Stage-1 Architecture Specification

Dual-Platform mRNA Therapy for X-Linked Adrenoleukodystrophy (ALD)
(LNP + EV; Peripheral-First → CNS)

Method2Model

Abstract

This document specifies the Stage-1 architecture for a mechanistic, scenario-capable model of a dual-platform mRNA therapy for X-linked adrenoleukodystrophy (ALD). The model integrates lipid nanoparticles (LNP) and extracellular vesicles (EV) as delivery platforms in a peripheral-first → CNS strategy. The architecture is organized into seven interacting blocks: (A) Delivery & Exposure (PBPK/Transport), (B) Expression & Targeting (mRNA → ALDP@Peroxisome), (C) Disease / Pharmacodynamics (ALDP → VLCFA → Biomarkers → Function), (D) Safety / Immunogenicity, (E) CMC / Batch Variability, (F) Uncertainty & Scenario Engine, and (G) Defensibility & Decision Logic.

The goal of this specification is to provide a complete, explicit, and implementation-ready description of the model structure at the level of states, observables, input parameters, and inter-block interfaces, without relying on simplifying assumptions such as single-compartment PK or purely linear dose-response.

Contents

1 Scope and Design Intent

The architecture captures the mechanistic chain

$$\begin{aligned}\text{Carrier/Route/Dosing} &\longrightarrow \text{Tissue \& Cellular Exposure} \longrightarrow \text{mRNA Expression}\\ &\longrightarrow \text{ALDP at Peroxisomal Membranes} \longrightarrow \text{VLCFA Reduction}\\ &\longrightarrow \text{Biomarkers \& Function} \longrightarrow \text{Safety, CMC, and Decisions.}\end{aligned}$$

The model must support:

- Multi-route, multi-platform delivery: IV-LNP (peripheral), EV-IN, and route combinations with focused ultrasound (FUS) to modulate blood–brain barrier (BBB) permeability.
- Explicit modeling of ALDP expression and peroxisomal targeting (vs. mislocalization) in relevant cell types.
- Coupling between ALDP levels, peroxisomal β-oxidation capacity, and very-long-chain fatty acid (VLCFA) dynamics in plasma and CNS.
- Safety endpoints including innate cytokine responses, complement activation, hepatic toxicity (ALT/AST), and anti-carrier immunity.
- CMC-driven variability in LNP/EV potency, quality attributes, and stability.
- Uncertainty-driven scenario analysis to identify first-break mechanisms and regimen robustness.
- Generation of defensible Go/No-Go and Pivot decisions aligned with the pre-specified aims of the programme.

2 Indices, Phenotypes, and State Classes

2.1 Indices

We define the following indices:

- Species: $s \in \{\text{mouse}, \text{human-projected}\}$.
- Phenotype / clinical window: $p \in \{\text{AMN}, \text{very-early cALD}, \text{early cALD}\}$, where AMN denotes adrenomyeloneuropathy and cALD denotes cerebral ALD.
- Carrier type: $k \in \{\text{LNP-std}, \text{LNP-brain}, \text{EV}\}$.
- Administration route: $r \in \{\text{IV}, \text{IN}, \text{IV+FUS}, \text{EV+FUS}\}$.
- Compartments:

$$\begin{aligned}c \in \{&\text{plasma}, \text{liver}, \text{spleen}, \text{adrenal}, \text{other periphery},\\ &\text{CNS-blood side}, \text{CSF}, \text{CNS-parenchyma}\}.\end{aligned}$$

- CNS cell types: $\text{cell} \in \{\text{endothelium}, \text{astrocyte}, \text{neuron}, \text{oligodendrocyte}, \text{microglia}\}$.

2.2 Classes of States

For each block we distinguish three categories of variables:

- **Simulated states** (S_{sim}): latent or internal states that the model evolves over time, but that may or may not be directly measurable (e.g. per-cell mRNA counts, β-oxidation capacity, anti-PEG titer).
- **Observable variables** (S_{obs}): variables that correspond to measurable quantities in experiments or clinical data (e.g. plasma VLCFA, DTI metrics, NfL, Loes score, ALT).
- **Policy/decision variables** (S_{pol}): derived variables that encode decisions, flags, or classifications (e.g. Go/No-Go labels, first-break mechanism, safety violations).

