4.4)

Samples and tests

None stated. Proposed: pouch integrity and concentration read after transfer IN-PROCESS.

Made Scientific position. Prepared in the Grade A media-preparation area on the process-buffer schedule; the pouch transfer is added to the aseptic process simulation. The MS-1 to MS-2 change is a starting-material change that Resolution owns; Made Scientific asks whether MS-1 lots can be supplied for the pilot and engineering runs so the site-transfer data are not confounded by an mRNA change (D-8).

For discussion. When does MS-2 material exist at clinical scale, and is the nucleoside modification unchanged? What is the maximum pouch hold before use?

2 Day 0 set-up and starting-material assessment

D0.1 to D0.2 · RTx 0.5 h + 0.5 h · Grade C · Prodigy, NC-202, BD FACSLyric · 1 operator

What happens

1. The leukapheresis product is received within 48 h of collection (Tuesday and Wednesday collections for the pivotal, arrival the next afternoon) and Day 0 starts within 24 h of receipt.
2. The Prodigy is set up with the CD14 selection tubing set and reagents.
3. The cellular starting material is assessed: CD45+ count on the NC-202 and %CD14+ by flow cytometry, and the product is volume-adjusted into the Prodigy input envelope.

Controls and records

Item	Requirement
Receipt clock	Within 48 h of collection; Day 0 within 24 h of receipt (RTx)
Input envelope	MATCH precedent: at or below 20×10^9 WBC, below 400×10^6 WBC/mL, at or below 3.5×10^9 CD14, 50 to 300 mL; RTx limits to be shared
Donor note	Cirrhotic donors commonly above 30% monocytes, so CD14 load can approach column capacity; the EMERALD eligibility text refers to filgrastim, and mobilization use is to be confirmed

Samples and tests

Sample	Test	Role
Leukapheresis product	CD45+ count (NC-202); %CD14+ (FACSLyric)	IN-PROCESS

Made Scientific position. Receipt, identity and chain-of-custody at the dock, with the flow-cytometry IPC in the QC laboratory and cell counts in-suite as discussed in June. The FACSLyric is a procurement item for RTX-001 and will be installed and qualified before the January 2027 analytical transfer; until then the qualified CytoFlex is used for feasibility only, with a paired bridge to the FACSLyric once it is live (4.8). Day 0 is Prodigy-bound at about 12 h, so one Prodigy supports one start per day; the capacity model treats Prodigy count as the first constraint.

For discussion. Is filgrastim mobilization used in the collection protocol? What are the RTx input limits and the action if the CD14 load exceeds them (split, or cap)?

3 CD14+ enrichment on the CliniMACS Prodigy

D0.3 to D0.4 · RTx 3.33 h prep + 6.5 h run (split approximate) · Grade C · Prodigy · 1 then 0.5 operator

What happens

1. Pre-enrichment preparation: loading and priming of the tubing set and the product.
2. CD14 microbead selection and wash on the Prodigy (MATCH precedent: CliniMACS CD14 microbeads, TS510 tubing set, LP-14 program).
3. The positive fraction is counted before seeding.

Controls and records

Item	Requirement
Time	RTx about 10 h for prep plus run; the sub-step split is approximate
Purity precedent	MATCH: about 20.5% monocytes pre-sort to 95.9% in the positive fraction; yield criterion at least 40% of input CD14+; selection performance was highly reproducible across donations
Utilities	Electrical and gas per Prodigy; the East Norriton ballroom concept places one Prodigy per workstation

Samples and tests

Sample	Test	Role
Positive fraction	CD45+ count post-enrichment; %CD14+ purity	IN-PROCESS

Made Scientific position. No concerns on the Prodigy itself: the platform, utilities and operator training are routine at Made Scientific. The step is transferred as written; the point of attention is the input envelope for high-monocyte cirrhotic donors, which sets whether one leukapheresis can ever need two selection runs.

For discussion. Has Resolution seen CD14 loads above the column capacity in EMERALD, and what was done?

4 Seeding into up to 10 MACS1000 bags via the Prodigy formulation bar

D0.5 · RTx about 1 h (TBC) · Grade C · formulation bar, NC-202, welder, balance, CO_2 incubator · 2 operators **C1 C2 C3**

What happens

1. Monocytes are seeded from the Prodigy through the formulation bar into up to 10 MACS1000 differentiation bags, replacing up to 18 to 20 MACS500 bags filled by CellConnect or Cue (changes C1, C2, C3).
2. A custom application (CAP) from Miltenyi is required; the bag number per run is limited and filtered air is required over the bar.
3. Bags are moved to the CO_2 incubator. Seeding density precedent: 2×10^6 monocytes per cm^2 and per mL (MATCH; RTx patent).

Controls and records

Item	Requirement
Time	RTx about 1 h, TBC; Day 0 hands-on about 11.5 h including Prodigy monitoring
Bag count	Up to 10 MACS1000; incubators per batch 1 to 1.5, measured on 28 September
CAP	Bag limit per run and the filtered-air requirement to be stated

Samples and tests

Sample	Test	Role
Post-seeding pool	Cell count end of Day 0 (NC-202)	IN-PROCESS

Made Scientific position. Supportive: removing the Cue removes the most equipment-intensive item of the earlier commercial model and the formulation bar keeps Day 0 closed. Made Scientific has limited direct experience with the formulation bar and asks for the CAP and intended workflow from Resolution and Miltenyi to assess bag capacity, tubing, fill accuracy, filtered air, footprint and qualification. Work Package D-5 measures the shelf fit at working volume, the fill accuracy per bag, the bag-position effect and the harvest recovery of the larger bag; the capacity model carries bags per shelf and shelves per unit as inputs and defaults to the conservative 1.5 incubators per batch until measured.

For discussion. What is the CAP's bag limit per run and the filtered-air requirement? MACS1000 dimensions and working volume? Can the bar fill in parallel with the enrichment wash?

5 Monocyte-to-macrophage differentiation, four days

Days 1 to 4 · 96 h · Grade C CO_2 incubator **C4**

What happens

1. Bags rest in the incubator for four days (was five; change C4) in serum-free medium with M-CSF (MATCH precedent: TexMACS plus 100 ng/mL M-CSF with 50% feeds on days 2 and 4 in a 7-day culture).
2. The feed schedule for the 4-day culture is not stated in the CP-2 narrative.
3. Resolution's rationale is a plateau in differentiation markers with omics work ongoing at scale; Resolution itself classes shorter maturation as a major change.

