

CAREFULNESS: a study design to integrate circulating biomarkers into lung cancer screening

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INTRODUCTION

Lung cancer (LC) screening programs using low-dose CT (LDCT) have proven to be effective among heavy current and former tobacco users. Current challenges include improving risk stratification, implementing personalized follow-up strategies, and minimizing unnecessary diagnostic procedures. Liquid biopsy may contribute to this evolving framework. Here, we report preliminary accrual and sampling data from an ancillary study within a LDCT screening program.

METHODS

Since September 2022, a LC screening program has been implemented at San Luigi Gonzaga University Hospital (Orbassano, Turin) for heavy (≥ 30 pack-years) current or former (within 15 years) tobacco users aged 55–75 years (**Fig.1**).

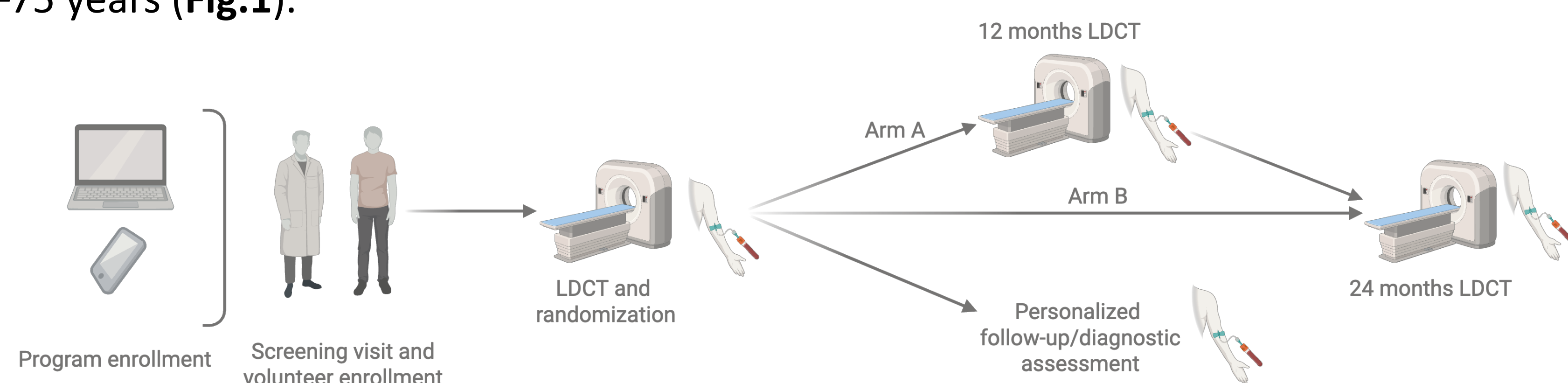


Figure 1: lung cancer screening program design. Radiological findings are classified according to Lung-RADS criteria. Randomization is stratified according to sex.

The CAREFULNESS is an ancillary study evaluating the feasibility of longitudinal blood collection and biobanking within a LDCT screening program, with the long-term aim of developing circulating biomarker-based risk stratification.

Volunteers were invited to participate through optional blood sampling prior to LDCT. Radiological assessment was performed according to Lung-RADS criteria. Blood samples (plasma, PBMC, and whole blood) were processed locally by centrifugation and stored at -80°C for plasma and whole blood, while PBMC were cryopreserved in liquid nitrogen (**Fig.2**).

