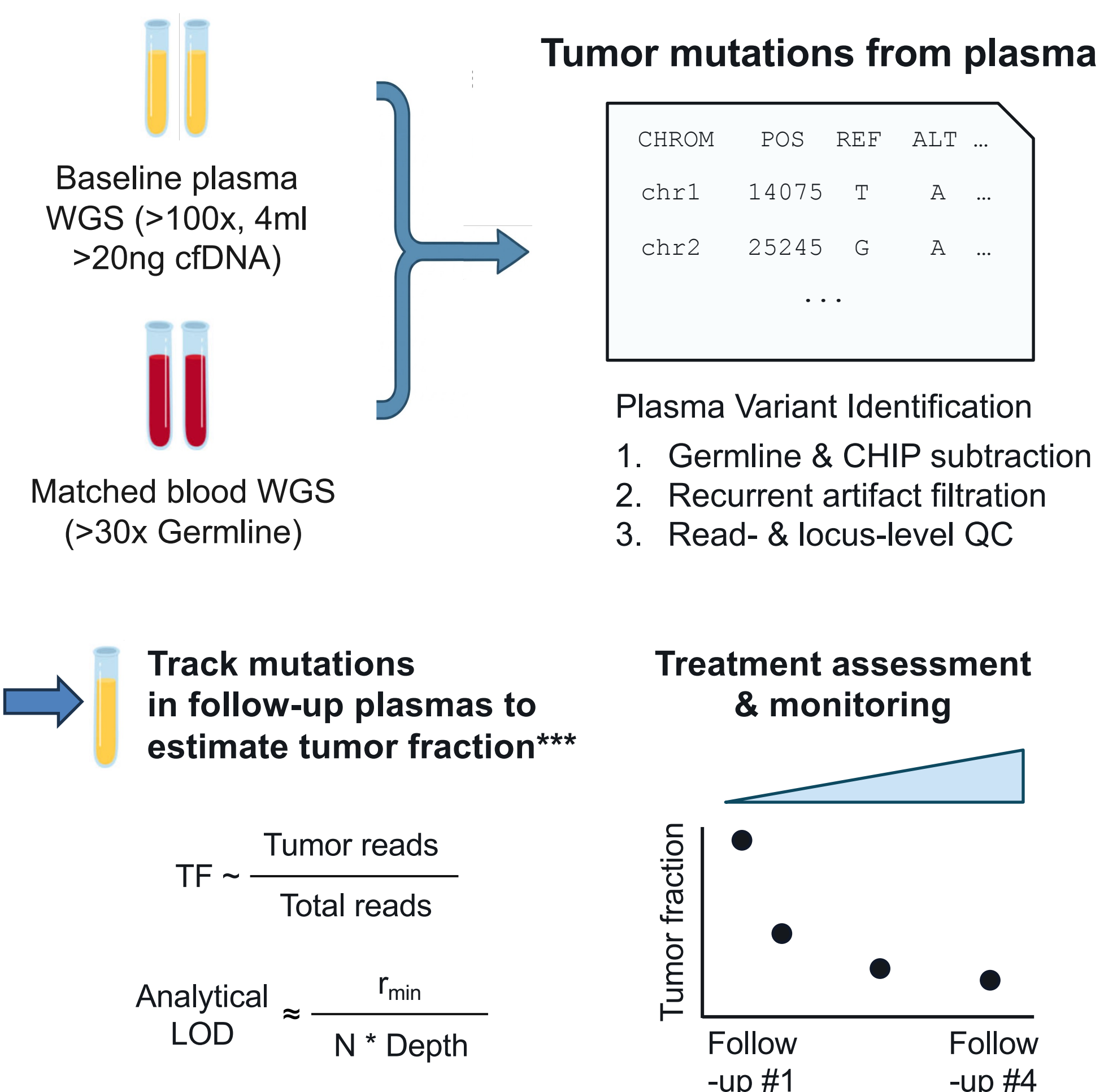


Motivation

Minimal residual disease (**MRD**) testing has rapidly emerged as an important clinical tool for assessing treatment response and longitudinal monitoring in patients with lung cancer. When matched tumor tissue is available, tumor-informed assays that combine whole-genome tumor profiling with cell-free DNA (**cfDNA**) sequencing, such as **MRDVision™**, can achieve ultra-high sensitivity, with a limit of detection as low as 1 part per million (**ppm**). However, many patients with advanced lung cancer lack the required baseline tumor tissue, since surgical resection or biopsy are not part of standard care*. This leaves tumor-naïve assays, which generally have lower sensitivity (>300 ppm), as the fallback option. To enable sensitive, tissue-free MRD monitoring, we developed the plasma tumor-informed MRD assay (**pTIMA**). pTIMA uses high-fidelity Ultima Genomics paired plus-minus sequencing (**ppmSeq**) to identify somatic mutations in baseline plasma cfDNA from samples with sufficient tumor fraction (**TF**). These mutations are unique to each patient and provide the targets for MRD monitoring in follow-up plasma.

