BRIEF REPORT

Identifying Novel Germline Mutations and Copy Number Variations in Patients With SCLC

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ABSTRACT

Introduction: SCLC has traditionally been considered to arise from toxic exposure factors, such as smoking. Recent evidence has revealed that germline mutations may also affect the development of SCLC; however, these alterations remain understudied. We sought to identify novel germline mutations in SCLC including germline copy number variations (CNVs) in our cohort of patients.

Methods: We designed a custom hybrid-capture gene panel to evaluate germline alterations in 192 cancer-predisposition and frequently mutated genes in SCLC. We applied this panel to germline analysis of a treatment-naive cohort of 67 patients with SCLC at our institution. Subsequently, we annotated the variants using the American College of Medical Genetics criteria and further classified variants of uncertain significance using a set of in silico tools, including DeepMind AlphaMissense, MutationTaster, SIFT, and Polyphen2.

Results: We identified American College of Medical Genetics pathogenic or likely pathogenic alterations in seven of 67 patients. Five (71%) were novel alterations (*BCORL1*, *FANCC*, *ATR*, and *BBC3*) and a novel CNV (*SLFN11*) with two (29%) previously described mutations (*CHEK1* and *BRIP1*). We also identified 191 variants of uncertain significance in 60 of 67 patients, of which, depending on the in silico tool, 5% to 14% were predicted to be pathogenic. Patients with SCLC with the seven pathogenic alterations were observed to have a numerically longer overall survival (hazard ratio = 0.50) and progression-free survival (hazard ratio = 0.45) though not statistically significant compared with the remaining cohort.

Conclusions: Our study identifies novel germline alterations, including a CNV, and provides additional evidence that germline factors could be important contributing factors to the development of SCLC.

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Introduction

SCLC is an aggressive cancer associated with tobacco smoking.[1] Genetically, SCLC tumors are defined by loss of *TP53* and *RB1*,[2] both associated with increased cancer risk when germline mutated.[3] A 2021 seminal study identified germline mutations in 38 of 80 patients with SCLC, including in *BRCA1*, *BRCA2*, *MUTYH*, and *TP53*.[4] These findings challenge the idea that SCLC is acquired primarily through smoking and raises questions about germline alterations in SCLC oncogenesis. Although this study has the potential to radically reshape our understanding of predisposing factors, the germline landscape of SCLC remains understudied. Germline copy number variations (CNVs) have been implicated in many cancers, especially in cancer-predisposition genes, such as *BRCA1*, *BRCA2*, and *APC*, and mismatch repair genes,[5] but have not been identified in SCLC.

We sought to identify germline variants that may contribute to the development of SCLC. By analyzing known cancer-predisposition and frequently mutated genes in SCLC,[2,6–9] we identified patients with novel pathogenic germline variants including a novel CNV involving the DNA damage repair gene, *SLFN11*.

Materials and Methods

Patient Enrollment

A total of 67 patients with treatment-naive SCLC were enrolled at the Princess Margaret Cancer Centre in Toronto, Canada. After institutional Research Ethics Board approval, all patients provided written informed consent to participate.

Panel Design

The hybrid capture panel included protein-coding regions of 192 genes frequently mutated in SCLC and genes implicated in cancer predisposition (Supplementary Table 1). We used Predesigned Gene Capture Pools (Integrated DNA Technologies Canada, Inc. xGen, 96rxns, coding region mutations) using GRCh37 as reference.

Genomic DNA Extraction

Genomic DNA extracted from peripheral blood leukocytes in the buffy coat was sheared and sonicated to a median size of 200 base pairs using the Covaris M220 and verified using the Agilent 2100 Bioanalyzer.

Hybrid Capture Panel Protocol

For each patient, 10 ng of sheared, sonicated genomic DNA was used for preparation of indexed libraries. Indexed libraries were subjected to hybrid capture using the custom xGen panel, per manufacturer's instructions. Captured libraries were amplified and sequenced at a depth of 70 to 80 million reads per sample, with coverage ranging from 13,000 to 20,000×.

Germline Variant Calling

Sequenced reads were aligned using BWA (0.7.12) and quality assessed using SAMtools (1.2), GATK (3.3-0), and PICARD (1.130). Single nucleotide variant and small insertion or deletion calls were performed using VarScan2 (2.3.8) and Pindel (0.2.5b8), respectively. CNVs were assessed using DeCON (1.0.2). Calls were annotated according to standardized classification recommended by the American College of Medical Genetics (ACMG)[10] using gnomeAD (release 2.11), ESP6500 (ESP6500SI-V2), 1000Genomes, ExAC (release 0.3), COSMIC (v70), dbSNP (build 147), and ClinVar (NCBI ClinVar 20190305). Variants of uncertain significance (VUS) were annotated with in silico tools, including MutationTaster, LRT, Polyphen2 HDIV, Polyphen2 HVAR, SIFT, PROVEAN, and Deepmind AlphaMissense.[11] Concordance between the various in silico tools was also evaluated.

