

TECHNICAL EVALUATION & CDMO FRAME

XIAP — Autologous CRISPR-Edited CD34+ HSC

MADE technical evaluation and CDMO frame

PI	MAYO SITE	ON NUMBER	MODALITY	VERSION	PREPARED
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1. At a glance

Program	Autologous CRISPR-edited CD34-positive hematopoietic stem cell drug product for XIAP deficiency
Modality	Gene-modified hematopoietic stem cell — ex vivo CRISPR edit
Phase	Phase 1, IND-enabling
Regulatory status	Pre-IND. MADE proposal ON23155-2026 issued 29 July 2026, valid to 27 October 2026
Starting material	Autologous apheresis — single patient, single collection, no cell bank
Drug substance	Edited CD34-positive cell suspension after post-edit handling, harvest and wash
Drug product	Formulated viable CD34-positive dose; fresh or cryopreserved is not yet settled — see §8
Where MADE would manufacture	Princeton, NJ
MADE reference	ON23155-2026
MADE technical fit	Direct on process and analytical development. No GMP process exists to transfer — the process is built

This is a technical assessment, not a proposal. A proposal is already with you; this document is the technical thinking behind it and what has developed since.

2. Program understanding

Your program requires establishing a manufacturing process where none exists — no GMP process or protocol is being transferred. Our proposal builds the autologous CD34-positive process from a Casgevy-class reference approach, opening with a structured gap assessment and risk register and following a phase-appropriate staged development path. That inverts the usual CDMO conversation: this is process development first and technology transfer second.

The four priorities your team set out map exactly onto what the field's evidence says the real risks are, and we think that is worth saying back to you, because it is not the usual case.

Your stated priority	What the evidence says
Establish a process where none exists	Programs without a documented source process need the artifacts created, not transferred — that work is real and should be scoped rather than assumed
De-risk electroporation, with significant expected post-electroporation cell loss	30–50% viability loss on electroporation is common and must be carried into the dose calculation, not treated as a deviation
Protect a single patient's material — one apheresis, no bank, every step irreplaceable	The yield chain compounds: CD34 selection runs 40–70% recovery, then electroporation loss on top — see §4

Your stated priority	What the evidence says
Reach IND-enabling GMP while managing a client-supplied editing reagent on the critical path	GMP Cas9 and guide RNA lead times are a recognized critical-path risk in this modality, on the same footing as viral vector

This is a well-diagnosed program, and our scope was built around those four rather than around a generic template. One thing is upstream of all of it: as of June the in vivo versus ex vivo decision was still open, with a decision expected within roughly two months. Our proposal scopes the ex vivo path, and that is the first thing to confirm.

3. Regulatory position and pathway

Current filing status	Pre-IND. The engagement is IND-enabling; the ex vivo path is the one scoped
Designations held	Not established — see §15
What the CMC package must support	Phase 1: the process described with critical parameters identified; identity by flow; viability; sterility, endotoxin and mycoplasma; editing efficiency or vector copy number; transgene expression; and a biologically relevant, quantitative potency assay, qualified rather than validated
Where the flexibility sits	FDA's January 2026 flexible-CMC position applies — before Phase 2/3 manufacture, 21 CFR Part 211 compliance is not expected, IND specifications may be permissive, and there is no requirement for three PPQ lots
Where it does not	Off-target characterization. It is a safety characterization for a permanent genomic modification, and it is the first-listed recurring FDA deficiency in this modality
Recurring FDA deficiencies, this modality	Off-target editing characterization; engraftment potency assay design; conditioning regimen compatibility with the product; vector clearance and residual impurity characterization
What a site change implies	Nothing is being transferred, so there is no prior GMP site to bridge from. The comparability burden runs forward instead — development runs, then engineering runs, then GMP. See §10
Long-term follow-up	Fifteen years applies to integrating vectors. A non-integrating editing approach does not automatically carry that expectation, and the applicable obligation should be settled with the agency rather than assumed
Applicable guidance	FDA CBER, <i>Long-Term Follow-Up After Administration of Human Gene Therapy Products</i> , January 2020; FDA CBER flexible-CMC announcement, 11 January 2026

Delivery modality is a real risk differentiator and worth stating precisely rather than by implication. Hematologic malignancy developed in 7 of 67 patients treated with an integrating lentiviral gene-addition therapy for cerebral adrenoleukodystrophy, with onset from 14 to 92 months after treatment, driven by vector integration into a proto-oncogene. A program using non-integrating delivery does not carry that specific risk. It still has to characterize its own dominant risk, which is off-target editing.