This classification is explicit in the block-by-block specification below and is important for implementation, calibration, and reporting.

3 Block A: Delivery and Exposure (PBPK / Transport)

3.1 States and Observables

For each carrier k, route r, compartment c, and cell type:

- S_{sim}:

$$\begin{aligned} C_{k,c}(t) &: \text{carrier concentration in compartment } c \text{ at time } t, \\ U_{k,c,\text{cell}}(t) &: \text{cumulative uptake of carrier } k \text{ into a given cell type in } c, \\ P_{\text{BBB}}(t) &: \text{effective BBB permeability at time } t. \end{aligned}$$

- S_{obs} (if measured):
  - Plasma and tissue PK curves (e.g. LC–MS/MS, fluorescence).
  - CNS tracer uptake (e.g. radiotracers, imaging).
- S_{pol}:
  - Qualitative classification of CNS exposure: “adequate” / “borderline” / “inadequate” for a given scenario.

3.2 Inputs

- Dose schedule: $D_{k,r}(t_j)$ (e.g. mg mRNA/kg or particles/kg at administration times t_j).
- FUS timing and parameters (if applicable).
- PBPK parameters: compartment volumes, blood flows, clearances CL_c, and baseline BBB permeability P_{baseline}, each with uncertainty ranges.
- Batch potency multipliers from Block E.

3.3 Dynamics

For each compartment c, the carrier concentration satisfies:

$$\frac{\mathrm{d}C_{k,c}(t)}{\mathrm{d}t} = \sum_{c'} Q_{c'\to c}\big(C_{k,c'}(t)\big) - \sum_{c''} Q_{c\to c''}\big(C_{k,c}(t)\big) - CL_c\big(C_{k,c}(t)\big) - \mathrm{Uptake}_c\big(C_{k,c}(t)\big), \quad (1)$$

where $Q_{c'\to c}$ are inter-compartment flows and Uptake_c represents loss into cells.

Transport across the BBB is modeled as

$$Q_{\mathrm{plasma}\to\mathrm{CNS}}(t) = P_{\mathrm{BBB}}(t)\, A_{\mathrm{BBB}}\,\big(C_{\mathrm{plasma}}(t) - C_{\mathrm{CNS}}(t)\big), \quad (2)$$

with

$$P_{\mathrm{BBB}}(t) = P_{\mathrm{baseline}} \times \begin{cases} M_{\mathrm{FUS}}(\omega), & t \in [t_{\mathrm{FUS}}, t_{\mathrm{FUS}} + \Delta t], \\ 1, & \text{otherwise}, \end{cases} \quad (3)$$

where $M_{\mathrm{FUS}}(\omega)$ is a random multiplicative factor (e.g. in the range 5–20) capturing the magnitude of FUS-induced BBB opening, and Δt is the open-window duration.

Cellular uptake is described generically by

$$\frac{\mathrm{d}U_{k,c,\mathrm{cell}}(t)}{\mathrm{d}t} = f_{k,r,c,\mathrm{cell}}\Big(C_{k,c}(t), \theta_{\mathrm{surface}}, \mathrm{ligands}\Big), \quad (4)$$

where $f_{k,r,c,\mathrm{cell}}$ encodes route-specific and cell-type specific uptake efficiency, including effects of surface receptors and ligand decoration.

3.4 Outputs and Interfaces

Block A provides:

- Trajectories $C_{k,c}(t)$ and $U_{k,c,\mathrm{cell}}(t)$ as inputs to Block B.
- Classification of CNS exposure quality as part of S_{pol} feeding into Block G (defensibility) and Block F (scenario outcomes).

4 Block B: Expression and Peroxisomal Targeting (mRNA → ALDP@Peroxisome)

4.1 States and Observables

For each relevant cell type (e.g. hepatocytes, oligodendrocytes):

- S_{sim}:

$$\begin{aligned} m(t) &: \text{intracellular mRNA copy number for ABCD1,} \\ P_{\mathrm{ALDP,tot}}(t) &: \text{total ALDP protein,} \\ P_{\mathrm{ALDP,peri}}(t) &: \text{ALDP localized at peroxisomal membranes,} \\ P_{\mathrm{ALDP,mis}}(t) &: \text{mislocalized ALDP (e.g. ER, aggregates),} \\ \theta_{\mathrm{target}}(t) = \frac{P_{\mathrm{ALDP,peri}}(t)}{P_{\mathrm{ALDP,tot}}(t)} &: \text{targeting efficiency.} \end{aligned}$$

- S_{obs}:
  - Immunofluorescence (IF): colocalization of ALDP with peroxisomal markers (e.g. PEX14, PMP70).

