



DESIGNING BRAIN PENETRANT THERAPEUTICS WITH REDUCED PERIPHERAL TOXICITY FOR NEURODEGENERATION

Kannan Vishwanath* & Lloyd Zulu**

* Fellow, Albert Schweitzer International Foundation, Karl-Löwe-Gasse 22/12, A-1120 Wien, Vienna, Austria
 ** World Christian University, Lusaka, Zambia

Cite This Article: Kannan Vishwanath & Lloyd Zulu, "Designing Brain Penetrant Therapeutics with Reduced Peripheral Toxicity for Neurodegeneration", Indo American Journal of Multidisciplinary Research and Review, Volume 6, Issue 2, Page Number 224-235, 2022.

Copy Right: © IAJMRR Publication, 2022 (All Rights Reserved). This is an Open Access Article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract:

This study examines how treatment intensity influences clinical safety in neurodegenerative drug development, addressing the global challenge of balancing therapeutic exposure and tolerability. A secondary analysis of published aggregate safety data from the multinational EPOCH randomized trial included 1,957 treated participants with mild to moderate Alzheimer's disease across placebo, 12 mg, and 40 mg daily verubecestat assignments. Binomial risk estimation, Cochran Armitage trend testing, Pearson contingency analysis, and Fisher exact comparisons assessed treatment differences. Adverse event risks increased from 81.62% under placebo to 89.26% and 92.18% under active treatment, representing absolute increases of 7.64 and 10.55 percentage points. Both active treatments differed significantly from placebo, whereas their direct comparison remained statistically inconclusive ($p = 0.0854$). Alternative dose scoring supported the overall ordered association. The findings distinguish treatment associated safety burdens from incremental dose effects without attributing adverse events to unmeasured biological mechanisms. This distinction refines exposure-based safety interpretation and supports more rigorous dose selection and regulatory benefit risk assessment internationally.

Key Words: Neurodegenerative Diseases; Verubecestat; Adverse Event Risk; Dose Response Relationship; Randomized Clinical Trial.

1. Introduction:

The development of therapeutics for neurodegenerative diseases faces a fundamental pharmacological challenge: achieving sufficient activity within the central nervous system while avoiding adverse effects that compromise clinical tolerability. This challenge is particularly consequential in Alzheimer's disease, where the blood-brain barrier restricts the entry of many therapeutic molecules, and increasing systemic drug exposure does not necessarily translate into improved clinical outcomes. Experimental advances in receptor-mediated transport have demonstrated that molecular binding characteristics can substantially influence brain uptake, yet improved delivery and acceptable safety remain distinct requirements for successful therapeutic development (Yu et al., 2011; Couch et al., 2013; Bien-Ly et al., 2014). Clinical investigations of amyloid-targeting treatments have further exposed the difficulty of balancing pharmacological activity, treatment exposure and adverse-event burden. In the randomized EPOCH trial, verubecestat, a beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1) inhibitor, did not produce meaningful clinical benefit in patients with mild-to-moderate Alzheimer's disease despite its intended action on amyloid-related pathways (Egan et al., 2018). The resulting scientific problem extends beyond whether a candidate treatment reaches or affects its biological target. It concerns how treatment intensity relates to clinical safety and how that relationship should inform the interpretation of therapeutic development strategies. Distinguishing dose-associated safety burdens from the molecular mechanisms governing brain penetration is therefore essential to understanding the limitations of exposure-based approaches to neurodegenerative therapy.

The theoretical basis for improving brain delivery rests partly on receptor-mediated transcytosis, through which therapeutic molecules interact with transport receptors at the blood-brain barrier. However, receptor binding does not produce a uniformly positive relationship with brain uptake. Yu et al. (2011) demonstrated that reducing the affinity of an antibody for its transcytosis target increased brain uptake, challenging the assumption that stronger receptor engagement necessarily improves delivery. Bien-Ly et al. (2014) subsequently showed that transferrin receptor trafficking determines the brain uptake of antibody affinity variants, with high-affinity binding promoting intracellular sorting processes that can restrict productive transport. Complementing these findings, Niewoehner et al. (2014) demonstrated that a monovalent molecular shuttle increased brain penetration and therapeutic potency in an experimental setting. Together, these studies establish that delivery efficiency depends on the configuration and intracellular consequences of receptor engagement rather than simply on the strength of molecular binding. Their significance for therapeutic development lies in identifying molecular strategies that may alter the relationship between systemic exposure and central nervous system delivery. Nevertheless, these experimental findings do not establish that comparable mechanisms determine the

clinical adverse-event profile of small-molecule treatments such as verubecestat. The distinction between molecular transport efficiency and clinical treatment safety must therefore remain explicit.

Peripheral safety introduces an additional constraint because therapeutic interactions outside the brain may generate adverse consequences independently of central target engagement. Couch et al. (2013) demonstrated that transferrin receptor-targeting bispecific antibodies could produce safety liabilities associated with their molecular properties and that modifying Fc effector function ameliorated particular adverse effects in experimental models. This evidence indicates that therapeutic safety depends not only on the amount of drug administered but also on the biological interactions produced by its molecular architecture. Accordingly, improved brain penetration and reduced peripheral toxicity should not be treated as interchangeable outcomes. Molecular modifications that improve transport may influence systemic safety, but the existence, direction and magnitude of such effects require direct evaluation within the relevant therapeutic platform. These findings provide a mechanistic context for considering why systemic exposure may create safety costs without guaranteeing proportional therapeutic benefits. In contrast, the clinical analysis of verubecestat addresses a narrower, directly measurable relationship between assigned daily dose and the probability of experiencing an adverse event. Maintaining this distinction prevents experimental mechanisms derived from antibody transport studies from being incorrectly attributed to clinical observations involving BACE1 inhibition.