Controls and records

Item	Requirement
Culture clock	96 h from seeding to harvest set-up
Incubator	Occupancy per batch 1 to 1.5 units; gas supply per incubator
Identity precedent	MATCH release: 25F9 and CD206 MFI at least 5-fold day-0 monocytes; CD45+/CD14+; viability above 80%
Variance	CD14-to-macrophage conversion efficiency is the variable term (MATCH phase 2), not the selection

Samples and tests

None stated by Resolution for Days 1 to 4. Proposed: a D2 to D3 conversion-efficiency read (count and 25F9/CD206 trajectory) as a for-information sample on the pilot and engineering runs IN-PROCESS.

Made Scientific position. Low implementation risk and favorable for throughput; Resolution must confirm that the Day 4 product maintains phenotype, function and CQAs. The analytical question is whether the plateau starts at D4 or D5, which needs a D3 to D6 time course on at least 3 donors with post-electroporation samples compared at matched recovery time (D-4). Expected direction at D4: CD163, CD206, CD16 and 25F9 lower; CCR2-positive monocytes and lymphoid carry-over higher. A D2 to D3 conversion read would let the schedule allocate doses, fill positions and freezer loads two days before harvest.

For discussion. Does the plateau start at D4 or D5 in the at-scale data, and on how many donors? Is there a D2 feed in the 4-day culture, and at what M-CSF concentration?

6 Day 4 harvest set-up, harvest and pooling

D4.1 to D4.2 · RTx 0.33 h + 2.75 h · MADE estimate 1.75 h · Grade C · BSC, welder, NC-202 · 2 operators C3

What happens

1. Harvest set-up in the safety cabinet.
2. Macrophages are harvested from up to 10 MACS1000 bags and pooled by gravity and welder, or by peristaltic pump if preferred to manual pooling.
3. Resolution's note on the 7 h harvest-and-wash figure: it was MACS500 time and is expected to be less with MACS1000; Resolution is testing more robust harvest methods before the Rotea.

Controls and records

Item	Requirement
Time	RTx 0.33 h + 2.75 h; MADE working estimate 1.75 h for harvest (to be confirmed at the rehearsal)
Adherence	Serum-free culture makes macrophages less adherent and improves harvest recovery (MATCH)
Operators	2 (scheduling model)

Samples and tests

Sample	Test	Role
Pooled harvest	Cell count start of Day 4 (NC-202)	IN-PROCESS

Made Scientific position. Day 4 is the day the eight changes concentrate: harvest, Rotea, electroporation, recovery, a second harvest and Rotea, formulation, fill, inspection and freeze now sit in one session that exceeds a 12-hour shift under every estimate. Made Scientific staffs it as a standing second and third shift and proposes two waves of harvest starts offset by 4 to 6 h so the shared Day 4 equipment serves two batches. The harvest time itself is set by the training runs; per-bag counts at harvest come free with D-5.

For discussion. Which harvest method is Resolution converging on, and what recovery does it give from a MACS1000 bag?

7 Rotea wash and buffer exchange into electroporation buffer

D4.3 · RTx 4.25 h · MADE estimate 1.25 h · Grade C · Rotea · 1 operator · hold before at most 0.5 h (model)

What happens

1. Automated, continuous wash and buffer exchange of the pooled macrophages into electroporation buffer on the Rotea.
2. Resolution notes the wide time range likely reflects variable cell loads.
3. A count after buffer exchange confirms the cell load before transfection.

Controls and records

Item	Requirement
Time	RTx 4.25 h; MADE working estimate 1.25 h (to be confirmed)
Hold	Pre-electroporation hold in EP buffer is a hold point with no data (4.4)
Equipment	The Rotea is used twice per batch on Day 4 and is the queue in the scheduling model

Samples and tests

Sample	Test	Role
Post-Rotea suspension	Cell count after buffer exchange (NC-202)	IN-PROCESS

Made Scientific position. Transferred as written. Because the Rotea runs twice per batch with the electroporation and the recovery hold between the two runs, Made Scientific sizes workstations with two Rotea per Prodigy in the model default and shows the sensitivity live; a second Rotea is cheaper than a missed hold. The longest Day 4 queue before electroporation is the design point for the pre-EP hold study.

For discussion. What cell-load range drives the 4.25 h figure, and what is the longest pre-electroporation hold Resolution has data for?

8 Transfection set-up: cell, mRNA and buffer preparation

D4.4 · RTx 1.5 h · Grade C with BSC steps in Grade B · balance, welder, NC-202 · 2 operators

What happens

1. The cell suspension is adjusted to the target concentration (patent: 50 to 100 × 10^6/mL at small and medium scale, 200 × 10^6/mL for large-scale fluidics).
2. The Flowfect cartridge is loaded; the mRNA volume is calculated for the mRNA:cell ratio (patent: 8 µg per 10^6 cells for the IL-10-MMP9 construct) and welded in from the storage pouch.
3. The two RTx unit tables disagree on grade for this step (B in one, C in the other); syringe volume adjustments were requested in a Grade B safety cabinet in June.

Controls and records

Item	Requirement
Time	RTx 1.5 h inside a 1.75 h transfection block
Grade	To be confirmed by Resolution: Grade B for the open syringe steps, Grade C otherwise
Concentration	Cell count before transfection sets the concentration and the mRNA volume; a gating IPC if the Flowfect range is narrow (D-6)

Samples and tests

Sample	Test	Role
Pre-EP suspension	Cell count before transfection (NC-202)	IN-PROCESS

Made Scientific position. Open manipulations in a Grade B safety cabinet within the Grade C suite, matching the June request, with the step covered by the aseptic process simulation. The cell-count-before-transfection IPC becomes a gating control with an action limit if D-6 shows the Flowfect operating range is narrower than the run-to-run variation in Day 4 cell concentration.

For discussion. Which grade table is current? Is the mRNA:cell ratio fixed, or does it scale with the post-Rotea count?

9 Electroporation on the Kytopen Flowfect

D4.5 · RTx 0.17 h (about 5 min run + 5 min close-out) · MADE estimate 0.75 h · Grade C · Flowfect · 2 operators C5

What happens

1. Continuous-flow, closed, bag-compatible electroporation replaces the Lonza 4D-Nucleofector LV (change C5, "to be confirmed").
2. Resolution reports the concept demonstrated with optimization pending; the program and operating range are to be finalized with Kytopen before transfer.
3. Published context: Kytopen reported over 90% delivery and over 90% viability in monocyte-derived macrophages for an unnamed customer; peer-reviewed mRNA electroporation of primary myeloid cells ranges from 45 to 72% to platform claims of 80 to 99%; the RTx patent names Lonza and CliniMACS electroporators, so the device change is itself a comparability event.