  - Protease-protection assays on peroxisomal fractions.
  - PTS1/PTS2 import reporter readouts.
  - Western blots: peroxisomal vs total ALDP.

- S_{pol}:
  - Regime label: "sub-therapeutic", "within therapeutic window", "overexpression-risk".

4.2 Inputs

- Uptake trajectories $U_{k,c,\text{cell}}(t)$ from Block A (converted to intracellular mRNA input rate).
- mRNA features: cap structure, nucleoside modifications, UTRs, codon optimization, which influence translation rate k_{tr} and innate sensing.
- Peroxisomal biogenesis capacity: PEX19/PEX3 levels and saturation characteristics.

4.3 Dynamics

mRNA kinetics.

$$\frac{\mathrm{d}m(t)}{\mathrm{d}t} = k_{\text{in}}(t) - k_{\text{deg,m}}\, m(t), \tag{5}$$

where $k_{\text{in}}(t)$ is determined by cellular uptake and endosomal escape, and $k_{\text{deg,m}}$ is the effective degradation rate of mRNA.

Translation.

$$\frac{\mathrm{d}P_{\text{ALDP,tot}}(t)}{\mathrm{d}t} = k_{\text{tr}}\, m(t) - k_{\text{deg,p}}\, P_{\text{ALDP,tot}}(t), \tag{6}$$

where k_{tr} is the translation rate and $k_{\text{deg,p}}$ is the protein degradation rate.

Peroxisomal targeting vs mislocalization.

$$\frac{\mathrm{d}P_{\text{ALDP,peri}}(t)}{\mathrm{d}t} = k_{\text{ins}}\Big(\text{PEX19/PEX3}, P_{\text{ALDP,tot}}(t), \text{crowding}\Big) - k_{\text{turnover,peri}}\, P_{\text{ALDP,peri}}(t), \tag{7}$$

with

$$P_{\text{ALDP,mis}}(t) = P_{\text{ALDP,tot}}(t) - P_{\text{ALDP,peri}}(t). \tag{8}$$

The insertion rate k_{ins} is modeled as a saturating function of total ALDP and the available PEX19/PEX3 capacity; at high overexpression levels, saturation leads to an increased fraction of $P_{\text{ALDP,mis}}$ and potential peroxisome/ER stress.

4.4 Therapeutic Window and Stress Zone

Let $P_{\text{ALDP,WT}}$ denote the peroxisomal ALDP level in a wild-type cell.

- **Therapeutic region:** $P_{\text{ALDP,peri}} \approx (1\text{–}3.5) \times P_{\text{ALDP,WT}}$, sufficient to restore a large fraction of peroxisomal β-oxidation capacity and to drive $\geq 50\%$ reduction in VLCFA in vitro.
- **Sub-therapeutic region:** $P_{\text{ALDP,peri}} < 0.5 \times P_{\text{ALDP,WT}}$.
- **Overexpression-risk region:** $P_{\text{ALDP,tot}} > 3\text{–}4 \times P_{\text{ALDP,WT}}$, associated with increased risk of mislocalization and peroxisomal/ER stress.

These regions are encoded as sets in the $(P_{\text{ALDP,peri}}, P_{\text{ALDP,mis}})$ plane rather than as single thresholds.

4.5 Targeting Score

We define a composite targeting score:

$$S_{\text{target}} = f_{\text{IF}} \cdot f_{\text{PTS}} \cdot f_{\text{protease}}, \tag{9}$$

where each factor is normalized to $[0, 1]$ based on:

- f_{IF}: quality and extent of ALDP colocalization with peroxisomal markers in IF.
- f_{PTS}: efficiency of PTS1/PTS2 cargo import.
- f_{protease}: degree of protection of ALDP in protease-protection assays of peroxisomal fractions.

4.6 Outputs and Interfaces

Block B outputs:

- Trajectories $P_{\text{ALDP,peri}}(t)$, $\theta_{\text{target}}(t)$, and $S_{\text{target}}(t)$.
- State classifications (therapeutic vs sub-therapeutic vs overexpression-risk).
- An effective peroxisomal capacity signal for Block C (via β-oxidation capacity $V_\beta(t)$).