Clinical evidence from BACE1 inhibitor development reinforces the importance of evaluating pharmacological activity and tolerability separately. Egan et al. (2018) reported the outcomes of a randomized, placebo-controlled trial of verubecestat in patients with mild-to-moderate Alzheimer's disease, providing comparative evidence on treatment assignment, clinical outcomes and adverse events. Subsequent analyses of the EPOCH trial further characterized its safety findings, demonstrating the importance of examining adverse-event patterns rather than interpreting pharmacological exposure as an isolated indicator of therapeutic performance (Egan et al., 2019a). In a separate randomized trial involving patients with prodromal Alzheimer's disease, Egan et al. (2019b) reported that verubecestat did not improve clinical outcomes, indicating that the limitations observed in the treatment programme were not confined to patients with established dementia. Evidence from other BACE1 inhibitors similarly illustrates the separation between biological activity, tolerability and clinical benefit. Wessels et al. (2020) reported that lanabecestat failed to slow cognitive or functional decline in the AMARANTH and DAYBREAK-ALZ trials despite a generally tolerable safety profile, while Novak et al. (2020) documented safety concerns during atabecestat treatment alongside changes in amyloid-related biomarkers. Sperling et al. (2021) additionally reported efficacy, safety and biomarker findings from a truncated atabecestat trial in cognitively normal individuals with elevated brain amyloid. Collectively, these investigations show that central pharmacological activity, clinical efficacy and treatment safety represent related but empirically distinct dimensions of therapeutic evaluation. They also establish the relevance of treatment-specific and population-specific comparisons rather than assuming that observations from different BACE1 inhibitors or disease stages represent a common exposure-response relationship.

A central measurement issue in evaluating therapeutic safety concerns the distinction between the overall occurrence of adverse events and the identification of specific toxicological mechanisms. The proportion of participants experiencing at least one adverse event provides a clinically interpretable measure of the burden associated with treatment assignment. However, this binary outcome does not distinguish the anatomical origin, severity, duration or biological cause of individual events. Nor does it identify whether an event reflects central pharmacological activity, peripheral injury or another process occurring during treatment. This limitation is especially important in Alzheimer's disease trials because adverse events also occur among participants receiving placebo, making an untreated comparison essential for separating background event occurrence from excess risk associated with active treatment. The EPOCH trial provides an appropriate empirical basis for this comparison because it includes concurrent placebo and active-treatment groups within a common randomized trial framework (Egan et al., 2018). Absolute risk differences quantify the additional proportion of participants experiencing an event, whereas risk ratios express the corresponding proportional increase relative to placebo. Both measures are informative when background event probabilities are substantial. Their combined interpretation allows the safety consequences of treatment assignment to be assessed without incorrectly equating overall adverse-event occurrence with confirmed peripheral toxicity.

The relationship between assigned dose and adverse-event probability can be understood through pharmacological exposure theory, under which increasing treatment intensity may alter the probability of unwanted biological effects. Greater administered doses can increase exposure to pharmacologically active compounds, although the translation from assigned dose to actual tissue exposure depends on drug-specific pharmacokinetic and pharmacodynamic processes. Consequently, a positive dose-adverse-event association is theoretically plausible but should not be assumed to follow a linear or continuously increasing function. Differences in biological response, exposure duration and the characteristics of individual adverse events may produce nonlinear patterns. Moreover, the presence of adverse events in placebo recipients requires attention to the distinction between an overall ordered treatment-group relationship and a statistically distinguishable difference between successive active doses. These are separate empirical questions: an ordered association across placebo and active assignments may be

supported even when the incremental difference between active treatment levels remains uncertain. Randomization strengthens the interpretation of contrasts between assigned treatment groups, but aggregate safety data cannot directly identify individual pharmacological exposure or the biological mechanism responsible for each event. The appropriate analytical objective is therefore to evaluate the observed relationship between assigned dose and overall adverse-event risk without extending the inference to unmeasured molecular pathways.

Although Egan et al. (2018) established the principal clinical efficacy and safety findings of the EPOCH trial, and Egan et al. (2019a) provided additional characterization of its safety outcomes, the distinction between an ordered dose association and independently distinguishable active-dose contrasts warrants explicit analytical attention. This distinction matters because a higher observed adverse-event proportion at a greater assigned dose does not, by itself, demonstrate a statistically reliable incremental increase in harm. The reviewed evidence establishes the clinical importance of verubecestat-associated adverse events but does not resolve every interpretation that can be drawn from the published aggregate treatment-group counts. In particular, the relationship between the overall dose ordering, absolute excess risk relative to placebo and uncertainty surrounding the direct comparison of active doses requires a consistent quantitative framework. The present study addresses this bounded interpretive issue through a secondary analysis of published EPOCH safety data. Its contribution is not the discovery of a previously undocumented adverse effect or the experimental validation of a new brain-delivery mechanism. Rather, it provides a reproducible evaluation of how different statistical comparisons qualify the interpretation of dose-associated clinical safety within an established randomized trial.

The empirical setting strengthens the relevance of this question because EPOCH was conducted across 238 centres in 21 countries, with trial activities extending from November 2012 through April 2017 (Egan et al., 2018). The present analysis uses published aggregate safety records for 1,957 treated participants distributed across placebo, 12 mg and 40 mg daily verubecestat assignments. Its unit of analysis is the treatment-arm adverse-event count and corresponding safety denominator, rather than individual patient-level exposure observations. The primary outcome is the proportion of participants experiencing at least one adverse event during the reported treatment period. The analytical approach combines descriptive event proportions, absolute risk differences, risk ratios and a Cochran-Armitage test of the ordered dose relationship. Pearson contingency testing and Fisher exact comparisons provide complementary assessments of differences among treatment groups. This design permits evaluation of the association between assigned treatment intensity and overall adverse-event probability using a consistent clinical population and endpoint definition. It does not estimate receptor-mediated transport, brain drug concentration, peripheral tissue exposure or interaction effects, and it does not combine experimental antibody observations with clinical trial participants. The multinational setting supports relevance to therapeutic development across healthcare systems, while the interpretation remains specific to the studied treatment, population, assignments and observation period.

This study aims to evaluate the relationship between assigned daily verubecestat dose and overall adverse-event risk among patients with mild-to-moderate Alzheimer's disease, using published aggregate safety data from the EPOCH randomized trial. The central research question is whether higher assigned daily verubecestat doses are associated with a greater probability of experiencing at least one adverse event and whether the observed pattern supports statistically distinguishable differences between active treatment assignments. Specifically, the study assesses adverse-event proportions across the three treatment groups, quantifies absolute and relative safety differences between active treatment and placebo, evaluates the ordered dose-risk association and examines the consistency of the findings using alternative statistical comparisons. By distinguishing the overall treatment ordering from uncertainty surrounding incremental active-dose differences, the analysis refines the interpretation of exposure-associated safety within neurodegenerative drug development. Its theoretical contribution is to clarify the evidentiary boundary between assigned dose, observed clinical safety and the molecular mechanisms proposed to improve brain delivery. Its practical contribution lies in providing a transparent basis for interpreting dose comparisons when evaluating therapeutic tolerability, without treating increased pharmacological activity or improved delivery as sufficient evidence of clinical benefit. The remainder of the article presents the research hypotheses in Section 2, the data in Section 3, and the methods in Section 4. Section 5 presents and interprets the findings, Section 6 discusses their significance, and Section 7 sets out the conclusions and implications.

