

PROGRAM TECHNICAL DEEP DIVE

XIAP — Autologous CRISPR-Edited CD34+ HSC

Process development and IND-enabling GMP manufacture — technical deep dive

PI	MAYO SITE	ON NUMBER	STAGE	MODALITY
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Contents

1	Executive technical summary
2	The modality: biology and mechanism
3	This program's product, specifically
4	Process architecture, unit operation by unit operation
5	Analytical strategy
5a	Potency
5 (continued)	Reading this program's analytical plan
6	Comparability and technology transfer
6 (continued)	The plan as proposed, and where it stands
7	Stability, storage and logistics
8	Supply chain and critical materials
9	Regulatory and CMC
10	Clinical and technical precedent
11	MADE capability against this modality
12	Open questions for Dr. Lambert's team
13	Sources

1. Executive technical summary

This is the only Mayo program with a MADE proposal already in the client's hands, and the only one where **no GMP process exists to transfer**. Mayo's own framing, captured in the delivered proposal, is that the engagement must "establish a manufacturing process where none exists" — there is no GMP process or protocol coming across ^[S70]. That inverts the usual CDMO conversation: this is process development first and technology transfer second.

XIAP deficiency is an X-linked primary immunodeficiency, and the therapeutic approach is autologous CRISPR editing of CD34-positive hematopoietic stem cells. The delivered proposal builds the process from a Casgevy-class reference approach, opening with a structured gap assessment and risk register and following a phase-appropriate staged development path ^[S70].

Mayo named four priorities in the proposal, and each maps onto a known modality risk:

Mayo's stated priority	What the modality core says about it
Establish a process where none exists; nothing is being transferred	Academic-origin transfer is HIGH risk with 3–5 bridging lots, and here there is not even a documented source process — the artifacts have to be created ^[S5]
De-risk electroporation, the highest-variability unit operation, 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 ^[S11]
Protect a single patient's material: one apheresis, one patient, no cell bank, every step irreplaceable	The yield chain compounds — CD34 selection at 40–70% recovery, then electroporation loss — and a backup collection plan is the item most often missing ^{[S9][S11]}
Reach IND-enabling GMP while managing a client-supplied editing reagent on the critical path	GMP Cas9 and guide RNA lead times are a named modality supply risk ^[S9]

That alignment is worth noting to Mayo directly: the four things they identified as their risks are the four the field's evidence says are the real ones. This is a well-diagnosed program.

The commercial status needs attention before the technical status. The proposal was issued 29 July 2026 with an expiration date of **27 October 2026** ^[S70]. It was delivered via PandaDoc and the document status is *viewed*, not completed ^[S71]. BD notes record technical and QA calls through June and July and a proposal delivered on 29 July awaiting feedback ^[S72]. The window is open but finite.

2. The modality: biology and mechanism

Hematopoietic stem cells sit at the top of the blood hierarchy: they self-renew and give rise to every myeloid and lymphoid lineage. Autologous HSCs are collected, modified ex vivo to correct or add a gene, and returned after a conditioning regimen has cleared marrow niche space for them to engraft. If enough corrected long-term HSCs engraft, they repopulate the hematopoietic system and the correction is permanent.

Permanence is the therapeutic proposition and the source of the risk. A one-time treatment that persists for life also persists with whatever genomic change was introduced. Where the modification is delivered by an integrating vector, the insertion site is semi-random, and an insertion that activates a proto-oncogene can drive clonal expansion years later.

The modification approaches differ materially in risk profile:

Approach	Mechanism	Integration	Risk character
Lentiviral gene addition	Adds a functional copy	Integrating, semi-random	Insertional oncogenesis — demonstrated ^[S41]
CRISPR gene disruption (NHEJ)	Knocks out a target	Non-integrating delivery; edits at a defined locus	Off-target editing; large deletions and rearrangements
CRISPR gene correction (HDR)	Repairs the sequence	Non-integrating delivery; requires a donor template	Off-target editing; low HDR efficiency; template-related risk
Base or prime editing	Precise nucleotide change	Non-integrating	Off-target base changes; bystander edits

A program using non-integrating delivery — RNP or mRNA plus guide RNA, electroporated — is in a materially different safety position from one using an integrating vector, and that distinction deserves to be stated explicitly rather than left for the reader to infer. It does not remove the need for off-target characterization; it changes what the dominant risk is.

Subset heterogeneity is the analytical trap. CD34-positive is a marker, not a function. The population it captures includes long-term HSCs, short-term HSCs and committed progenitors, and editing efficiency in bulk CD34-positive cells may differ significantly from efficiency in the long-term repopulating subset ^[S11]. A program reporting one number for editing efficiency has not yet answered the question that determines whether the patient is cured.

