

Stage-2 Model Explainer for Medical and Translational Researchers

Dual-Platform mRNA Therapy in X-Linked ALD
(LNP + EV, Peripheral-First \rightarrow CNS)

Method2Model Framework

Why This Document Exists

This explainer is written *for medical and translational researchers* who do **not** need to read every equation in the Stage-2 Formula Pack, but still need to understand:

- What this model does in practical terms.
- What questions each model block answers.
- What the input and output files mean at the protocol level.
- How Stage-2 protects the trial from fragile assumptions.

If you are a PI, clinical scientist, pharmacologist, or CMC lead, this document is intended as your “front door” to the model. The detailed equations live in:

- **Stage-2 Formula Pack:** full mathematical formulation.
- **Stage-2 I/O Contract:** technical file schemas.

You do not need to be a mathematician to use this model *as a decision tool*. This explainer keeps equations to a minimum and focuses on how the pieces fit together and what they mean in practice.

1 Big Picture: What the Model Is For

The model represents a dual-platform mRNA therapy for X-linked adrenoleukodystrophy (ALD), with:

- **Two delivery platforms:** lipid nanoparticles (LNP) and extracellular vesicles (EV).
- **Two targets:** peripheral tissues and central nervous system (CNS).
- **Key disease axis:** how restoring ALDP (peroxisomal ABCD1) affects VLCFA levels, neuroinflammation, axonal injury, and clinical markers (NfL, Loes).

Stage-2 answers questions such as:

- “If we change dose, schedule, or FUS settings, how does that change CNS exposure and downstream biomarkers?”

- “Given realistic uncertainty and CMC variability, how often does the design meet our efficacy and safety criteria?”
- “Where are we fragile to assumptions (e.g. CNS delivery, microglial activation, batch-to-batch potency)?”

The outputs are *decision-ready*, not just curves: they include probabilities of success and explicit Go/No-Go/Pivot regions.

2 How the Model Is Structured (Blocks A–G)

The model is organised into seven blocks. You can think of them as modules in a pipeline.

Block A: Delivery & Exposure (PK / Distribution)

Clinical question: *Where do the LNP and EV actually go in the body and CNS, over time, for a given dose, route, and FUS protocol?*

- Inputs:
 - Dose level and schedule (per kg, per route).
 - Whether FUS is used, at what time, and for how long.
 - Basic physiology (volumes, blood flows).
 - Batch-level CMC information (encapsulation, size, potency).
- Outputs:
 - Time-course of LNP and EV exposure in plasma, liver, periphery, and CNS.
 - How much cargo each cell type (hepatocytes, oligodendrocytes, etc.) actually internalises.

If you ask: “How much mRNA reaches the CNS under this protocol compared to peripheral tissues?” — you are asking Block A.

Block B: Expression & Peroxisomal Targeting

Biological question: *Given the delivered mRNA, how much functional ALDP is produced in cells, how much reaches peroxisomes, and how much is mislocalized?*

- Inputs:
 - Cellular uptake over time from Block A.
 - Assumptions about endosomal escape and translation efficiency.
 - Parameters for peroxisomal insertion and PEX capacity.
- Outputs:
 - Time-course of ABCD1 mRNA per cell.
 - Total ALDP protein, peroxisomal ALDP, mislocalized ALDP.
 - Effective peroxisomal β -oxidation capacity $V_{\beta}(t)$.

If you ask: “Does this dosing strategy plausibly restore peroxisomal function to near wild-type levels in relevant cells?” — you are asking Block B.

Block C: Disease / Pharmacodynamics

Block C has two branches: **mouse** and **human-projected**.

Mouse branch. Preclinical question: *In Abcd1-deficient mice, how does changing ALDP capacity change VLCFA levels, inflammation, axonal injury, and behavioural readouts?*

- Inputs: $V_{\beta}(t)$ from Block B (mouse parameters), baseline disease state (phenotype and age).
- Outputs:
 - Plasma and CNS VLCFA trajectories.
 - CNS inflammation and axonal injury over time.
 - Mouse NfL, DTI-FA, and Rotarod performance as modelled readouts.

