

Stage-2 Formula Pack v4.0

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

Method2Model Framework

Purpose of this Formula Pack (Stage 2)

This document provides the *full mathematical formulation* of the architecture defined in Stage 1 for the dual-platform mRNA therapy in X-linked adrenoleukodystrophy (ALD), covering:

- Block A: Delivery & Exposure (PBPK / transport).
- Block B: Expression & Peroxisomal Targeting.
- Block C: Disease / Pharmacodynamics (mouse + human-projected).
- Block D: Safety & Immunogenicity.
- Block E: CMC / Manufacturing Variability.
- Block F: Uncertainty & Scenario Engine.
- Block G: Decision Logic & Go/No-Go Surfaces.

The goal of Stage 2 is to remove all ambiguity in:

- State variables and their dynamics (ODEs or algebraic maps).
- Parameters, constraints, and boundary/initial conditions.
- Functional dependencies between blocks A–G.

No numerical values are fixed here; those belong to Stage 3 (calibration and implementation). This document is designed to be sign-off-ready for a *Formula Lock*, after which coding can start with a stable I/O contract.

1 Global Notation and Index Sets

We define the following index sets and variables, used across blocks.

1.1 Index Sets

- **Time:**

$$t \in [0, T] \subset \mathbb{R}_{\geq 0}.$$

- **Platforms (carriers):**

$$k \in \mathcal{K} = \{\text{LNP}, \text{EV}\}.$$

- **Routes:**

$$r \in \mathcal{R} = \{\text{IV}, \text{IN}, \text{IV+FUS}, \text{EV+FUS}, \dots\}.$$

- **Compartments:**

$$c \in \mathcal{C} = \{\text{plasma, liver, periphery, CNS, CSF}\}.$$

- **Disease phenotype:**

$$p \in \mathcal{P} = \{\text{AMN, very-early cALD, early cALD}\}.$$

- **Species branch:**

$$s \in \mathcal{S} = \{\text{mouse, human}\}.$$

- **Cell types (for expression/targeting):**

$$\text{cell} \in \mathcal{C}_{\text{cell}} = \{\text{hepatocyte, oligodendrocyte, astrocyte, microglia, \dots}\}.$$

- **Dose index:**

$$j = 1, \dots, J,$$

with dose administration times t_j .

1.2 Dose Specification

For each platform k and route r , we define the nominal dose administered at time t_j as

$$D_{k,r}(t_j) \quad [\text{amount per body weight}],$$

with route-specific target compartment $c_r \in \mathcal{C}$.

1.3 Kronecker Delta and Dirac Delta

We use the Kronecker delta

$$\delta_{c,c_r} = \begin{cases} 1, & c = c_r, \\ 0, & c \neq c_r, \end{cases}$$

and the Dirac delta $\delta(t - t_j)$ to represent instantaneous dosing events.

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

Block A describes systemic and CNS exposure of mRNA carriers (LNP, EV), their distribution across compartments, and uptake into relevant cells.

2.1 State Variables in Block A

For each carrier $k \in \mathcal{K}$ and compartment $c \in \mathcal{C}$:

$C_{k,c}(t)$	[amount/volume]	Carrier concentration in compartment c ,
$U_{k,c,\text{cell}}(t)$	[amount]	Cumulative uptake into cell type cell in compartment c .

Blood-brain barrier (BBB) permeability:

$$P_{\text{BBB}}(t) \quad [\text{length/time}].$$

2.2 Parameters in Block A

- V_c [volume]: Volume of compartment c .
- $Q_{c'c}$ [volume/time]: Blood flow from compartment c' to c .
- CL_c [volume/time]: Clearance from compartment c .
- A_{BBB} [area]: Effective BBB area.
- P_{baseline} [length/time]: Baseline BBB permeability.
- M_{FUS} [–]: BBB permeability multiplier during FUS.
- $t_{\text{FUS}}, \Delta t_{\text{FUS}}$ [time]: FUS window.
- $V_{\text{max},k,c,\text{cell}}$ [amount/time]: Maximal uptake rate into cell type cell in compartment c .
- $K_{m,k,c,\text{cell}}$ [amount/volume]: Michaelis–Menten constant for uptake in that cell/compartment.
- θ_{surface} and ligand-related parameters: Control the surface binding term ϕ_{surface} .

2.3 PBPK Dynamics for Carrier Concentrations

For each $k \in \mathcal{K}$ and $c \in \mathcal{C}$, the carrier concentration $C_{k,c}(t)$ evolves according to:

$$\begin{aligned} \frac{dC_{k,c}(t)}{dt} = & \underbrace{\sum_{c' \neq c} \frac{Q_{c'c}}{V_c} C_{k,c'}(t)}_{\text{inflow from other compartments}} - \underbrace{\sum_{c'' \neq c} \frac{Q_{cc''}}{V_c} C_{k,c}(t)}_{\text{outflow to other compartments}} \\ & - \underbrace{\frac{CL_c}{V_c} C_{k,c}(t)}_{\text{clearance from compartment } c} - \underbrace{\frac{1}{V_c} \sum_{\text{cell} \in \mathcal{C}_{\text{cell}}} v_{k,c,\text{cell}}^{\text{uptake}}(t)}_{\text{cellular uptake}} + I_{k,r,c}(t). \end{aligned} \quad (1)$$

The dosing input term $I_{k,r,c}(t)$ is:

$$I_{k,r,c}(t) = \sum_{j=1}^J \frac{D_{k,r}(t_j) \delta_{c,c_r}}{V_c} \delta(t - t_j), \quad (2)$$

where c_r is the dosing compartment for route r , and the units are chosen such that $I_{k,r,c}(t)$ has dimensions consistent with $dC_{k,c}/dt$.

