TR2 and TR3 Studies:

Progressive Topical Evaluation of TR-Series Candidates Across Quantitative Trichometry, Follicular Enumeration, and Longitudinal Zone-Level Outcomes

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Abstract

This manuscript documents the TR2 and TR3 studies performed under the Trichological Revolution (TR) program to evaluate topical candidates for hair growth support using standardized scalp-zone mapping, quantitative trichometry, and follicular-level enumeration. TR2 is presented as a downstream translational extension of the TR1 validation pipeline, incorporating short-interval follicular changes and medium-interval density outcomes in mapped scalp zones. TR3 extends the platform to a multi-subject cohort spanning several hair-loss classes, integrating automated and manual trichometric systems, zone-specific summary outcomes, and longitudinal tracking with telogen-adjusted estimation of net new hairs. Together, these studies provide a structured, measurement-forward framework for iterative candidate evaluation while preserving internal formulation parameters.

Key Terms

Trichological Revolution; TR2; TR3; scalp-zone mapping; TrichoScan; Folliscope; phototrichography; vellus hair; terminal hair; telogen-adjusted net new hairs; follicular unit tracking.

1 Introduction

Hair follicle cycling and miniaturization are governed by a tightly regulated interplay between epithelial stem cell compartments, mesenchymal signaling (including dermal papilla behavior), and local microenvironmental cues (Blanpain and Fuchs 2006; Cotsarelis and Millar 2001; Morris et al. 2004). While many interventions focus on pharmaceuticals or procedures, the TR program was established to build a structured discovery-to-translation workflow for natural candidates, combining ex vivo and cellular assays with reproducible clinical measurement systems.

TR2 and TR3 were designed as progressive steps after initial TR-series validation work (TR1) to interrogate: (i) whether topical application could produce measurable changes in hair density and shaft caliber in mapped scalp zones, (ii) whether follicular-level enumeration could detect short-interval emergence and thickening signals, and (iii) whether a multi-subject cohort could demonstrate zone-specific trends over time with conservative accounting for normal hair-cycle dynamics. For botanical research programs, a systematic candidate selection and prioritization approach is commonly used to focus efforts on plausible leads (Kala, Farooquee, and Dhar 2004), and plant-derived extracts can be produced and evaluated using established laboratory methods (Zhang

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et al. 2005; Sokmen et al. 2004). The TR platform extends these concepts into a trichology-specific, measurement-driven clinical workflow.

These studies build directly on the TR1 validation pipeline described in the internal TR1 report (**TR1StudyInternal**).

2 Study Design Overview

2.1 Program Evolution Schematic (TR1 → TR2 → TR3)

Figure 1 summarizes the evolution of the TR research framework. TR1 established the foundational screening-to-validation pipeline; TR2 and TR3 extended this platform into increasingly structured topical and longitudinal designs while maintaining consistent measurement definitions and zone mapping.

