Document 1 — Study Protocol (NAID-F)

Cover Page - Official Study Title: Risk of Anemia Development and Clinical Effects of Oral Iron Therapy in Women (18–55 Years) With Non-Anemic Iron Deficiency - NCT Number: [To be assigned] - Document Date: October 28, 2025

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1. Study Identification - Organization's Unique Protocol ID: 2024-KAEK-22 - Brief Title: Risk of Anemia and Effects of Oral Iron Therapy in Non-Anemic Iron-Deficient Women (18–55 Years) - Acronym: NAID-F - Study Type: Interventional - Official Title: Risk of Anemia Development and Clinical Effects of Oral Iron Therapy in Women (18–55 Years) With Non-Anemic Iron Deficiency - Secondary IDs: - Other Identifier: E-14028348-302.14.02-985103 (Istanbul Univ.–Cerrahpasa Faculty of Medicine, Thesis Approval) - Other Identifier: E-15916306-604.01-281470566 (Istanbul Provincial Health Directorate, Institutional Approval)

2. Study Status - Record Verification Date: October 2025 - Overall Recruitment Status: Recruiting - Study Start Date: September 01, 2025 (Actual) - Primary Completion Date: January 01, 2026 (Anticipated) - Study Completion Date: February 28, 2026 (Anticipated)

3. Sponsors and Collaborators - Responsible Party: Principal Investigator - Investigator Name [Username]: Osman Demir, MD [odemir] - Investigator Official Title: MD - Investigator Affiliation: Istanbul University - Cerrahpasa - Name of Sponsor: Istanbul University - Cerrahpasa - Collaborators: Istanbul University - Cerrahpasa

4. Oversight - U.S. FDA-Regulated Drug: No - U.S. FDA-Regulated Device: No - IND/IDE: No - Human Subjects Protection Review: Approved - Board Name: Istanbul University – Cerrahpaşa Clinical Research Ethics Committee - Approval Number: 2024-KAEK-22 - Board Affiliation: Istanbul University – Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Clinical Research Ethics Committee - Board Contact: +90 (212) 414 32 52, kliniketik@iuc.edu.tr

5. Study Description - Brief Summary: This study investigates anemia risk and clinical effects of oral iron therapy in women aged 18–55 years with non-anemic iron deficiency. Conducted in two phases at Kağıthane 5 No'lu Family Health Center, Istanbul. - Phase 1 (Observational Nutrition Intervention, 2 months): All participants receive dietary guidance to improve iron intake and absorption. Biweekly phone monitoring. Participants classified into five subgroups (ARM1-0 to ARM1-4) based on hematologic outcomes after 2 months. - Phase 2 (Experimental Oral Iron Therapy, 1 month): Participants in ARM1-1 to ARM1-4 receive oral ferrous sulfate 80 mg/day. ARM1-0 participants maintain normal iron status and do not receive therapy.

6. Conditions - Non-Anemic Iron Deficiency - Risk of Anemia Development - Clinical Effects of Oral Iron Therapy - Keywords: Iron Deficiency Without Anemia, Women 18–55 years, Hemoglobin, Ferritin, Symptom Scores, Prospective Cohort, Experimental Intervention, Iron Supplementation, Nutritional Intervention, Study Design

7. Study Design - Primary Purpose: Treatment - Study Phase: N/A - Interventional Study Model: Single Group Assignment - Number of Arms: 1 - Masking: None (Open Label) - Allocation: Non-Randomized - Enrollment: 60 (Anticipated) - Arms and Interventions: - Experimental: ARM 1 – Nutritional Intervention with Post-Intervention Subgrouping (All participants receive 2-month dietary intervention; subgrouping based on lab outcomes; persistent iron deficiency participants receive 1-month oral elemental iron 80

mg/day). - Other: Dietary Intervention (2 months) – iron-rich foods, absorption enhancement strategies, biweekly follow-up. - Drug: Oral Elemental Iron (Ferrous Sulfate) 80 mg/day – 1 month for persistent iron deficiency.