2.4 BBB Transport and FUS Modulation

The flux across the BBB (e.g. between plasma and CNS) for carrier k is modeled as:

$$Q_{k,\text{plasma} \rightarrow \text{CNS}}(t) = P_{\text{BBB}}(t) A_{\text{BBB}} (C_{k,\text{plasma}}(t) - C_{k,\text{CNS}}(t)). \quad (3)$$

The effective BBB permeability $P_{\text{BBB}}(t)$ is:

$$P_{\text{BBB}}(t) = P_{\text{baseline}} \times \begin{cases} M_{\text{FUS}}, & t \in [t_{\text{FUS}}, t_{\text{FUS}} + \Delta t_{\text{FUS}}], \\ 1, & \text{otherwise.} \end{cases} \quad (4)$$

The BBB flux term (3) appears in the $C_{k,\text{plasma}}$ and $C_{k,\text{CNS}}$ equations as inflow/outflow contributions divided by the respective compartment volumes.

2.5 Cellular Uptake Dynamics

Cellular uptake in compartment c for platform k and cell type cell is modeled as a saturable process:

$$v_{k,c,\text{cell}}^{\text{uptake}}(t) = V_{\max,k,c,\text{cell}} \frac{C_{k,c}(t)}{K_{m,k,c,\text{cell}} + C_{k,c}(t)} \phi_{\text{surface}}(\theta_{\text{surface}}, \text{ligands}), \quad (5)$$

where $\phi_{\text{surface}} \in (0, 1]$ captures modulation due to surface ligands, receptor density, and other targeting design features.

The cumulative uptake in cell type cell satisfies:

$$\frac{dU_{k,c,\text{cell}}(t)}{dt} = v_{k,c,\text{cell}}^{\text{uptake}}(t). \quad (6)$$

2.6 Initial Conditions for Block A

Unless otherwise stated:

$$\begin{aligned} C_{k,c}(0) &= C_{k,c}^{\text{baseline}} \approx 0, \\ U_{k,c,\text{cell}}(0) &= 0, \\ P_{\text{BBB}}(0) &= P_{\text{baseline}}. \end{aligned}$$

3 Block B: Expression and Peroxisomal Targeting

Block B converts cellular uptake of carriers into intracellular mRNA, ALDP protein, and peroxisomal targeting capacity that controls β -oxidation capacity $V_{\beta}(t)$.

We formulate the block for a generic cell type; in implementation, parameters differ for hepatocytes, oligodendrocytes, astrocytes, etc.

3.1 State Variables in Block B

For each relevant cell type:

$m(t)$	ABCD1 mRNA copies (effective amount per cell),
$P_{\text{ALDP,tot}}(t)$	Total ALDP protein in the cell (all locations),
$P_{\text{ALDP,peri}}(t)$	ALDP localized to peroxisomal membranes,
$P_{\text{ALDP,mis}}(t)$	Mislocalized ALDP (e.g. ER, aggregates),
$V_{\beta}(t)$	Peroxisomal β -oxidation capacity,
$\theta_{\text{target}}(t) = \frac{P_{\text{ALDP,peri}}(t)}{P_{\text{ALDP,tot}}(t)}$	Peroxisomal targeting fraction.

3.2 Parameters in Block B

- $\eta_{\text{esc}} [-]$: Endosomal escape efficiency (fraction of internalized cargo leading to cytosolic mRNA).
- $\alpha_{k,c,\text{cell}} [-]$: Allocation of uptake in compartment c and platform k to this cell type (normalization factor).
- $k_{\text{deg,m}} [1/\text{time}]$: mRNA degradation rate.
- $k_{\text{tr}} [1/\text{time}]$: Translation rate from mRNA to ALDP.

- $k_{\text{deg,p}}$ [1/time]: Protein degradation rate (all ALDP pools).
- $k_{\text{ins,max}}$ [1/time]: Maximum peroxisomal insertion rate.
- K_{ins} [same units as $P_{\text{ALDP,tot}}$]: Saturation constant for insertion versus total ALDP.
- C_{PEX} [same units as $P_{\text{ALDP,tot}}$]: Effective capacity of PEX import machinery.
- $k_{\text{turnover,peri}}$ [1/time]: Turnover of peroxisomal ALDP.
- V_{β}^{WT} [capacity units]: β -oxidation capacity in wild-type cells.
- $P_{\text{ALDP,WT}}$: Reference peroxisomal ALDP in wild-type.
- K_{β} [dimensionless]: Half-saturation parameter in the Hill function.
- γ [dimensionless]: Hill exponent controlling steepness.

3.3 Input Flux from Block A

The effective mRNA input rate $k_{\text{in}}(t)$ (mRNA per cell per unit time) is linked to cellular uptake from Block A:

$$k_{\text{in}}(t) = \eta_{\text{esc}} \sum_{k \in \mathcal{K}} \sum_{c \in \mathcal{C}} \alpha_{k,c,\text{cell}} \frac{dU_{k,c,\text{cell}}(t)}{dt}. \quad (7)$$

Units are chosen such that $k_{\text{in}}(t)$ is consistent with the units of $m(t)$ and the ODE for mRNA.