2. Theory, Conceptual Framework and Hypothesis Development:

This analysis draws on receptor mediated transport and pharmacological exposure theory to explain the relationship between brain delivery and therapeutic safety. The blood brain barrier restricts antibody entry, while interactions with transport receptors determine whether a therapeutic reaches brain tissue or remains within vascular cells. Yu and colleagues in 2011 demonstrated that reducing transferrin receptor binding affinity improved antibody delivery into mouse brain. This finding establishes a mechanism through which molecular design can improve delivery without relying solely on greater systemic dosing.

Receptor engagement can also constrain delivery. Strong binding may increase receptor capture while limiting release into brain tissue. Bien-Ly and colleagues in 2014 showed that high affinity

antibodies promoted lysosomal trafficking and transferrin receptor degradation, reducing subsequent transport capacity. Binding strength therefore does not have a uniformly positive relationship with brain exposure. Its consequences depend on intracellular sorting and receptor availability.

Antibody architecture provides another route to modifying this constraint. Niewoehner and colleagues in 2014 demonstrated that monovalent transferrin receptor engagement improved brain target engagement relative to the parent antibody in a mouse model. Their findings support the theoretical expectation that transport efficiency can improve through changes in binding configuration. The mechanism concerns delivery efficiency rather than a general increase in administered dose.

Peripheral interactions introduce a competing safety mechanism. A therapeutic that engages transport receptors outside the brain may affect circulating cells or activate immune pathways. Couch and colleagues in 2013 reported that removing Fc effector function ameliorated acute clinical signs and partly rescued reticulocyte reductions in mice. These findings indicate that safety depends on molecular interactions as well as delivery. Efficient brain transport and peripheral tolerability therefore represent related but distinct design objectives.

The empirical framework concentrates on administered dose because the clinical records provide comparable treatment groups, adverse event counts and denominators. Dose denotes the assigned daily amount of verubecestat. The outcome is the proportion of participants experiencing at least one adverse event during the reported treatment period. This outcome measures overall clinical safety. It does not isolate peripheral toxicity.

The theoretical expectation is that increasing dose may increase adverse event risk by intensifying pharmacological activity across central and peripheral tissues. A competing expectation is that the response may plateau when relevant biological pathways approach saturation. Adverse events also occur without active treatment, making the concurrent placebo group necessary for interpreting treatment associated differences.

Egan and colleagues in 2018 evaluated two verubecestat doses against placebo in a randomized trial involving patients with mild to moderate Alzheimer disease. Their report documents clinical adverse events alongside central biomarker activity, providing a basis for examining the safety consequences of dose escalation. Randomization supports comparisons between assigned treatment groups, although the aggregate records do not identify the biological pathway responsible for each event.

The resulting conceptual framework links assigned daily dose to overall adverse event risk within a single trial population. Both variables are observed measures. The framework does not construct a latent therapeutic performance index or combine animal findings with clinical observations. Treatment group event counts and denominators permit a binomial comparison and an ordered dose trend test without requiring individual participant records.

H1: Higher assigned daily verubecestat dose is associated with a higher probability of experiencing at least one adverse event among patients with mild to moderate Alzheimer disease. This hypothesis is exploratory because its formulation followed inspection of the published aggregate data. Its evaluation concerns the observed dose and safety relationship. The molecular transport findings supply the theoretical setting rather than additional statistically estimated pathways.

3. Data:

This analysis consolidates published experimental and clinical evidence on brain delivery and therapeutic safety. It preserves source provenance, separates evidence types and constructs an outcome specific sample for evaluating the relationship between assigned dose and adverse event risk.

3.1 Data Sources, Population, and Sample Construction:

The source collection contains six primary publications within the eligible period of 2010 to 2021. Four experimental publications examine transferrin receptor binding, antibody architecture, brain delivery and safety mechanisms. These comprise Yu et al. in 2011, Couch et al. in 2013, Bien-Ly et al. in 2014 and Niewoehner et al. in 2014. Their extracted findings provide mechanistic context rather than independent clinical observations.

The clinical sources are the EPOCH report by Egan et al. in 2018 and the EARLY report by Sperling et al. in 2021. The former concerns patients with mild to moderate Alzheimer disease, while the latter concerns cognitively normal adults with elevated brain amyloid. These populations remain separate throughout the analysis.

The workbook assigns source identifiers S01 to S06 and records publication year, DOI, extraction URL and source location. Access routes include publisher pages, PubMed and PubMed Central. The source versions are the identified journal publications. The workbook does not record an extraction date or a separate dataset release number. Public accessibility does not establish an unrestricted reuse licence, and publisher terms govern the underlying articles.

The target population comprises therapeutic interventions intended to improve brain delivery while limiting toxicity in neurodegeneration. The accessible frame comprises the six selected publications. It is a targeted evidence collection rather than an enumerated population of all eligible interventions. Selection therefore supports a focused secondary analysis rather than population representative inference.

The consolidated collection contains 32 clinical endpoint records and seven experimental findings. A clinical record represents one trial arm and one endpoint at its reported assessment. An experimental

record represents one extracted design finding. The data form a collection of aggregate experimental and trial results rather than an annual panel. Publication years identify sources rather than observation frequency.

The hypothesis testing sample uses the EPOCH safety population. Its setting covers 238 centres in 21 countries, with trial conduct from November 2012 through April 2017 and a planned treatment period of 78 weeks. Three treatment arms provide the relevant dose variation. One participant in the published primary population received no treatment and was excluded from the safety denominator.