8. Outcome Measures - **Primary Outcome:** 1. Change from Baseline in Patient-Reported Iron Deficiency Symptom Scores (Baseline, Week 8, Week 12) - **Secondary Outcomes:** 2. Hemoglobin and RBC indices (Baseline, Week 8, Week 12) 3. Serum Iron and TIBC (Baseline, Week 8, Week 12) 4. Incidence of progression to anemia after 2-month nutritional intervention (Week 8) 5. Hematologic response to 1-month oral iron therapy (Hb increase ≥1 g/dL; Week 8–12) 6. Ferritin response to 1-month oral iron therapy (Week 8–12) - **Pre-specified Outcomes:** 7. Adherence to Nutritional Intervention (Week 0–8) 8. Adherence to Oral Iron Therapy (Week 8–12) 9. Incidence of Treatment-Related Adverse Events (Week 8–12)

9. Statistical Analysis Plan - Data will be analyzed using repeated measures ANOVA for longitudinal comparisons of laboratory values and symptom scores. - Participants will be stratified into five subgroups (ARM1-0 to ARM1-4) based on hematologic outcomes after 2 months. - ANOVA comparisons will be performed across these five groups to assess differences in outcomes including hemoglobin, ferritin, RBC indices, serum iron, TIBC, and patient-reported symptom scores. - Within-group changes over time will be analyzed using paired t-tests. - Categorical data (proportions and percentages) will be analyzed using Chi-Square tests. - Continuous variables will be summarized as mean ± SD; categorical variables as counts and percentages. - Subgroup analyses will focus on ARM1-1 to ARM1-4 participants who receive oral iron therapy. - Sample size of 60 determined via G*Power analysis (α=0.05, 80% power). - Missing data handled via multiple imputation if >5% missing.

10. Eligibility - Accepts Healthy Volunteers: No - Sex: Female - Age Limits: 18–55 years - Inclusion Criteria: Female, 18–55 years, non-anemic iron deficiency (ferritin <15 µg/L), normal Hb, B12, folate, TSH, sT4, CRP <5 mg/L, informed consent. - Exclusion Criteria: Pregnancy/postpartum, infection, malignancy, chronic disease, depression, chronic fatigue, kidney/heart disease, hematologic disorders, ongoing iron therapy.

11. Contacts and Locations - Central Contact Person: Osman Demir (+90 532 291 4470, osman.demir@iuc.edu.tr) - Central Contact Backup: Ayşen Fenercioğlu (+90 537 964 5751, aysen.fenercioglu@iuc.edu.tr) - Study Officials: Osman Demir (Principal Investigator), Kağıthane No.5 Family Health Center, Istanbul, Turkey; Ayşen Fenercioğlu (Study Director), Istanbul University – Cerrahpaşa. - Location: Kağıthane No.5 Family Health Center, Istanbul, Turkey (Recruiting)

12. IPD Sharing Statement - Plan to Share IPD: Undecided; to be determined post-study in accordance with ethics and participant privacy.

13. References and Additional Information 1. World Health Organization. Guideline on the Use of Ferritin Concentrations to Assess Iron Status in Individuals and Populations. Geneva: WHO; 2020. 2. Soppi ET. Iron deficiency without anemia: a clinical challenge. Clin Case Rep. 2018;6:1082–1086. https://doi.org/10.1002/ccr3.1529 3. Balendran S, Forsyth C. Non-anaemic iron deficiency. Aust Prescr. 2021;44(6):193–196. doi:10.18773/austprescr.2021.052 4. World Health Organization & FAO. Guidelines on Food Fortification with Micronutrients. Geneva: WHO Press; 2006. 5. Skolmowska D, Glabska D, Kolota A, Guzek D. Effectiveness of dietary interventions to treat iron-deficiency anemia in women: a systematic review. Nutrients. 2022;14:2724. https://doi.org/10.3390/nu14132724 6. Clenin G. Treatment of iron deficiency without anaemia. Swiss Med Wkly. 2017;147:w14434. 7. British Society for Haematology. Guideline for the Laboratory Diagnosis of Iron Deficiency in Adults and Children. Br J Haematol. 2022;196:523–529. 8. Houston BL, Hurrie D, Graham J, et al. Efficacy of iron supplementation

on fatigue in non-anemic iron-deficient adults: systematic review. BMJ Open. 2018;8:e019240. 9. Miles LF, Litton E, Imberger G, Story D. Intravenous iron therapy for non-anaemic, iron-deficient adults. Cochrane Database Syst Rev. 2019;12:CD013084. 10. da Silva Lopes K, Yamaji N, Rahman MO, et al. Nutrition-specific interventions for preventing and controlling anaemia: overview of systematic reviews. Cochrane Database Syst Rev. 2021;9:CD013092.