Controls and records

Item	Requirement
Time	RTx 0.17 h; MADE working estimate 0.75 h including cartridge handling (to be confirmed)
Equipment	Flowfect is a procurement item for RTX-001; installed and qualified before the process transfer; 10-week order-to-install lead time
Hold	At most 0.5 h before the step (model)

Samples and tests

Sample	Test	Role
Post-EP suspension	Post-transfection cell count (NC-202)	IN-PROCESS

Made Scientific position. Medium risk. Individual Made Scientific scientists have prior hands-on Kytopen experience; the platform is new to the site and will be procured, installed and qualified for RTX-001. The broad waveform and parameter space can give variable results without a bounded operating range, so D-6 screens energy by cell concentration by dose and then runs a split-donor Flowfect-versus-Nucleofector LV comparability on at least 3 donors so the device change can be carried into the CP-1 to CP-2 argument. No footprint or workflow concern for manufacturing.

For discussion. Which Flowfect configuration and cartridge, and is the program fixed before December? Does Flowfect reduce the IL-10 spread across donors seen with the LV?

10 Recovery medium addition and recovery culture

D4.6 to D4.7 · RTx 1.17 h + about 4 h (TBC; window 2 to 6 h) · Grade C incubator · 2 then 0.5 operators C6

What happens

1. Recovery medium is added and the cells are transferred to up to 4 MACS bags (the RTx diagram shows about 3 MACS500 recovery bags) and returned to the incubator.
2. Recovery is about 4 h (was overnight on a magnetic stirrer with freezing on Day 6); the window discussed is 2 to 6 h. T0 is the final Flowfect step complete.
3. Resolution's rationale: mRNA residency decays and IL-10 secretion is cumulative to about 16 h, so earlier freezing preserves mRNA in the drug product and reduces the risk of OOS at the IL-10 lower limit; the root-cause analysis lists stress, EP parameters, hold times and mRNA with no single cause. Resolution calls this one of the riskiest changes.

Controls and records

Item	Requirement
Window	T0 (Flowfect complete) to T4 (CRF initiated): about 4 h target; recovery, second harvest, Rotea, formulation, fill and cassette inside it; Resolution to confirm the start and end points
Bags	About 3 to 4 MACS500 recovery bags; the incubator math for the hold uses 4 MACS500, not 10 MACS1000
Stress markers	Membranes leaky shortly after EP; Annexin V and DRAQ7 transiently up; JC-1 down and ROS up within hours; HSP70 up after 2 to 6 h; read viability after recovery

Samples and tests

None stated. Proposed end-of-recovery viability and stress read (NC-202; Annexin V/DRAQ7) IN-PROCESS CHARACTERIZATION.

Made Scientific position. Medium risk and the highest analytical risk in CP-2 together with the maturation plateau. Made Scientific proposes the T0-to-T4 definition above, runs the GMP time-and-motion study that sets each hold window inside it, and offers D-3: recovery hold 2, 4, 6 h and overnight by mRNA dose low, reference and high on matched donors with fresh and post-thaw readouts, then confirmation across donors with realistic processing-time excursions. One question to settle first: the RTx patent claims an overnight IL-4/IL-13 plus M-CSF conditioning immediately before cryopreservation; is that step in CP-2, and if not, what replaces it for cryoresilience? Operations teams value a 4-hour gap for paperwork, staging and preparation; the hold is an asset for the schedule if its window is defined.

For discussion. Confirm T0 and T4. Is 4 h a maximum, a target or a minimum? Is any recovery data patient-derived? Is IL-4/IL-13 conditioning in CP-2?

11 Second harvest set-up, harvest and pooling into the harvest bag

D4.8 to D4.9 · RTx 0.5 h + 1 h · Grade C (ISO 8 in the narrative, C in the table) · BSC · 2 operators

What happens

1. Harvest set-up after the recovery hold.
2. The recovery bags are harvested and pooled into the harvest bag.
3. The RTx narrative labels this unit operation Grade C / ISO 8 and the table Grade C; the total for harvest, formulation and cryopreservation is given as 6 to 7 h hands-on.

Controls and records

Item	Requirement
Time	RTx 0.5 h + 1 h; no MADE estimate differs
Clock	Inside the T0-to-T4 window
Operators	2 (scheduling model)

Samples and tests

Sample	Test	Role
Pooled harvest	Post-harvest count (NC-202)	IN-PROCESS

Made Scientific position. Transferred as written. In a two-wave Day 4, the second wave's harvest lands here for the first wave, which is the point at which the shared Rotea and Ares are handed between batches; the hold windows at this hand-off are set by the time-and-motion study.

For discussion. Confirm the grade for this unit operation (ISO 8 vs C in the two RTx tables).

12 Rotea wash and concentrate into saline; TruCount and dose setting

D4.10 to D4.11 · RTx 0.33 h + 1 h wait · MADE estimate 1.25 h for the Rotea · Grade C · Rotea, BD FACSLyric · 1 operator

What happens

1. The harvest is washed and concentrated into saline in an intermediate bag on the Rotea; Resolution notes this is much shorter than the Day 4 (pre-EP) Rotea.
2. A post-Rotea count is taken and a stained TruCount sample goes to the flow cytometer; the product waits (about 1 h) for the TruCount result.
3. Dose setting is performed on drug substance: the TruCount viable macrophage count gates the fill.

Controls and records

Item	Requirement
Time	RTx 0.33 h Rotea + 1 h wait; MADE working estimate 1.25 h for the Rotea (to be confirmed)
Counting	Bead-based absolute count by flow, because impedance counters under-counted macrophages in the MATCH development
Hold	DS in saline awaiting the count: a hold point with no data (4.4)

Samples and tests

Sample	Test	Role
Post-Rotea DS	Post-Rotea count (NC-202); TruCount viable macrophage enumeration (FACSLyric)	IN-PROCESS dose-setting

Made Scientific position. The TruCount wait is the one Day 4 step where the product idles for an analytical result inside the four-hour window; the flow cytometer is therefore sited and staffed for a guaranteed turnaround on Day 4 evenings and nights, and the DS-in-saline hold window is set by data. A D2 to D3 conversion read (step 5) would let the fill plan be drafted before this count rather than after it.