3.4 mRNA and Protein Dynamics

The mRNA and total ALDP protein follow:

$$\frac{dm(t)}{dt} = k_{\text{in}}(t) - k_{\text{deg,m}} m(t), \quad (8)$$

$$\frac{dP_{\text{ALDP,tot}}(t)}{dt} = k_{\text{tr}} m(t) - k_{\text{deg,p}} P_{\text{ALDP,tot}}(t). \quad (9)$$

3.5 Peroxisomal Insertion and Mislocalization

The peroxisomal insertion rate (into $P_{\text{ALDP,peri}}$) is modeled as a saturable function of total ALDP and PEX capacity:

$$k_{\text{ins}}(P_{\text{ALDP,tot}}(t)) = k_{\text{ins,max}} \frac{P_{\text{ALDP,tot}}(t)}{K_{\text{ins}} + P_{\text{ALDP,tot}}(t)} \cdot \frac{C_{\text{PEX}}}{C_{\text{PEX}} + P_{\text{ALDP,tot}}(t)}. \quad (10)$$

Peroxisomal ALDP dynamics:

$$\frac{dP_{\text{ALDP,peri}}(t)}{dt} = k_{\text{ins}}(P_{\text{ALDP,tot}}(t)) - k_{\text{turnover,peri}} P_{\text{ALDP,peri}}(t). \quad (11)$$

Mislocalized ALDP is:

$$P_{\text{ALDP,mis}}(t) = P_{\text{ALDP,tot}}(t) - P_{\text{ALDP,peri}}(t). \quad (12)$$

3.6 Peroxisomal β -Oxidation Capacity

The capacity $V_{\beta}(t)$ is expressed as a Hill-type function of the peroxisomal ALDP relative to wild-type:

$$V_{\beta}(t) = V_{\beta}^{\text{WT}} \cdot g(x(t)), \quad x(t) = \frac{P_{\text{ALDP,peri}}(t)}{P_{\text{ALDP,WT}}}, \quad (13)$$

where

$$g(x) = \frac{x^{\gamma}}{x^{\gamma} + K_{\beta}^{\gamma}}, \quad x \geq 0. \quad (14)$$

3.7 Initial Conditions for Block B

In untreated ALD cells (e.g. ABCD1-deficient):

$$\begin{aligned} m(0) &\approx 0, \\ P_{\text{ALDP,tot}}(0) &\approx 0, \\ P_{\text{ALDP,peri}}(0) &\approx 0, \\ V_{\beta}(0) &\ll V_{\beta}^{\text{WT}}. \end{aligned}$$

4 Block C: Disease / Pharmacodynamics

Block C maps peroxisomal capacity $V_{\beta}(t)$ into VLCFA levels, inflammation, axonal injury, and clinically relevant endpoints. It has two branches:

- Mouse branch (for preclinical Aim 2).
- Human-projected branch (for translation, Loes and NFL).

4.1 Mouse Branch: C_{mouse}

4.1.1 State Variables (Mouse)

$V_{\beta}(t)$	Imported from Block B (mouse cells),
$C_{26}^{\text{plasma}}(t)$	Plasma VLCFA (e.g. C26:0),
$C_{26}^{\text{CNS}}(t)$	CNS VLCFA,
$\text{Inflamm}_{\text{CNS}}(t)$	CNS inflammation state variable,
$\text{AxonInjury}(t)$	Cumulative axonal injury (mouse).

4.1.2 Parameters (Mouse)

- $P_{\text{prod,plasma}}(p, s, t)$, $P_{\text{prod,CNS}}(p, s, t)$ [amount/(volume·time)]: VLCFA production rates in plasma and CNS, dependent on phenotype p and species $s = \text{mouse}$.
- $V_{\text{max,C26}}$, $K_{m,C26}$: β -oxidation parameters for VLCFA.
- $k_{\text{clear,plasma}}$, $k_{\text{clear,CNS}}$: Non- β -oxidation clearance rates.
- k_{cross} : Exchange rate between plasma and CNS.
- κ_{infl} , κ_{resolve} : Inflammation induction and resolution rates.
- α_{inj} , β_{inj} , K_I , γ_C , γ_I : Parameters for axonal injury dynamics.
- $NfL_{\text{baseline,mouse}}$, α_{NFL} : Baseline and sensitivity for NFL.
- $\text{FA}_{\text{baseline}}$, α_{FA} : Baseline and sensitivity for DTI FA.
- $\text{Rotarod}_{\text{baseline}}$, α_{Rot} : Baseline and sensitivity for Rotarod performance.

4.1.3 VLCFA Dynamics (Mouse)

We use a Michaelis–Menten form for consumption:

$$h(C_{26}) = \frac{C_{26}}{K_{m,C26} + C_{26}}. \quad (15)$$

Plasma VLCFA:

$$\frac{dC_{26}^{\text{plasma}}(t)}{dt} = P_{\text{prod,plasma}}(p, s = \text{mouse}, t) - V_{\beta}(t) h(C_{26}^{\text{plasma}}(t)) - k_{\text{clear,plasma}} C_{26}^{\text{plasma}}(t). \quad (16)$$

CNS VLCFA:

$$\frac{dC_{26}^{\text{CNS}}(t)}{dt} = P_{\text{prod,CNS}}(p, s = \text{mouse}, t) - V_{\beta}(t) h(C_{26}^{\text{CNS}}(t)) - k_{\text{clear,CNS}} C_{26}^{\text{CNS}}(t) + k_{\text{cross}} (C_{26}^{\text{plasma}}(t) - C_{26}^{\text{CNS}}(t)). \quad (17)$$

4.1.4 Inflammation and Axonal Injury (Mouse)

Inflammation:

$$\frac{d\text{Inflamm}_{\text{CNS}}(t)}{dt} = \kappa_{\text{infl}} \phi_{\text{infl}}(C_{26}^{\text{CNS}}(t)) - \kappa_{\text{resolve}} \text{Inflamm}_{\text{CNS}}(t), \quad (18)$$

where ϕ_{infl} is a non-negative increasing function, e.g. a Hill function.