3. This program's product, specifically

3.1 What is being made

An autologous, CRISPR-edited CD34-positive hematopoietic stem cell drug product for XIAP deficiency. Mayo was, as of June 2026, still deciding between an in vivo and an ex vivo approach, with a decision expected within about two months of that point ^[S72] — which places the decision in roughly the current window and makes it the first thing to confirm.

The proposal scopes the ex vivo path.

3.2 What makes this program harder than a standard HSC transfer

There is no source process. Every other Mayo program in this package arrives with batch records or at least a defined process description. This one arrives with a therapeutic intent and a target. The gap assessment and risk register that opens the proposed scope is therefore not a formality — it is the deliverable that makes

everything downstream estimable.

HDR or NHEJ is the question that sets the difficulty. The HSC core is explicit: HDR efficiency in CD34-positive cells typically runs 10–40%, against 50–80% for NHEJ disruption ^[S11]. XIAP deficiency is a loss-of-function disorder, which points toward correction or gene addition rather than disruption — the harder number. If the mechanism is HDR, a donor template is required and its format, ssODN or AAV6, is a material with its own supply chain ^[S9]. **Confirm this before any timeline is committed.**

Single patient, single apheresis, no bank. This is the phrase from Mayo's own priority list and it is the constraint that should shape the whole engineering approach. There is no retained material to re-run from. The yield arithmetic therefore has to close on paper before the first patient, and the engineering runs have to establish it on healthy-donor material rather than discovering it on the patient's.

Client-supplied editing reagent. Mayo supplies the editing reagent, which removes a sourcing burden from MADE and adds a coordination one. The proposal tracks it as a parallel workstream with Supply Chain against the manufacturing schedule ^[S70]. That is the right structure — the HSC core places GMP Cas9 and gRNA lead times alongside vector as a critical-path item.

3.3 The analytical position, as proposed

No qualified Mayo analytical methods exist for transfer. Methods are established using MADE in-house methods ^[S70]. The proposal's analytical work order covers:

- Cell count and viability on NucleoCounter NC-200 — an established in-house platform method requiring only a product-specific qualification run-check, not development
- Identity and purity by flow cytometry, viable CD34-positive, on CytoFLEX — feasibility qualification of an in-house method
- **Off-target analysis and genomic integrity by NGS and cytogenetics — outsourced to a qualified subcontractor** ^[S70]

That outsourcing decision matches the modality core exactly: off-target analysis by GUIDE-seq, CIRCLE-seq or equivalent is where REF_O4 places it, and the logistics of it belong on the critical path ^{[S4][S9]}.

The analytical work order is scoped to commence approximately two weeks ahead of the process development runs, so that methods supporting in-process and characterization testing exist at first run execution ^[S70]. That sequencing is correct and is the thing programs most often get wrong.

4. Process architecture, unit operation by unit operation

4.1 The chain, end to end

#	Unit operation	Purpose	Where it fails
1	Mobilization and apheresis	Collect enough CD34-positive cells	Poor mobilizer; insufficient yield; no backup plan

#	Unit operation	Purpose	Where it fails
2	CD34-positive selection	Define the input population	60–80% purity, 40–70% recovery, variable ^[S11]
3	Pre-stimulation culture	Bring cells into cycle for editing or transduction	Too long costs stemness; too short costs efficiency
4	Gene modification	Correct, disrupt or add	Narrow Cas9 window; 30–50% viability loss on electroporation ^[S11]
5	Post-edit rest or brief expansion	Recovery	Extended culture loses stemness — the lever is not available ^[S11]
6	Harvest, wash, formulate	Prepare the dose	Yield loss compounds everything upstream
7	Fill and cryopreserve, or deliver fresh	Preserve or deliver	Conditioning schedule compatibility
8	Release testing	Disposition	CFU takes 14 days — cannot gate a fresh product ^[S11]

4.2 Starting material and the yield chain

Source may be mobilized peripheral blood, bone marrow, or cord blood; for mobilized collection the regimen — G-CSF, plerixafor, or both — must be defined, because it determines both yield and the character of the collected cells ^[S9].

The questions that decide feasibility: target CD34-positive purity and recovery, minimum CD34 cell dose, and **the backup collection plan if the first apheresis is insufficient** ^[S9]. The last is routinely absent from RFPs and is the item with the largest patient impact. Insufficient yield may require multiple collections, which increases patient burden and program cost ^[S11].