Survival Analysis and Descriptive Statistics

Kaplan-Meier survival analysis and univariate Cox proportional-hazards regressions were performed to evaluate differences in overall survival (OS, time from diagnosis to death or censored at date last known to be alive) and progression-free survival (PFS, time from diagnosis date to death, disease progression, or censored at date of last follow-up). Continuous variables were tested using Kruskal-Wallis H test and categorical variables using Fisher's exact test.

Results

Patient Demographics

Characteristics of the 67 patients are described in Table 1. All patients were treatment naive at the time of testing. Median age was 68 years, 64% were male, 89% were current or former smokers, and 58% were had extensive-stage disease. Furthermore, 63% self-reported a family history of cancer (Table 1), of which 13 reported family history of lung cancer and 11 with breast cancer. In addition, 16% self-reported a personal history of cancer. Self-reported ethnicity was as follows: 13% opted not to self-report, 65% were White, 6% East Asian,

Table 1. Patient demographics and clinical characteristics. Continuous variables were tested using Kruskal-Wallis test and categorical variables were tested using Fisher exact test

Total N (%)	Demographic Characteristics	No Pathogenic Variant Detected N = 60 (89.6)	Pathogenic Variant Detected N = 7 (10.4)	Total N = 67	p
Age (years)	Median (IQR)	68.0 (60.8 to 75.5)	62.2 (58.9 to 79.3)	68.0 (60.5 to 75.5)	0.886
Sex	Female	23 (38.3)	1 (14.3)	24 (35.8)	0.407
	Male	37 (61.7)	6 (85.7)	43 (64.2)	
SCLC diagnosis	De Novo SCLC	57 (95.0)	7 (100.0)	64 (95.5)	1.000
	Other Transformed SCLC	1 (1.7)	0 (0.0)	1 (1.5)	
	Transformed SCLC (previously EGFR)	2 (3.3)	0 (0.0)	2 (3.0)	
Self-reported ethnicity	White	38 (63.3)	6 (85.7)	44 (65.7)	0.485
	East Asian	4 (6.7)	-	4 (6.0)	
	Southeast Asian	4 (6.7)	-	4 (6.0)	
	Black	2 (3.3)	1 (14.3)	3 (4.5)	
	Latin American	2 (3.3)	-	2 (3.0)	
	Middle Eastern	1 (1.7)	-	1 (1.5)	
	Not provided	9 (15.0)	-	9 (13.4)	
Smoking status	Current smoker	27 (45.0)	2 (28.6)	29 (43.3)	0.613
	Former smoker	27 (45.0)	4 (57.1)	31 (46.3)	
	Never smoker	6 (10.0)	1 (14.3)	7 (10.4)	
Smoking Pack Years	Median (IQR)	40.0 (30.0 to 50.0)	65.0 (59.2 to 77.5)	40.0 (30.0 to 59.2)	0.013
VA Staging at Diagnosis	Extensive-stage	35 (58.3)	4 (57.1)	39 (58.2)	1.000
	Limited-stage	25 (41.7)	3 (42.9)	28 (41.8)	
Family History of Lung Cancer	Yes	18 (30.0)	2 (28.6)	20 (29.8)	1.000
	No	40 (66.7)	5 (71.4)	45 (67.2)	
	Not provided	2 (3.3)	0	2 (3.0)	
Family History of Cancer	Yes	37 (61.7)	5 (71.4)	42 (62.7)	1.000
	No	21 (35.0)	2 (28.6)	23 (34.3)	
	Not provided	2 (3.3)	0	2 (3.0)	
Relatives with Cancer	First-degree relatives	26 (43.3)	4 (57.1)	30 (44.8)	1.000
	More than two first-degree relatives	9 (15.0)	2 (28.5)	11 (16.4)	
	Second-degree relatives	9 (15.0)	1 (14.3)	10 (14.9)	

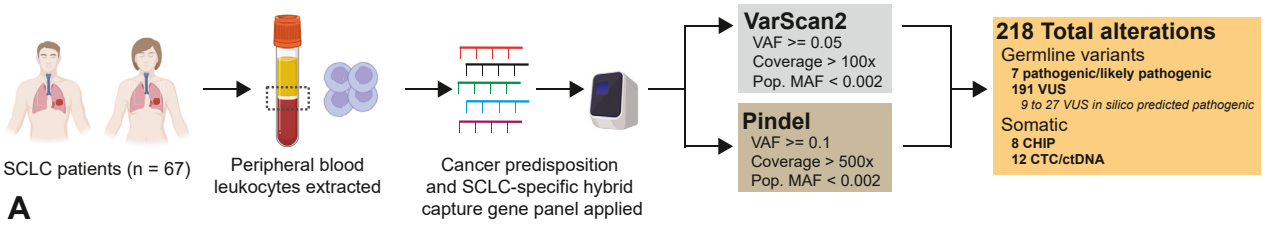
6% Southeast Asian, 5% Black, 3% Latin American, 2% Middle Eastern.