4. Starting material

Source	Mobilized peripheral blood apheresis. The regimen — G-CSF, plerixafor, or both — determines both the yield and the character of the collected cells, and is not yet established (§15)
Collection	One patient, one apheresis, no cell bank. Every step is irreplaceable and there is no retained material to re-run from
Acceptance criteria	Not established — target CD34-positive purity, recovery and minimum cell dose are open (§15)
Fresh or cryopreserved on receipt	Not established — see §15
Time from collection to processing	Not established — see §15
Chain of identity and custody	End to end across all in-house unit operations, from apheresis receipt through cryopreservation and QA disposition, as one continuous sequence in one building
On a failed or insufficient collection	Not established, and it is the single most patient-impacting gap in the program. Insufficient yield may require repeat collection, which increases patient burden. A backup collection plan is the item most often missing in this modality

The yield chain compounds, and the arithmetic has to close on paper before the first patient. CD34-positive selection runs 60–80% purity at 40–70% recovery, variable with material quality. Electroporation then costs 30–50% viability. If selection recovers 55% and electroporation costs 40%, a collection has to start well above the minimum dose to land above it. That arithmetic belongs in the process description, and the engineering runs should establish it on healthy-donor material rather than discovering it on your patient's.

The chain of identity and custody commitment matters more here than in most programs. For a single-patient, single-apheresis product with no bank, running every unit operation in one building as a single continuous sequence is the control that matters most — and it is what our proposal commits to.

5. Process flow

Unit operation	Your approach	MADE capability	Read
Mobilization and apheresis	Regimen and target CD34 dose not established	Standard receipt, chain of identity and custody maintained as one continuous sequence	The backup collection plan is the open item
CD34-positive selection	Not established	CliniMACS Plus and CliniMACS Prodigy in service — see §6	60–80% purity, 40–70% recovery. The first term in the yield chain
Pre-stimulation culture	Not established	Process development activity in the proposed scope	Long enough to bring cells into cycle for editing, short enough to preserve stemness

Unit operation	Your approach	MADE capability	Read
Gene modification by electroporation	Client-supplied editing reagent; correction or disruption not established	Platform down-selection across available development systems is explicitly scoped, with post-electroporation viability, recovery and editing performance characterized before GMP commitment	The highest-variability step. Pre-complexed Cas9-guide ribonucleoprotein degrades at room temperature and must be freshly prepared within a defined hold time, and the Cas9 concentration window is narrow — more gives more on-target and more off-target editing at once
Post-edit rest or brief recovery	Not established	Process design accounts for the constraint	Extended culture costs stemness — you cannot grow your way to dose. This inverts the usual logic: expansion here degrades a good product rather than rescuing a marginal one
Harvest, wash, formulate	Not established	Standard	Yield compounds from every step upstream
Fill and cryopreserve, or deliver fresh	Not established — see §8	Controlled-rate freezing and vapor-phase liquid nitrogen storage in service	The choice interacts with release: a fourteen-day colony-forming unit result cannot gate a fresh product
Release testing and QA disposition	Panel established on MADE in-house methods	See §7	Off-target analysis is outsourced and sits on the critical path

Where the mechanism is correction rather than disruption, efficiency in CD34-positive cells typically runs 10–40% against 50–80% for disruption, and a donor template is required — ssODN or AAV6 — which is itself a GMP material with its own supply chain. That single question sets the difficulty of the whole process, which is why it opens §15.