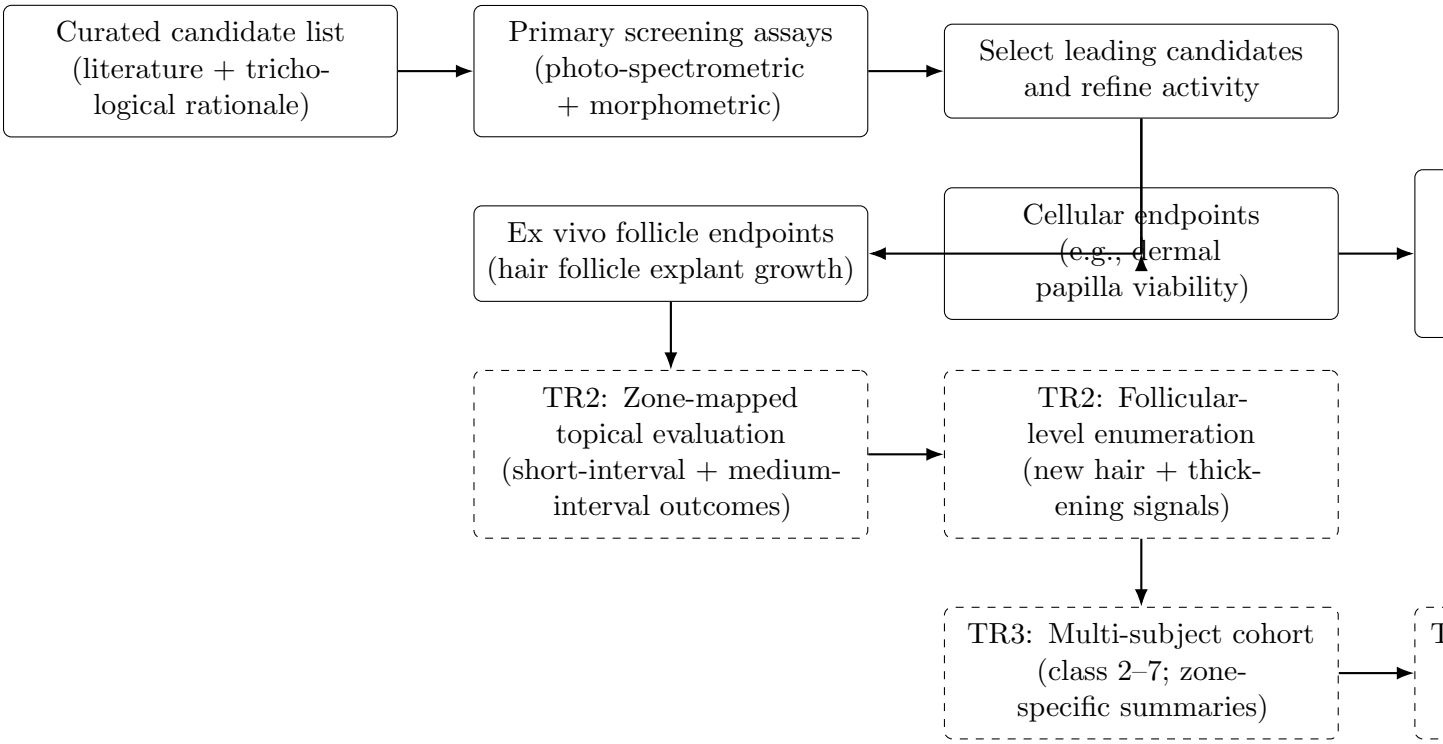

Figure 1: Evolution of the Trichological Revolution (TR) research framework from TR1 through TR3. Core TR1 steps established screening-to-validation endpoints; TR2 extended topical evaluation with zone-mapped measurement and follicular enumeration; TR3 scaled the platform to a multi-subject cohort with longitudinal zone summaries and conservative estimation of net new hairs accounting for hair-cycle dynamics.

2.2 Scalp Zone Mapping

The scalp was mapped into pre-specified zones to standardize sampling and longitudinal measurement (Figure 2). Zones included lateral and midline regions and were used for both automated and manual trichometric measurements.

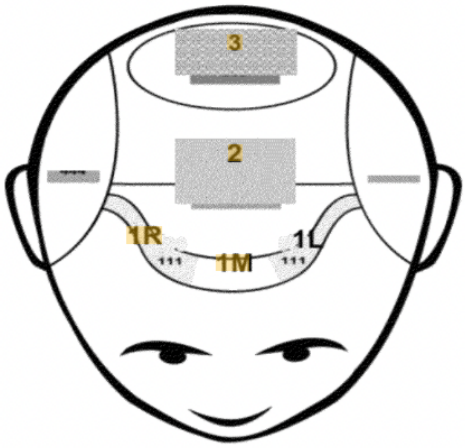

Figure 2: Scalp zones evaluated for standardized measurements and longitudinal tracking. Zone mapping enabled consistent selection of measurement areas across time points and across subjects.