Table 1: Construction of the analytical sample

Stage	Units	Observations	Reason or Rule
Consolidated source collection	6 publications	39 extracted records	Published experimental and clinical evidence
Experimental evidence retained outside estimation	4 publications	7 findings	Different species, interventions and measurement structures
Clinical evidence collection	2 trials and 6 arms	32 endpoint records	Published treatment group summaries
EARLY retained outside the hypothesis test	1 trial and 3 arms	14 endpoint records	Different drug, population and exposure pattern
EPOCH clinical collection	1 trial and 3 arms	18 endpoint records	Common trial setting
Other EPOCH endpoints retained outside the primary test	3 arms	15 endpoint records	Primary test uses any adverse event
Final hypothesis testing sample	3 arms	3 event count records	Complete dose, event count and denominator

The final three records represent 1,957 treated participants, rather than three individual participants. The source collection and endpoint selection were assembled after the published findings were available. The resulting hypothesis test is exploratory.

3.2 Data Integration, Cleaning, and Quality Assurance:

Integration uses source identifiers, trial names, treatment labels, doses, endpoint names and assessment periods. Source identifiers link extracted records to bibliographic entries. Trial, treatment, dose, endpoint and assessment jointly distinguish clinical observations. Records describing different endpoints within the same arm remain separate because they measure different outcomes.

The clinical and experimental tables are not merged into a common estimation dataset. This prevents animal findings, qualitative mechanisms and clinical event counts from entering the analysis as interchangeable observations. The safety comparison table contains eight calculations derived from clinical records and contributes no additional participants or independent observations.

Daily doses are recorded in milligrams. Placebo receives a dose value of zero, which denotes absence of active treatment. Assessment labels preserve the reported week, month or treatment period. No conversion to annual frequency, interpolation across assessments or alignment of unequal treatment durations was performed.

Quality checks compare extracted event counts and denominators with the cited source locations. Counts must be nonnegative; denominators must be positive and event counts cannot exceed denominators. Calculated proportions must lie between zero and one. Endpoint denominators take precedence over nominal arm sizes when laboratory coverage differs.

The clinical collection contains event counts and denominators for 18 of its 32 records. The remaining 14 records report continuous estimates or published proportions without both count fields. Confidence interval bounds are populated for two cognitive effect estimates. Empty fields remain unavailable or structurally inapplicable and are never interpreted as zero.

The three records used for the primary test contain no missing dose values, event counts or denominators. No observations were removed for missing controls, lags or differencing because these elements are absent from the implemented analysis. No imputation, winsorisation, trimming or statistical outlier exclusion was applied.

The analysis uses published aggregate findings rather than information available sequentially during trial conduct. It therefore evaluates a historical relationship and does not construct a forecasting exercise. Population differences and unequal exposure periods prevent direct pooling of the two clinical trials.

3.3 Variable Measurement, Construction, and Validation:

The empirical predictor is assigned daily verubecestat dose. The primary outcome is the proportion of participants experiencing at least one adverse event during the reported safety period. This measure captures overall clinical safety rather than peripheral toxicity alone. Equation 1 defines the event proportion.

$$p_g = \frac{E_g}{N_g}$$

Here, p_g is the observed event proportion in treatment group g , E_g is the number of participants experiencing the event and N_g is the corresponding safety denominator. Multiplication by 100 expresses the proportion as a percentage. Equation 2 defines the absolute comparison with placebo.

$$RD_g = 100[p_g - p_0]$$

Here, RD_g is the risk difference in percentage points, and p_0 is the placebo event proportion. Positive values indicate greater event risk in the active treatment group. Equation 3 defines the relative comparison.

$$RR_g = \frac{p_g}{p_0}$$

Here, RR_g is the risk ratio. Values above one indicate greater risk relative to placebo. All placebo denominators required for the implemented comparisons are positive.

Table 2: Variable dictionary

Variable Or Indicator	Analytical Role	Definition And Construction	Source and Identifier	Unit and Frequency	Transformation and Direction	Missing Value Rule	Coverage and Limitation
Assigned daily dose	Predictor	Published treatment assignment	EPOCH, S05	Milligrams daily	Placebo coded zero. Higher values indicate greater assigned dose	Require reported dose	Three arms. Does not measure adherence or individual exposure
Any adverse event count	Outcome numerator	Participants with at least one adverse event	EPOCH, S05, Table 3	Participants over the reported safety period	Count used directly	Require observed count	Three arms. Does not identify event location or mechanism
Safety denominator	Outcome denominator	Participants included in each reported safety group	EPOCH, S05, Table 3	Participants	Count used directly	Require positive denominator	Three arms. Differs from the primary randomized population
Adverse event proportion	Primary outcome	Event count divided by safety denominator	EPOCH, S05	Proportion over the safety period	Higher values indicate greater overall event risk	Calculate only from complete counts	Three arms. Does not measure event frequency per participant
Risk difference	Descriptive contrast	Active proportion minus placebo proportion, multiplied by 100	EPOCH, S05	Percentage points	Positive values indicate excess risk	Require both proportions	Two comparisons share the placebo group
Risk ratio	Descriptive contrast	Active proportion divided by placebo proportion	EPOCH, S05	Ratio	Values above one indicate excess relative risk	Require positive placebo proportion	Two comparisons share the placebo group

Validation concerns transcription accuracy, units, denominators and arithmetic consistency. No latent constructs or composite indices were constructed. Factor analysis and internal consistency statistics therefore do not apply.

Table 3: Descriptive statistics for the hypothesis testing records

Variable	Valid Records	Mean	Standard Deviation	Median	Minimum	Maximum
Daily dose, milligrams	3	17.33	20.53	12.00	0.00	40.00
Adverse event percentage	3	87.69	5.45	89.26	81.62	92.18

These statistics describe treatment arms with equal arm weighting. They are not individual participant means or estimates of variation between patients.

3.4 Analytical Framework, Identification, and Reproducibility:

The implemented analysis evaluates the association between assigned daily dose and overall adverse event risk within EPOCH. A Cochran Armitage trend test uses dose scores of zero, 12 and 40 milligrams, together with group event counts and safety denominators. The test evaluates an ordered relationship without estimating a multivariable exposure model.

A Pearson contingency test evaluates differences between the three event proportions without imposing a linear dose trend. Separate Fisher exact tests compare each active treatment group with placebo. These analyses provide exploratory checks against dependence on a single testing approach. They use the same safety population and require no additional sample exclusions.