For discussion. What is the TruCount turnaround Resolution achieves today, and is the 1 h wait the target or the observed value?

13 Drug substance then final drug product formulation on the Ares X20

D4.12 · RTx 2.5 h ("TBC for Ares") · MADE estimate 1.25 h with fill · Grade C · Ares X20 · 2 operators C1 C7

What happens

1. The DS is diluted in DS formulation buffer to the dose concentration set from the TruCount result.
2. The FDP is formulated by CryoStor CS10 addition on the Xiogenix Ares X20 (change C7; replaces the Cue or manual formulation, change C1). Resolution has completed water and buffer runs and is testing cells.
3. QC samples for release and characterization are taken here; cell count results are recorded.

Controls and records

Item	Requirement
Time	RTx 2.5 h marked TBC for Ares; MADE working estimate 1.25 h for formulation and fill (to be confirmed)
Formulation	CS10 (DMSO); DMSO hypersensitivity is an EMERALD exclusion criterion; monocyte cryopreservation precedent 7.5 to 10% DMSO
Hold	DP before freeze in CS10: a hold point with no data; maximum DMSO exposure to be set (D-7)

Samples and tests

Sample	Test	Role
DS and FDP	Release panel and characterization (4.2, 4.3)	RELEASE CHARACTERIZATION

Made Scientific position. Low to medium risk. Made Scientific has demonstrated the Ares X20 and is planning to acquire the platform; it is not yet installed or qualified at Made Scientific and first-hand GMP operating experience is built during the training and pilot runs. Bag configuration, fill performance, cell recovery, aseptic integration and qualification are confirmed in D-7. One Ares serves one daily start unless harvests are staggered, which is the argument for the two-wave Day 4.

For discussion. Which Cue limitation drove the change? Is the Ares used at any Day 0 or Day 4 transfer step, or only here? Where does QC sample aliquoting happen, QC or manufacturing?

14 Fill: dose bags 1 to 20 and QC vials

D4.13 · RTx 0.25 h set-up + 0.33 h bags + 0.08 h vials · Grade C · Ares X20, heat sealer · 2 operators C7

What happens

1. Container preparation and Ares set-up, then bag filling into up to 20 positions, then QC vial filling.
2. Drug product: 10 mL fill in a CryoMACS 50 bag at 1×10^7 cells/mL; 4 to 9 bags per batch on the RTx diagram; about 20 × 1 mL QC vials.
3. The narrative asks whether visual inspection can run concurrently with the fill.

Controls and records

Item	Requirement
Time	RTx 0.67 h in total
Uniformity	Fill weight and viable count first vs last container: proposed IPC, acceptance to be agreed (is uniformity an Ares acceptance criterion?)
Vials	Filled after the bags, so a longer CS10 exposure and a different surface-to-volume ratio than the bag
Storage sizing	DP bag count and size drive the LN_2 storage sizing

Samples and tests

Sample	Test	Role
First, middle and last bag; vials	Fill weight; TruCount (proposed, D-7)	IN-PROCESS

Made Scientific position. D-7 tests fill uniformity at 4, 9 and 20 positions, the bag-versus-vial representativeness post-thaw on at least 3 runs (frozen in the same CRF runs with product probes in both, together with D-1), the vial fill order, and the pre-freeze hold in CS10. Concurrent visual inspection is adopted in the time-and-motion study. The QC-vial allocation is written against validated sample volumes (4.7).

For discussion. How many DP bags per batch and which CryoMACS size? Is fill uniformity an Ares acceptance criterion?

15 Visual inspection, label, overwrap, vacuum seal and cassette

D4.14 · RTx 0.25 h · MADE estimate 0.75 h · Grade C · VI light box, vacuum sealer, fridge · 2 operators C8

What happens

1. Each bag is visually inspected, labeled, over-wrapped, vacuum-sealed and placed in the metal cassette (the cassette change is pending at Resolution), then held in a temporary fridge until the freezer is loaded.
2. Appearance is a release test (USP <790>).

Controls and records

Item	Requirement
Time	RTx 0.25 h; MADE working estimate 0.75 h for VI, label and cassette (to be confirmed)
Cassette	Proposed metal cassette; fit, orientation, bag protection, labeling and overwrap, cryogenic handling and thaw interface assessed in D-1 stage 3
Hold	Temporary fridge before CRF load: inside the DP-before-freeze window (D-7)

Samples and tests

Sample	Test	Role
Each bag	Visual inspection USP <790>	RELEASE

Made Scientific position. Made Scientific's visual inspection program is applied with criteria aligned to Resolution's. The cassette engineering assessment is part of D-1; a cassette change by itself does not alter the freezer's program, but it changes the product cooling curve, which is why the matched-product comparability arm is the minimum evidence.

For discussion. What is the current cassette and the intended metal cassette (supplier, part number, bags per cassette)?

16 Controlled-rate freeze and transfer to vapor-phase LN_2

D4.15 · RTx 0.33 h · MADE estimate 1.0 h · Grade C · CryoMed LN_2 CRF (Planer historically), metal cassette · 1 operator **C8**

What happens

1. The safety cabinet is closed; cassettes are transferred to the controlled-rate freezer; the CRF program runs; product is transferred to vapor-phase LN_2. T4 is the CRF cycle initiated.
2. Change C8: the Planer used historically gives way to a Thermo CryoMed LN_2 CRF (or other) with a metal cassette, for CDMO availability and broader access; temperature mapping and equipment suitability have not been started; Resolution has no controlled-rate freezer of its own, its laboratory uses an electric ViaFreeze and EMERALD manufacturing used an LN_2-driven CRF, so the move creates a three-way matrix.
3. Published context: frozen monocytes fail (45.5% viable vs 75.2% fresh, MATCH development) while cryopreserved differentiated, conditioned macrophages recover 87% viable post-thaw with markers and phagocytosis retained and fresh equivalent to thawed in vivo.