6. Key equipment

Instrument	Your platform	MADE's installed position	Onboarding required
CD34-positive selection	Not established	CliniMACS Plus × 5 in service at Princeton; CliniMACS Prodigy × 14 in service across our two sites	None
Electroporation	Not established — down-selection is scoped	Three platforms in service: Thermo Neon × 2 and Lonza 4D-Nucleofector × 1	None. Three platforms gives the down-selection real alternatives to compare rather than a single option
Electroporation, other platforms	If your development work specifies one	Thermo CTS Xenon is on site at Princeton but Inactive; MaxCyte is not held	Acquisition and qualification, on a stated timeline
Editing efficiency by ddPCR	Not established	Bio-Rad QX200 × 5 in service — 1 Princeton, 4 East Norriton	Assay development per locus
Identity and purity by flow	Not established	Beckman Coulter CytoFLEX 13-color × 4 in service at Princeton	Feasibility qualification of an in-house method

Instrument	Your platform	MADE's installed position	Onboarding required
Count and viability	Not established	NucleoCounter NC-200 × 7 in service at Princeton	A product-specific qualification run-check, not development
Orthogonal digital PCR	Not established	Thermo QuantStudio × 3 in service	None
Colony-forming unit readout	Not established	Colony counter × 1 in service at East Norriton	Scoring standardization across the QC group is the real question here, not the instrument
Controlled-rate freezing and LN2 storage	Not established	Controlled-rate freezers × 9 in service across both sites	None

7. Analytical panel and release

No qualified Mayo methods exist to transfer, so methods are established using MADE in-house methods.

Attribute	Your method	MADE position	In house or outsourced
Identity and purity — viable CD34-positive	Not established	Flow cytometry on CytoFLEX; also the input specification	In house — feasibility qualification
Strength — viable CD34-positive cell dose per kg	Not established	The clinically governing number	In house
Viability	Not established	Automated counter with 7-AAD; post-electroporation losses land here	In house — established platform method, product-specific qualification run-check
Editing efficiency, or vector copy number	Not established — ddPCR per locus or NGS amplicon is an open choice	ddPCR is more GMP-friendly and needs assay development per locus; NGS amplicon is more accurate and needs a bioinformatics pipeline	In house on Bio-Rad QX200 — assay development per locus
Transgene expression, where a gene is added	Not established	qPCR or flow	In house
Engraftment potency	Colony-forming unit assay	Primary, and not sufficient on its own — see §7a	In house
Off-target editing and genomic integrity	Not established	NGS and cytogenetics — GUIDE-seq, CIRCLE-seq or DISCOVER-seq, in practice a combination, with top off-target sites characterized and a defined frequency of reassessment	Outsourced to a qualified subcontractor
Residual editing reagent or vector	Not established	qPCR, ELISA	In house

Attribute	Your method	MADE position	In house or outsourced
Sterility, mycoplasma, endotoxin	Standard	Standard	In house

The analytical work order commences approximately two weeks ahead of the process development runs, so that methods supporting in-process and characterization testing exist at first run execution. That sequencing is deliberate and it is the thing programs most often get backwards.

7a. Potency

The colony-forming unit assay is the standard primary engraftment-potency measure for this modality, and it has documented limits: it measures progenitor function rather than long-term engraftment potential, takes fourteen days, and is semi-subjective to score, with high inter-operator variability across BFU-E, CFU-GM and CFU-GEMM without standardized training. Engraftment potency assay design is the second-listed recurring FDA deficiency in this modality, so a program relying on an unaugmented colony count should expect the question.

The sharpening question is whether the assay also measures **edit or transgene status in the colonies**. A colony count proves cells are functional; only the augmented version proves the *corrected* cells are, which is the actual product claim. Whether that belongs in the Phase 1 scope or in characterization is a judgment call — but it should be a deliberate one, and it is in §14.