3 Materials and Methods

3.1 Definitions and Measurement Systems

Hair was classified by shaft diameter into vellus/miniaturized hair (VH; diameter $< 40\,\mu$m) and terminal hair (TH; diameter $> 40\,\mu$m). Hair density (hairs per cm^2) and caliber categories were quantified using standardized phototrichographic systems. Two trichometric systems were used depending on zone and hair-loss class:

- **TrichoScan** (automated phototrichography system).
- **Folliscope** (manual system for follicular-level evaluation and/or specific zones).

3.2 TR2 Study Design

3.2.1 TR2: Zone-Level Density Outcomes

A defined area within a mapped zone was trimmed to a standardized hair length for baseline trichometric capture. Hair density and caliber composition (VH, TH, total VH+TH) were measured at baseline and after a medium-interval treatment period using a phototrichographic system. The primary endpoint was the change in terminal hair density (TH) over time. Treatment followed internal topical application procedures; formulation parameters are intentionally omitted in this manuscript.

3.2.2 TR2: Follicular-Level Enumeration (Short-Interval)

For short-interval evaluation, treated zones were trimmed to a standardized hair length at baseline, imaged, and re-imaged after a short treatment interval. Individual follicular units were manually enumerated at high magnification for:

- **New hairs:** hairs not visible at baseline that became visible at follow-up.
- **Thickened hairs:** hairs demonstrating an increase in caliber category or visually evident thickening relative to baseline.

Where present, bracketed numbers indicate the number of new hairs per follicular unit; markings may denote special cases (e.g., entirely new hairs). This enumeration was used to generate percent-of-follicles estimates for new hair and thickening signals.

3.3 TR3 Study Design

TR3 was evaluated across **20 subjects** spanning hair-loss classes **2 through 7**. Zone-level measurements were captured at baseline (Month 0), Month 1 (M1), and Month 3 (M3). TrichoScan was used for classes 2–6 across zones, with additional manual measurements in selected zones; for class 7, manual measurement was used. Outcomes were summarized as percent change in VH, TH, and total hair (VH+TH) per cm^2 across zones.

3.3.1 Follicular Tracking and Telogen-Adjusted Net New Hairs

In selected subjects/zones, individual follicles were tracked longitudinally and categorized as thicker hairs, new hairs, and telogen-associated losses. Because reappearance of hairs can occur due to normal cycling, net new hairs attributable to treatment were conservatively estimated by adjusting total new hairs by observed telogen-associated losses over the same interval:

$$\text{Net new hairs} = (\text{Total new hairs over interval}) - (\text{Telogen-associated losses over interval}).$$

3.4 Hair Follicle Viability / Growth Assays (Comparative)

Hair follicle viability/growth assays comparing TR-series candidates were performed using standardized internal protocols. Summary results are presented as relative changes compared to control conditions; formulation parameters and concentrations are intentionally omitted.

3.5 Ethics and Consent

All clinical imaging and trichometric measurements were collected under internal clinical documentation standards with patient consent for photography and analysis. Where applicable, images were de-identified and used for research documentation and retrospective reporting. This manuscript is intended for archival and scientific documentation and does not disclose internal formulation parameters.

4 Results

4.1 Comparative Hair Follicle Viability/Growth Across TR-Series Candidates

Figure 3 shows comparative hair follicle viability across TR-series candidates relative to control. Figure 4 summarizes comparative outcomes across three internal test conditions; test-condition labels are intentionally generalized.