Controls and records

Item	Requirement
Time	RTx 0.33 h; MADE working estimate 1.0 h including the CRF run start (to be confirmed)
Freezer	Multiple CryoMed units at Made Scientific supporting other programs; unit selection and cassette/load configuration for RTX-001 to be confirmed; 1 CRF per daily start unless harvests are staggered
Storage	Vapor-phase LN_2; long-term stability schedule proposed (T0, 3, 6, 12, 18, 24 months), no data yet

Samples and tests

Sample	Test	Role
CRF chamber and product-simulating probes	Temperature profile against the recipe	IN-PROCESS
QC vials (from step 13)	Release panel; sterility 7 days	RELEASE

Made Scientific position. Medium risk and an admitted gap on Resolution's side, so Made Scientific offers D-1 as the Q2a exemplar: readiness review; surrogate thermal mapping at minimum, nominal and maximum loads with eight probes and three repeats per load (no cells needed, can start before process lock); cassette engineering assessment; a matched-product comparability in a 2 by 2 of freezer by cassette on at least 3 paired donors, in both the 10 mL bag and the 1 mL vial, with Biofreeze and BioGenics 2101 as backups; confirmation and load diagram. Preliminary estimate 8 to 12 weeks after equipment, cassette, material and assay readiness. Mapping alone is not accepted as evidence that the change preserves product quality. A CRF mapping run is started on the morning of 28 September with a readout in the room.

For discussion. Which profile is the reference, ViaFreeze or the EMERALD LN_2 CRF? Recipe, current cassette, intended metal cassette, and minimum, nominal and maximum bags per load? Thaw method and post-thaw hold at the site (the collection network partner performs controlled thawing and just-in-time delivery)?

4. Analytical methods

4.1 The in-process controls by unit operation

The in-process controls by unit operation, from the CP-2 narrative, with the decision each one gates and the Made Scientific proposals marked. No sample volume is stated in any shared document, so every volume is set in the analytical transfer gap assessment; no IPC action limit has been shared.

Day	Step	Unit operation	Sample and test	Instrument	Role	Decision it gates
-15 to -1	prep	Buffer and reagent preparation; mRNA pouch	Pouch integrity and concentration (proposed)	NanoDrop; RT-qPCR	IPC	Pouch hold to maximum pre-use time
0	D0.2	Starting-material assessment	CD45+ count; %CD14+	NC-202; FACSLyric	IPC	Volume adjustment into the Prodigy input envelope
0	D0.4	CD14+ enrichment	CD45+ count post-enrichment; %CD14+ purity	NC-202; FACSLyric	IPC	Go to seeding (MATCH precedent: yield at least 40% of input CD14+)
0	D0.5	Seeding via formulation bar	Cell count end of Day 0	NC-202	IPC	Seeding density and bag count
1 to 4	culture	Differentiation	None stated; proposed D2 to D3 count and 25F9/CD206	NC-202; flow	IPC proposed	Early dose allocation; go/no-go rule to be agreed
4	D4.2	Harvest and pooling	Cell count start of Day 4	NC-202	IPC	Harvest recovery; input to Rotea
4	D4.3	Rotea buffer exchange	Cell count after buffer exchange	NC-202	IPC	Cell load before transfection
4	D4.4	Transfection set-up	Cell count before transfection	NC-202	IPC	Target concentration; mRNA volume; gating if the Flowfect range is narrow (D-6)
4	D4.5	Electroporation	Post-transfection cell count	NC-202	IPC	Recovery bag count and medium volume
4	D4.7	Recovery	End-of-recovery viability and stress read (proposed)	NC-202; flow	IPC / CHR	Confirms the T0-to-T4 window in the record
4 into 5	D4.9	Second harvest	Post-harvest count	NC-202	IPC	Input to the second Rotea
4 into 5	D4.10	Rotea wash and concentrate	Post-Rotea count; TruCount viable macrophage enumeration	NC-202; FACSLyric	IPC	Dose set on DS; the TruCount result gates the fill
4 into 5	D4.12	DS then FDP formulation	Release and characterization samples; cell count	Per panel	REL CHR	Release sampling point; QC vial allocation
4 into 5	D4.13	Fill	Fill weight; viable count first vs last (proposed)	Balance; FACSLyric	IPC proposed	Dose uniformity (D-7)
4 into 5	D4.14	Inspection and cassette	Visual inspection USP <790>	VI light box	REL	Bag disposition before CRF
4 into 5	D4.15	CRF	Chamber and product-simulating probes	CRF logger	IPC	Freeze-cycle acceptance (D-1)

Other tests such as pH, osmolality and cell density may be performed in-process as determined during execution (CP-2 narrative). The CSV companion to this table is sampling_plan.csv.

4.2 The release panel, turnaround and critical path

The release panel as listed by Resolution, with the published precedent or patent target where one exists; Resolution's specifications and acceptance limits have not been shared and none is stated here as such.

Test	Method and platform	TAT	Precedent or note
Macrophage identity	Flow (BD FACSLyric)	1 to 2 d	MATCH release: CD45+/CD14+; 25F9 and CD206 MFI at least 5-fold day-0 monocyte MFI
Viable macrophages (dose)	Flow TruCount	1 to 2 d	Bead-based absolute count; impedance counting under-counted macrophages in MATCH

Transgene identity	RT-qPCR or ddPCR	2 to 3 d	QuantStudio 7 Flex or QX600 at Resolution; QuantStudio 6 Pro accepted as suitable by Resolution (November 2025); QX200 assumed equivalent
Transfection efficiency (purity)	RT-qPCR or ddPCR	2 to 3 d	The narrative and the earlier proposal disagree on whether TE or myofibroblast deactivation is the TBD ddPCR method
IL-10 and MMP-9 protein	ELLA (ProteinSimple)	2 to 3 d	Patent targets: IL-10 at least 10,000 pg/mL at 4 × 10^6 cells/mL; MMP9 at least 200 ng/mL; OOS risk at the IL-10 lower limit is the stated driver for change C6
MMP-9 activity	FRET (VarioSkan LUX)	2 to 3 d	Patent: activity at least 1.5 × untransfected
Appearance	Visual inspection USP <790>	same day	
Sterility	BacT/ALERT 3D (rapid method under evaluation)	7 d	Longest test; growth-based methods need antibiotic-free medium (MATCH rejected AIM-V on this point)
Mycoplasma	RT-qPCR / BioFire	1 d	Sample at Day 4 harvest, pre-wash
Endotoxin	LAL, Endosafe Nexgen-PTS, USP <85>	1 d	

Critical path. Release samples are drawn at DS/FDP formulation on Day 4 into 5. Sterility at 7 days sets the earliest release at Day 4 plus 7 days, about Day 11 to 12 after start; the 2-to-3-day tests finish inside that window. The earlier program KPI was a vein-to-vein turnaround at or below 25 days, at or below 21 after batch 30. If a validated rapid sterility method replaces BacT/ALERT, transgene identity, TE and ELLA become the critical path. Because the product is cryopreserved, release gates shipment to the site and the collection network's controlled thaw, not the infusion timing of a fresh product.

