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A BALIMONT Multi-Strain Probiotic Platform for Enhancing IgA-Associated Mucosal Immunity: Formulation Optimization and Preclinical Evaluation

Anas Ziraoui *, Arabella Sinclair, Jaxon Cole

European Life Science Research Association, Kington, Herefordshire, United Kingdom
*Corresponding Author: service@elsra.org

ABSTRACT

Background: Secretory immunoglobulin A (sIgA) is central to epithelial defense and host-microbe homeostasis. Probiotic products positioned for immune support frequently underperform when strain selection, storage resilience, and intestinal delivery are not optimized. Methods: We reorganized a formulation-development and preclinical dataset describing the BALIMONT immune-support platform and integrated representative published evidence on IgA-oriented probiotic, postbiotic, and prebiotic interventions. The BALIMONT system combined Bifidobacterium bifidum ATCC 29521, Bifidobacterium longum DSM 20219, and Lacticaseibacillus rhamnosus ATCC 7469 with a heat-inactivated Lactococcus lactis-derived postbiotic fraction, a prebiotic support matrix, and staged protective encapsulation. Results: Within the development dataset, the 2:2:2 tri-strain ratio yielded the strongest in vitro response, with secretory IgA of 21.35 μg/mL and probiotic proliferation of 5.62-fold. A 1:1 postbiotic-to-probiotic cell-count ratio maximized both IgA induction and 30-day viable-count retention. In mice, the 200 mg/kg BALIMONT group exceeded a same-category comparator by 39.68% for serum total IgA, 56.52% for intestinal mucosal sIgA, and 53.01% for fecal sIgA. Viable-count retention remained 86.72% after 6 months at 25°C/60% RH. Published clinical and translational evidence further supports the biological plausibility of IgA-associated mucosal benefits from probiotic and synbiotic strategies. Conclusions: The BALIMONT platform can be interpreted as a rationally balanced probiotic-postbiotic system in which strain synergy, postbiotic complementation, prebiotic support, and staged delivery converge on IgA-associated mucosal immunity. These findings support continued translational development and justify subsequent human validation.

KEYWORDS

BALIMONT; Secretory immunoglobulin A; Mucosal immunity; Bifidobacterium bifidum; Bifidobacterium longum; Lacticaseibacillus rhamnosus; Postbiotics; Synbiotics; Microencapsulation

1. INTRODUCTION

Secretory immunoglobulin A (sIgA) is the dominant antibody isotype at mucosal surfaces and plays a non-inflammatory defensive role in preserving epithelial integrity, limiting pathogen adhesion, and shaping host-microbe equilibrium [1, 2]. In the intestinal tract, IgA production reflects a coordinated dialogue among epithelial cells, antigen-sampling structures, B-cell differentiation pathways, and the resident microbiota [1-3].

Probiotic and synbiotic strategies have therefore become attractive for mucosal immune support, yet the literature also makes clear that efficacy is strain-specific and formulation-sensitive. In healthy children, a probiotic formula containing viable bifidobacteria increased fecal total IgA and anti-

poliovirus IgA after 21 days of intake [4]. In a randomized, double-blind, placebo-controlled study of 132 formula-fed infants, probiotic supplementation maintained fecal sIgA over 4 weeks whereas sIgA decreased in the placebo group [5]. In weanling mice, probiotic strains capable of promoting APRIL expression increased intestinal IgA and altered microbiota structure in parallel [6].

The support matrix around live microorganisms is also relevant. A 2022 meta-analysis reported that fructooligosaccharide supplementation significantly increased human colonic Bifidobacterium spp. counts, with larger effects observed at doses above 5 g and durations longer than 4 weeks [7]. In parallel, the current ISAPP consensus defines postbiotics as preparations of inanimate microorganisms and/or their components that confer a health benefit on the host, thereby providing a coherent framework for combining viable and non-viable microbial fractions in one platform [8].