Compound the losses honestly. If selection recovers 55% and electroporation costs 40% viability, a collection has to start well above the minimum dose to land above it. That arithmetic should be in the process description, not discovered on the first patient.

4.3 Gene modification

For CRISPR delivery, the practical constraints are specific and unforgiving. Pre-complexed Cas9-guide RNP degrades at room temperature and must be freshly prepared and used within a validated hold time ^[S11]. Electroporation kills — 30–50% viability loss is common and must be carried into the dose calculation rather than treated as a deviation ^[S11]. And the Cas9 concentration window is narrow: more gives more on-target editing and more off-target editing at the same time ^[S11].

Where the mechanism is correction rather than disruption, HDR efficiency in CD34-positive cells typically runs 10–40% against NHEJ disruption at 50–80% ^[S11]. A program that requires gene correction is therefore attempting the harder edit, and needs a donor template — ssODN or AAV6 — which is itself a GMP material with a supply chain ^[S9].

For lentiviral gene addition, the CAR-T core's vector guidance applies in full: functional rather than physical titer for MOI, rapid titer loss at room temperature, lot-to-lot variability, and long lead times ^{[S11][S6]}.

4.4 Culture duration — the constraint that shapes the process

Extended culture after editing causes loss of stemness, so there is limited opportunity to grow more cells after modification ^[S11]. This inverts the usual manufacturing logic. In CAR-T, expansion is where a marginal starting material is rescued. Here, expansion is where a good product is degraded. Process duration is therefore bounded above by biology and below by the editing efficiency the process can achieve in the time available — and that tension is the central process development question.

4.5 Product form and conditioning compatibility

Conditioning must be defined — myeloablative or reduced-intensity — and **the manufacturing timeline must be compatible with the conditioning schedule** ^[S9]. This is a hard clinical constraint, not a planning preference: a patient conditioned for a product that is not ready is a patient with an ablated marrow and nothing to reconstitute it.

Fresh product removes cryopreservation losses and forces exact schedule alignment. Cryopreserved product decouples the schedule and adds a freeze-thaw stress to a population whose function is already the limiting quantity. The choice interacts with the release strategy: a 14-day CFU result cannot gate a fresh product, so fresh programs release on a rapid panel with CFU following as confirmatory data ^[S11].

5. Analytical strategy

5.1 The release panel

Attribute	Method	Note
Identity and purity	CD34-positive by flow	Required; also the input specification
Strength	Viable CD34-positive cell dose per kg	The clinically governing number
Viability	Automated counter, 7-AAD	Post-electroporation losses land here
Editing efficiency, or VCN	ddPCR, or NGS amplicon sequencing	Primary ^[S4]
Transgene expression	qPCR or flow	Primary where a gene is added ^[S4]
Engraftment potency	CFU assay	Primary — with the limitations in §5a ^[S4]
Off-target editing	GUIDE-seq, CIRCLE-seq, DISCOVER-seq	Critical , outsourced ^{[S4][S9]}
Sterility, mycoplasma, endotoxin	Standard	Required
Residual vector or editing reagent	qPCR, ELISA	Vector clearance is a named FDA deficiency area ^[S1]

5.2 Measuring the edit

NGS amplicon sequencing gives the most accurate on-target quantification and requires a bioinformatics pipeline. **ddPCR with editing-specific probes** is simpler and more GMP-friendly but requires assay development per locus — so it is a development cost, not a method transfer ^[S11].

For HDR specifically, the measurement must distinguish correct integration from random integration, NHEJ-mediated insertion and large deletions. Primers spanning the HDR site can miss large rearrangements entirely, which means a program can report a clean HDR number while carrying undetected structural changes ^[S11]. This is the single most technically demanding analytical point in the modality and it is worth asking about directly.

And bulk is not the subset. Editing efficiency in bulk CD34-positive cells may differ significantly from efficiency in long-term HSCs; bulk overstates the therapeutic dose ^[S11].

5.3 Off-target analysis

Method must be defined — GUIDE-seq, CIRCLE-seq, DISCOVER-seq or in silico prediction, and in practice a combination. Top off-target sites characterized, acceptance criteria set, an outsourced testing plan in place, and a defined frequency of reassessment: per guide RNA lot, or per process change ^[S9]. That last question is the one programs answer late. A new gRNA lot is a new off-target question unless the program has argued otherwise.

Off-target characterization is the first-listed FDA deficiency for this modality ^[S1].