Human-projected branch. Translational question: *If we achieved similar ALDP restoration in humans, what might happen to VLCFA, microglial activation, axonal injury, NfL, and Loes score over a clinically relevant horizon?*

- Inputs: CNS ALDP restoration (human), phenotype, and baseline Loes/NfL values.
- Outputs:
 - Predicted evolution of CNS VLCFA.
 - Microglial activation and axonal injury (projection).
 - NfL trajectory and Loes progression.

If you ask: “Under this regimen, do we expect meaningful slowing of Loes progression in early cALD?” — you are asking Block C (human).

Block D: Safety & Immunogenicity

Safety question: *Given exposure and disease context, are we likely to trigger unacceptable cytokine storms, liver enzyme elevations, or anti-drug antibodies?*

- Inputs:
 - Exposure from Block A (especially in liver and immune organs).
 - Disease burden from Block C (VLCFA, inflammation).
 - Dose schedule and repeat dosing pattern.
- Outputs:
 - Time-course of cytokine response.
 - ALT/AST dynamics as markers of hepatic stress.
 - ADA formation against LNP and EV.

This block supports early safety boundary-setting before actual animals or patients are exposed.

Block E: CMC / Batch-to-Batch Variability

CMC question: *How do realistic batch-to-batch differences (encapsulation, particle size, potency) affect effective dose and endosomal escape?*

- Inputs: CMC metrics per batch (encapsulation efficiency, size, PDI, potency, impurities).
- Outputs:
 - Effective potency factors applied to each batch.
 - Adjusted endosomal escape efficiencies and related parameters.

This block makes CMC part of the model, not a separate afterthought.

Block F: Uncertainty & Scenario Engine

Risk question: *How robust are our conclusions when key parameters are uncertain or pessimistic scenarios occur?*

- Inputs:
 - Priors for uncertain parameters (PK, PD, safety, CMC).
 - Scenario definitions (e.g. “CNS delivery-limited”, “immune-sensitive repeat dosing”).
- Outputs:
 - Distributions of outcomes (not just single trajectories).
 - For each design and scenario: probability of meeting efficacy and safety criteria.

This is where the model stops being a “best guess” and becomes a *risk tool*.

Block G: Decision Logic (Go / No-Go / Pivot)

Program question: *Given the model and uncertainty, should we proceed, stop, or pivot the design?*

- Inputs:
 - Summary metrics (VLCFA reduction, NfL change, Loes change, safety peaks) from Block C–E.
 - Scenario-wise probabilities from Block F.
- Outputs:
 - For each design and scenario: p_{eff} , p_{safe} .
 - Region classification: Go, No-Go, or Pivot.

This is what you show in a governance meeting, a DSMB prep, or an investment discussion.

3 What the Input Files Mean in Plain Language

The I/O Contract lists many files. Here we describe them in practical terms for a medical researcher.

3.1 dosing_schedule.csv

What you put here: the protocol's dose and schedule, per arm.

- Which regimen belongs to which design or trial arm.
- For each dose: time, platform (LNP or EV), route (IV, IN, with/without FUS), and dose in mg/kg.
- Which CMC batch is used for that dose (to tie into Block E).

What the model does with it: turns each dose into an instantaneous injection in Block A, then propagates through all downstream blocks.

3.2 fus_schedule.csv

What you put here: when FUS is applied, for which arm, and with what intensity.

- Start and end of each FUS window.
- The fold-increase in BBB permeability you want to assume.

What the model does with it: modulates BBB permeability in Block A only during the defined window.

3.3 params_fixed.csv and params_prior.csv

What you put here: your best current knowledge and uncertainty about key biological and PK/PD parameters.

- `params_fixed.csv`: single values you consider fairly stable (organ volumes, some baseline rates).
- `params_prior.csv`: parameters you are uncertain about (e.g. endosomal escape, Hill exponents). Here you specify distributions, not just point values.

What the model does with them: uses fixed parameters directly, and samples from the priors when running uncertainty and scenario simulations (Block F).