Inference treats participants in different treatment arms as independent and the event indicator as binary. The two active treatment comparisons share a placebo group and are not independent replications. No clustering adjustment was applied because the workbook contains no centre specific event counts. No controls, interactions, fixed effects or mediating variables enter the analysis.

Random assignment supports interpretation of treatment group contrasts, but the extracted safety population excludes participants who received no treatment. The analysis does not recover a complete intention to treat estimand. It also cannot distinguish greater biological toxicity from differences in treatment exposure or establish the mechanism underlying an adverse event.

The workbook preserves original counts, source locations, record identifiers and formula-based risk comparisons. These elements allow the reported proportions and contrasts to be reconstructed. Definitions and denominators remain consistent within each comparison, while experimental findings and results from different clinical populations remain outside the primary test.

4. Methods:

This study employs a retrospective secondary analysis of published randomized clinical trial data to evaluate the relationship between assigned treatment intensity and overall adverse-event risk among patients with mild-to-moderate Alzheimer's disease. The methodological framework integrates treatment-arm comparisons, binomial risk estimation, ordered trend testing, and complementary exact inference. The analysis distinguishes evidence of an overall dose-related safety pattern from evidence of statistically distinguishable differences between active treatment assignments, while preserving the inferential boundaries of aggregate clinical data.

4.1 Research Design and Identification Strategy:

The study adopts a quantitative, comparative design based on aggregate safety observations from the randomized, placebo-controlled EPOCH trial reported by Egan et al. (2018). The analytical population comprises 1,957 participants who received treatment across three assignments: placebo, 12 mg, and 40 mg daily verubecestat. The unit of analysis is the treatment arm, represented by its adverse-event count and corresponding safety denominator. The outcome is the probability of experiencing at least one adverse event during the reported safety observation period, within a trial planned for 78 weeks.

The primary estimands are the differences in adverse-event probability between treatment assignments, expressed as absolute risk differences and risk ratios. The analysis additionally evaluates whether the observed probabilities exhibit an ordered association with assigned daily dose. Random assignment in the original trial provides a defensible basis for comparing treatment groups because assignment precedes the observed safety outcomes and reduces systematic baseline differences between groups. However, the secondary analysis uses the treated safety population rather than the complete randomized population. Consequently, the estimated contrasts describe the analysed safety groups and do not recover a complete intention-to-treat estimand.

Identification relies on variation in assigned treatment intensity within the same clinical trial, rather than differences across studies, populations, or observation periods. The concurrent placebo group establishes the background frequency of adverse events, allowing active-treatment risks to be interpreted relative to events occurring without active verubecestat exposure. Because all comparisons originate from the same trial, the design avoids confounding arising from pooling fundamentally different interventions and clinical populations. Nevertheless, aggregate records do not permit adjustment for individual treatment adherence, actual pharmacological exposure, treatment discontinuation, or participant characteristics. The resulting inference therefore concerns assigned-dose comparisons and observed safety outcomes, not the identification of specific biological toxicity mechanisms.

4.2 Model Specification and Measurement Alignment:

The empirical framework uses the observed adverse-event proportions defined in Equation 1, together with the absolute and relative treatment comparisons established in Equations 2 and 3 of Section 3. These measures retain their original definitions, denominators, and interpretation. The primary inferential specification evaluates whether the probability of experiencing an adverse event change systematically across the ordered treatment assignments. The Cochran-Armitage trend statistic is expressed as:

$$Z = \frac{\sum_{j=1}^3 w_j (x_j - n_j \hat{p})}{\sqrt{\hat{p}(1 - \hat{p}) \left[\sum_{j=1}^3 n_j w_j^2 - \frac{(\sum_{j=1}^3 n_j w_j)^2}{\sum_{j=1}^3 n_j} \right]}}$$

Where j indexes the three treatment groups; w_j represents the assigned daily dose score of 0, 12, or 40 mg; x_j denotes the number of participants experiencing at least one adverse event; n_j is the corresponding safety denominator; and $\hat{p} = \frac{\sum_j x_j}{\sum_j n_j}$ is the pooled event proportion. Under the null hypothesis of no ordered association, the statistic is evaluated against the standard normal distribution using a two-sided test.

The test assesses whether the observed safety pattern is systematically related to the assigned dose scores. It does not estimate a continuous pharmacological dose-response function or establish that each incremental dose increase produces a proportional change in risk. Because the available observations represent only three treatment assignments, the specification does not introduce nonlinear dose parameters, individual-level covariates, interaction terms, or unobserved biological pathways.

To evaluate treatment-group differences without imposing an ordered trend, the Pearson contingency statistic is specified as:

$$\chi^2 = \sum_{j=1}^3 \sum_{k=1}^2 \frac{(O_{jk} - E_{jk})^2}{E_{jk}}$$

Where O_{jk} denotes the observed frequency in treatment group j and adverse-event category k , and E_{jk} denotes the corresponding expected frequency under independence. The two categories distinguish participants who experienced at least one adverse event from those who did not. The test has two degrees of freedom and evaluates the null hypothesis that event probabilities are equal across the three assignments.

The measurement framework follows the variable dictionary in Table 2. Assigned dose is measured in milligrams per day, while safety is measured as a binary event occurrence aggregated within treatment groups. This outcome provides a direct measure of overall adverse-event risk but does not identify peripheral toxicity, event severity, or event frequency per participant. No composite indices or latent constructs are estimated, making factor analysis and internal-consistency reliability testing inapplicable.

4.3 Estimation, Statistical Inference, and Analytical Procedures:

Estimation proceeds from the cleaned clinical records described in Section 3. The three eligible EPOCH safety records contain complete treatment assignments, event counts, and denominators. The analysis retains all three groups without imputation, interpolation, winsorisation, or exclusion of valid extreme observations. Experimental findings concerning antibody transport remain outside the statistical estimation sample because their biological systems, interventions, and measurement structures differ from those of the clinical trial.

For each treatment assignment, the observed adverse-event proportion is calculated using Equation 1. Wilson score confidence intervals quantify uncertainty around the individual binomial proportions. Absolute risk differences and risk ratios are then calculated using Equations 2 and 3. Confidence intervals for absolute differences use independent binomial variance estimates, whereas confidence intervals for risk ratios use the logarithmic scale. These procedures preserve the original safety denominators and provide complementary interpretations of treatment-associated risk.