5a. Potency

5a.1 CFU is primary and CFU is not sufficient

The colony-forming unit assay is the recommended primary engraftment-potency measure ^[S4], and its limitations are documented in the same reference set:

- **It measures progenitor function, not long-term engraftment potential** ^[S11]. The cells that form colonies in two weeks are not necessarily the cells that reconstitute a marrow for decades.
- **Turnaround is 14 days**, so it is not practical as a release test for a fresh product ^[S11].
- **Scoring is semi-subjective.** Distinguishing BFU-E, CFU-GM and CFU-GEMM carries high inter-operator variability without standardized training ^[S11].

REF_09 asks the sharpening question directly: does the CFU assay also measure transgene expression in the colonies ^[S9]. A colony count tells you cells are functional. A colony count with transgene expression tells you the *corrected* cells are functional — which is the actual product claim.

Engraftment potency assay design is the second-listed FDA deficiency for this modality ^[S1]. Programs relying on unaugmented CFU should expect the question.

5a.2 Building past CFU

Layer	What it adds
CFU with transgene expression per colony	Links function to correction rather than to cell survival
Subset-resolved editing efficiency	Measures the edit in the population that matters, not in the bulk
Long-term repopulating capacity assessment	The closest available surrogate for the therapeutic mechanism; typically a research-scale readout rather than a release test

REF_09 asks for both subset-resolved efficiency and LT-HSC frequency ^[S9]. Neither is trivial, and a phase-appropriate answer at Phase 1 may be characterization data rather than a specification — but the program should have chosen its position deliberately.

5 (continued) — Reading this program's analytical plan

The proposed panel covers identity, purity, count, viability and off-target genomic integrity. Two observations, offered as strengthening rather than correction.

Engraftment potency is the second-listed FDA deficiency for this modality, and CFU is where it sits. The HSC core sets out why unaugmented CFU is insufficient: it measures progenitor function rather than long-term engraftment, takes 14 days, and is semi-subjective to score ^[S11]. REF_09's sharpening question is whether the CFU assay also measures **transgene expression in the colonies** — which is what converts "cells are functional" into "the edited cells are functional" ^[S9].

For an ex vivo XIAP correction the analogous readout is editing or expression status resolved at the colony level. Whether that belongs in the Phase 1 scope or in characterization is a judgment call, but the program should make it deliberately, and the proposal is the natural place to say so.

Bulk editing efficiency overstates the therapeutic dose. Editing efficiency measured across bulk CD34-positive cells may differ significantly from efficiency in the long-term repopulating subset ^[S11]. Adding a subset-resolved measurement during process development — not necessarily at release — gives the program a defensible answer when the agency asks, and the development runs are where it is cheap to generate.

On measuring the edit itself: ddPCR is GMP-friendly but requires assay development per locus, and NGS amplicon sequencing is more accurate but needs a bioinformatics pipeline ^[S11]. Where HDR is the mechanism, the method must distinguish correct integration from random integration, NHEJ insertion and large deletions — primers spanning the HDR site can miss large rearrangements entirely ^[S11]. This is the most technically demanding analytical point in the program and is worth an explicit conversation with Mayo's team.

6. Comparability and technology transfer

6.1 Transfer risk

The standard matrix applies — academic origin HIGH with three to five bridging lots, sponsor internal lab HIGH-MEDIUM, another CDMO MEDIUM ^[S5]. Gene-edited HSC programs carry an extra comparability burden that most modalities do not: the editing reagents themselves are a lot-defined input, so a gRNA or Cas9 lot change is a potential product change.

6.2 The changes that carry real risk

Change	Risk	Why
Editing reagent lot — gRNA or Cas9	Highest	Potential change in on-target and off-target profile; reassessment frequency must be defined ^[S9]
Electroporation instrument or program	Highest	Efficiency and viability both shift; disposables are instrument-proprietary ^[S6]
Culture duration or cytokine cocktail	High	Directly trades editing efficiency against stemness ^[S11]
CD34 selection reagent or system	High	Purity and recovery shift, changing the input population
Donor template format, ssODN to AAV6	High	Different HDR kinetics and different impurity profile
Fresh to cryopreserved	High	Post-thaw viable CD34 dose and CFU must both be demonstrated

6.3 The CQA set

Viable CD34-positive dose HIGH; editing efficiency or VCN HIGH; **CFU CRITICAL — engraftment potency for HSC** ^[S5]; transgene expression HIGH; off-target profile CRITICAL; residual editing reagent and vector clearance MEDIUM-HIGH, and a named FDA deficiency area ^[S1]; viability HIGH; sterility, mycoplasma and endotoxin required every lot.