5 Block C: Disease / Pharmacodynamics (ALDP $\rightarrow$ VLCFA $\rightarrow$ Biomarkers $\rightarrow$ Function)

Block C has two branches: a mouse branch C_{mouse} and a human-projected branch C_{human}. The former is used for design and interpretation of preclinical studies; the latter is used for translational reasoning and risk mapping in humans.

5.1 Mouse Branch C_{mouse}

5.1.1 States and Observables

- S_{sim}:

$$\begin{aligned} V_\beta(t) &: \text{peroxisomal } \beta\text{-oxidation capacity,} \\ C_{26}^{\text{plasma}}(t),\ C_{26}^{\text{CNS}}(t) &: \text{VLCFA concentrations,} \\ \text{Inflamm}_{\text{CNS}}(t) &: \text{CNS inflammatory tone,} \\ \text{AxonInjury}(t) &: \text{latent axonal injury index.} \end{aligned}$$

- S_{obs}:
  - Plasma and CNS VLCFA levels (e.g. C26:0 and C26:0/C22:0).
  - DTI metrics (FA, MD, RD) in relevant brain regions.
  - Rotarod performance.
  - Histological measures (e.g. demyelination, microgliosis).
- S_{pol}:
  - Aim-2 success/failure classification.
  - Progression vs stabilization labels based on combined VLCFA and functional readouts.

5.1.2 Inputs

- Peroxisomal ALDP signal from Block B: $P_{\mathrm{ALDP,peri}}(t)$.
- Phenotype p (e.g. AMN-like vs early cALD-like), which influences VLCFA production and baseline inflammation.

5.1.3 Dynamics

ALDP to β-oxidation capacity.

$$V_\beta(t) = V_\beta^{\mathrm{WT}} \cdot g\Big(\frac{P_{\mathrm{ALDP,peri}}(t)}{P_{\mathrm{ALDP,WT}}}\Big), \tag{10}$$

where g is a saturating or threshold-like function with steep response at low ALDP levels (partial rescue can yield substantial functional restoration) and a plateau near wild-type.

VLCFA dynamics. For each relevant compartment (plasma or CNS), we consider:

$$\frac{\mathrm{d}C_{26}(t)}{\mathrm{d}t} = P_{\mathrm{prod}}(p,s,t) - V_\beta(t)\, h\big(C_{26}(t)\big), \tag{11}$$

where $P_{\mathrm{prod}}(p,s,t)$ is the VLCFA production rate (phenotype- and age-dependent) and h is a substrate-dependent function (e.g. of Michaelis–Menten type).

Axonal injury and imaging/behavior. A latent axonal injury index evolves according to

$$\frac{\mathrm{dAxonInjury}(t)}{\mathrm{d}t} = F_{\mathrm{axon}}\Big(C_{26}^{\mathrm{CNS}}(t), \mathrm{Inflamm}_{\mathrm{CNS}}(t)\Big), \tag{12}$$

and DTI metrics and Rotarod performance are modeled as functions of $\mathrm{AxonInjury}(t)$ plus measurement noise and sensitivity characteristics specific to each assay.

5.1.4 Endpoints and Aim-2 Criteria

Example Aim-2 criteria (preclinical):

- Plasma C26:0 reduction $\geq 35\%$ at week 4 versus untreated control.
- CNS C26:0 reduction $\geq 25\%$ at week 4.
- Functional improvement:
  - Rotarod performance improvement $\geq 20\%$, and/or
  - DTI fractional anisotropy (FA) increase $\geq 10\%$ (or RD decrease) in predefined regions.

Block C_{mouse} aggregates these into S_{pol} (Aim-2 Go / No-Go / borderline).