The primary hypothesis is evaluated using the Cochran-Armitage trend test with actual milligram dose scores. A two-sided significance threshold of 0.05 is applied. The Pearson contingency test separately assesses whether the treatment-group event proportions differ without requiring a specified ordering. Pairwise comparisons are evaluated using two-sided Fisher exact tests, including each active treatment assignment against placebo and the direct comparison between active doses. Fisher exact inference is appropriate for the binary event counts and does not rely on the large-sample chi-square approximation for the individual two-group comparisons.

The analyses assume independent participants across randomized treatment arms and a binary adverse-event indicator for each participant within the reported safety period. The two active-treatment comparisons share the same placebo group and therefore do not constitute independent replications. No centre-level clustering adjustment is implemented because the available records do not contain centre-specific event counts. The calculations use published aggregate observations rather than a fitted multivariable regression model; consequently, there are no iterative optimization procedures, convergence criteria, or estimated regression residuals. The available analytical documentation does not identify the statistical software or package versions used, so these implementation details cannot be independently specified.

4.4 Diagnostic Assessment, Robustness, and Reproducibility:

Methodological validation focuses on the integrity of the extracted counts, consistency of treatment definitions, appropriateness of the binomial comparisons, and sensitivity of inference to alternative representations of treatment ordering. Source-level checks verify that adverse-event counts are nonnegative, denominators are positive, and event counts do not exceed the corresponding denominators. Calculated proportions are restricted to the interval from zero to one. These checks support arithmetic and measurement consistency without implying validation of unobserved biological mechanisms.

Robustness assessment uses three complementary approaches. First, the Pearson contingency test examines whether the observed differences persist without imposing an ordered dose relationship. Second, an exploratory Cochran-Armitage analysis replaces actual milligram values with equally spaced rank scores, distinguishing sensitivity to treatment ordering from sensitivity to unequal dose spacing. Third, Fisher exact comparisons evaluate whether the overall pattern is supported by statistically distinguishable differences between individual assignments. These procedures use the same safety population, ensuring that differences in inference arise from analytical choices rather than changes in sample composition.

The primary hypothesis and supplementary analyses are exploratory because they were formulated after inspection of published aggregate findings. Accordingly, statistical significance is interpreted as evidence within the analysed treatment groups rather than as independent confirmatory validation. The available data cannot establish the shape of a continuous dose-response curve, identify individual pharmacological exposure, or distinguish central from peripheral adverse-event mechanisms. Similarly, the absence of participant-level and centre-level records prevents assessment of patient heterogeneity, within-centre dependence, and differential exposure duration.

Reproducibility is supported by the preservation of source identifiers, publication details, original event counts, safety denominators, treatment labels, and formula-based risk comparisons. The published EPOCH report provides the primary clinical source, while the analytical workbook documents the extracted observations and derived comparisons. Together, these materials permit reconstruction of the

descriptive risks and treatment contrasts, subject to the availability of the underlying source publications. The methodological contribution lies in the transparent integration of established statistical procedures to distinguish an overall ordered safety association from uncertainty surrounding incremental active-dose differences, without claiming a new estimator or an independently identified biological mechanism.

5. Findings:

This analysis evaluates whether higher assigned verubecestat dose is associated with greater adverse event risk. It presents treatment group estimates, tests the ordered dose relationship and assesses sensitivity to alternative comparisons. The findings concern overall clinical safety within the analysed trial population.

5.1 Analytical Sample and Observed Risks:

The analytical sample comprises three EPOCH treatment arms representing 1,957 participants who received treatment. Daily assignments were placebo, 12 milligrams and 40 milligrams. The outcome identifies participants experiencing at least one adverse event during the reported safety period. All three records contain complete dose values, event counts and denominators. The underlying trial concerned patients with mild to moderate Alzheimer disease and a planned treatment period of 78 weeks, as reported by Egan et al. in 2018.

Table 4: Adverse event risks by assigned daily dose

Treatment	Participants With an Event	Safety Denominator	Event Risk, Percent	95 Percent Confidence Interval
Placebo	533	653	81.62	78.47 to 84.41
Verubecestat, 12 milligrams	582	652	89.26	86.65 to 91.41
Verubecestat, 40 milligrams	601	652	92.18	89.86 to 94.00

Adverse event risk increased across the three ordered assignments. The high placebo risk makes absolute differences important for interpretation. The active treatment groups exceeded an already substantial background event risk rather than shifting the outcome from rare to common.

5.2 Dose Comparisons and Hypothesis Assessment:

The 12 milligram assignment exceeded placebo by 7.64 percentage points, while the 40-milligram assignment exceeded placebo by 10.55 percentage points. These differences correspond to approximately eight and eleven additional participants experiencing an adverse event per 100 treated over the reported observation period.

Table 5: Calculated treatment contrasts

Comparison	Risk Difference, Percentage Points	95 Percent Confidence Interval	Risk Ratio	95 Percent Confidence Interval	Fisher Exact p Value
12 milligrams versus placebo	7.64	3.84 to 11.44	1.094	1.045 to 1.144	0.000112
40 milligrams versus placebo	10.55	6.94 to 14.17	1.129	1.082 to 1.179	$\backslash(1.66\backslash\times 10^{\{-8\}}\backslash)$
40 versus 12 milligrams	2.91	-0.23 to 6.06	1.033	0.997 to 1.069	0.0854

Note: Risk difference intervals use independent binomial variance estimates. Risk ratio intervals use the logarithmic scale. Fisher exact tests are two sided. These are calculated intervals rather than reproductions of the published interval procedure. Both active treatment contrasts indicate greater adverse event risk than placebo. Their confidence intervals exclude zero for absolute differences and one for risk ratios. Relative increases are approximately 9.4 percent and 12.9 percent, respectively. These relative changes should be interpreted alongside the absolute differences because the outcome is common.

The comparison between active doses is less precise. Its interval includes a small negative difference and an increase of approximately six percentage points. The estimate therefore does not establish that the 40-milligram assignment carries greater risk than the 12-milligram assignment. The Cochran Armitage test using actual dose scores produced $Z = 5.316$, with a two-sided probability of $p = 1.06 \times 10^{-7}$. At the five percent significance level, this result supports H1 that higher assigned daily dose is associated with greater adverse event probability across the three treatment assignments. Support concerns the overall ordered pattern, including placebo. It does not establish a statistically distinguishable increase between every adjacent dose. The hypothesis assessment remains exploratory because the relationship was formulated after inspection of published aggregate findings.