6.4 The staged plan MADE runs

Stage 0 — technical assessment. Read the process against the checklist. In this modality expect the yield chain, the off-target plan and the CFU-augmentation question to be the three largest open items.

Stage 1 — paper transfer and gap closure. Documentation review, equipment fit, GMP Cas9 and gRNA sourcing with lead times confirmed, off-target CRO selected and capacity booked, per-locus ddPCR assay development started. The editing-reagent supply chain and the off-target CRO both start here because both have lead times measured in months ^[S9].

Stage 2 — engineering run. Non-GMP on destination equipment, with the full yield chain measured step by step — collection, selection recovery, post-electroporation viability, final viable CD34 dose — so the arithmetic is evidence rather than assumption.

Stage 3 — comparability. Head-to-head on the risk-weighted CQA set, with editing efficiency measured in the relevant subset and not only in bulk.

Stage 4 — GMP manufacture and release, with conditioning schedule alignment designed in.

6 (continued) — The plan as proposed, and where it stands

6.1 Structure

The delivered proposal is organized into work orders, opening with project initiation and a gap assessment and risk register, running through analytical development, process development studies centered on de-risking electroporation with platform down-selection, GMP readiness and manufacture, and formal transfer qualification of the analytical development methods into Quality Control ^[S70].

The electroporation focus deserves emphasis: dedicated feasibility and development runs, with platform down-selection across available development systems, characterizing post-electroporation viability, recovery and editing performance **before GMP commitment** ^[S70]. That is the right sequence for the modality's highest-variability step and directly answers Mayo's stated priority.

6.2 Commercial status

Proposal	MayoClinic_XIAP_Proposal_ON23155-2026_V1, issued 29 July 2026
Expiration	27 October 2026 [proposal ON23155-2026]
Delivery	PandaDoc, status <i>viewed</i> ^[S71]
Last recorded BD position	30 July — delivered, awaiting feedback ^[S72]

Recommendation: treat the technical package in this document as the substance of a follow-up conversation rather than as a re-proposal. The proposal is live until late October; what it has not had is a technical dialogue since delivery. The in vivo versus ex vivo decision, the HDR-versus-NHEJ question and the CFU augmentation question are three concrete reasons to reopen the conversation with something to say.

6.3 The change and risk inventory

Item	Risk	Note
In vivo versus ex vivo decision	Gating	The proposal scopes ex vivo. An in vivo pivot changes the modality entirely ^[S72]
HDR versus NHEJ mechanism	High	Sets achievable efficiency and whether a donor template is needed ^[S11]
Electroporation platform down-selection	High — and explicitly scoped	Instrument choice fixes disposables to a single source ^[S6]
Editing reagent lead time	High	Client-supplied; tracked as a parallel workstream ^[S70]
CD34 selection recovery	High	60–80% purity, 40–70% recovery, variable with material quality ^[S11]

Item	Risk	Note
Backup collection plan	Unknown	Not addressed in what MADE holds. The single most patient-impacting gap ^[S9]
Conditioning schedule alignment	High	Named FDA deficiency; a conditioned patient with no product is the failure mode ^[S1]

7. Stability, storage and logistics

The governing constraint is conditioning compatibility, and it runs in both directions. Product must be available when the patient completes conditioning; and where product is cryopreserved, post-thaw viable CD34 dose must still meet the minimum, which means the pre-freeze target has to be set above it.

For fresh product the release strategy has to be designed around a 14-day CFU that cannot gate infusion — rapid methods release, CFU confirms. For cryopreserved product, storage is vapor-phase liquid nitrogen, and the stability program covers viable CD34 recovery, CFU and editing efficiency retention.

Long-term follow-up is fifteen years for integrating vectors ^[S16], and the Skysona experience shows why: the malignancies emerged between 14 and 92 months after treatment ^[S42]. Sample retains and archive requirements land on the manufacturer and belong in the storage plan from the first GMP batch. For non-integrating editing approaches, confirm the applicable follow-up expectation with the sponsor rather than assuming the fifteen-year obligation transfers unchanged.