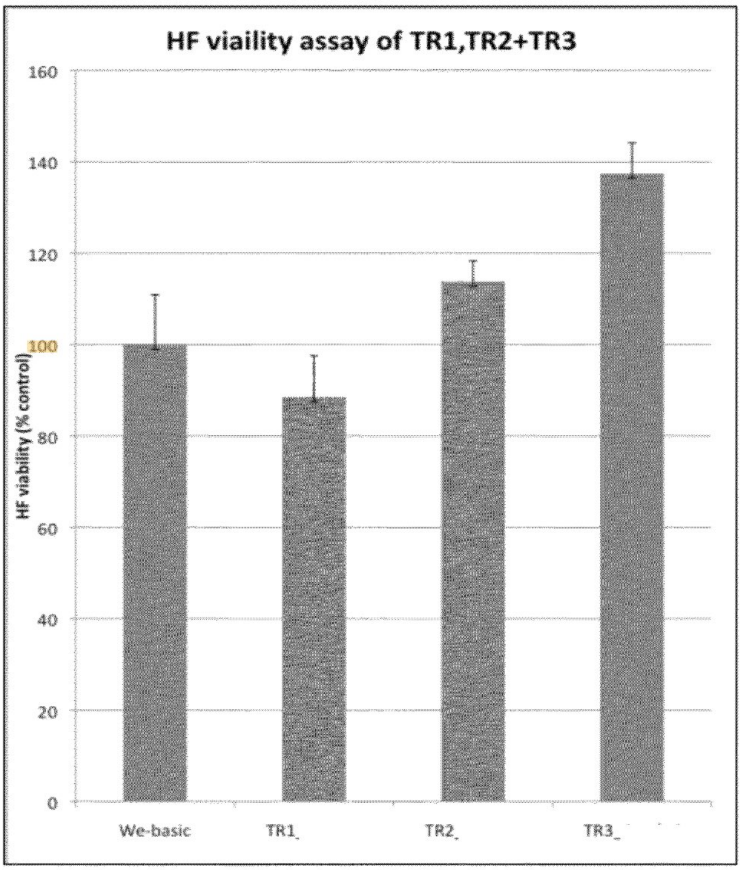

Figure 3: Hair follicle viability assay comparing TR-series candidates versus control under standardized internal assay conditions.

Concentration	Dose 1	Dose 2	Dose 3
TR1	88.5 ± 9.03	109.6 ± 4.2	69.7 ± 16.1
TR2	113.8 ± 4.5	95.9 ± 4.03	95.5 ± 8.4
TR3	137.3 ± 6.7	103.4 ± 4.6	83.7 ± 9.9

'Represents Mean ± Standard deviation of Mean (SEM) for 4-6 replicates derived from HF
'iability/growth assays of 3-8 donors.

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Figure 4: Summary table of hair follicle viability/growth outcomes across TR-series candidates across three internal test conditions (labels intentionally generalized). Values represent mean ± SEM as reported in the source figure.

4.2 TR2: Medium-Interval Increase in Terminal Hair Density

TR2 topical evaluation produced measurable improvement in terminal hair density over a medium-interval follow-up in a mapped zone. The observed shift is directionally consistent with follicular caliber support and/or recruitment of hairs into terminal categories, as defined by standardized diameter thresholds (Cotsarelis and Millar 2001).

4.3 TR2: Short-Interval Follicular Emergence and Thickening Signals

Figure 5 shows representative before/after phototrichographic fields following short-interval TR2 topical treatment. Follicular-level enumeration demonstrated a measurable fraction of follicles exhibiting thickening and a measurable fraction exhibiting new hair emergence (Figure 6).

