**PATHFINDER 2: A Prospective Study to Evaluate Safety and Performance of a Multi-Cancer Early Detection Test in a Population Setting**

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**Background:** Multi-cancer early detection (MCED) tests are being established as a novel approach to screen for multiple cancer types with one test. A blood-based MCED test using cell-free DNA targeted methylation patterns to detect a shared cancer signal and predict a cancer signal origin (CSO) has demonstrated feasibility. An initial return of results study (PATHFINDER; NCT04241796) reported performance of a refined version of the MCED test, including a 43.1% positive predictive value (PPV), 98.5% negative predictive value (NPV), 99.5% specificity, and 88.0% CSO accuracy. To build on this work, the PATHFINDER 2 study (NCT05155605) will evaluate safety and performance of the MCED test in larger, more diverse populations.

**Methods:** PATHFINDER 2 is a prospective, multicenter, interventional study. Participants ≥50 years of age will be enrolled, with specific targets to improve diversity (age, sex, race/ethnicity) and few exclusions due to comorbid conditions. Exclusion criteria include current clinical suspicion of cancer or recent cancer/treatment (within 3 years). Participants will undergo blood draw for MCED testing, followed by return of results (cancer signal detection and CSO) to the investigator and diagnostic evaluations if a cancer signal is detected. A confirmatory PET-CT scan will be performed when CSO-directed workups do not result in a cancer diagnosis. Participants will be followed for approximately 3 years. Primary endpoints include 1) MCED test safety in terms of diagnostic testing triggered by a positive result (number/type of procedures and adverse events) and 2) test performance (PPV, NPV, specificity, sensitivity, CSO accuracy, cancer detection rate and number needed to screen). Secondary endpoints include participant reported outcomes (eg, anxiety), utilization of guideline-recommended cancer screening, and cancer detection rate of confirmatory PET-CT, amongst others.

The study plans to enroll approximately 35,000 participants across North America. Sites were selected based on geographic location, catchment area demographics, and practice setting (academic vs non-academic). Enrollment targets for age, sex and race/ethnicity (White [non-Hispanic] 72%; Hispanic or Latino 11%; African American or Black 11%; Asian, Native Hawaiian or Other Pacific Islander 6%; American Indian/Alaska Native 1%) were derived from US Census data. Strategies to promote diverse recruitment include translation of participant-facing documents across multiple languages, participant and healthcare provider educational material, and site-led community outreach campaigns.
MCED testing is an emerging, potentially paradigm-changing strategy in cancer screening, and PATHFINDER 2 is designed to characterize the safety, performance, and clinical implementation of the MCED test as a screening tool in a broad and representative population.