Proposed Method**



Dataset

Cohort. Feasibility of pTIMA was assessed retrospectively on 153 plasma samples with ppmSeq whole-genome sequencing (median depth 75x), available at Inocras.

Reference measurement. Tumor fraction was determined independently by MRDVision™. Plasma-derived variants were called *de novo*, without using tumor tissue.

Data composition

- Healthy controls (n = 52)
- Cancer patients (n = 101)
 - TF > 1%: (n = 31)
 - TF 0.1–1%: (n = 21)
 - TF 0.01–0.1%: (n = 24)
 - TF < 0.01%: (n = 25)

Cancer types — ovarian, lung, breast, pancreatic and others, including advanced lung cancer cohorts from Seoul National University Hospital (n = 10) and Severance Hospital (n = 9).

Plasma-derived variants are tumor-specific and scale with tumor fraction

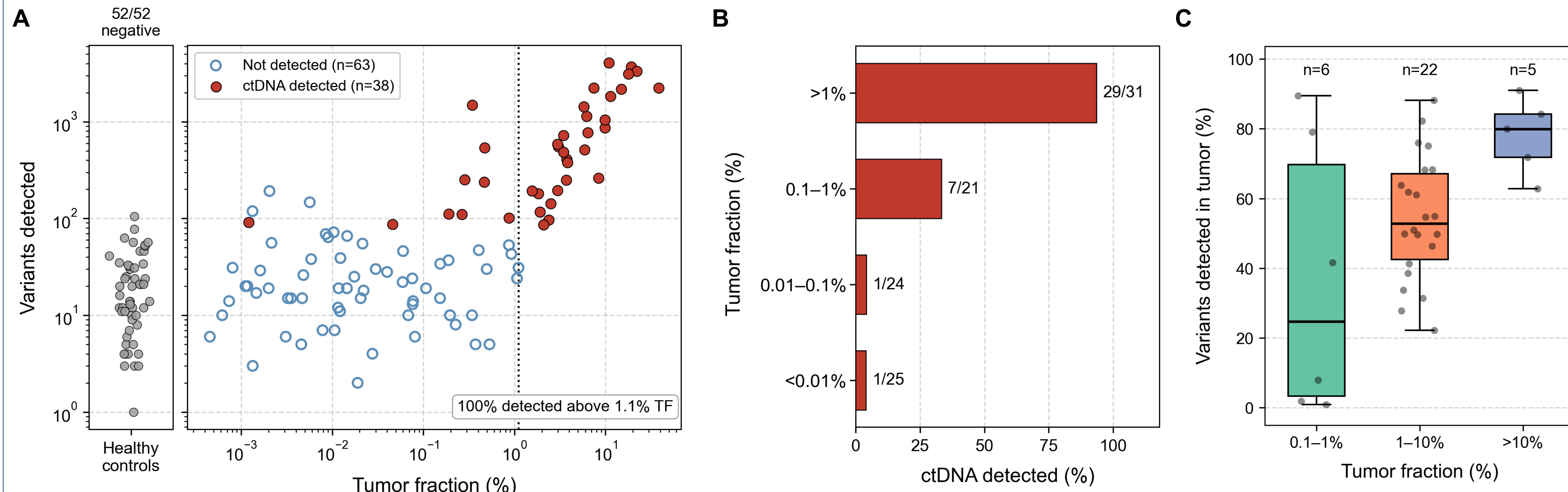


Figure 1. ctDNA detection and variant precision across tumor fractions. (A) Plasma-derived variant counts vs tumor fraction (MRDVision™). Samples were called ctDNA-positive (filled) when counts exceeded the 99.5th percentile of a depth-matched negative binomial model fitted to healthy controls ($p < 0.005$). Controls (grey) are shown separately. Dotted line: lowest tumor fraction with 100% detection. (B) Detection rate by tumor fraction band. (C) Proportion of plasma-derived variants confirmed somatic in matched tumor tissue, among detected samples.

