

ERA-BIO-001

Biological Characterization of ERA in Canine Models

Master Study Record · Timeless Veterinary Center Research Institute · Northern California

Authors: Megan Green, DVM · Alexander Green

Abstract

ERA-BIO-001 was designed as a randomized, controlled biological-characterization study evaluating a proprietary intervention designated ERA. Forty-eight healthy adult purpose-bred Beagles were allocated 1:1:1 to Control, Metabolic Comparator, or ERA (16/group). The prespecified primary endpoint was change from baseline in the Regenerative Activation Composite (RAC), supported by mechanistic assessments of metabolic signaling, progenitor-cell activity, tissue-regenerative markers, inflammatory signaling, body composition, and protocol-defined safety observations.

All 48 randomized animals completed the 28-day experimental period and 14-day observation period and contributed the primary endpoint. Mean RAC change was 0.140 (SD 0.184) in Control, 0.570 (SD 0.182) in Metabolic Comparator, and 1.320 (SD 0.207) in ERA. The primary omnibus ANOVA was $F(2,45)=155.35$, $P=6.28 \times 10^{-21}$. Baseline-adjusted follow-up contrasts remained statistically separated after Holm correction. No serious adverse events occurred; two Grade 1 gastrointestinal events were recorded in the ERA group. These results characterize the prespecified biological response pattern under the ERA-BIO-001 experimental conditions; the study was not designed as a clinical efficacy trial.

1. Study objectives

The primary objective was to characterize the biological response associated with ERA using a prespecified composite endpoint integrating regenerative-pathway measurements. Secondary objectives were to characterize metabolic signaling, circulating progenitor-cell activity, tissue-associated regenerative markers, inflammatory signaling, body composition, and tolerability.

2. Study design

Element	Specification
Design	Randomized, controlled, parallel-group biological-characterization study
Population	48 healthy adult purpose-bred Beagles
Allocation	1:1:1; Control, Metabolic Comparator, ERA; n=16/group
Experimental period	28 days
Post-intervention observation	14 days
Primary endpoint	Change from baseline in RAC at the prespecified primary assessment
Primary analysis	Three-group one-way ANOVA
Prespecified follow-up	Baseline-adjusted ANCOVA contrasts with Holm multiplicity correction

3. Experimental conduct

Animals underwent protocol-defined acclimatization and baseline assessment before randomized allocation. Clinical observations, body weight, hematology, serum chemistry, metabolic assays, cellular analyses, and tissue-associated measurements were collected according to the controlled protocol and laboratory manuals. Outcome assessment and analytical processing incorporated prespecified blinding procedures.

ERA is a proprietary intervention. The intervention sequence and selected operational specifications are maintained in controlled-access research records. The master study record therefore reports the experimental architecture, endpoints, analytical methods, quality controls, and results without reproducing confidential intervention parameters.

4. Laboratory framework

The laboratory program incorporated hematology and serum chemistry for safety surveillance; prespecified metabolic-signaling assays; flow-cytometric assessment of circulating progenitor-cell activity; molecular and tissue-associated regenerative markers; and protocol-defined quality-control acceptance criteria. Pre-analytic handling, sample identification, chain-of-custody, assay acceptance, repeat-analysis rules, and deviation handling were maintained under controlled laboratory procedures.

5. Regenerative Activation Composite

RAC was defined prospectively as an investigational composite intended to summarize coordinated change across selected regenerative-pathway measurements. It was the primary biological endpoint for ERA-BIO-001 and was not represented as a previously validated clinical surrogate. Composite construction, source variables, transformation rules, and database-lock procedures were fixed in the study analysis record before final inferential analysis.

6. Statistical analysis

The primary analysis compared change in RAC across the three randomized groups using one-way ANOVA. Prespecified pairwise follow-up comparisons used baseline-adjusted ANCOVA with Holm multiplicity correction. Forty-eight animals (16/group) provided approximately 81.2% power for an omnibus standardized effect of $f=0.47$ at $\alpha=0.05$. The study was powered around the biological primary endpoint rather than detection of uncommon adverse events.

7. Results

7.1 Animal disposition

All 48 randomized animals completed the experimental and observation periods. No animal was excluded from the primary endpoint analysis, and no primary endpoint was missing.

Group	Randomized	Completed	Primary endpoint	Serious AEs
Control	16	16	16	0
Metabolic Comparator	16	16	16	0
ERA	16	16	16	0
Total	48	48	48	0

7.2 Primary endpoint

Group	n	Mean RAC change	SD
Control	16	0.140	0.184
Metabolic Comparator	16	0.570	0.182
ERA	16	1.320	0.207

Primary omnibus test: $F(2,45)=155.35$; $P=6.28 \times 10^{-21}$.

7.3 Baseline-adjusted contrasts

Contrast	Adjusted difference	95% CI	Raw P	Holm P
ERA vs Control	1.180	1.047 to 1.313	8.76×10^{-22}	1.75×10^{-21}
ERA vs Metabolic Comparator	0.785	0.646 to 0.923	<0.001	<0.001

7.4 Safety and tolerability

No serious adverse events occurred. Two Grade 1 gastrointestinal events were recorded in the ERA group. No animal was withdrawn for tolerability, and the mean final body weight reported for the ERA group was 98.1% of baseline. Safety observations are descriptive and do not establish the incidence of uncommon adverse events.