8. Fill, finish and presentation

Container closure	Not established — see §15
Fill volume	Not established — see §15
Dose form	Formulated viable CD34-positive cell suspension, dosed per kg
Labeling	Not established — see §15
Fresh or cryopreserved	Not established, and it is a design decision rather than a detail. Fresh removes cryopreservation losses and forces exact alignment with the conditioning schedule; cryopreserved decouples the schedule and adds a freeze-thaw stress to a population whose function is already the limiting quantity
Controlled-rate freeze	Controlled-rate freezers × 9 in service across both sites
Storage	Vapor-phase liquid nitrogen where the product is cryopreserved

Container closure, fill volume and labeling all follow from the fresh-versus-cryopreserved decision, which is why they are open here rather than assumed.

9. Stability, storage and shipping

Stability status	Not established — no stability program exists yet. For a cryopreserved product it has to cover viable CD34 recovery, colony-forming units and editing-efficiency retention
Storage condition	Vapor-phase liquid nitrogen
Post-thaw requirement	Post-thaw viable CD34 dose must still meet the minimum, which means the pre-freeze target has to be set above it
Shipper	Not established — see §15
Qualification status	Not established — see §15
Chain of custody, outbound leg	Maintained in house through QA disposition. The onward leg to the treating site is not established
Governing constraint	Conditioning compatibility, and it runs in both directions. The product must be available when the patient completes conditioning, and a conditioned patient with no product is the failure mode
Retains and archive	Long-term follow-up places sample retains and archive requirements on the manufacturer, and they belong in the storage plan from the first GMP batch

10. Comparability

There is no prior GMP process to bridge to, so comparability here does not mean matching a predecessor. It runs forward instead. Development runs establish the yield chain and the editing performance; the engineering run measures the whole chain step by step on destination equipment; and the GMP lots are then read against that evidence base. Academic-origin transfer would normally call for three to five bridging lots — here there is not even a documented source process, so the artifacts are created rather than compared, and that work should be scoped rather than assumed.

The editing reagents add a burden most modalities do not carry. They are a lot-defined input, so a guide RNA or Cas9 lot change is a potential product change, and the reassessment frequency has to be defined — per lot, or per process change.

Change	Risk	Why
Editing reagent lot — guide RNA or Cas9	Highest	Potential change in the on-target and off-target profile
Electroporation instrument or program	Highest	Efficiency and viability both shift, and disposables are instrument-proprietary
Culture duration or cytokine cocktail	High	Directly trades editing efficiency against stemness
CD34 selection reagent or system	High	Purity and recovery shift, changing the input population
Donor template format, ssODN to AAV6	High	Different repair kinetics and a different impurity profile
Fresh to cryopreserved	High	Post-thaw viable CD34 dose and colony-forming units must both be demonstrated

The analytical bridge reads against a risk-weighted attribute set: off-target profile and colony-forming potency as critical; viable CD34-positive dose, editing efficiency or vector copy number, transgene expression and viability as high; residual editing reagent and vector clearance medium to high and a named FDA deficiency area; sterility, mycoplasma and endotoxin required every lot. Editing efficiency should be read in the relevant subset and not only in bulk — see §14.

11. Technology transfer approach

Stage	What happens	Where the gate sits
Stage 0 — technical assessment	This document, the equipment position above, and the questions in §15. Expect the yield chain, the off-target plan and the potency augmentation to be the three largest open items	The in vivo versus ex vivo decision
Stage 1 — gap closure	Project initiation with a structured gap assessment and risk register — the deliverable that makes everything downstream estimable. GMP Cas9 and guide RNA sourcing with lead times confirmed; the off-target subcontractor selected and capacity booked; per-locus ddPCR assay development started. Both the editing-reagent supply chain and the subcontractor start here because both carry lead times measured in months	Gap assessment and risk register accepted
Analytical development, in parallel	Commences approximately two weeks ahead of the process development runs, so methods exist at first run execution	Methods available before the first run
Stage 2 — process development and engineering runs	Non-GMP on destination equipment, centered on de-risking electroporation with platform down-selection, and measuring the full yield chain step by step — collection, selection recovery, post-electroporation viability, final viable CD34 dose — so the arithmetic is evidence rather than assumption	Electroporation platform down-selected before GMP commitment
Stage 3 — comparability	Head to head on the risk-weighted attribute set, with editing efficiency measured in the relevant subset	Comparability data reviewed jointly
Stage 4 — GMP readiness, manufacture and release	Conditioning schedule alignment designed in, and formal transfer qualification of the analytical development methods into Quality Control	A stage-gate readiness assessment before GMP activity begins