Axonal injury:

$$\frac{d\text{AxonInjury}(t)}{dt} = F_{\text{axon}}(C_{26}^{\text{CNS}}(t), \text{Inflamm}_{\text{CNS}}(t)), \quad (19)$$

with a typical parametric form:

$$F_{\text{axon}}(C, I) = \alpha_{\text{inj}} \frac{C^{\gamma_C}}{C^{\gamma_C} + K_C^{\gamma_C}} \frac{I^{\gamma_I}}{I^{\gamma_I} + K_I^{\gamma_I}} - \beta_{\text{inj}} \text{AxonInjury}(t). \quad (20)$$

4.1.5 Mouse Endpoints: NfL, DTI, Rotarod

NfL:

$$\text{NfL}_{\text{mouse}}(t) = NfL_{\text{baseline,mouse}} + \alpha_{\text{NfL}} \text{AxonInjury}(t) + \epsilon_{\text{NfL}}(t). \quad (21)$$

DTI FA:

$$\text{FA}(t) = \text{FA}_{\text{baseline}} - \alpha_{\text{FA}} \text{AxonInjury}(t) + \epsilon_{\text{FA}}(t). \quad (22)$$

Rotarod performance:

$$\text{Rotarod}(t) = \text{Rotarod}_{\text{baseline}} - \alpha_{\text{Rot}} \text{AxonInjury}(t) + \epsilon_{\text{Rot}}(t). \quad (23)$$

Here $\epsilon_{\text{NfL}}(t)$, $\epsilon_{\text{FA}}(t)$, and $\epsilon_{\text{Rot}}(t)$ are noise terms capturing measurement variability.

4.1.6 Initial Conditions (Mouse)

Typical untreated ABCD1-deficient baseline:

$$\begin{aligned} C_{26}^{\text{plasma}}(0) &= C_{26,\text{baseline}}^{\text{plasma}}, \\ C_{26}^{\text{CNS}}(0) &= C_{26,\text{baseline}}^{\text{CNS}}, \\ \text{Inflamm}_{\text{CNS}}(0) &= \text{Inflamm}_{\text{baseline}}, \\ \text{AxonInjury}(0) &= \text{AxonInjury}_{\text{baseline}}. \end{aligned}$$

4.2 Human-Projected Branch: C_{human}

4.2.1 State Variables (Human-Proj)

$V_{\beta}^{\text{human}}(t)$	Peroxisomal capacity in human CNS-relevant cells,
$C_{26,\text{human}}^{\text{plasma}}(t)$	Plasma VLCFA (human projection),
$C_{26,\text{human}}^{\text{CNS}}(t)$	CNS VLCFA (human projection),
$\text{MicroglialAct}(t)$	Microglial activation state,
$\text{AxonInjury}_{\text{human}}(t)$	Cumulative axonal injury (human projection),
$\text{NfL}_{\text{human}}(t)$	Human-projected NfL,
$\text{Loes}(t)$	Loes MRI score (human projection).

4.2.2 Parameters (Human-Proj)

Analogous to the mouse branch, but with human-relevant values:

- $V_{\beta,\text{WT}}^{\text{human}}, P_{\text{ALDP,WT}}^{\text{CNS,human}}$.
- $P_{\text{prod,plasma}}^{\text{human}}(p, t), P_{\text{prod,CNS}}^{\text{human}}(p, t)$.
- $h_{\text{human}}(\cdot)$ parameters: $V_{\text{max,C26}}^{\text{human}}, K_{m,\text{C26}}^{\text{human}}$.
- $k_{\text{clear,plasma}}^{\text{human}}, k_{\text{clear,CNS}}^{\text{human}}, k_{\text{cross}}^{\text{human}}$.
- $\kappa_{\text{micro}}, \kappa_{\text{micro,resolve}}$, plus parameters for ϕ_{micro} .
- Parameters of $F_{\text{axon,human}}, G_{\text{NfL}}, G_{\text{Loes}}$.

4.2.3 Linking ALDP to Human V_{β}

Let $P_{\text{ALDP,peri}}^{\text{CNS,human}}(t)$ denote peroxisomal ALDP in human-projected CNS cells, imported from a human version of Block B. Then:

$$V_{\beta}^{\text{human}}(t) = V_{\beta,\text{WT}}^{\text{human}} g_{\text{human}}(x_{\text{human}}(t)), \quad x_{\text{human}}(t) = \frac{P_{\text{ALDP,peri}}^{\text{CNS,human}}(t)}{P_{\text{ALDP,WT}}^{\text{CNS,human}}}, \quad (24)$$

where g_{human} is a Hill-type function analogous to (14), with its own $(K_{\beta}^{\text{human}}, \gamma^{\text{human}})$.

4.2.4 VLCFA Dynamics (Human-Proj)

We define:

$$h_{\text{human}}(C_{26}) = \frac{C_{26}}{K_{m,\text{C26}}^{\text{human}} + C_{26}}. \quad (25)$$

Plasma VLCFA:

$$\frac{dC_{26,\text{human}}^{\text{plasma}}}{dt} = P_{\text{prod,plasma}}^{\text{human}}(p, t) - V_{\beta}^{\text{human}}(t) h_{\text{human}}(C_{26,\text{human}}^{\text{plasma}}) - k_{\text{clear,plasma}}^{\text{human}} C_{26,\text{human}}^{\text{plasma}}. \quad (26)$$

CNS VLCFA:

$$\frac{dC_{26,\text{human}}^{\text{CNS}}}{dt} = P_{\text{prod,CNS}}^{\text{human}}(p, t) - V_{\beta}^{\text{human}}(t) h_{\text{human}}(C_{26,\text{human}}^{\text{CNS}}) - k_{\text{clear,CNS}}^{\text{human}} C_{26,\text{human}}^{\text{CNS}} + k_{\text{cross}}^{\text{human}} (C_{26,\text{human}}^{\text{plasma}} - C_{26,\text{human}}^{\text{CNS}}). \quad (27)$$

4.2.5 Microglial Activation and Axonal Injury (Human-Proj)

Microglial activation:

$$\frac{d\text{MicroglialAct}}{dt} = \kappa_{\text{micro}} \phi_{\text{micro}}(C_{26,\text{human}}^{\text{CNS}}(t)) - \kappa_{\text{micro,resolve}} \text{MicroglialAct}(t), \quad (28)$$

where ϕ_{micro} is an increasing function of CNS VLCFA.