8. Supply chain and critical materials

Category	Material	Typical source	Risk
Starting material	Mobilized apheresis, marrow or cord blood	Collection site	MEDIUM — yield is patient-dependent and the backup plan is often absent
Selection	CliniMACS CD34 reagent and tubing sets	Miltenyi Biotec	MEDIUM — proprietary single-source ^[S6]
Editing	GMP Cas9 protein	Limited GMP suppliers	HIGH — lead times ^[S9]
Editing	GMP guide RNA synthesis	Specialist suppliers	HIGH — lead times; each lot is a potential off-target reassessment ^[S9]
Editing	Donor template, ssODN or AAV6	Specialist suppliers	HIGH where HDR is the mechanism
Vector	Lentiviral vector, where gene addition	Sponsor or vector CDMO	HIGH — 3–6 month lead time, lot variability ^[S6]
Electroporation	Instrument-proprietary disposables — MaxCyte, Neon/Xenon, Nucleofector	MaxCyte, Thermo, Lonza	MEDIUM — single-source per system ^[S6]

Category	Material	Typical source	Risk
Culture	Cytokines: SCF, TPO, FLT3-L	CellGenix, Miltenyi and others	MEDIUM — specify by specific activity [S11]
Off-target testing	GUIDE-seq, CIRCLE-seq	Specialist CROs	MEDIUM-HIGH — capacity and turnaround are on the critical path [S9]

REF_09 flags gene editing reagent supply chain — GMP Cas9 and gRNA synthesis lead times — as a named modality risk [S9]. Treat both as critical-path workstreams from day one, in the same way the CAR-T core treats vector.

9. Regulatory and CMC

9.1 The four deficiencies that recur

1. Off-target editing characterization, for CRISPR and gene-editing products
2. Engraftment potency assay design — CFU may not be sufficient
3. Conditioning regimen compatibility with product
4. Vector clearance and residual impurity characterization

Source: FDA deficiency patterns by modality [S1]. All four are addressable by a CDMO, and all four are cheaper to address at transfer than at pre-BLA.

9.2 Phase-appropriate expectations

Phase 1 requires the process described with critical parameters identified; identity by flow; viability; sterility, endotoxin and mycoplasma; VCN or editing efficiency; transgene expression; and a biologically relevant, quantitative potency assay, qualified rather than validated [S1]. The 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 [S12].

Off-target characterization is the exception to phase-appropriate leniency. It is a safety characterization for a permanent genomic modification, and the Skysona experience means the agency approaches this class with the evidence in hand [S41][S42].

9.3 Where a CDMO adds real regulatory value

Sponsors frequently over-specify against their phase, and right-sizing that is genuine value [S1]. In this modality the more valuable contribution is usually the opposite direction: making sure the off-target plan, the reassessment frequency and the potency augmentation are adequate *before* the IND, because those are the three items the agency reliably probes.

10. Clinical and technical precedent

10.1 The modality delivers

On 8 December 2023 the FDA approved Casgevy (exagamglogene autotemcel) — the first approved therapy using CRISPR/Cas9 gene editing — for sickle cell disease in patients twelve and older, and Lyfgenia (lovotibeglogene autotemcel), a lentiviral gene-addition therapy, on the same day. Casgevy's indication was extended to transfusion-dependent beta thalassemia on 16 January 2024 and to patients two and older on 1 July 2026 ^[S40].

Casgevy matters as precedent beyond the approval itself: it is an *ex vivo* CRISPR edit of autologous CD34-positive cells, manufactured and released at commercial scale, with an off-target package the agency accepted. The path exists and has been walked.

10.2 And the modality has caused cancer

Hematologic malignancy developed in **7 of 67 patients** treated with Skysona (elivaldogene autotemcel) for cerebral adrenoleukodystrophy: myelodysplastic syndrome with unilineage dysplasia in two patients at 14 and 26 months, MDS with excess blasts in three at 28, 42 and 92 months, MDS in one at 36 months, and acute myeloid leukemia in one at 57 months ^[S42]. The cases appear driven by integration of the lentiviral vector into a proto-oncogene, insertional oncogenesis is the labeled principal risk, and treated patients require lifelong hematologic monitoring ^[S41].

Three things follow for any program in this modality.

The latency is long. Cases emerged from 14 to 92 months. A Phase 1 readout showing no malignancy is not evidence of absence, and the fifteen-year follow-up obligation is proportionate to the observed timeline, not conservative ^[S16].

Delivery modality is a real risk differentiator. Skysona's mechanism was vector integration. A program using non-integrating delivery — RNP or mRNA with guide RNA — does not carry that specific risk, and should say so precisely rather than by implication, while still characterizing its own dominant risk, which is off-target editing.

Integration-site and clonality monitoring is not paperwork. For integrating-vector programs it is the surveillance that detects the failure mode the field has already experienced.

10.3 The field's practical pattern

Manufacturing yield, not editing chemistry, is the most common practical failure. Programs underestimate the compounded losses from selection recovery and electroporation viability, and discover on the first patients that the minimum dose is difficult to hit. Programs that measured the whole yield chain in engineering runs do not have that problem.