4.3 Extended characterization and potency candidates

Extended characterization as listed: collagen degradation ELISA; IL-10 activity (ELISA or HEK-Blue bioassay); a TBD ddPCR method. Resolution describes three tiers: release, for-information-only during the pivotal (building potency assurance), and an extended-characterization comparability suite not continued during the pivotal. The potency assay still has to be developed, and Resolution has published its own reading of the FDA draft potency guidance for an mRNA gene-modified cell therapy.

Candidate (RTx MoA suite)	Arm	Readout	Made Scientific note
Phagocytosis, pHrodo Red Zymosan by flow	Anti-inflammatory	% phagocytic cells and kinetics	Proposed by Made Scientific's analytical development group as a potency candidate for the polarized macrophage. Caveat from the record: in the MATCH development, pHrodo bead uptake was identical between monocytes and day-7 macrophages, so it failed as a release assay; cryopreserved conditioned macrophages phagocytosed faster than naive macrophages (30 vs 40 min). A kinetic readout with an engineered-vs-non-engineered or conditioned-vs-naive contrast is the defensible design.
Monocyte recruitment	Anti-inflammatory	Transwell PBMC migration	Tier 2 or 3; higher variance
Naive-macrophage polarization	Anti-inflammatory	Flow panel on responder cells	Tier 2
MMP activity, FRET	Anti-fibrotic	Fold over untransfected	Already on the release panel
Collagen degradation; myofibroblast deactivation	Anti-fibrotic	ELISA; ddPCR (TBD)	Extended characterization; method TBD for the ddPCR
IL-10 capture by flow; MSD V-PLEX 10-plex secretome	Both	Per-cell IL-10; cytokine panel	Patent suite; the secretome panel is a candidate "does not turn M1" safety-side assay (TNF-alpha plus poly I:C raised IL-10, IL-1Ra and VEGF but not IL-12; LPS plus IFN-gamma raised IL-12, MATCH)

4.4 Hold points and the stability plan

What exists is a proposed schedule only: long-term LN_2 vapor at T0, 3, 6, 12, 18 and 24 months; in-use post-thaw at 2 to 8 C at 0, 2, 4, 6 and 8 h; commercial T0, 6, 12 and 24 months; stability may use engineering-run healthy-donor material. There are no data, no DS hold-time data and no post-thaw in-use claim. Each hold point below becomes a defined window so the Day 4 schedule can flex without a deviation.

Hold point	CP-2 condition	Readouts	Design	Data status
mRNA after thaw	Pouch, Day -15 to -1	Integrity, concentration	Hold to maximum pre-use time	None shared

Incoming leukapheresis	Transit and hold before selection; receipt within 48 h	Viability, CD14+ count	Shortest and longest transit, at least 3 donors	None shared; the MATCH 48 h ambient window was for the fresh final product, a different material
Pre-electroporation	Post-Rotea, in EP buffer	Viability, downstream TE	Longest Day 4 queue	None
Post-EP rest	2 to 6 h window	Viability, transgene mRNA, IL-10	Freeze at 2, 4 and 6 h (D-3)	Small-scale healthy-donor data at Resolution
DP before freeze	CS10; 10 mL bags and 1 mL vials	Post-thaw viability, potency	Maximum DMSO exposure, bag and vial (D-7)	None
DP long term	LN_2 vapor	Viability, count, identity, potency, sterility	Engineering and PPQ lots, 5 time points	Schedule only
Post-thaw and shipping	Thaw to dose; dry shipper	Viability, count, potency	Maximum in-use time; simulated shipment	Precedent: apheresis-derived monocytes/macrophages held 1 to 6 C for up to 4 h post-thaw with function retained (2003)

4.5 Rapid safety methods and their validation

Rapid safety methods and their validation: the three methods are routine in Made Scientific QC; only matrix suitability is new for RTX001 and it is done on healthy-donor lots. Sequence: feasibility in AD (matrix screen, sample volume set); suitability in QC on 3 healthy-donor lots under QA; routine with system suitability and trending. The rapid-sterility decision should be made before the January 2027 analytical transfer so the suitability study is run once.

Test	Method	TAT	Basis	RTX001 validation
Sterility	BacT/ALERT 3D	7 days	21 CFR 610.12; USP <71>, <1223>; Ph. Eur. 2.6.27	Bacteriostasis and fungistasis in the CS10 matrix
Mycoplasma	BioFire PCR	about 1 day	USP <63>; Ph. Eur. 2.6.7	LOD at or below 10 CFU/mL; Day 4 harvest, pre-wash
Endotoxin	LAL, Nexgen-PTS	same day	USP <85>	Inhibition and enhancement; MVD

4.6 Reference standards and controls

One hierarchy from the Resolution primary standard: primary standard, a Resolution-supplied lot held in LN_2 at Made Scientific; working standard, Made Scientific engineering-run material qualified against the primary; run controls, working-standard aliquots on every run. New lots are bridged in at least 3 runs; Westgard trending; standards on LN_2 stability; tier 3 potency data trended against the same standard.

Assay	Control material	Every-run suitability
Flow (FACSLyric)	Macrophage control cells; TruCount beads	CS&T pass; control in the trended range
RT-qPCR / dPCR	IVT RNA standard; reference-lot RNA; transgene-specific design	R^2 at least 0.98, efficiency 90 to 110%; NTC and no-RT negative
ELLA	Reference DP media; mock-EP baseline	Controls in range; cartridge lot bridged
MMP-9 FRET	Recombinant MMP-9; inhibitor control	Signal-to-background met; inhibitor suppresses the signal

4.7 QC fill, retains and stability sampling for about 20 × 1 mL vials

Does the 1 mL vial represent the 10 mL bag? The fill volume and surface-to-volume ratio differ, so freeze and thaw kinetics differ; vials fill after the bags, so they see a longer CS10 exposure before the freeze. Made Scientific's answer: freeze bags and vials in the same CRF runs with product probes in both (D-1), pair bag against vial on at least 3 runs for viability, recovery, identity and potency (D-7), and if the vial diverges, move to mini-bags for viability and potency and keep the vial for identity, safety and retains.

Philosophy: patient batches give up only what release, retest and retain need; stability pulls come from engineering and PPQ lots only; the final split is set from validated sample volumes.