3.4 cmc_batches.csv

What you put here: realistic CMC data for each batch.

- Encapsulation efficiency (%).
- Particle size and PDI.
- Potency readouts from assays.
- Impurity levels.

What the model does with it: computes effective potency factors and endosomal escape adjustments per batch, which are then applied to dose and expression in Blocks A & B.

3.5 cohort_definition.csv

What you put here: the phenotype and baseline disease state for the cohorts you care about.

- Whether we are simulating AMN, very-early cALD, early cALD.
- Baseline VLCFA levels, inflammation/axonal injury, NfL, and Loes.

What the model does with it: sets initial conditions in Block C (mouse and human branches).

3.6 Observed Data Files (obs_mouse.csv, obs_human.csv, obs_safety.csv)

What you put here: the actual data you already have.

- Mouse data: C26, NfL, FA, Rotarod over time.
- Human data: NfL, Loes, VLCFA where available.
- Safety data: cytokines, ALT/AST, ADA.

What the model does with them: uses these as calibration/validation targets to adjust uncertain parameters and assess whether the model reproduces the observed patterns.

3.7 scenarios.json

What you put here: named “what if” worlds.

- Baseline scenario.
- Pessimistic CNS delivery scenario.
- Immune-sensitive repeat dosing scenario.
- CMC-stressed scenario, etc.

What the model does with it: overlays these scenario assumptions over the priors to see how your design behaves in each case.

4 What the Output Files Tell You

4.1 sim_timeseries.h5

For modelers: contains all the time series needed for deep technical debugging and inspection.

For you: you may not need to open this file directly. Plotting scripts or dashboards will typically read from this file to generate figures such as:

- VLCFA time-courses.
- NfL and Loes trajectories under different designs.
- Safety curves (cytokines, ALT/AST).

4.2 `sim_summary.csv`

What you see here: for each design, scenario, and parameter draw (run):

- Change in VLCFA relative to baseline.
- Change in NfL and Loes.
- Peak safety biomarkers.

This is the main input to decision analysis and is easier to read than full time series.

4.3 `decision_metrics.csv`

This is the governance-facing output.

For each design and scenario, you see:

- p_{eff} : probability that the design meets the efficacy thresholds (e.g. VLCFA reduction, NfL/Loes conditions).
- p_{safe} : probability that safety constraints are respected (cytokines and liver enzymes below pre-specified limits).
- **decision**: Go / No-Go / Pivot, based on pre-agreed cut-offs.

This is the file that becomes a slide: a 2D map of $(p_{\text{eff}}, p_{\text{safe}})$ with regions coloured as Go, No-Go, or Pivot.

5 How Stage-2 Prevents Scope Creep

Stage-2 is not just “more equations”. It creates a contract between the scientific team and the implementation team:

- **Formula Pack** fixes what the model is allowed to do: which states and relationships exist and which do not.
- **I/O Contract** fixes how information flows in and out: file names, fields, units, and meanings.
- **Formula Lock** means: *we will not change the science while we are coding and calibrating*, unless we explicitly version the model.

For a medical investigator, this means:

- You can review and challenge assumptions at Stage-2, rather than discover them at the end of Stage-3.
- You can map model inputs and outputs directly to protocol elements, endpoints, and CMC documents.
- You can trust that, once Stage-2 is locked, any change in the science is traceable and versioned.

How to Use This Explainer in Practice

1. Start with this explainer to understand the roles of Blocks A–G and the meaning of key input and output files.
2. Use the I/O Contract as a checklist when:
 - drafting or updating a protocol;
 - preparing CMC and cohort information;
 - planning which data are needed for calibration.
3. Use the Formula Pack when you or your modelling collaborator need to inspect or modify the exact mathematical form.
4. Use the decision outputs (especially `decision_metrics.csv`) for governance and external communication (e.g. grant applications, investor decks, internal R&D reviews).

With these three documents together (Formula Pack, I/O Contract, and this Explainer), Stage-2 is not only mathematically complete but also *narratively and operationally accessible* to the broader team.