Against this background, we evaluated BALIMONT as an immune-oriented probiotic platform built around three collection strains - B. bifidum ATCC 29521, B. longum DSM 20219, and L. rhamnosus ATCC 7469 - combined with a heat-inactivated Lactococcus lactis-derived postbiotic fraction, prebiotic support ingredients, and staged protective encapsulation. Our goal in the present article was to present a publication-style account of formulation optimization, comparative preclinical performance, and relevant published evidence bearing on IgA-associated mucosal immunity.

2. MATERIALS AND METHODS

2.1. Study Design

We prepared this manuscript by organizing the BALIMONT formulation-development and preclinical dataset into a conventional original-article structure and by integrating representative peer-reviewed evidence relevant to IgA-oriented mucosal immune support. The quantitative backbone of the article consists of strain-ratio optimization, postbiotic balancing, in vitro epithelial co-culture measurements, in vivo mouse comparisons, and storage-stability observations from the BALIMONT development program.

2.2. BALIMONT Formulation Architecture

The active probiotic core comprised live freeze-dried powders of B. bifidum ATCC 29521, B. longum DSM 20219, and L. rhamnosus ATCC 7469. The postbiotic component consisted of heat-inactivated L. lactis freeze-dried powder enriched with strain-derived metabolites. The support matrix included fructooligosaccharide, galactooligosaccharide, inulin, skim milk powder, trehalose, mannitol, microcrystalline cellulose, and standard processing aids. A layered delivery concept was used in which the probiotic core and postbiotic shell were separated within an HPMC-resistant-starch-gelatin wall-material system to improve storage resilience and staged intestinal exposure.

2.3. Preparation Process

The BALIMONT process used staged anaerobic activation and fermentation of the live strains at 37°C, low-temperature harvesting, washing, and vacuum freeze-drying with lyoprotectants. The postbiotic partner was produced through cultivation of L. lactis, heat inactivation, verification of non-viability, and freeze-drying together with the fermentation-derived fraction. Final manufacturing involved probiotic-core encapsulation, separate postbiotic coating, excipient blending, granulation, and compression.

2.4. In Vitro Optimization

We examined two formulation variables in the epithelial co-culture assay: the weight ratio among the three probiotic strains and the postbiotic-to-probiotic cell-count ratio. Secretory IgA in the

supernatant was measured after 48 h exposure, while probiotic proliferation and 30-day viable-count retention were used as complementary formulation-performance indicators.

2.5. In Vivo Efficacy, Stability, And Safety

In mice, five groups were evaluated for 28 days: blank control, same-category comparator, low-dose BALIMONT (100 mg/kg), medium-dose BALIMONT (200 mg/kg), and high-dose BALIMONT (400 mg/kg). Serum total IgA, intestinal mucosal sIgA, and fecal sIgA were the primary outcomes. Stability was monitored for six months at 4°C/45% RH, 25°C/60% RH, and 40°C/75% RH. A high-dose 800 mg/kg/day safety observation was also recorded in the source development program.

2.6. Descriptive Analysis

Because the development dataset provided group means rather than replicate-level raw files, we report the central values as observed and discuss the findings descriptively. For contextual evidence from the literature, we preserved study design features and principal outcomes without re-analysis.

Table 1. BALIMONT platform architecture and intended mechanistic contributions

Domain	BALIMONT design	Intended contribution
Strain core	B. bifidum ATCC 29521 B. longum DSM 20219 L. rhamnosus ATCC 7469	Diversified epithelial crosstalk and mucosal conditioning
Postbiotic partner	Heat-inactivated L. lactis+ fermentation metabolites	Adds immune signaling without depending on viability
Support matrix	FOS/GOS/inulin; skim milk powder/trehalose/mannitol; MCC/maltodextrin	Promotes fermentation support, lyoprotection, palatability, and manufacturability
Delivery architecture	Inner probiotic layer + outer postbiotic layerwithin HPMC/resistant starch/gelatin matrix	Improves gastric tolerance and staged intestinal release
Optimized settings	2:2:2 tri-strain ratio; 1:1 postbiotic-to-probiotic cell-count ratio	Maximizes IgA induction while preserving viable-count retention