Axonal injury (human-projected):

$$\frac{d\text{AxonInjury}_{\text{human}}}{dt} = F_{\text{axon,human}}(C_{26,\text{human}}^{\text{CNS}}(t), \text{MicroglialAct}(t)). \quad (29)$$

4.2.6 NfL and Loes Dynamics (Human-Proj)

NfL:

$$\frac{d\text{NfL}_{\text{human}}(t)}{dt} = G_{\text{NfL}}(C_{26,\text{human}}^{\text{CNS}}(t), \text{AxonInjury}_{\text{human}}(t)) - k_{\text{NfL,clear}} \text{NfL}_{\text{human}}(t), \quad (30)$$

with G_{NfL} typically increasing in both arguments.

Loes score:

$$\frac{d\text{Loes}(t)}{dt} = G_{\text{Loes}}(C_{26,\text{human}}^{\text{CNS}}(t), \text{MicroglialAct}(t)), \quad (31)$$

where G_{Loes} is non-negative and reflects lesion expansion rate.

4.2.7 Initial Conditions (Human-Proj)

Baseline for given phenotype p :

$$\begin{aligned} C_{26,\text{human}}^{\text{plasma}}(0) &= C_{26,\text{baseline,plasma}}^{(p)}, \\ C_{26,\text{human}}^{\text{CNS}}(0) &= C_{26,\text{baseline,CNS}}^{(p)}, \\ \text{MicroglialAct}(0) &= \text{MicroglialAct}_{\text{baseline}}^{(p)}, \\ \text{AxonInjury}_{\text{human}}(0) &= \text{AxonInjury}_{\text{baseline}}^{(p)}, \\ \text{NfL}_{\text{human}}(0) &= \text{NfL}_{\text{baseline}}^{(p)}, \\ \text{Loes}(0) &= \text{Loes}_{\text{baseline}}^{(p)}. \end{aligned}$$

5 Block D: Safety and Immunogenicity

Block D collects safety-relevant dynamics including cytokines, liver enzymes, and anti-drug immune responses.

5.1 State Variables in Block D

$S_{\text{cytokine}}(t)$	Composite cytokine response (e.g. IL-6, TNF- α),
$S_{\text{ALT}}(t)$	ALT level,
$S_{\text{AST}}(t)$	AST level,
$\text{ADA}_k(t)$	Anti-drug antibodies against platform $k \in \mathcal{K}$.

5.2 Parameters in Block D

- $\kappa_{\text{cyt,ind}}, \kappa_{\text{cyt,decay}}$: Induction and decay of cytokines.
- $\kappa_{\text{ALT,ind}}, \kappa_{\text{ALT,decay}}, \kappa_{\text{AST,ind}}, \kappa_{\text{AST,decay}}$: Induction and decay of liver enzymes.
- $\phi_{\text{cyt}}, \phi_{\text{hep}}$: Functions mapping exposure and dose features to safety stress.
- $\kappa_{\text{ADA,ind},k}, \kappa_{\text{ADA,decay},k}$: Induction and decay rates of ADA for platform k .
- $E_{\text{safe,cyt}}, E_{\text{safe,ALT}}, E_{\text{safe,AST}}$: Safe bounds for safety endpoints (used later in Block G).

5.3 Exposure-Driven Cytokine Response

We define a scalar *safety exposure* driver $E_{\text{cyt}}(t)$ based on Block A (e.g. peak or cumulative exposure in immune-relevant compartments such as spleen, liver, and blood):

$$E_{\text{cyt}}(t) = \phi_{\text{cyt}}\left(\{C_{k,c}(t)\}_{k \in \mathcal{K}, c \in \mathcal{C}_{\text{immune}}}, \{D_{k,r}(t_j)\}_{k,r,j}\right), \quad (32)$$

where $\mathcal{C}_{\text{immune}}$ is a subset of compartments (e.g. plasma, spleen) and ϕ_{cyt} is a non-negative function.

Cytokine dynamics:

$$\frac{dS_{\text{cytokine}}(t)}{dt} = \kappa_{\text{cyt,ind}} E_{\text{cyt}}(t) - \kappa_{\text{cyt,decay}} S_{\text{cytokine}}(t). \quad (33)$$

5.4 Liver Enzyme Responses (ALT/AST)

We define a *hepatic stress* driver $E_{\text{hep}}(t)$, e.g. based on LNP exposure in liver and possibly VLCFA burden:

$$E_{\text{hep}}(t) = \phi_{\text{hep}}\left(C_{\text{LNP,liver}}(t), C_{26}^{\text{plasma}}(t), C_{26}^{\text{CNS}}(t)\right), \quad (34)$$

with ϕ_{hep} non-negative and increasing in its arguments.

ALT dynamics:

$$\frac{dS_{\text{ALT}}(t)}{dt} = \kappa_{\text{ALT,ind}} E_{\text{hep}}(t) - \kappa_{\text{ALT,decay}} S_{\text{ALT}}(t). \quad (35)$$

AST dynamics:

$$\frac{dS_{\text{AST}}(t)}{dt} = \kappa_{\text{AST,ind}} E_{\text{hep}}(t) - \kappa_{\text{AST,decay}} S_{\text{AST}}(t). \quad (36)$$

5.5 Anti-Drug Antibody (ADA) Dynamics

For each platform $k \in \mathcal{K}$, we define an ADA state $\text{ADA}_k(t)$ driven by exposure in immune-relevant compartments and cumulative dosing:

$$E_{\text{ADA},k}(t) = \phi_{\text{ADA},k}\left(\{C_{k,c}(t)\}_{c \in \mathcal{C}_{\text{immune}}}, \{D_{k,r}(t_j)\}_j\right), \quad (37)$$

with $\phi_{\text{ADA},k} \geq 0$.