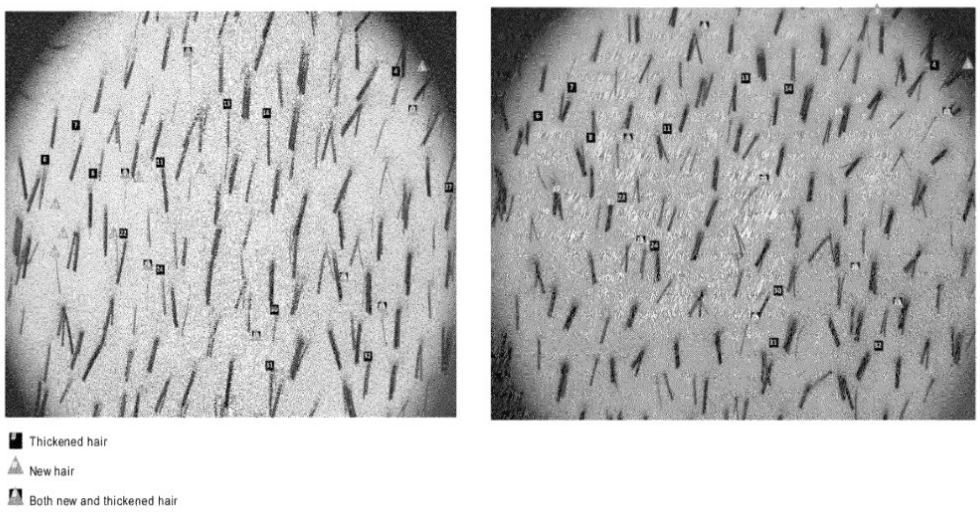

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Figure 5: Representative TR2 phototrichographic fields (baseline vs follow-up). Symbols indicate thickened hairs, new hairs, and hairs exhibiting both changes, as defined by standardized follicular-level enumeration.

Hair	Changes		Increased thickness
follicle number	Increased thickness	Increased number	and number
1	■	▲ (3)	1
2		▲ (1)	
3		▲ (1)	
4	■		
5		▲ (2)	
6	■		
7	■		
8	■		
9	■	▲ (1)	1
10		▲ (1)*	
11	■		
12		▲ (1)	
13	■		
14	■		
15		▲ (1)	
16		▲ (1)	
17	■	▲ (1)	1
18		▲ (1)	
19		▲ (1)*	
20		▲ (1)	
21		▲ (1)	
22	■		
23	■	▲ (1)	1
24	■		
25		▲ (1)*	
26	■	▲ (1)	1
27	■		
28		▲ (1)	
29	■	▲ (1)	1

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Figure 6: Follicular-level enumeration summary for TR2 short-interval evaluation. Individual follicles are marked for thickening and/or new hair emergence. Bracketed numbers indicate the number of new hairs at a follicle; special markings may denote entirely new hairs.

4.4 TR3: Zone-Level Outcomes Across a Multi-Subject Cohort

TR3 was evaluated across 20 subjects (hair-loss classes 2–7) using standardized zone mapping and repeated measures at baseline, Month 1, and Month 3. Figure 7 presents cumulative zone-specific outcomes after 3 months.

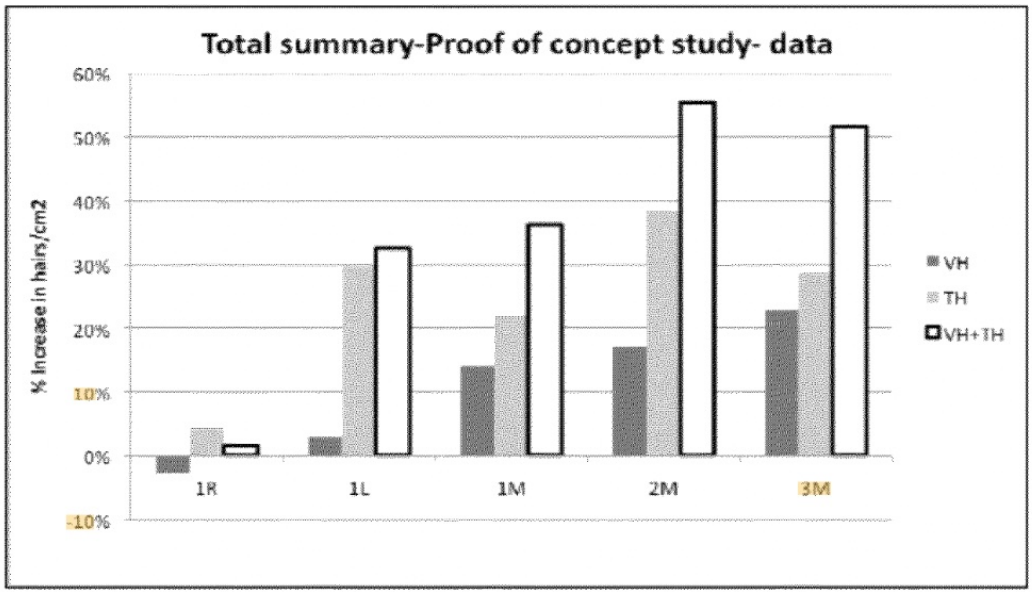

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Figure 7: Cumulative TR3 results by scalp zone after three months of treatment (averaged across 20 subjects). Percent changes are shown for vellus/miniaturized hair (VH), terminal hair (TH), and total hair (VH+TH) per cm^2.