5.2 Human-Projected Branch C_{human}

5.2.1 States and Observables

- S_{sim}:

$$\begin{aligned} V_\beta^{\mathrm{human}}(t) &: \text{projected human peroxisomal capacity,} \\ C_{26,\mathrm{human}}^{\mathrm{plasma}}(t),\ C_{26,\mathrm{human}}^{\mathrm{CNS}}(t) &: \text{human VLCFA states,} \\ \mathrm{AxonInjury}_{\mathrm{human}}(t) &: \text{latent human axonal injury,} \\ \mathrm{MicroglialAct}(t) &: \text{microglial activation level.} \end{aligned}$$

- S_{obs}:
  - Plasma VLCFA (e.g. C26:0, ratios).
  - Neurofilament light chain (NfL).
  - **Loes score** (MRI-based severity index; *only present in this human-projected branch, not in the mouse branch*).
  - Clinical functional scales (e.g. neurologic or disability scores).
- S_{pol}:
  - Projected stabilization vs progression vs improvement with respect to natural history or standard-of-care.

5.2.2 Dynamics

The structure mirrors the mouse branch but with human-specific time scales and scaling relations. For example:

$$\frac{\mathrm{d}C^{\text{CNS}}_{26,\text{human}}(t)}{\mathrm{d}t} = P^{\text{human}}_{\text{prod}}(p,t) - V^{\text{human}}_{\beta}(t)\, h_{\text{human}}\big(C^{\text{CNS}}_{26,\text{human}}(t)\big), \tag{13}$$

$$\frac{\mathrm{dNfL}(t)}{\mathrm{d}t} = G_{\text{NfL}}\Big(C^{\text{CNS}}_{26,\text{human}}(t),\ \text{AxonInjury}_{\text{human}}(t)\Big), \tag{14}$$

$$\frac{\mathrm{dLoes}(t)}{\mathrm{d}t} = G_{\text{Loes}}\Big(C^{\text{CNS}}_{26,\text{human}}(t),\ \text{MicroglialAct}(t)\Big). \tag{15}$$

The functions G_{NfL} and G_{Loes} encode relationships between VLCFA burden, inflammatory/axonal injury, and biomarkers. They are parameterized using human data from natural history and relevant interventions (e.g. HSCT, gene therapy), subject to uncertainty quantification in Block F.

5.3 Outputs and Interfaces

Block C exports:

- Simulated trajectories of VLCFA, NfL, DTI/Rotarod (mouse), and NfL/Loes (human-projected).
- Aim-1 and Aim-2 success probabilities (preclinical) and qualitative translational implications (stabilization vs progression).
- Inputs to Block G for defensibility and decision-making.

6 Block D: Safety and Immunogenicity

6.1 States and Observables

- S_{sim}:

$$\begin{aligned} \text{Cytokine}_i(t)&: \text{innate cytokines (e.g. IL-6, TNF-}\alpha\text{)},\\ \text{sC5b9}(t)&: \text{complement activation},\\ \text{ALT}(t),\ \text{AST}(t)&: \text{liver enzyme states},\\ Ab_{\text{PEG}}(t)&: \text{anti-PEG (or anti-lipid) antibody titer}. \end{aligned}$$

- S_{obs}:
  - Measured cytokine profiles, complement markers.

  - ALT/AST laboratory values.
  - CRS grades, clinical signs.
  - Imaging evidence of FUS-related events (e.g. microbleeds).

- S_{pol}:
  - Safety violation flags: liver, CRS, FUS-related, etc.

6.2 Inputs

- Dose history for each carrier/route.
- mRNA and carrier features (e.g. dsRNA content, endotoxin, nucleoside modifications, lipid composition).
- Individual or strain-level baseline immune sensitivity.

6.3 Dynamics

Innate cytokine and complement response.

$$\text{Cytokine}_{i,\max} = f_{\text{innate},i}\Big(D_{\text{effective}}, \text{dsRNA}, \text{modifications}, s\Big), \tag{16}$$

$$\text{sC5b9}_{\max} = f_{\text{comp}}\Big(D_{\text{effective}}, \text{lipid composition}, s\Big), \tag{17}$$

where $f_{\text{innate},i}$ and f_{comp} are carrier- and species-specific response functions, modulated by nucleoside chemistry and formulation.

Liver enzymes.

$$\frac{\mathrm{dALT}(t)}{\mathrm{d}t} = f_{\text{hepatotox}}\Big(C_{\text{LNP,liver}}(t), \text{repeat-dose history}\Big), \tag{18}$$

with a similar expression for AST.