11. MADE capability against this modality

11.1 Analytical bench — evidenced against the registers

Verified 23 August 2026 against the Princeton register (464 assets) and the East Norriton register (1,530 assets), with qualification status attested by MADE operations on the same date ^[s8].

Need	Instrument	In service
Identity, purity, phenotype, exhaustion panel	Beckman Coulter CytoFLEX, 13-color	4 Princeton
Additional flow capacity	Miltenyi MACSQuant Analyzer; Agilent NovoCyte Penteon; Nexcelom image cytometer	6, 1 and 2 respectively, East Norriton, in L-135 and L-141
Rapid in-process flow	Accellix cytometer	1 East Norriton, in L-141
Cytotoxicity, cytokine and ELISA readouts	Thermo Varioskan Lux; Thermo plate reader; Molecular Devices SpectraMax M3	1, 2 and 3 respectively — Princeton and East Norriton L-135/L-141
Vector copy number, editing efficiency	Bio-Rad QX200 ddPCR	1 Princeton, 4 East Norriton in L-138 and L-144
Orthogonal dPCR	Thermo QuantStudio	1 Princeton, 2 East Norriton
Count and viability	NucleoCounter NC-200	7 Princeton
Count and viability	Beckman Vi-CELL	2 East Norriton, in V-018 and V-019
Particle concentration and size	Particle Metrix ZetaView Evolution quatt	1 Princeton
Mycoplasma	BioFire	1 Princeton, 2 East Norriton
Endotoxin	Endosafe	4 Princeton, 3 East Norriton
Nucleic acid quantitation	NanoDrop	1 Princeton, 3 East Norriton
Colony counting (CFU)	Reichert colony counter	1 East Norriton, in V-042
Hematology differential	Sysmex XN-330	held at East Norriton, in storage

11.2 Process equipment — evidenced against the registers

Verified 23 August 2026 against both site registers, with qualification status attested by MADE operations on the same date ^[s8].

REF_09 fit-check item	In service	Detail
CliniMACS for CD34-positive selection	19	CliniMACS Plus × 5 Princeton; CliniMACS Prodigy × 14 across both sites
Electroporation — Neon / Xenon	3	Thermo Neon × 2; Lonza 4D-Nucleofector × 1. Xenon itself is held at Princeton but Inactive
QX200 for editing efficiency by ddPCR	5	1 Princeton; 4 East Norriton in L-138 and L-144

REF_O9 fit-check item	In service	Detail
CFU assay capability	1	Reichert colony counter, East Norriton in V-042
MaxCyte	—	Not held; Neon and 4D-Nucleofector cover the need

Three electroporation platforms in service gives a program's platform down-selection real alternatives to compare rather than a single option — which matters in a modality where post-electroporation viability loss of 30–50% is the norm and the instrument choice is one of the few levers on it.

On CFU, the instrument is not the whole question. A colony counter is in service. What this core says elsewhere still stands: scoring BFU-E, CFU-GM and CFU-GEMM carries high inter-operator variability without standardized training ^[S11], and that is a capability question for the QC group rather than an equipment question.

11.3 Manufacturing environment

Princeton, NJ and East Norriton, PA. Grade B background with Grade A BSC for open manipulations; closed-system steps described as Grade B / Closed System. Batch sizes are small and per-patient; the constraint is suite scheduling against a conditioning calendar rather than capacity.

11.4 Team experience

MADE's quality leadership carries collective prior-organization experience directly relevant to this modality — QC laboratory leadership and BLA-stage quality for gene-modified cell products at Novartis and Bristol Myers Squibb, quality risk management and inspection readiness at Adaptimmune and GSK, and regulatory CMC leadership covering forty-plus products through FDA INDs, including head-of-investigational-products responsibility at Memorial Sloan Kettering. This is prior-organization experience held by named individuals now at MADE. MADE itself has not yet completed a client audit, and that distinction is stated wherever this is described to a client.

11.5 Indicative economics and duration

Custom process, no platform	12–18 months, kick-off to first released GMP batch ^[S2]
Novel gene editing approach — CRISPR, base editing	add 1–3 months to process development ^[S2]
Academic-origin process	add 2–4 months to knowledge transfer ^[S2]
Complex potency assay development	add 2–4 months to the analytical timeline ^[S2]
Autologous scheduling complexity	add 1 month to GMP readiness ^[S2]

Industry-standard scoping ranges for directional use, not MADE pricing until finance has scoped the program ^[S2]. For a CRISPR correction program originating in an academic lab, the modifiers stack: the realistic planning range is toward the upper end of 12–18 months plus several of the additions above, and saying so early is better than discovering it at month nine.