The delivered proposal is organized into work orders on exactly this shape — project initiation and gap assessment, analytical development, process development studies centered on de-risking electroporation, GMP readiness and manufacture, and transfer qualification of methods into Quality Control — each carrying its own deliverables, assumptions and exclusions, with Mayo's deliverables stated alongside ours.

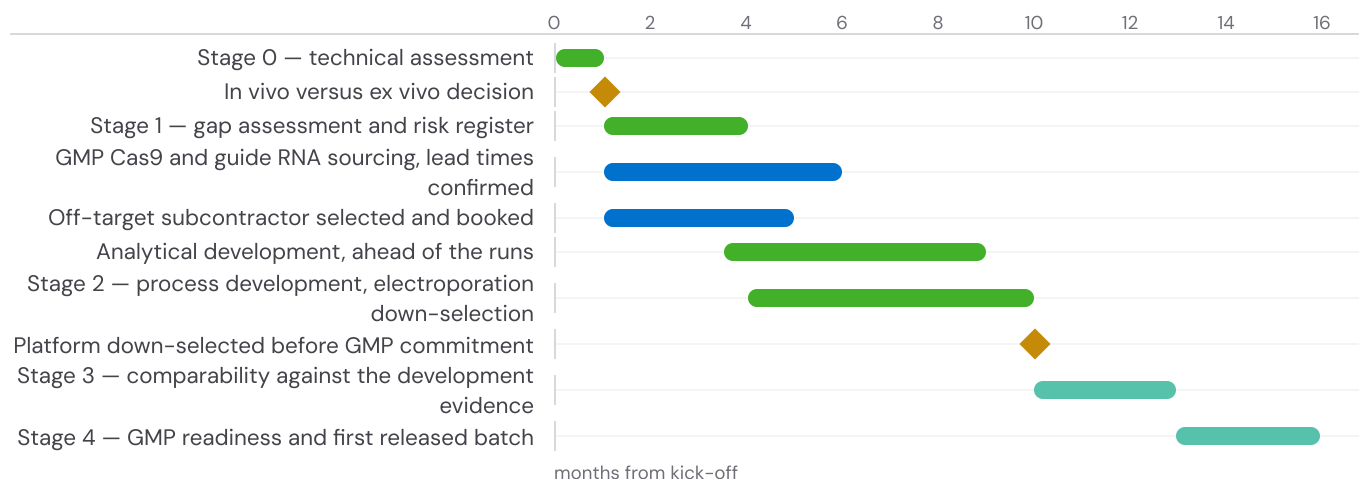
12. Indicative timeline

The delivered proposal carries the committed scope and timeline. For context, the independent industry planning ranges behind it:

Custom process, no platform	12–18 months to first released GMP batch [industry range]
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Novel gene-editing approach	add 1–3 months to process development [industry range]
No documented source process	add 2–4 months to knowledge transfer [industry range]
Autologous scheduling complexity	add 1 month to GMP readiness [industry range]

The modifiers stack on this program more than on most, and we would rather say so than present a timeline that assumes none of them apply.



The bars show sequence and dependency against the industry planning range above, not committed dates — those are in the proposal. The two supply-chain lanes are drawn from Stage 1 deliberately, because both are measured in months and both sit on the critical path.

13. MADE experience that supports this

On building a process from nothing. Your first stated priority is precisely the case where an integrated development organization contributes more than a manufacturing slot. Our proposal scopes process and analytical development running together rather than sequentially.