Anti-PEG and effect on PK.

$$\frac{\mathrm{d}Ab_{\text{PEG}}(t)}{\mathrm{d}t} = f_{\text{B-cell}}\Big(D_{\text{LNP}}(t)\Big) - k_{\text{decay}}\, Ab_{\text{PEG}}(t). \tag{19}$$

The level $Ab_{\text{PEG}}(t)$ feeds back into Block A by effectively increasing clearance of LNPs and potentially modifying uptake patterns.

6.4 Policy and Safety Rules

Typical policy rules encoded in S_{pol} include:

- If ALT or AST exceeds 3× the upper limit of normal (ULN), then raise a “liver safety violation” flag.
- If CRS grade ≥ 2, then raise a “CRS violation” flag.
- If FUS-induced imaging abnormalities (e.g. microbleeds, edema) are detected, then raise a “FUS safety violation” flag and recommend stopping or de-escalating FUS in that arm.

7 Block E: CMC and Batch Variability

7.1 States and Observables

For each batch of LNP:

- S_{sim}:

  Size, PDI, EE%, RNAInt, $\text{Pot}_{\text{in vitro}}$, Endotoxin, pH, Osm

  $\text{Pot}(t)$: effective potency over time,

  where $\text{Pot}(t)$ encodes the fraction of nominal dose that remains functionally active given storage time and handling.

- S_{obs}:
  - Measured size/PDI/EE% (e.g. DLS, HPLC).
  - RNA integrity (e.g. RIN-like indices).
  - In vitro potency assays (e.g. reporter expression, VLCFA rescue in cells).
- S_{pol}:
  - Batch classification: "in-spec", "borderline", or "out-of-spec".

For EV-based products, analogous variables include particle count, size distribution, mRNA copies per 10^{10} EV, translation competency, and relevant markers.

7.2 Role in the Model

Hard constraints. If critical quality attributes (CQA) fall outside pre-defined specification limits (e.g. PDI > 0.2, excessive endotoxin), the batch is considered unusable.

Potency as a random variable. For in-spec batches, we model a batch-specific potency multiplier $\text{Pot}_{k,\text{batch}}$ as a random variable with a distribution informed by empirical data. The effective dose entering Block A and Block B is:

$$D_{\text{effective}} = D_{\text{nominal}} \cdot \text{Pot}_{k,\text{batch}}. \tag{20}$$

Stability and freeze–thaw. Time- and handling-dependent decay of potency is captured by:

$$\text{Pot}(t) = \text{Pot}_0 \cdot e^{-k_{\text{decay}}\, t} \cdot f_{\text{FT}}\big(\#\text{freeze–thaw}\big), \tag{21}$$

where k_{decay} is a storage-dependent decay constant and f_{FT} accounts for additional potency loss per freeze–thaw cycle.

7.3 Outputs and Interfaces

Block E provides:

- Effective potency multipliers and quality states per batch to Block A and Block B.
- Batch classification for Block G (defensibility) and Block F (scenario generation).

8 Block F: Uncertainty and Scenario Engine

8.1 Uncertain Parameters

Block F treats the following as random parameters with prior distributions:

- Block A: CNS uptake fractions per route (IV-LNP, EV-IN, EV+FUS), blood flows, clearances CL_c, FUS multiplier M_{FUS}.
- Block B: Translation rates k_{tr}, insertion rates k_{ins}, mRNA and protein half-lives ($k_{\text{deg,m}}, k_{\text{deg,p}}$), PEX19/PEX3 capacity.
- Block C: VLCFA production rates P_{prod}, functional forms and parameters of g and h, sensitivities mapping VLCFA/injury to NfL/DTI/Loes.
- Block D: Innate response sensitivities, dose–response of cytokines and complement, hepatotoxicity response, anti-PEG kinetics.
- Block E: Batch potency distributions, decay k_{decay}, and freeze–thaw sensitivity.

8.2 Scenario Dimensions

Scenarios combine:

- Phenotype: AMN vs very-early cALD vs early cALD.
- Route/platform: IV-LNP (peripheral), EV-IN, IV+FUS, EV+FUS, and combinations.
- Dosing regimens: weekly vs every-two-weeks (Q2W), duration 4–8 weeks, dose levels.
- Batch qualities: high-, medium-, low-potency batches consistent with Block E.
- Immune backgrounds: innate "sensitive" vs "non-sensitive", prior exposure effects.