Use (illustrative)	Vials
Post-thaw release tests	6
Tier 3 information-only assays	4
Retest reserve	4
Retain	4
Investigation spare	2
Total	**about 20**

4.8 Instrument bridges

Resolution platform	Made Scientific platform	Bridge

BD FACSLyric	BD FACSLyric, procured for RTX-001 and qualified before the analytical transfer; the qualified CytoFlex for feasibility only	Resolution declined the CytoFlex as a substitute (November 2025). Paired-sample CytoFlex-to-FACSLyric comparison once the FACSLyric is live, with the acceptance criteria already proposed (%positive within 20 points, MFI within 30%, CV at or below 10 and 15%), so early feasibility data carry forward; release identity and TruCount dose run on the FACSLyric only.
QuantStudio 7 Flex	QuantStudio 6 Pro (accepted as suitable by Resolution)	Verification, not validation: paired-sample comparison on the IVT RNA standard curve (efficiency 90 to 110%, R^2 at least 0.98 on both) and at least 3 lots across the range, regression bias at the limits.
Bio-Rad QX600	QX200 (assumed equivalent)	Same droplet chemistry, different multiplex capacity; the transgene assay is verified on the QX200 with the same paired design.
ProteinSimple ELLA; NanoDrop Eight	ELLA (procured); NanoDrop One (accepted)	Resolution declined a manual ELISA in place of ELLA; cartridge-lot bridging is part of the run-control lifecycle.

4.9 The analytical transfer sequence

Phase 1, analytical transfer from January 2027. Ten steps in two blocks. Analytical development laboratory: (1) knowledge transfer of methods, reports, historical data and critical reagents; (2) gap assessment of equipment, reagents, software and matrix; (3) BOM and acquisition with equipment IQ/OQ; (4) feasibility on shared samples against Resolution's results; (5) transferred to AD when the pre-agreed comparability is met; (6) AD qualification, with the extent set by the data Resolution already holds (validated method: confirmatory accuracy and intermediate precision; qualified method: partial; development-stage method: full phase-appropriate; compendial safety method: verification and matrix suitability). QC: (7) QC transfer protocol with training built in; (8) GMP SOP and forms drafted from the AD method; (9) QC execution under QA oversight; (10) QC transfer report referencing the AD qualification report. Successful execution of the QC transfer protocol completes the transfer. All data are shared with Resolution weekly and the next stage is agreed before it starts.

Three tiers by method. Tier 1 (release) moves to matched platforms by comparative testing; tier 2 (comparability-only) stays in the Resolution laboratory and Made Scientific samples are tested there; tier 3 (information-only potency) is co-validated. Statistics: site transfer on at least 3 lots across the range plus the reference standard, 2 laboratories, at least 2 analysts, at least 3 runs each, TOST equivalence with the 90% confidence interval inside the margin; method change on retained EMERALD and CP-2 lots including near-limit samples, old against new on the same aliquots the same day; two methods for one attribute by Bland-Altman.

Phase 2, process transfer from April 2027. The methods are in place before the first training run so pilot-run and engineering-run samples are tested on transferred methods; comparability (D-8) then runs on bridged methods, because methods must be shown comparable first or an assay difference reads as a process difference. The impurity ELISA (D-2) is the one method that does not exist yet and is developed in parallel from the first quarter of 2027 so it is qualified before the engineering runs.

5. Questions for the workshop, by unit operation

Questions for the workshop, by unit operation: placed against the step they decide, with the Resolution question (Q1 to Q4) and the work package (D-n) each one feeds. "Settles" names what an answer lets Made Scientific fix.

Step	Question	What we need from Resolution	Q	Settles
1	When does MS-2 mRNA exist at clinical scale, and can MS-1 lots be supplied for the pilot and engineering runs? Is the nucleoside modification unchanged?	Supply timeline; MS-1 availability; the MS-1 to MS-2 comparison plan	Q4	D-8
2	Is filgrastim mobilization used in the collection protocol, and what are the Resolution input limits into the Prodigy?	Collection protocol; the input envelope and the action above it	Q1	Capacity model
3	Has any EMERALD leukapheresis exceeded the CD14 column capacity?	EMERALD selection data	Q1	Batch record
4	What is the formulation-bar CAP's bag limit per run and the filtered-air requirement? MACS1000 part, dimensions and working volume? Can the bar fill in parallel with the enrichment wash?	CAP scope from Miltenyi; consumable specification	Q1	D-5; Day 0 clock
4	How many recovery bags return to the incubator after the Day 4 harvest, and are they MACS500 or MACS1000?	The recovery bag count and size	Q1	Incubator math
5	Does the maturation plateau start at D4 or D5 in the at-scale data, and on how many donors? Is there a D2 feed?	The maturation time-course data; the CP-2 feed schedule	Q4	D-4
6	Which harvest method is Resolution converging on, and what recovery does it give from a MACS1000 bag?	Harvest method data	Q1	D-5; Day 4 clock
7	What cell-load range drives the 4.25 h Rotea figure, and what is the longest pre-electroporation hold with data?	Rotea run data; hold data	Q1	Hold windows
8	Which grade table is current for transfection set-up? Is the mRNA:cell ratio fixed or scaled to the count?	Confirmation of the grade and the dosing rule	Q1	Batch record
9	Which Flowfect configuration and cartridge, and is the program fixed before December? Does Flowfect reduce the IL-10 spread seen with the LV?	The Kytopen program and operating range; TE and IL-10 spread by donor	Q1, Q4	D-6
10	Confirm T0 and T4. Is 4 h a maximum, a target or a minimum? Is any recovery data patient-derived? Is the IL-4/IL-13 conditioning of the patent in CP-2?	The window definition; the recovery data set; the conditioning status	Q1	D-3
11	Confirm the grade for the second harvest (ISO 8 vs C in the two tables).	Confirmation	Q1	Batch record
12	What TruCount turnaround does Resolution achieve, and is the 1 h wait a target or an observation?	Flow turnaround data	Q2b	QC staffing
13	Which Cue limitation drove the change? Is the Ares used at any Day 0 or Day 4 transfer, or only at formulation and fill? Where does QC aliquoting happen?	The Ares scope; the QC sampling location	Q1, Q2b	D-7; layout
14	How many DP bags per batch, which CryoMACS size, and is fill uniformity an Ares acceptance criterion?	Bag count and size; the Ares acceptance criteria	Q1, Q2b	D-7; LN_2 sizing
15	What is the current cassette and the intended metal cassette (supplier, part number, bags per cassette)?	Cassette specification	Q2a	D-1
16	Which profile is the reference, ViaFreeze or the EMERALD LN_2 CRF? Recipe, and minimum, nominal and maximum bags per load? Thaw method and post-thaw hold at the site?	The freeze recipe and any paired ViaFreeze vs LN_2 data; the thaw interface specification	Q2a	D-1; stability
4.2	Specifications and acceptance limits for every release test, and the IPC action limits Study 4 refers to.	The specification	Q2b, Q4	Transfer plan
4.2	Sample volumes per test, and which method is the TBD ddPCR (TE or myofibroblast deactivation)?	Sample volumes; method identity	Q2b	Vial allocation