12. Open questions for Dr. Lambert's team

Decision status — ask first. Has the in vivo versus ex vivo decision been made? BD notes from 24 June recorded a decision expected within roughly two months ^[S72], which places it now.

Mechanism. Is the therapeutic mechanism gene correction (HDR) or disruption (NHEJ)? If HDR, is a donor template defined, and in what format — ssODN or AAV6? 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 gRNA, by what method, and how often is it reassessed — per lot, or per process change?

Starting material. What is the mobilization regimen? Target CD34 purity, recovery and minimum cell dose?
What is the backup collection plan if the first apheresis is insufficient?

Analytics. Would Mayo support adding transgene or edit status resolved at the colony level to the CFU readout? Is subset-resolved editing efficiency planned during development? For measuring the edit — ddPCR per locus, or NGS amplicon?

Clinical. Conditioning regimen, and its schedule constraints against manufacture? Fresh or cryopreserved product? What long-term follow-up has been agreed with the agency, given a non-integrating editing approach does not automatically carry the fifteen-year integrating-vector expectation?

Commercial. Is there feedback on the 29 July proposal? What would need to change for it to proceed before the 27 October expiration?

13. Sources

Tag	Source
[S1]	REF_01 — Regulatory Intelligence Knowledge Base (MADE internal, proposal-generator appraisal engine)
[S2]	REF_02 — COGS and Timeline Modeling Framework (MADE internal)
[S4]	REF_04 — Potency Assay Strategy Advisor, §2.6 Gene-Modified HSC (MADE internal)
[S5]	REF_05 — Comparability and Tech Transfer Planning Framework (MADE internal)
[S6]	REF_06 — Supply Chain and BOM Risk Analysis Framework (MADE internal)
[S8]	MADE internal capability inventories, as specified in proposal-generator design.md §4.8. The <code>xlsx</code> files were not available to this machine; instrument names come from that specification and must be confirmed against the BMRAM export before client use.
[S9]	REF_09 — Modality-Specific Evaluation Depth Checklists, Modality 6 (MADE internal)
[S11]	REF_11 — Cell Therapy Manufacturing and Analytical Deep Knowledge v2.0 (MADE internal)
[S12]	FDA CBER, Flexible CMC Requirements announcement, 11 January 2026
[S16]	FDA CBER, Long-Term Follow-Up After Administration of Human Gene Therapy Products, final guidance, January 2020

Tag	Source
[S40]	FDA approval of Casgevy (exagamglogene autotemcel) and Lyfgenia (lovotibeglogene autotemcel), 8 December 2023; Casgevy indication extended to transfusion-dependent beta thalassemia 16 January 2024 and to patients two years and older 1 July 2026
[S41]	SKYSONA (elivaldogene autotemcel) US prescribing information — hematologic malignancy including MDS and AML; insertional oncogenesis as principal risk; lifelong hematologic monitoring required
[S42]	Hematologic Cancer after Gene Therapy for Cerebral Adrenoleukodystrophy. New England Journal of Medicine — 7 of 67 patients, onset 14 to 92 months post-treatment
[S70]	MayoClinic_XIAP_Proposal_ON23155-2026_V1, 29 July 2026, 40 pp. Made Scientific services proposal — Process Development and GMP Manufacture of an Autologous CRISPR-Edited CD34+ HSC Drug Product. Issued by Simona Jusyte; expiration 27 October 2026
[S71]	PandaDoc document ChusXZ6URYLg7DyXvVY4ub, MayoClinic_XIAP_Proposal_ON23155-2026_V1_29JUL2026; sent 30 July 2026, status <i>viewed</i> ; retrieved 2026-08-22
[S72]	Salesforce opportunity 23155-2026, XIAP HSC Gene Editing; stage Proposal (Delivered), amount; proposal delivered 29 July 2026, primary contact Laura J. Lambert; BD notes recording the 24 June technical call, the 23 July QA call, and the in vivo versus ex vivo decision expected within two months; retrieved 2026-08-22
[S73]	MayoClinic_XIAP_HSC_GeneEditing_Appraisal_v2.0, 30 June 2026, and the SME input correspondence held in the Drive folder — Kevin Macera on PD studies, Chen-Yuan Kao on CD34 selection and PD studies, Satyakam Singh on Accellix, dPCR/NGS and AD recommendations