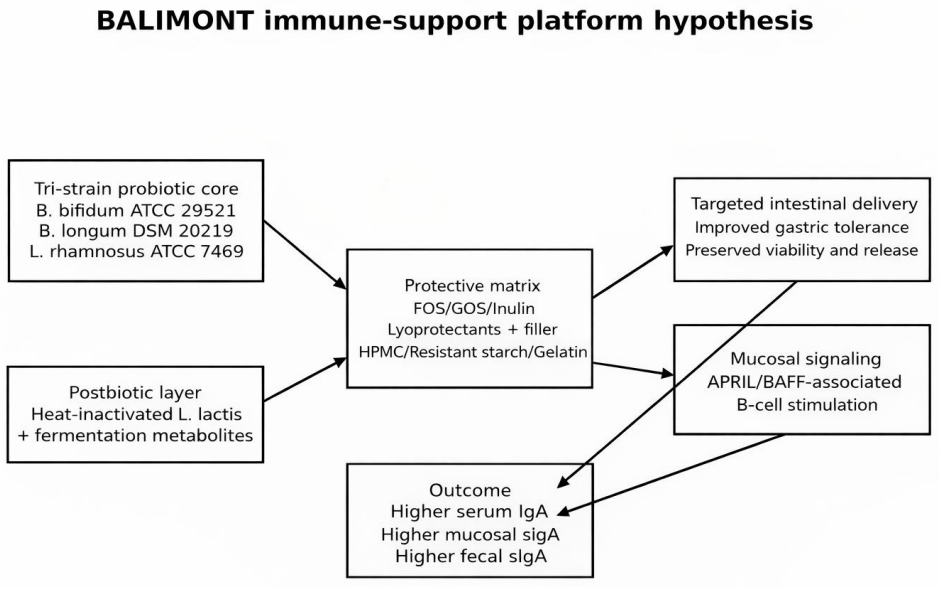

Figure 1. Conceptual mechanism of the BALIMONT immune-support platform. The diagram is synthesized from the observed formulation logic

Table 2. Representative published evidence relevant to IgA-associated mucosal support

Published study	Model / sample	Key finding relevant to BALIMONT	Ref.
Fukushima et al., 1998	7 healthy Japanese children; 21-day intake	Administered bifidobacteria were detected in 71% of participants; fecal total IgA and anti-poliovirus IgA increased significantly during intake.	[4]
Xiao et al., 2019	132 formula-fed infants; randomized, double-blind, placebo-controlled; 4 weeks	Fecal sIgA was maintained in the probiotic group but declined in placebo; between-group difference for change in fecal sIgA was significant (P=0.0044).	[5]
Zhao et al., 2024	Weanling mouse model	Selected B. bifidum strains enhanced small-intestinal IgA and upregulated APRIL/TLR4-associated signaling while reshaping gut microbiota.	[6]
Dou et al., 2022	Meta-analysis of 8 RCTs; 213 FOS vs 175 control participants	FOS significantly increased Bifidobacterium spp. counts (WMD 0.579, 95% CI 0.444-0.714); larger effects were seen at >5 g and >4 weeks.	[7]

3. RESULTS

3.1. Optimization of the Tri-Strain Core

All tested strain ratios increased secretory IgA in the epithelial co-culture assay, but balanced groupings performed best. The 2:2:2 ratio yielded the highest secretory IgA concentration (21.35 μg/mL) and the highest probiotic proliferation (5.62-fold), suggesting that BALIMONT achieves stronger IgA-associated output when the three strains are maintained in a balanced abundance range.