4.5 TR3: Longitudinal Follicular Tracking With Telogen-Adjusted Net New Hairs

Figure 8 summarizes follicular tracking in a representative zone over 3 months, including thicker hairs, total new hairs, telogen-associated losses, and conservative estimation of net new hairs. This analysis demonstrates that a meaningful portion of observed emergence persists even after accounting for hair-cycle dynamics (Blanpain and Fuchs 2006).

Zone 3M (S.L): Study of 140 Hair Follicular Units (HF) in Zone 3M containing 266 hairs over 3 months of treatment with TR3

thicker hairs	new hairs	telogen hairs	net new hairs
Month-1			
31	45	29	16
Month-3			
52	49	26	23
Month-3 (Cumulative)			
83 (31%)	94	55	39 (15%)

Net effects:
Hair thickening: 83 hairs (31%)
Net New hairs (total new hairs – telogen hairs): 39 hairs (15%)
New hairs + Hair thickening: 46%

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Figure 8: Representative TR3 longitudinal follicular tracking (Zone 3M): thicker hairs, total new hairs, telogen-associated losses, and telogen-adjusted net new hairs over 3 months. Percentages reflect the study-area totals reported in the source figure.

5 Discussion

TR2 and TR3 extend the TR platform from early translational validation into progressively more structured topical and cohort-based evaluation.

5.1 TR2: Early Translational Signal at the Follicular-Unit Level

TR2 demonstrates that short-interval follicular enumeration can detect measurable changes—*new hair emergence* and *thickening*—within a defined measurement field. This matters because early response to topical interventions may present first as caliber modulation and follicular recruitment rather than immediate, field-wide density changes.

5.2 TR3: Scaling to Zone-Level and Multi-Class Evaluation

TR3 advances the program by introducing a multi-subject cohort, zone-specific summaries, and a conservative adjustment for cycling via telogen-associated losses when estimating net new hairs. The

telogen-adjusted approach strengthens interpretability because it explicitly acknowledges hair-cycle phase transitions and avoids attributing all reappearance to treatment (Cotsarelis and Millar 2001).

5.3 Positioning Within the TR Program (Linkage to TR1)

These studies were designed as downstream extensions of TR1 rather than isolated experiments. TR1 established the core screening and validation logic; TR2 and TR3 demonstrate how that logic can be carried forward into repeatable clinical measurement systems (**TR1StudyInternal**).

6 Limitations

- Portions of TR2 and TR3 are observational and may not include randomized controls.
- Imaging, trimming, and lighting standardization may vary across time points and subjects.
- Zone-level averages can obscure individual heterogeneity and baseline severity differences.
- Telogen-adjusted net new hair estimation is conservative but still relies on visible cycling proxies rather than direct phase labeling.
- Internal formulation parameters are intentionally omitted, limiting external reproducibility in this draft.

7 Conclusion

TR2 and TR3 provide a structured, measurement-forward extension of the TR platform into topical and longitudinal evaluation. TR2 demonstrates follicular-unit signals (new hair emergence and thickening) and medium-interval density gains within mapped zones. TR3 scales the approach across a multi-subject cohort and introduces conservative telogen-adjusted net new hair estimation. Together, these studies strengthen the translational evidence base supporting continued TR-series development.

Acknowledgements

Internal clinical and research staff who supported trichometric capture, data tabulation, and longitudinal documentation.

Data Availability

This manuscript is based on internal documentation and representative figures. Raw trichometric outputs and full subject-level tables may be appended for internal archival versions.

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