4.3	The full tiered assay list (release; for information; comparability-only).	The list owed since the pre-briefing	Q2b, Q4	Transfer tiers
4.5	Can the rapid sterility decision be made before January 2027?	Resolution's evaluation status	Q2b	Suitability study
D-2	Which interleukin is the impurity analyte, and what medium level is expected?	Analyte, level, any data	Q2a	D-2
4.2	Is IL-10 secretion measured on DS (as the briefing chart shows) or on DP post-thaw for release?	The release sampling definition	Q2b	Release panel
D-8	Would Resolution add a Study 5 (CP-2 at laboratory scale vs CP-2 at Made Scientific at scale on matched donors), and can a fresh split be executed with both arms at one site?	A view on the design and on the starting-material format	Q4, Q3	D-8

6. Sources

Sources for this document. Resolution documents are shared under the confidentiality agreement and cited by title and date.

1. Resolution Therapeutics. *US CDMO pivotal process: detailed process flow diagram and narrative for CP-2* (key changes highlighted), 3 to 4 September 2026; with the embedded process flow diagram and the reviewer comments on harvest time and the Ares formulation time.
2. Resolution Therapeutics. *RTx CDMO workshop briefing pack*, September 2026: slides 4 to 9 (process updates, updated PFD, summary of proposed changes, drivers for reducing post-electroporation time, ongoing development), 10 to 13 (transition to pivotal, IND readiness, comparability strategy and Studies 1 to 4), 14 to 18 (questions Q1 to Q4).
3. Resolution Therapeutics and Made Scientific. *Process workshop pre-briefing*, 21 September 2026: comparability run count and equivalence basis, controlled-rate freezer status, post-electroporation window, final container, tiered assays.
4. Made Scientific. *Ruby process canonical unit-operation model* (ruby_process.json v1.0, 26 September 2026), the Day 4 scheduling model (day4model2) and the hour-by-hour occupancy maps; every "MADE estimate" in this document is from these and is to be confirmed by the Day 4 rehearsal and the training runs.
5. Made Scientific. *Development positions for the 28 September 2026 workshop*: four-hour window definition and study design, controlled-rate freezer and cassette strategy, analytical transfer, comparability basis and statistics, impurity ELISA, rapid safety, reference standards, QC fill and retains, stability-indicating markers; and *Development Work Packages, DOE Plans*, Version 01, D-1 to D-8.
6. Fraser AR, et al. Development, functional characterization and validation of methodology for GMP-compliant manufacture of phagocytic macrophages: a novel cellular therapeutic for liver cirrhosis. *Cytotherapy* 2017;19(9):1113-1124 (PMC5571439): Prodigy input envelope, selection purity and yield, seeding density, TexMACS plus M-CSF, release criteria, TruCount, 48 h ambient stability, frozen-monocyte failure, antibiotic-free medium.
7. Brennan PN, et al. Autologous macrophage therapy for liver cirrhosis: a phase 2 randomized controlled trial (MATCH). *Nature Medicine* 2025 (PMC11922741): reproducible selection, variable conversion efficiency, dose and minimum release dose.
8. Resolution Therapeutics. *Therapeutic macrophages*, WO2024074376A1 (priority 27 September 2023): day5 and day7 differentiation methods, electroporation devices and cell concentrations, mRNA doses, expression window, IL-10 and MMP9 targets, cryoresilience conditioning, assay suite.
9. Starkey Lewis PJ, et al. Cryopreserved human alternatively activated macrophages promote resolution of acetaminophen-induced liver injury in mouse. *npj Regenerative Medicine* 2025 (PMC11754469): post-thaw viability, marker retention, phagocytosis kinetics, fresh equals thawed in vivo.
10. Kytopen Corp. Press release, 22 October 2024 (monocyte-derived macrophage engineering on Flowfect); Van Tendeloo VF, et al. *Blood* 2001;98:49 (mRNA vs plasmid electroporation); Smits E, et al. *Methods Mol Biol* 2016 (mRNA electroporation platform); *Cytokine* 2018 (PMID 29579547); *Sci Rep* 2023 (PMC10133335, continuous-flow electroporation); *Sci Rep* 2020 (PMC7060354) and *J Leukoc Biol* 2024 (DOI 10.1093/jleuko/qiae059) on nucleotide modification and innate activation.
11. Cryopreservation precedents: *J Leukoc Biol* 1980 (PMID 6935476, DMSO 7.5 to 10%); *Transfusion* 2003 (PMID 12631303, post-thaw hold 1 to 6 C for 4 h); *RSC Appl Polym* 2025 (PMC12147442, macromolecular cryoprotectants and ice nucleation); *Bio-protocol* 2026 (PMC13265409, thaw protocol).
12. Public Resolution Therapeutics releases: enrollment complete in EMERALD, 11 September 2026; partnership with Blood Centers of America, 1 September 2026; ASGCT 2025 process and testing-suite disclosure, 14 May 2025; ClinicalTrials.gov NCT06823713; Resolution Therapeutics, *Interpreting the new FDA draft potency guidance: an mRNA gene modified cell therapy perspective*, Cell & Gene Therapy Insights, 2 December 2024.
13. Regulatory basis: ICH Q5E; FDA CBER draft guidance on manufacturing changes and comparability for human cellular and gene therapy products (July 2023); FDA CBER draft guidance on potency assurance for cellular and gene therapy products (December 2023); ICH Q2(R2) and Q14; ICH Q5C; ICH Q6B; ICH Q9(R1); USP <1224>, <1226>, <1210>, <1043>, <1046>, <790>, <71>, <1223>, <63>, <85>; 21 CFR 610.12.

Version 01 · 27 September 2026 · Prepared for the Resolution Therapeutics and Made Scientific pivotal process workshop. Figures quoted from Resolution documents are quoted as shared and remain subject to Resolution's final data review before process lock in December 2026. Made Scientific estimates are working figures to be confirmed by the Day 4 rehearsal and the training runs. Made Scientific positions are proposals for discussion.