ADA dynamics:

$$\frac{d\text{ADA}_k(t)}{dt} = \kappa_{\text{ADA,ind},k} E_{\text{ADA},k}(t) - \kappa_{\text{ADA,decay},k} \text{ADA}_k(t). \quad (38)$$

ADA may feed back into Block A (e.g. by reducing effective exposure through accelerated clearance) or Block B (e.g. reducing effective *mRNA* input); such feedback is specified in the I/O contract.

5.6 Initial Conditions (Block D)

Baseline healthy or ALD values:

$$\begin{aligned} S_{\text{cytokine}}(0) &= S_{\text{cytokine,baseline}}, \\ S_{\text{ALT}}(0) &= S_{\text{ALT,baseline}}, \\ S_{\text{AST}}(0) &= S_{\text{AST,baseline}}, \\ \text{ADA}_k(0) &= 0 \quad \forall k \in \mathcal{K}. \end{aligned}$$

6 Block E: CMC / Manufacturing Variability

Block E maps batch-level CMC measurements (e.g. size distribution, encapsulation efficiency, potency assays) into effective model parameters, such as dose scaling and endosomal escape efficiencies.

6.1 Batch Index and CMC Measurements

We introduce a batch index $b = 1, \dots, B$. For each batch b and platform k , we observe a vector of CMC features:

$$\mathbf{Q}_{k,b} = (\text{EE}_{k,b}, \text{SizeMean}_{k,b}, \text{SizePDI}_{k,b}, \text{PotencyAssay}_{k,b}, \text{Impurity}_{k,b}, \dots).$$

6.2 Effective Potency and Dose Scaling

We define an effective *potency factor* $\phi_{k,b}$ derived from CMC features:

$$\phi_{k,b} = \Phi_k(\mathbf{Q}_{k,b}), \quad (39)$$

where Φ_k is a function mapping CMC metrics to a dimensionless scale factor.

The effective dose entering Block A for batch b is:

$$D_{k,r}^{\text{eff}}(t_j; b) = \phi_{k,b} D_{k,r}^{\text{nominal}}(t_j; b). \quad (40)$$

6.3 Endosomal Escape and Other Parameter Modulation

Similarly, we define an effective escape efficiency $\eta_{\text{esc},k,b}$ and possibly other parameters as functions of CMC metrics:

$$\eta_{\text{esc},k,b} = \Psi_k(\mathbf{Q}_{k,b}), \quad (41)$$

$$k_{\text{deg,m},b} = \Theta_{\text{mRNA}}(\mathbf{Q}_{k,b}), \quad \text{etc.} \quad (42)$$

These are algebraic relationships; they do not introduce new state variables but affect the parameters in Blocks A and B for batch b .

6.4 Constraints and Acceptability Criteria

CMC criteria for acceptable batches are represented as inequalities on $\mathbf{Q}_{k,b}$ and derived factors such as $\phi_{k,b}$, e.g.:

$$\phi_{k,b}^{\min} \leq \phi_{k,b} \leq \phi_{k,b}^{\max}, \quad (43)$$

$$\text{SizeMean}_{k,b}^{\min} \leq \text{SizeMean}_{k,b} \leq \text{SizeMean}_{k,b}^{\max}, \quad (44)$$

with analogous constraints for PDI and impurity. These inequalities are used in Block G as part of CMC-related decision logic.

7 Block F: Uncertainty and Scenario Engine

Block F formalizes parameter uncertainty and scenario definitions, allowing systematic exploration of how variability and design choices impact outcomes.

7.1 Uncertain Parameter Vector

We collect uncertain parameters into a vector

$$\boldsymbol{\theta} = (\theta_1, \dots, \theta_M),$$

which may include:

- PK parameters (e.g. P_{BBB} , M_{FUS} , CL_c).
- Expression parameters (e.g. η_{esc} , k_{tr} , $k_{\text{ins,max}}$).
- PD parameters (e.g. $V_{\text{max,C26}}$, $K_{m,C26}$, κ 's).
- Safety parameters (e.g. $\kappa_{\text{cyt,ind}}$).
- CMC-derived factors (e.g. $\phi_{k,b}$, $\eta_{\text{esc},k,b}$).

7.2 Prior Distributions

Each parameter θ_i is endowed with a prior distribution $\pi_i(\theta_i)$, often specified as log-normal, normal, or uniform within bounds:

$$\theta_i \sim \pi_i(\cdot), \quad i = 1, \dots, M. \quad (45)$$

The joint prior may factorize or include correlations:

$$\boldsymbol{\theta} \sim \pi(\boldsymbol{\theta}), \quad (46)$$

where π is a multivariate distribution summarizing prior knowledge.

7.3 Simulation Outputs as Functions of $\boldsymbol{\theta}$

Let \mathcal{Y} denote the set of model outputs of interest (e.g. End-of-study NfL, Loes, C26 reductions, safety peaks). For a given design d (dose, schedule, FUS setting) and scenario s , we denote the model solution as:

$$\mathbf{Y} = \mathcal{M}(d, s, \boldsymbol{\theta}), \quad (47)$$

where \mathcal{M} symbolizes the numerical solution of Blocks A–E given parameters $\boldsymbol{\theta}$.

7.4 Scenario Definitions

We define a finite set of scenarios $\mathcal{S}_{\text{scen}}$ (not to be confused with the species set \mathcal{S}), with each scenario $s \in \mathcal{S}_{\text{scen}}$ represented by:

$$s = (\boldsymbol{\theta}_{\text{fixed},s}, \mathcal{I}_s, \mathcal{D}_s), \quad (48)$$

where

- $\boldsymbol{\theta}_{\text{fixed},s}$: Parameters fixed or shifted in scenario s (e.g. “worst-case FUS”, “low CNS uptake”).
- \mathcal{I}_s : Index set of parameters held fixed versus varied.
- \mathcal{D}_s : Any design-specific modifications (e.g. presence/absence of CNS arm in the trial).