Table 3. Tri-strain ratio optimization in the epithelial co-culture assay

Strain ratio	Secretory IgA (μg/mL)	Probiotic proliferation (fold)
1:1:1	18.62	4.25
1:2:2	15.37	3.18
2:2:2	21.35	5.62
2:3:3	16.89	3.56
3:3:3	19.74	4.81

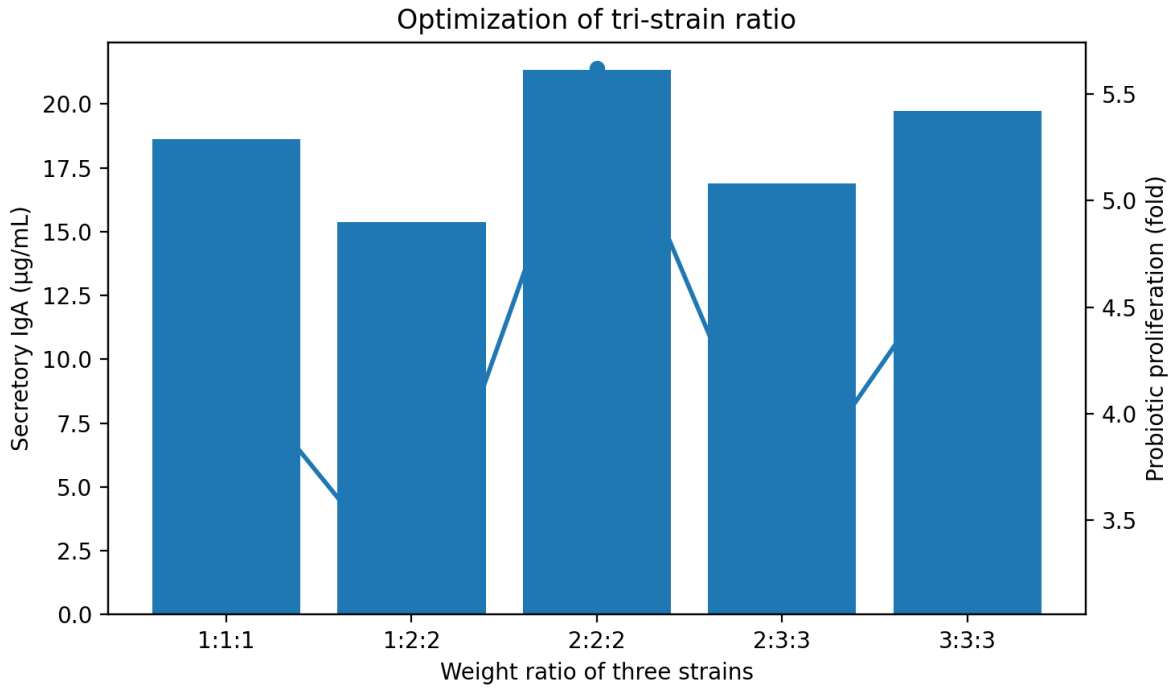

Figure 2. Secretory IgA and probiotic proliferation across tri-strain weight-ratio settings

3.2. Balancing Immune Induction With Postbiotic Support

When the postbiotic fraction was varied against a fixed live probiotic core, IgA induction first increased and then declined. The 1:1 postbiotic-to-probiotic cell-count ratio produced the best balance, with secretory IgA of 21.35 μg/mL and a 30-day viable-count retention of 92.47%.