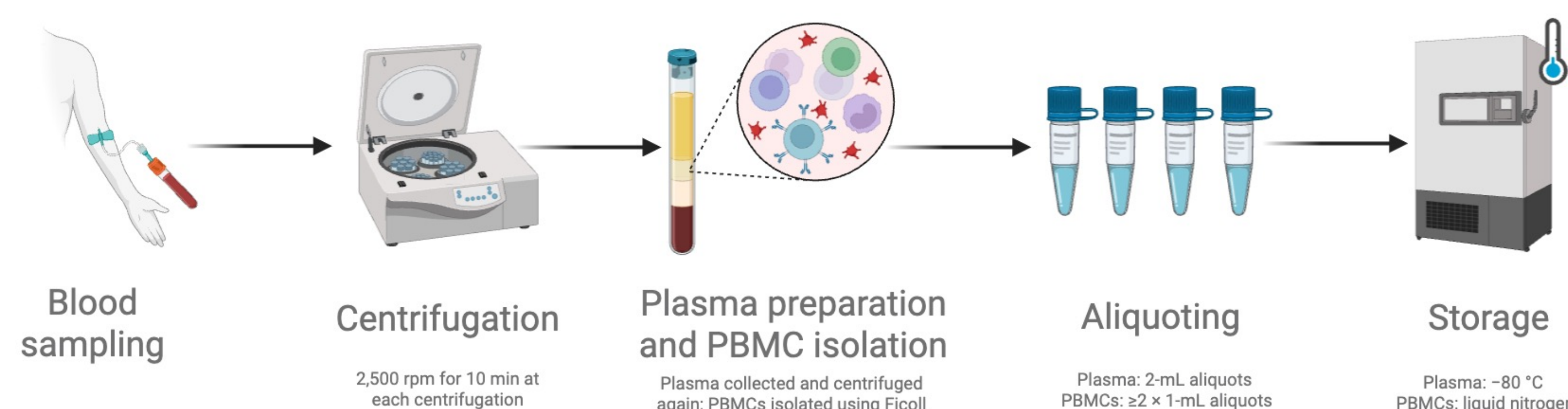


Figure 2: Description of the initial workflow for processing and storing blood samples in the local biobank.

HIGHLIGHTS

- Longitudinal blood sampling was successfully integrated into a LDCT-based lung cancer screening program.
- Baseline blood samples were collected in 74.6% of participants.
- Circulating biomarker profiling may support risk stratification and diagnostic escalation.



RESULTS

Overall, 1,003 participants were enrolled in the lung cancer screening program; 748 (74.6%) provided a baseline blood sample, and 381 additional samples were collected during subsequent recalls ($T \geq 1$). At baseline, the median age was 62 years, and 60.3% were male. Baseline LDCT findings were distributed as shown in **Table 1**. Overall, 16 participants diagnosed with lung cancer underwent blood sampling before treatment initiation, including 15 before surgery and 1 before systemic therapy. **Figure 3** illustrates a potential clinical scenario in which the risk stratification process could be applied.

	Timepoints				
Lung-RADS	T0	T1	T2	T3	T4
1	296 (39.6%)	20 (8.8%)	13 (11.8%)	3 (8.3%)	0
2	361 (48.3%)	114 (50.2%)	84 (76.4%)	32 (88.9%)	7 (87.5%)
3	53 (7.1%)	52 (22.9%)	2 (1.8%)	0	0
4 (A, B, X)	38 (5.1%)	41 (18.1%)	11 (10.0%)	1 (2.8%)	1 (12.5%)

Table 1: Distribution of samples according to timepoints and Lung-RADS findings.

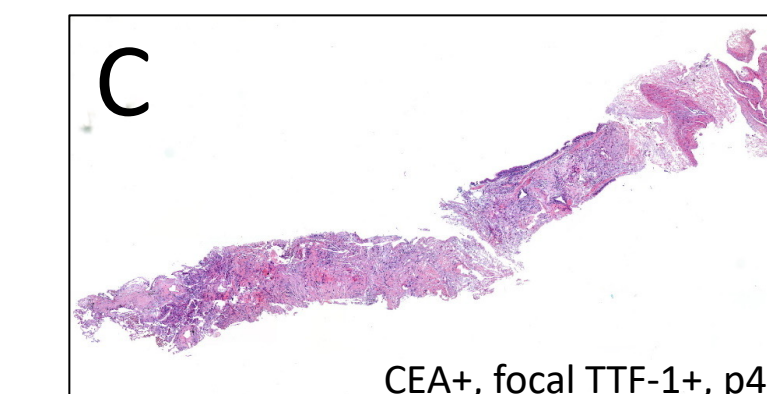


Figure 3: Potential scenario for biomarker-informed risk stratification.

Initial biopsy was negative (**A**); subsequent lesion growth prompted repeat biopsy (**B**), diagnosing *KRAS G12C*-mutated lung adenocarcinoma with mucinous features (**C**).

DISCUSSION AND CONCLUSIONS

These preliminary findings show the feasibility of integrating liquid biopsy within LDCT screening programs. Subsequent analyses will assess the potential benefit of biomarker-based profiling in individuals with indeterminate radiological findings, whose clinical management remains challenging.

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