Examples:

- $s = S0$: Baseline scenario (nominal parameters).
- $s = S1$: CNS delivery-limited scenario (low P_{BBB} , low M_{FUS}).
- $s = S2$: Overexpression/targeting risk (high ALDP expression in non-target cells).
- $s = S3$: Immune-sensitive repeat dosing.
- $s = S4$: CMC-driven potency variability scenario.
- $s = S5$: Translational scenario across phenotypes (AMN to early cALD).

7.5 Uncertainty Propagation

For a fixed design d and scenario s , the distribution of outcomes \mathbf{Y} induced by parameter uncertainty is:

$$\mathbf{Y} \mid d, s \sim \{\mathcal{M}(d, s, \boldsymbol{\theta}) : \boldsymbol{\theta} \sim \pi(\boldsymbol{\theta})\}. \quad (49)$$

Key summary statistics:

$$\mathbb{E}[\mathbf{Y} \mid d, s] = \int \mathcal{M}(d, s, \boldsymbol{\theta}) \pi(\boldsymbol{\theta}) d\boldsymbol{\theta}, \quad (50)$$

$$\text{Var}[\mathbf{Y} \mid d, s] = \int \|\mathcal{M}(d, s, \boldsymbol{\theta}) - \mathbb{E}[\mathbf{Y} \mid d, s]\|^2 \pi(\boldsymbol{\theta}) d\boldsymbol{\theta}, \quad (51)$$

and quantiles or probabilities of satisfying decision criteria (used in Block G).

8 Block G: Decision Logic and Go/No-Go Surfaces

Block G defines decision functions that map summary outputs of the model (Blocks A–F) into trial-level or program-level decisions.

8.1 Derived Quantities of Interest

We define a set of scalar quantities derived from the model over relevant time horizons, including but not limited to:

$$\begin{aligned} \Delta C_{26, \text{mouse}}^{\text{plasma}} &= \frac{C_{26}^{\text{plasma}}(t_{\text{end}}) - C_{26}^{\text{plasma}}(t_{\text{baseline}})}{C_{26}^{\text{plasma}}(t_{\text{baseline}})}, \\ \Delta C_{26, \text{mouse}}^{\text{CNS}} &= \frac{C_{26}^{\text{CNS}}(t_{\text{end}}) - C_{26}^{\text{CNS}}(t_{\text{baseline}})}{C_{26}^{\text{CNS}}(t_{\text{baseline}})}, \\ \Delta \text{NfL}_{\text{human}} &= \text{NfL}_{\text{human}}(t_{\text{end}}) - \text{NfL}_{\text{human}}(t_{\text{baseline}}), \\ \Delta \text{Loes} &= \text{Loes}(t_{\text{end}}) - \text{Loes}(t_{\text{baseline}}), \\ S_{\text{cytokine}}^{\text{peak}} &= \max_{t \in [0, T]} S_{\text{cytokine}}(t), \\ S_{\text{ALT}}^{\text{peak}} &= \max_{t \in [0, T]} S_{\text{ALT}}(t), \\ S_{\text{AST}}^{\text{peak}} &= \max_{t \in [0, T]} S_{\text{AST}}(t). \end{aligned}$$

Additional quantities can include measures of Rotarod performance change, DTI changes, or CMC acceptability metrics.

8.2 Decision Criteria and Surfaces

We represent decision rules via inequalities on these derived quantities. For example, an *efficacy* criterion:

$$\Delta C_{26,\text{mouse}}^{\text{plasma}} \leq -0.35, \quad (52)$$

$$\Delta C_{26,\text{mouse}}^{\text{CNS}} \leq -0.25, \quad (53)$$

and a *human projection* criterion:

$$\Delta \text{NfL}_{\text{human}} \leq 0, \quad (54)$$

$$\Delta \text{Loes} \leq \Delta \text{Loes}_{\text{max}}, \quad (55)$$

where $\Delta \text{Loes}_{\text{max}}$ is a tolerated lesion progression bound.

Safety criteria might require:

$$S_{\text{cytokine}}^{\text{peak}} \leq E_{\text{safe, cyt}}, \quad (56)$$

$$S_{\text{ALT}}^{\text{peak}} \leq E_{\text{safe, ALT}}, \quad (57)$$

$$S_{\text{AST}}^{\text{peak}} \leq E_{\text{safe, AST}}. \quad (58)$$

CMC acceptability is enforced via:

$$Q_{k,b} \in \mathcal{Q}_k^{\text{acceptable}} \quad \text{and} \quad \phi_{k,b} \in [\phi_{k,b}^{\min}, \phi_{k,b}^{\max}], \quad (59)$$

where $\mathcal{Q}_k^{\text{acceptable}}$ is defined by inequalities such as (44).