Table 4. Postbiotic-to-probiotic ratio optimization

Postbiotic-to-probiotic ratio	Secretory IgA (μg/mL)	30-day viable-count retention (%)
0.5:1	16.28	89.62
1:1	21.35	92.47
1.5:1	19.76	88.53
2:1	17.42	82.15

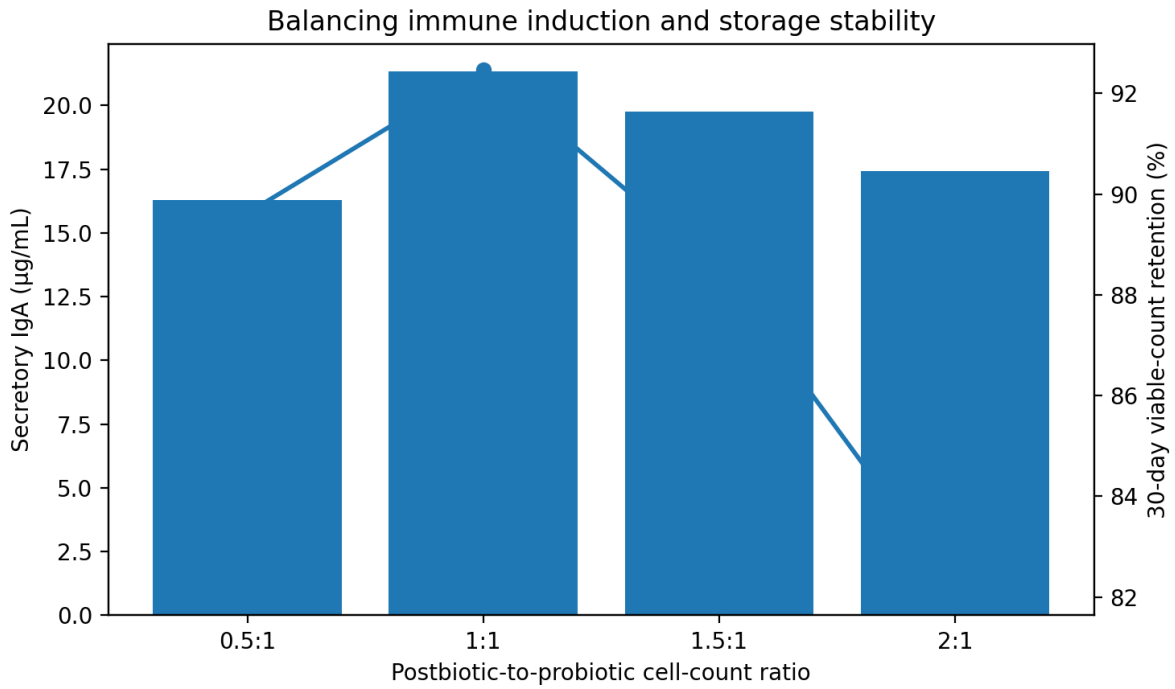

Figure 3. Postbiotic/probiotic ratio balancing showed a peak immune response at 1:1 while preserving storage stability

3.3. In Vivo Efficacy Against A Same-Category Comparator

In mice, BALIMONT demonstrated a dose-dependent increase in serum total IgA, intestinal mucosal sIgA, and fecal sIgA. At the matched 200 mg/kg comparison, the BALIMONT medium-dose group exceeded the same-category comparator by 39.68% for serum total IgA, 56.52% for intestinal mucosal sIgA, and 53.01% for fecal sIgA.

Table 5. In vivo IgA outcomes in mice after 28 days of administration

Group	Dose (mg/kg)	Serum total IgA (µg/mL)	Intestinal mucosal sIgA (µg/mg)	Fecal sIgA (µg/g)
Blank	0	78.62	2.35	12.47
Comparator	200	112.35	3.68	18.62
Low dose	100	125.78	4.12	21.35
Medium dose	200	156.93	5.76	28.49
High dose	400	198.46	7.23	36.72