5.3 Sensitivity of the Dose Relationship:

The Pearson contingency test rejected equality of event proportions across treatment arms, with $\chi^2 = 35.914$, two degrees of freedom and $p = 1.59 \times 10^{-8}$. This finding shows that the group differences do not depend on imposing an ordered dose trend. An additional exploratory trend test replaced actual milligram

values with equally spaced rank scores. It produced $Z = 5.802$ and $p = 6.55 \times 10^{-9}$. The positive relationship therefore persists when the analysis represents treatment ordering rather than the unequal spacing between assigned doses. The exact comparisons provide a narrower interpretation of this consistency. Each active assignment differs from placebo, while the direct comparison between active doses remains less precise. The evidence supports an overall treatment ordering but does not identify the shape of a continuous dose response curve. The shared placebo group also means that the two active treatment comparisons are not independent replications.

5.4 Empirical Interpretation:

The clinical estimates identify a safety cost associated with active verubecestat treatment in this population. They do not demonstrate that greater brain delivery caused the observed events or that a particular antibody design would prevent them. The experimental evidence addresses complementary mechanisms. Yu et al. in 2011 linked reduced transferrin receptor affinity to improved brain uptake, while Bien-Ly et al. in 2014 showed how strong receptor binding can redirect intracellular trafficking. Niewoehner et al. in 2014 demonstrated improved target engagement through monovalent receptor binding. Couch et al. in 2013 showed that modifying Fc effector function could ameliorate specific safety liabilities in mice. These findings provide mechanistic context but do not constitute statistical confirmation of those pathways in the clinical sample. The findings support H1 within the analysed safety population. Both active assignments increased overall adverse event risk relative to placebo, and the ordered association persisted under alternative dose scoring. The evidence supports a clinical safety relationship rather than a demonstrated solution for achieving brain penetration with reduced peripheral toxicity.

6. Discussion:

A significant dose trend does not establish that every dose increase produces a distinguishable increase in harm. Tables 4 and 5 reveal this distinction: both verubecestat assignments increased overall adverse event risk relative to placebo, while the direct comparison between active doses remained less precise. Equations 1 to 3 clarify the interpretation by separating event probability, absolute excess risk and relative risk. The contribution of this reanalysis is therefore an explicit distinction between evidence for an overall ordered association and evidence for incremental harm between active doses. It extends the interpretation of published safety findings rather than establishing a previously unknown adverse effect. Egan et al. in 2019a had already documented increased adverse events in EPOCH, including differences in how particular events accumulated over time.

The clinical relationship supports an exposure related safety concern, but its biological interpretation requires endpoint specific evidence. The aggregate outcome in Table 4 combines events that may arise through different central and peripheral pathways. It therefore identifies a clinical safety burden without assigning that burden to a single mechanism. Novak et al. in 2020 reported liver enzyme elevations during atabecestat treatment alongside reductions in cerebrospinal fluid amyloid markers. This comparison shows why central pharmacological activity and peripheral tolerability require separate evaluation. The theoretical implication is that evidence of target activity cannot serve as evidence of a favourable safety profile, even when both outcomes respond to treatment exposure.

The contrast structure in Table 5 also limits claims about pathway dominance or dose response shape. The larger differences occur between active treatment and placebo, while the smaller difference between active doses has greater uncertainty relative to its magnitude. This pattern is compatible with several exposure response relationships and does not establish a biological threshold or saturation mechanism. Equation 2 expresses the additional clinical burden, whereas Equation 3 places that burden against the high background event probability. Their joint interpretation prevents a modest relative increase from obscuring a meaningful absolute difference. The findings therefore refine the conceptual framework by treating assigned dose as an observed predictor of overall safety rather than a direct measure of brain exposure or a substitute for molecular design quality.

The practical challenge is to distinguish delivery, tolerability and therapeutic benefit when selecting doses for further development. The observed safety differences justify scrutiny of dose escalation, but they do not identify an optimal dose. Wessels et al. in 2020 reported that lanabecestat was generally well tolerated yet failed to slow cognitive or functional decline in two global trials. This differs from treating tolerability as sufficient evidence of therapeutic promise. Together, these observations favour decision criteria that evaluate clinical benefit and safety jointly. Development programmes should compare candidate doses against concurrent controls and retain separate assessments of event severity, peripheral injury and functional benefit rather than relying on a single aggregate measure.

The international relevance concerns a shared therapeutic development problem rather than a demonstrated difference between economies. Egan et al. in 2019b reported that verubecestat also failed to improve clinical outcomes in a multinational prodromal Alzheimer population, with some outcomes favouring placebo. That evidence extends the concern beyond patients with established dementia, although it does not establish identical risks across disease stages or countries. The present results contribute a reproducible interpretation of dose related safety that remains sensitive to uncertainty between active assignments. Future evaluations should determine whether improved delivery efficiency can preserve clinical benefit at lower systemic exposure, using measured brain exposure and endpoint

specific toxicity within the same comparative design. Such evidence would connect molecular optimisation to clinical outcomes more directly than an aggregate dose trend alone.

7. Conclusion and Implications:

The global pursuit of effective neurodegenerative therapies demands a fundamental distinction between pharmacological activity, therapeutic benefit, and clinical safety. This study shows that increasing treatment intensity is associated with a greater overall adverse-event burden, although the evidence does not establish a statistically distinguishable increase between successive active treatment levels. This distinction provides the study's central empirical contribution: an ordered safety relationship cannot automatically be interpreted as evidence of proportional incremental harm. The findings refine pharmacological exposure theory by demonstrating the importance of separating treatment-associated risk from assumptions about continuous biological responses. They further clarify that improvements in central nervous system delivery cannot independently establish peripheral tolerability or clinical effectiveness. For pharmaceutical developers, these insights support more discriminating dose-selection strategies that integrate absolute safety burdens, comparative uncertainty, and demonstrated therapeutic benefit. Regulatory authorities can strengthen benefit-risk evaluations by requiring transparent comparisons against concurrent controls and distinguishing overall adverse-event occurrence from clinically specific toxicity. At the operational level, integrating these distinctions into clinical development protocols can improve safety monitoring, resource allocation, and decisions about treatment advancement. The broader societal implication is that more rigorous interpretation of therapeutic safety can reduce avoidable patient exposure, support responsible pharmaceutical innovation, and strengthen evidence-based decision-making across international healthcare systems.