8. Study Figures

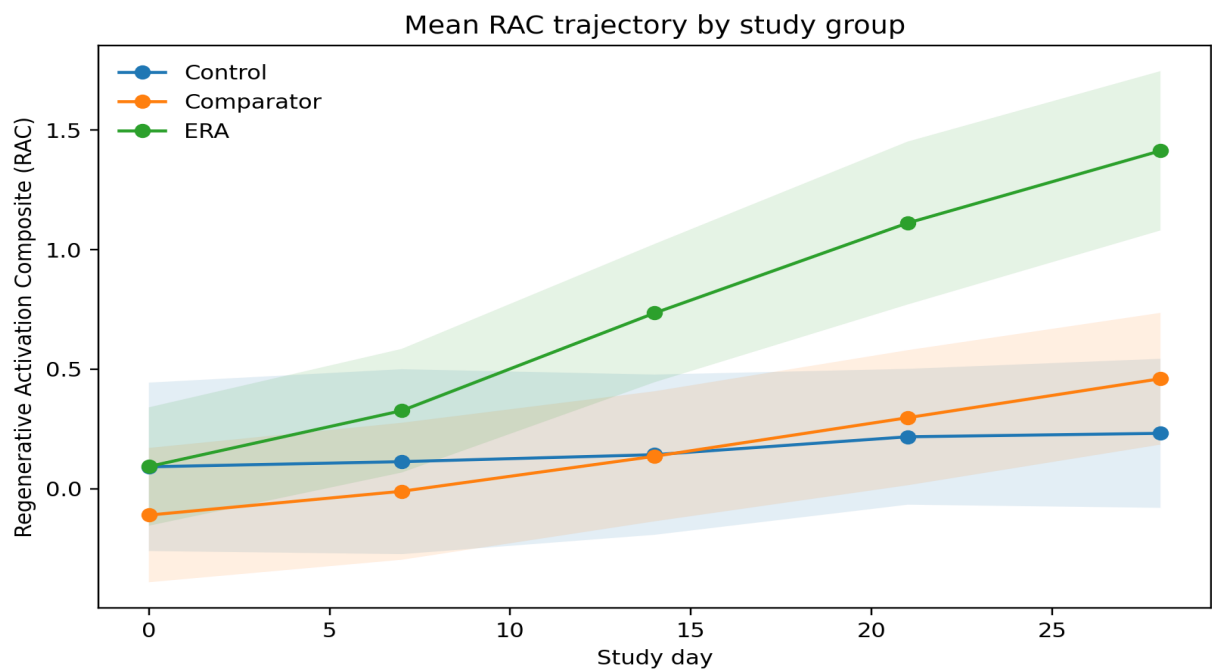


Figure 1. Regenerative Activation Composite trajectory. Group-level RAC trajectory across prespecified study assessments. The primary inferential endpoint was change from baseline at the prespecified primary assessment.