On the equipment this program needs. Five CliniMACS Plus units in service at Princeton for CD34–positive selection, alongside fourteen CliniMACS Prodigy units in service across our two sites. Three electroporation platforms in service, which gives the down-selection in our proposed scope real alternatives to compare. Edit measurement by ddPCR on Bio–Rad QX200, of which we hold five in service. A colony counter for the colony-forming unit readout is in service at East Norriton.

On the analytical bench. Beckman Coulter CytoFLEX 13–color flow cytometry, Bio–Rad QX200 ddPCR, Thermo QuantStudio, NucleoCounter NC–200, and multi–mode plate reading. Off–target analysis by sequencing and cytogenetics is outsourced, which is the standard industry position.

On quality systems for a gene–edited IND. MADE's quality and regulatory leadership carries collective experience from prior organizations taking gene–modified cell products through BLA and FDA pre–approval inspection, and regulatory CMC covering forty–plus products through FDA INDs including head–of–investigational–products responsibility at a major cancer center. This is experience held by individuals now at MADE; the company itself has not yet completed a client audit.

On facilities and capacity. Princeton, NJ and East Norriton, PA — Grade B background with Grade A biological safety cabinets for open manipulations, and Grade B / Closed System for closed steps. Our proposal confirmed immediate availability at the New Jersey facility, with resource readiness assessed across personnel, laboratory and cleanroom operations.

14. Guidance and suggestions

Three points, offered as strengthening.

On engraftment potency. Augment the colony-forming unit readout with edit or transgene status resolved at the colony level, as set out in §7a. Inadequate engraftment potency assay design is one of the recurring FDA observations in this modality, and we would rather design for it than answer for it.

On bulk versus subset. Editing efficiency measured across bulk CD34-positive cells can differ significantly from efficiency in the long-term repopulating subset, and bulk overstates the therapeutic dose. Adding a subset-resolved measurement during process development — not necessarily at release — gives you a defensible answer when the question is asked, and the development runs are where that data is cheapest to generate.

On measuring the edit, if the mechanism is correction rather than disruption. Where homology-directed repair is the therapeutic mechanism, the analytical method has to distinguish correct integration from random integration, from repair-mediated insertion, and from large deletions. Primers spanning the repair site can miss large rearrangements entirely — meaning a program can report a clean efficiency number while carrying undetected structural change. This is the most technically demanding analytical point in the program and we would want an explicit conversation about it with your team.

15. Open questions

Decision status — we would ask this first. Has the in vivo versus ex vivo decision been made? Our proposal scopes the ex vivo path, and an in vivo direction would change it substantially.

Mechanism. Is the therapeutic mechanism gene correction or gene disruption? If correction, is a donor template defined and in what format? What editing efficiency is targeted, and on what evidence?

Editing reagents. What is the source and lead time for GMP Cas9 and guide RNA? Is off-target assessment already performed on the current guide, by what method, and how often is it reassessed — per lot, or per process change?

Starting material. Mobilization regimen? Target CD34 purity, recovery and minimum cell dose? Is the collection processed fresh, and what is the time from collection to processing start? **What is the backup collection plan if the first apheresis is insufficient?**

Analytics. Would you support adding edit status resolved at the colony level to the colony-forming unit readout? Is subset-resolved editing efficiency planned during development?

Presentation and logistics. What container closure and fill volume do you intend, and what labeling requirements apply? Which shipper and lane would carry product to the treating site, and is either already qualified?

Regulatory. Are any designations held or planned for this program? What long-term follow-up has been agreed with the agency, given a non-integrating editing approach does not automatically carry the fifteen-year expectation that applies to integrating vectors?

Clinical. Conditioning regimen and its schedule constraints against manufacture? Fresh or cryopreserved product?

16. Suggested next step

A technical call on the three questions that have developed since the 29 July proposal was written: the potency augmentation described in §7a and §14, the subset-resolved editing measurement, and the homology-directed repair analytical point. None of those changes the proposed scope materially; all three strengthen the IND.