8.3 Probabilistic Decision Rules under Uncertainty

Under parameter uncertainty, we consider the probability of satisfying efficacy and safety criteria for a given design d and scenario s :

$$p_{\text{eff}}(d, s) = \mathbb{P}_{\theta \sim \pi(\theta)} [\text{Eqs. (52)–(55) hold} \mid d, s], \quad (60)$$

$$p_{\text{safe}}(d, s) = \mathbb{P}_{\theta \sim \pi(\theta)} [\text{Eqs. (56)–(58) hold} \mid d, s]. \quad (61)$$

8.4 Go/No-Go/Pivot Regions

We define three regions in the space of $(p_{\text{eff}}, p_{\text{safe}})$:

$$\mathcal{R}_{\text{Go}} = \left\{ (p_{\text{eff}}, p_{\text{safe}}) : p_{\text{eff}} \geq p_{\text{eff}}^{\min}, p_{\text{safe}} \geq p_{\text{safe}}^{\min} \right\}, \quad (62)$$

$$\mathcal{R}_{\text{NoGo}} = \left\{ (p_{\text{eff}}, p_{\text{safe}}) : p_{\text{eff}} < p_{\text{eff}}^{\text{no-go}} \text{ or } p_{\text{safe}} < p_{\text{safe}}^{\text{no-go}} \right\}, \quad (63)$$

$$\mathcal{R}_{\text{Pivot}} = \text{Complement of } \mathcal{R}_{\text{Go}} \cup \mathcal{R}_{\text{NoGo}}, \quad (64)$$

with thresholds p_{eff}^{\min} , p_{safe}^{\min} , $p_{\text{eff}}^{\text{no-go}}$, $p_{\text{safe}}^{\text{no-go}}$ defined by stakeholders.

Based on Monte Carlo estimates of $p_{\text{eff}}(d, s)$ and $p_{\text{safe}}(d, s)$ across scenarios $s \in \mathcal{S}_{\text{scen}}$, design d is classified as Go, No-Go, or Pivot.

Summary and Next Steps

This Stage-2 Formula Pack v4.0:

- Provides explicit state definitions, ODEs, and algebraic maps for Blocks A–G.
- Preserves and formalizes the Stage 1 architecture in a code-ready form.
- Clearly defines how CMC variability and uncertainty feed into outcomes and decisions.

Once this formulation is approved (Formula Lock), the next steps are:

1. Define the Stage-2 I/O Contract: explicit types, shapes, units, ranges, and file schemas for all inputs/outputs.
2. Implement and verify numerical solvers for Blocks A–E (Stage 3), plus uncertainty propagation (Block F).
3. Implement decision dashboards based on Block G for trial design and program-level decisions.

Appendix: State / Observable / Decision Summary

Block	Dynamic states (ODE / integral)	Observables / derived outputs	Decision variables / levers
A	$C_{k,c}(t)$, $U_{k,c,\text{cell}}(t)$, $P_{\text{BBB}}(t)$	Exposure profiles (time courses, AUC, C_{max}) in plasma, liver, periphery, CNS, CSF; cell-specific uptake metrics; BBB fluxes under FUS vs. non-FUS	Dose level per platform/route; dosing schedule; choice of route (IV, IN, IV+FUS, EV+FUS); FUS timing and window
B	$m(t)$, $P_{\text{ALDP,tot}}(t)$, $P_{\text{ALDP,peri}}(t)$, $V_{\beta}(t)$	ALDP expression levels (total and peroxisomal), mislocalized pool, targeting fraction $\theta_{\text{target}}(t)$, peroxisomal capacity $V_{\beta}(t)$	Target per-cell expression window; acceptable targeting range; constraints on overexpression / mislocalization in non-target cells
C	Mouse: $C_{26}^{\text{plasma}}(t)$, $C_{26}^{\text{CNS}}(t)$, $\text{Inflamm}_{\text{CNS}}(t)$, $\text{AxonInjury}(t)$; Human-proj.: $C_{26,\text{human}}^{\text{plasma}}(t)$, $C_{26,\text{human}}^{\text{CNS}}(t)$, $\text{MicroglialAct}(t)$, $\text{AxonInjury}_{\text{human}}(t)$, $\text{NfL}_{\text{human}}(t)$, $\text{Loes}(t)$	VLCFA time courses and percent change; NfL trajectories (mouse & human-proj.); Loes evolution; DTI FA and Rotarod readouts; integrated axonal injury	Efficacy thresholds in mouse (e.g. ΔC_{26} , Rotarod, FA) and human-proj. (ΔNfL , ΔLoes); phenotype-specific translation gates (AMN, early cALD, very-early cALD)
D	$S_{\text{cytokine}}(t)$, $S_{\text{ALT}}(t)$, $S_{\text{AST}}(t)$, $\text{ADA}_k(t)$	Cytokine peaks and time courses; ALT/AST profiles; ADA titers per platform; composite safety burden metrics	Safety boundaries (max. tolerated cytokine peak; ALT/AST envelopes); repeat-dosing feasibility; stopping rules and dose reductions
E	—	CMC QC metrics $\mathbf{Q}_{k,b}$ (EE, size, PDI, impurity, potency, ...), and <i>derived factors</i> $\phi_{k,b}$, $\eta_{\text{esc},k,b}$, $k_{\text{deg},m,b}$ etc. as algebraic functions of CMC	Batch acceptance / selection; linking specific CMC profiles to allowed dose bands or exclusion; CMC-driven scenario definitions used in Block F/G
F	— (uncertain parameters are not dynamic states)	Parameter priors and posteriors; distributions of outcomes \mathbf{Y} under $\boldsymbol{\theta} \sim \pi(\boldsymbol{\theta})$; sensitivity indices and scenario-wise summaries	Definition of uncertainty sets, priors, and scenarios $\mathcal{S}_{\text{scen}}$ (e.g. CNS-limited, immune-sensitive, CMC-variability); choice of which parameters are varied vs. fixed
G	—	Derived efficacy and safety metrics (e.g. ΔC_{26} , ΔNfL , ΔLoes , $S_{\text{cytokine}}^{\text{peak}}$, $S_{\text{ALT}}^{\text{peak}}$, $S_{\text{AST}}^{\text{peak}}$), plus probabilities $p_{\text{eff}}(d, s)$, $p_{\text{safe}}(d, s)$	Go / No-Go / Pivot classification; selection among candidate designs d (dose, schedule, route mix, FUS plan); prioritization of programs or arms to advance