Baseline plasma mutations enable MRD tracking

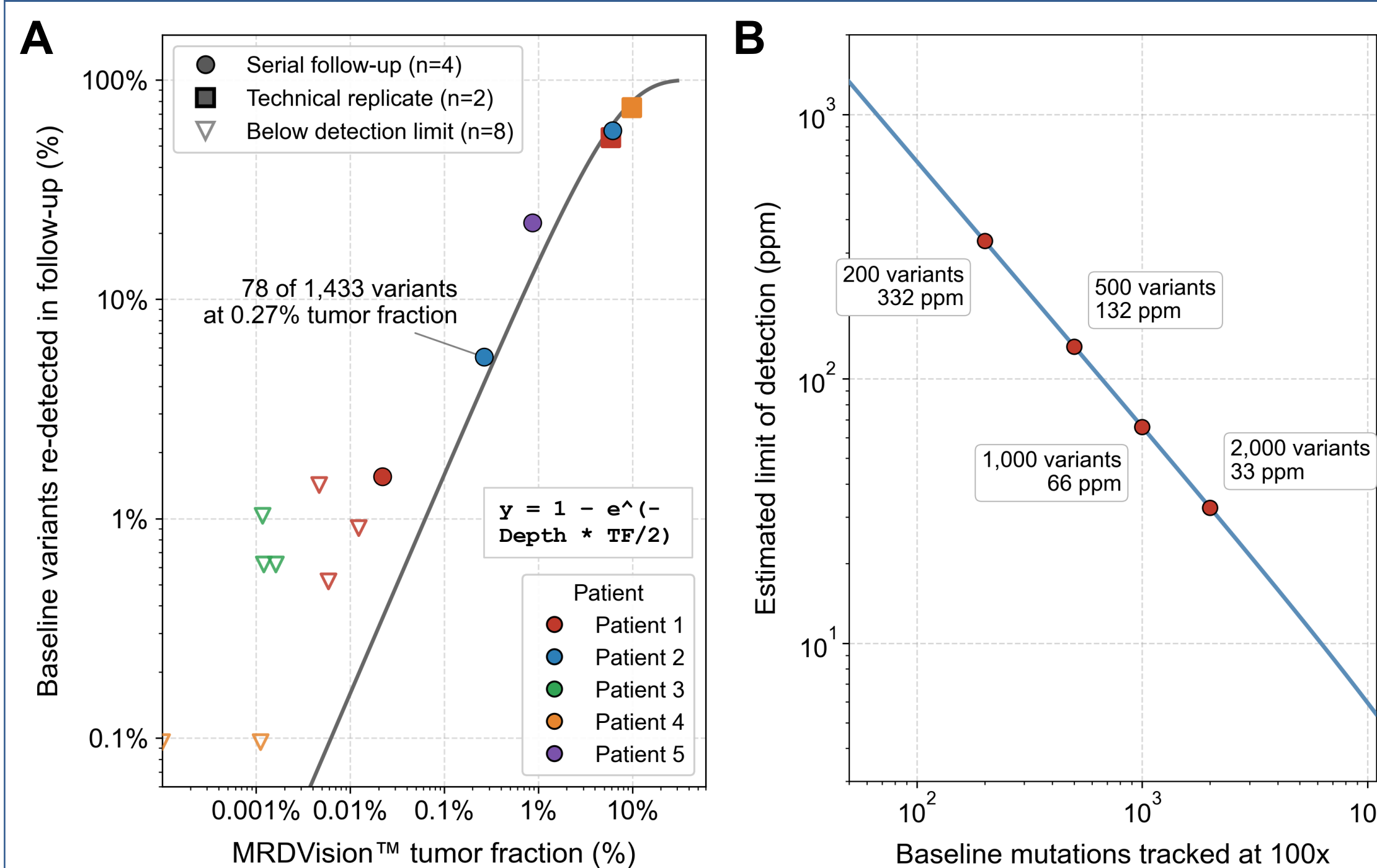


Figure 2. MRD tracking using baseline plasma. (A) Baseline mutations (486–1,433 variants, 5 patients) tracked in 14 follow-up samples. Grey line: re-detection expected from cfDNA molecule sampling at median follow-up depth (65x). (B) Projected LOD95 versus baseline variants tracked, at 100x depth. Binomial model, residual error 3.5×10^{-7} .