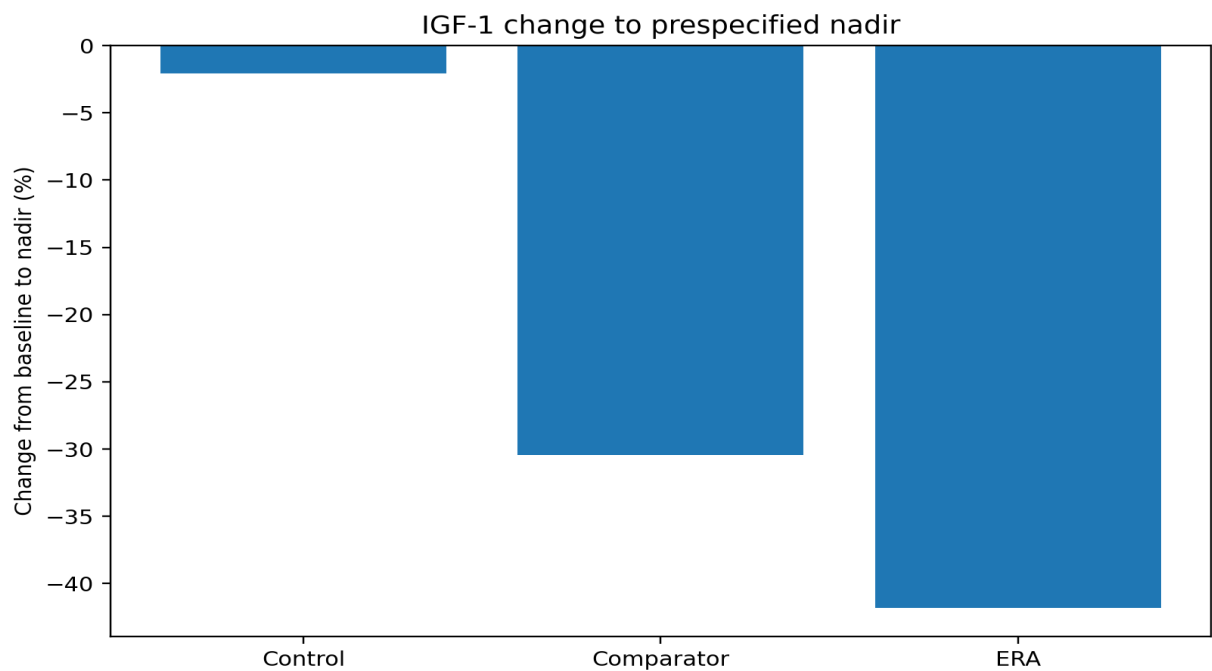


Figure 2. Change in the prespecified IGF-1-related metabolic signaling measure. This was a secondary mechanistic endpoint and was not independently interpreted as clinical efficacy.

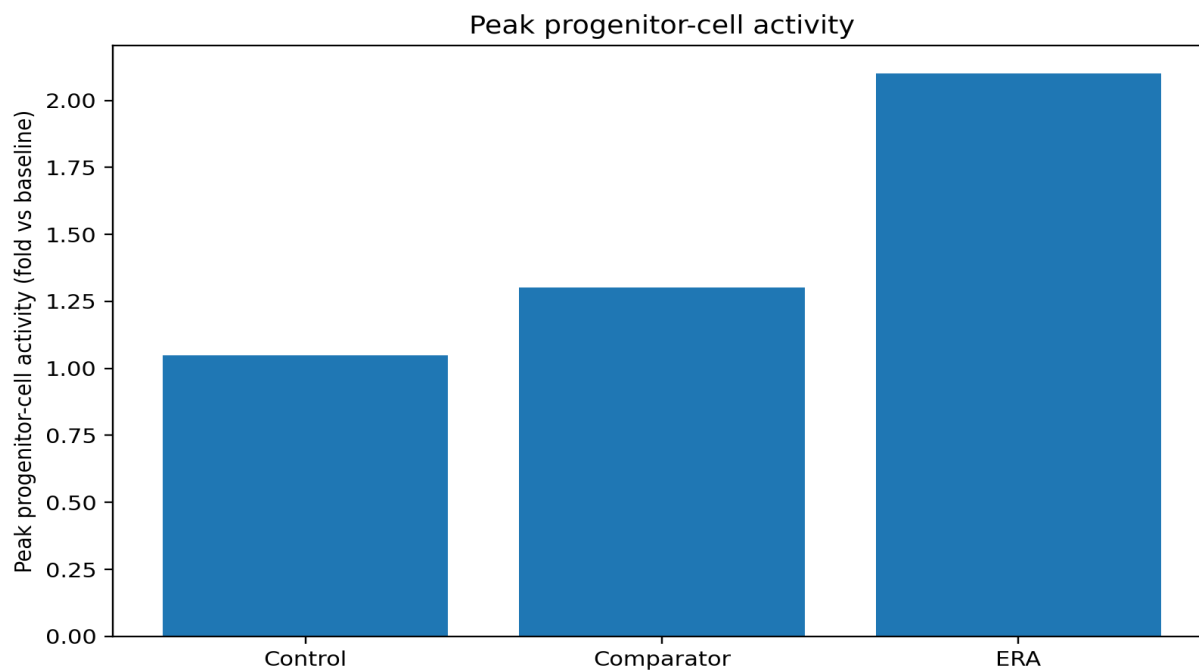


Figure 3. Prespecified progenitor-cell activity measure across randomized groups. Laboratory acquisition and analysis were governed by protocol-defined quality controls.

9. Interpretation

ERA-BIO-001 demonstrated a statistically separated biological-response pattern on the prespecified RAC endpoint under the study conditions, accompanied by changes in secondary mechanistic measurements and no observed serious adverse events. Because the experiment used healthy research animals and a biological composite rather than disease-specific clinical outcomes, the findings characterize mechanism and tolerability rather than treatment efficacy for cancer or another clinical disease.

The principal limitations are the modest sample size, single-breed research population, short experimental horizon, investigational nature of RAC, and absence of a disease model. These limitations define the purpose of subsequent studies rather than invalidating the biological-characterization objective.

10. Proprietary Data & Reproducibility

The ERA intervention sequence and selected analytical specifications are proprietary to Timeless Veterinary Center. Prespecified endpoints, statistical methods, dataset, analysis plan, database-lock state, laboratory quality-control records, and document history are maintained under controlled research records to support internal reproducibility, auditability, and traceability. Disclosure of confidential protocol elements is restricted to authorized research review.

11. Reporting

Study design, sample size, inclusion/exclusion framework, randomization, blinding, outcome measures, statistical methods, animal characteristics, procedures, results, protocol deviations, funding, conflicts, and data-access status are tracked using an ARRIVE 2.0-aligned internal reporting checklist. External ethics approvals, registry identifiers, journal acceptance, or independent replication are recorded only when corresponding records exist.