The study's reliance on published aggregate observations provides a reproducible foundation for evaluating treatment-associated safety while identifying opportunities for deeper investigation. Individual-level longitudinal data could clarify exposure duration, patient heterogeneity, and the temporal development of adverse events. Pharmacokinetic measurements and organ-specific safety assessments could establish whether observed clinical differences reflect systemic exposure, central activity, or peripheral injury. Future comparative studies across therapeutic compounds, disease stages, and international trial populations could assess the transferability of the observed relationship. Integrating molecular delivery measurements with clinical outcomes would further test whether improved brain penetration can reduce systemic exposure without compromising therapeutic effectiveness.

References:

1. Bien-Ly, N., Yu, Y. J., Bumbaca, D., Elstrott, J., Boswell, C. A., Zhang, Y., Luk, W., Lu, Y., Dennis, M. S., Weimer, R. M., Chung, I., & Watts, R. J. (2014). Transferrin receptor trafficking determines brain uptake of TfR antibody affinity variants. *Journal of Experimental Medicine*, 211(2), 233-244. <https://doi.org/10.1084/jem.20131660>
2. Couch, J. A., Yu, Y. J., Zhang, Y., Tarrant, J. M., Fuji, R. N., Meilandt, W. J., Solanoy, H., Tong, R. K., Hoyte, K., Luk, W., Lu, Y., Gadkar, K., Prabhu, S., Ordonia, B. A., Nguyen, Q., Lin, Y., Lin, Z., Balazs, M., Searce-Levie, K., ... Watts, R. J. (2013). Addressing safety liabilities of TfR bispecific antibodies that cross the blood-brain barrier. *Science Translational Medicine*, 5(183), Article 183ra57. <https://doi.org/10.1126/scitranslmed.3005338>
3. Egan, M. F., Kost, J., Tariot, P. N., Aisen, P. S., Cummings, J. L., Vellas, B., Sur, C., Mukai, Y., Voss, T., Furtek, C., Mahoney, E., Harper Mozley, L., Vandenberghe, R., Mo, Y., & Michelson, D. (2018). Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. *New England Journal of Medicine*, 378(18), 1691-1703. <https://doi.org/10.1056/NEJMoa1706441>
4. Egan, M. F., Kost, J., Voss, T., Mukai, Y., Aisen, P. S., Cummings, J. L., Tariot, P. N., Vellas, B., van Dyck, C. H., Boada, M., Zhang, Y., Li, W., Furtek, C., Mahoney, E., Harper Mozley, L., Mo, Y., Sur, C., & Michelson, D. (2019a). Randomized trial of verubecestat for prodromal Alzheimer's disease. *New England Journal of Medicine*, 380(15), 1408-1420. <https://doi.org/10.1056/NEJMoa1812840>
5. Egan, M. F., Mukai, Y., Voss, T., Kost, J., Stone, J., Furtek, C., Mahoney, E., Cummings, J. L., Tariot, P. N., Aisen, P. S., Vellas, B., Lines, C., & Michelson, D. (2019b). Further analyses of the safety of verubecestat in the phase 3 EPOCH trial of mild-to-moderate Alzheimer's disease. *Alzheimer's Research & Therapy*, 11, Article 68. <https://doi.org/10.1186/s13195-019-0520-1>
6. Niewoehner, J., Bohrmann, B., Collin, L., Urich, E., Sade, H., Maier, P., Rueger, P., Stracke, J. O., Lau, W., Tissot, A. C., Loetscher, H., Ghosh, A., & Freskgård, P. O. (2014). Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. *Neuron*, 81(1), 49-60. <https://doi.org/10.1016/j.neuron.2013.10.061>
7. Novak, G., Streffer, J. R., Timmers, M., Henley, D., Brashear, H. R., Bogert, J., Russu, A., Janssens, L., Tesseur, I., Tritsmans, L., Van Nueten, L., & Engelborghs, S. (2020). Long-term safety and tolerability of atabecestat, an oral BACE1 inhibitor, in early Alzheimer's disease spectrum patients: A randomized, double-blind, placebo-controlled study and a two-period extension study. *Alzheimer's Research & Therapy*, 12, Article 58. <https://doi.org/10.1186/s13195-020-00614-5>
8. Sperling, R., Henley, D., Aisen, P. S., Raman, R., Donohue, M. C., Ernststrom, K., Rafii, M. S., Streffer, J., Shi, Y., Karcher, K., Raghavan, N., Tymofeyev, Y., Bogert, J., Brashear, H. R., Novak,

- G., Thippawong, J., Saad, Z. S., Kolb, H., Rofael, H., Sanga, P., & Romano, G. (2021). Findings of efficacy, safety, and biomarker outcomes of atabecestat in preclinical Alzheimer disease: A truncated randomized phase 2b/3 clinical trial. *JAMA Neurology*, 78(3), 293-301. <https://doi.org/10.1001/jamaneurol.2020.4857>
9. Wessels, A. M., Tariot, P. N., Zimmer, J. A., Selzler, K. J., Bragg, S. M., Andersen, S. W., Landry, J., Krull, J. H., Downing, A. M., Willis, B. A., Shcherbinin, S., Mullen, J., Barker, P., Schumi, J., Shering, C., Matthews, B. R., Stern, R. A., Vellas, B., Cohen, S., ... Sims, J. R. (2020). Efficacy and safety of lanabecestat for treatment of early and mild Alzheimer disease: The AMARANTH and DAYBREAK-ALZ randomized clinical trials. *JAMA Neurology*, 77(2), 199-209. <https://doi.org/10.1001/jamaneurol.2019.3988>
10. Yu, Y. J., Zhang, Y., Kenrick, M., Hoyte, K., Luk, W., Lu, Y., Atwal, J., Elliott, J. M., Prabhu, S., Watts, R. J., & Dennis, M. S. (2011). Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. *Science Translational Medicine*, 3(84), Article 84ra44. <https://doi.org/10.1126/scitranslmed.3002230>