High TMB improves variant recovery in advanced lung cancer

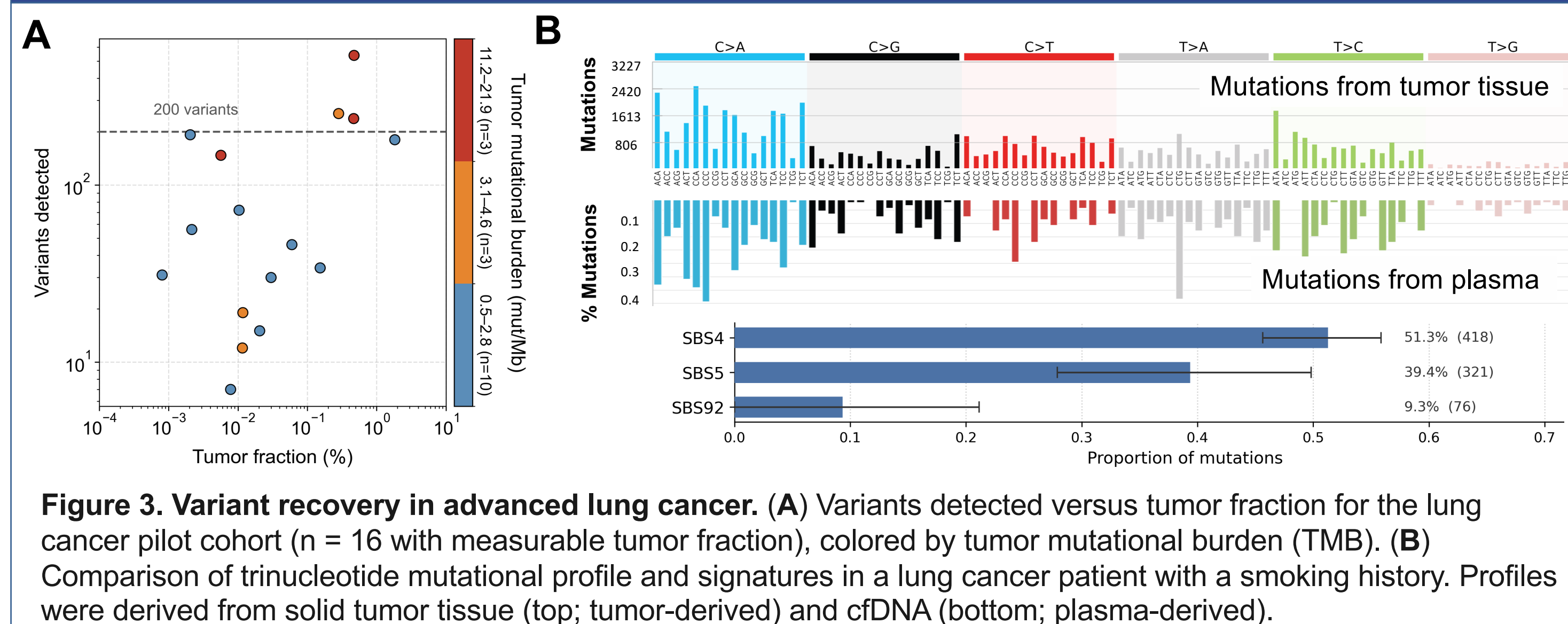


Figure 3. Variant recovery in advanced lung cancer. (A) Variants detected versus tumor fraction for the lung cancer pilot cohort (n = 16 with measurable tumor fraction), colored by tumor mutational burden (TMB). (B) Comparison of trinucleotide mutational profile and signatures in a lung cancer patient with a smoking history. Profiles were derived from solid tumor tissue (top; tumor-derived) and cfDNA (bottom; plasma-derived).

Key Results

ctDNA-positive samples yielded a median of **494** plasma-derived variants, 55% of which were tumor-informed. High mutational burden compensated for low tumor fraction. Tracking 486–1,433 baseline variants per patient identified follow-up mutations in five patients at tumor fractions as low as 0.012%. Detection limit depends on baseline variant count and follow-up sequencing depth.

Ongoing Work

- Prospective evaluation of a cohort of ~40 lung cancer patients, in partnership with AstraZeneca.
- Establishing the experimental limit of detection and assessing clinical feasibility of pTIMA.

Conclusions

- Somatic mutations can be identified *de novo* in baseline plasma without matched tissue, with the majority confirmed in the tumor.
- Plasma variant recovery scales with tumor fraction and mutational burden; high-TMB yield baselines < 1% TF.
- Plasma-derived baselines enable tissue-free MRD tracking, projected to 132 ppm (500 mutations, 100x depth).

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Footnotes

*Baseline tissue may be unavailable, insufficient, inaccessible, or biologically unrepresentative because it is old or derived from a different disease site.
**Baseline plasma for tumor mutation fingerprinting were collected from patients with metastatic lung cancer immediately before initiation of a subsequent line of systemic therapy. CHIP = Clonal Hematopoiesis of Indeterminate Potential.
***TF: fraction of reads supporting tracked mutations. LOD: smallest TF at which $r_{\min}$ supporting reads are expected across all N tracked mutations. $r_{\min}$ = minimum supporting reads required for 95% detection; N = baseline mutations tracked; Depth = usable depth per locus.

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AI Use Disclosure

Claude was utilized as a supportive tool for text revision and figure generation. The authors maintain full responsibility for the final content and scientific accuracy of this poster.