8.3 Outputs

Using Monte Carlo or Latin hypercube sampling across the parameter and scenario space, Block F yields:

- **First-break maps**: for each scenario, the distribution of which constraint or mechanism fails first (e.g. CNS exposure, peroxisomal targeting, PD effect, safety, CMC).
- **Robustness curves**: probability of satisfying Aim-1 and Aim-2 criteria as a function of uncertainty and regimen design.
- **Generalization matrices**: cross-scenario comparisons such as AMN vs cALD, LNP vs EV, and route-level performance.

These outputs feed directly into Block G for decision-making and into Method2Model Use Cases 1, 4, 5, and 6.

9 Block G: Defensibility and Decision Logic

9.1 Chain-of-Evidence Construction

Block G collects and organizes outputs from Blocks A–F into a defensible chain of evidence:

1. **Delivery & exposure:** CNS exposure trajectories, tissue distribution, and cell-type uptake for each route and platform.

2. **Targeting:** ALDP peroxisomal levels $P_{\text{ALDP,peri}}(t)$, targeting efficiency $\theta_{\text{target}}(t)$, and composite score S_{target}.
3. **Biochemistry:** VLCFA trajectories in plasma and CNS in mouse and human-projected branches.
4. **Function:**
   - Mouse: Rotarod performance, DTI metrics, histology.
   - Human-projected: NfL and *Loes score* (the latter present only in the human-projected branch).
5. **Safety:** Risks and violation probabilities for ALT/AST, CRS, and FUS safety.
6. **CMC:** Batch quality and stability, in-spec vs borderline vs out-of-spec status, and comparability to reference lots.

9.2 Decision Surfaces

Based on the chain-of-evidence and Block F outputs, Block G defines multi-dimensional decision surfaces:

- **Go:** high probability of meeting Aim-1 and Aim-2 thresholds with acceptable safety and CMC performance; first-break mechanisms manageable via tuning.
- **No-Go:** structural failures, such as:
  - CNS delivery remains inadequate even under optimistic parameter combinations, or
  - safety/CMC constraints are violated in ways that are not addressable by realistic adjustments.
- **Pivot:** intermediate cases where:
  - platform mix should shift (e.g. more EV, less LNP),
  - route should change (e.g. from IN to FUS),
  - phenotype priority should be adjusted (e.g. focus on AMN if CNS effect is insufficient for cALD),
  - endpoint strategy should refocus on more sensitive or robust biomarkers.

Block G also formalizes endpoint hierarchy (primary/secondary/exploratory) consistent with regulatory and reviewer expectations, and prepares standard outputs for IRB, grant, and sponsor review, including robustness plots, first-break maps, and generalization matrices.

10 Alignment with Method2Model Stage-1 Use Cases

The architecture directly supports Method2Model Stage-1 Use Cases:

- *Use Case 1 – Scan for protocol blind spots:* via Block F first-break maps and cross-block constraints (A–E).
- *Use Case 2 – Validate power against real-world noise:* by providing variance and uncertainty structures for key endpoints in Block C (mouse and human-projected branches).
- *Use Case 3 – Stop paying for low-information data:* by evaluating, via Block F, which endpoints actually change Go/No-Go decisions.

- *Use Case 4 – Stress-test regimen robustness:* by exploring dosing and scheduling scenarios in Block A and Block F.
- *Use Case 5 – Prevent "works here, fails there":* through the mouse vs human-projected dual branch in Block C and scenario generalization in Block F.
- *Use Case 6 – Simulate bottlenecks and drop-offs:* via CMC-driven constraints in Block E and their impact on exposure and efficacy.
- *Use Case 7 – Before you submit:* via Block G, which assembles the chain-of-evidence and decision surfaces into a reviewer-ready defensibility pack.

11 Conclusion

This Stage-1 architecture (Version 2) defines a mechanistic, multi-block model that:

1. Represents delivery, expression, disease dynamics, safety, and CMC as coupled but distinct subsystems.
2. Distinguishes simulated states, observable variables, and policy/decision variables for clarity and implementability.
3. Explicitly separates mouse and human-projected branches in Block C, with the Loes score confined to the human-projected branch.
4. Supports uncertainty-aware scenario analysis and structured Go/No-Go/Pivot decisions.

This specification is intended to be implementation-ready: in the next stage, each block can be mapped to software modules (e.g. Python classes) with parameter dictionaries and solver interfaces, while preserving the structure and semantics defined herein.