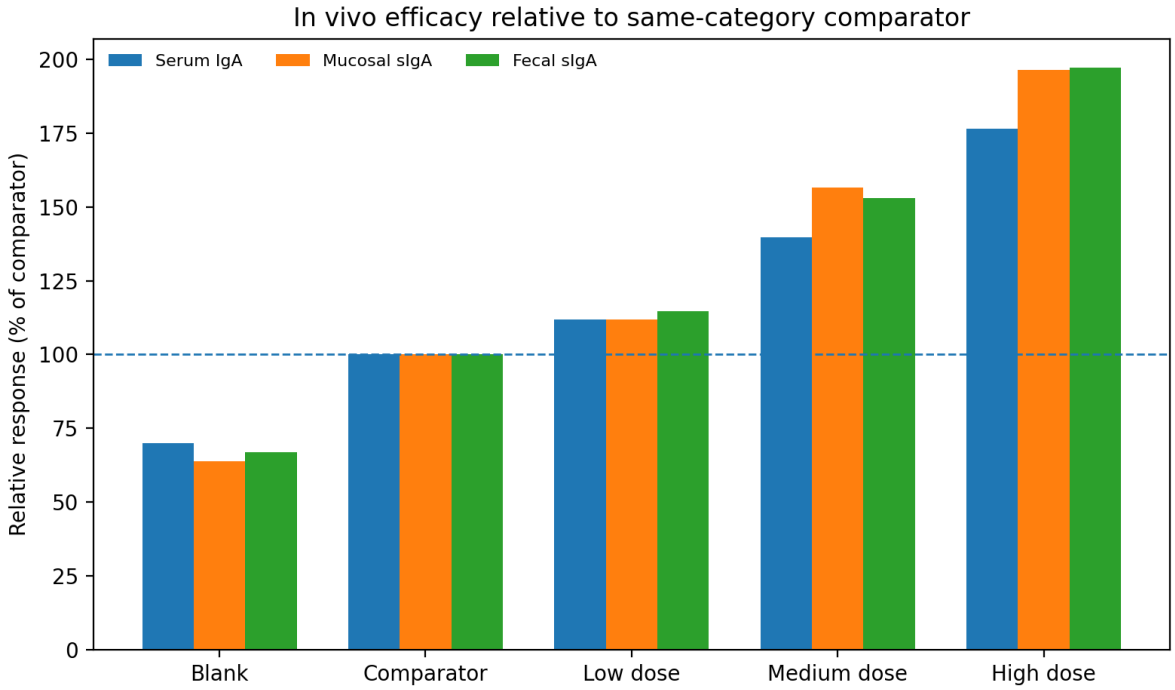

Figure 4. Relative in vivo efficacy of BALIMONT groups normalized to the same-category comparator (100%)

3.4. Stability and Safety

The BALIMONT formulation showed the best viable-count retention under 4°C/45% RH and acceptable retention under room-temperature conditions. After six months at 25°C/60% RH, viable-count retention remained 86.72%, supporting practical shelf-life feasibility. No mortality, overt toxicity, or relevant histopathological abnormalities were described during the high-dose 800 mg/kg/day safety observation.

Table 6. Storage stability of the BALIMONT formulation

Storage condition	1 month (%)	3 months (%)	6 months (%)
4°C / 45% RH	98.62	95.37	91.45
25°C / 60% RH	97.45	92.68	86.72
40°C / 75% RH	92.37	84.62	75.18

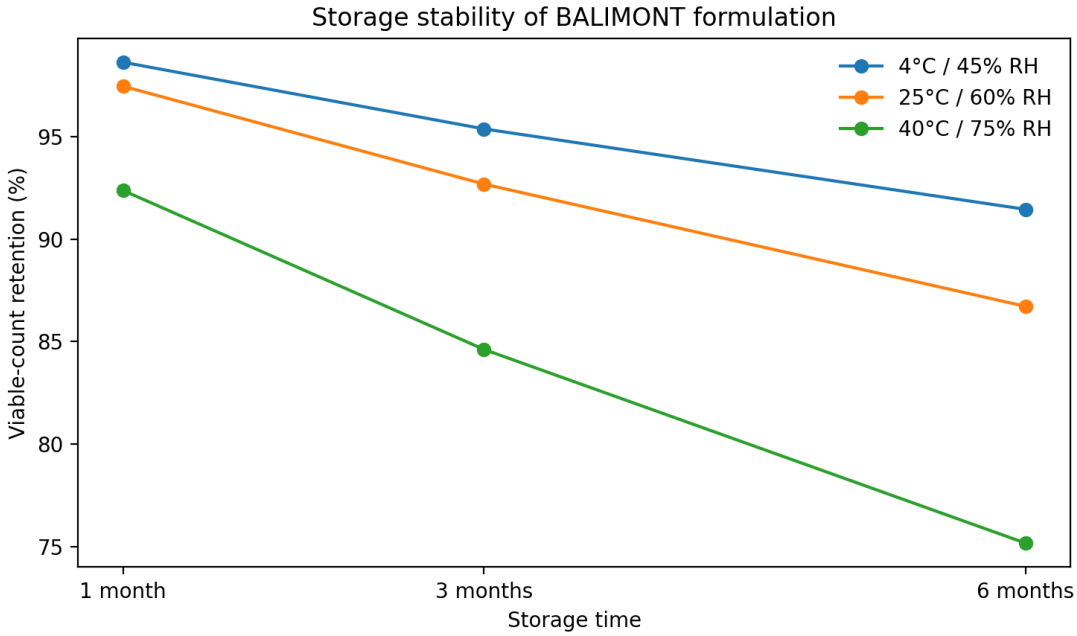

Figure 5. Viable-count retention over six months under three storage conditions

4. DISCUSSION

The BALIMONT dataset suggests that its most credible differentiator is balance rather than simple strain count. The equal-ratio 2:2:2 core performed better than the imbalanced tri-strain settings in the epithelial assay, which is biologically plausible because IgA-related mucosal conditioning depends on community-level signaling among microorganisms and the epithelial immune interface rather than on nominal taxonomic diversity alone [1-3, 6].

A second differentiator is the deliberate coupling of viable probiotics with an inactivated postbiotic partner. The BALIMONT optimization series showed a peak response at a 1:1 postbiotic-to-probiotic cell-count ratio, consistent with the broader postbiotic framework advanced by ISAPP and with the idea that non-viable microbial structures can complement live-cell signaling when incorporated in a controlled manner [8].

Third, the excipient and wall-material system adds translational value. The meta-analytic evidence for FOS-driven enrichment of bifidobacteria [7] aligns with BALIMONT's prebiotic support logic, while encapsulation literature indicates that protected delivery materially improves probiotic survival under simulated gastrointestinal stress and storage conditions [9, 10]. This combination of strain design, postbiotic complementation, and delivery protection helps explain why BALIMONT outperformed the same-category comparator under matched preclinical conditions.

From a translational perspective, the mouse data and the published human literature point in the same direction: probiotic and synbiotic systems can sustain or increase mucosal IgA-related outputs, but performance depends on strain choice, formulation quality, and the protection of viable counts throughout manufacturing and intestinal transit [4-7]. BALIMONT therefore appears best positioned as a scientifically rational mucosal-immune platform rather than as a generic high-count probiotic product.

The present article remains bounded by the available dataset. Full raw-data review, inferential re-analysis, definitive animal ethics identifiers, and controlled human clinical studies are still required before submission to a peer-reviewed journal. Nonetheless, the assembled evidence supports the biological plausibility and translational value of the BALIMONT platform.

5. CONCLUSION

We conclude that BALIMONT represents a rational multi-strain probiotic-postbiotic platform in which balanced strain architecture, a defined postbiotic partner, prebiotic support, and staged protective delivery jointly favor IgA-associated mucosal immune performance. Within the observed development dataset, BALIMONT delivered stronger in vitro IgA induction, higher matched-dose in vivo IgA outcomes than a same-category comparator, and acceptable six-month stability. These findings justify further validation in well-controlled human studies.

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