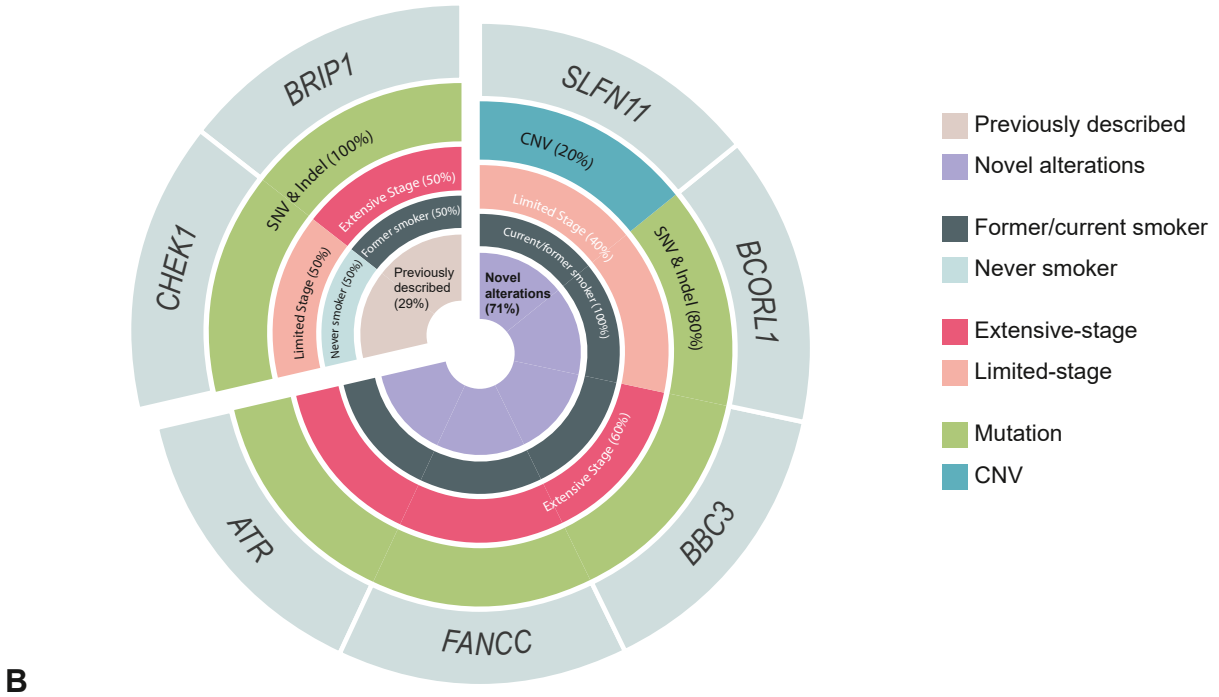
Germline Alterations Identified

After removal of known benign/likely benign variants, we identified a total of 218 high-confidence variants that were of interest (Fig. 1*A* and Supplementary Fig. 1). Using the ACMG criteria for interpreting high-confidence germline variants, we identified pathogenic or likely pathogenic germline alterations in seven patients (10%) in Table 2 and 191 variants of unknown significance in all 67 patients (VUS; Supplementary Table 2). The ACMG pathogenic and likely pathogenic variant alterations included mutations in *CHEK1*, *BCORL1*, *FANCC*, *BRIP1*, *ATR*, and *BBC3* (Fig. 1*B* and Table 2). In addition to Single nucleotide variants and insertion/deletions, we identified a novel deletion of exons 4 to 6 in *SLFN11* (Fig. 1*C*).

The median age of patients at diagnosis with pathogenic alterations was 62 (interquartile range [IQR]: 58.9–79.3) years, whereas the median was 68 (IQR: 60.8–75.5) years for patients without pathogenic alterations (Table 1). For the patients with pathogenic or likely pathogenic variants, six of seven were male, six of seven former or current smokers, four of seven had extensive-stage disease, three of seven had limited-stage disease, six of seven were White, and one of seven was Black. Two patients had both maternal and paternal histories of cancer. Compared with the rest of the cohort, patients with pathogenic mutations had a greater smoking history (median = 65 pack-years [IQR: 59.2–77.5] versus median = 40 pack-years [IQR: 30.0–50.0]) and family history of cancer (five of seven [71%] versus 37 of 60 [62%]).

Although our sample size is small, we observed a not significant trend toward numerically longer OS (hazard





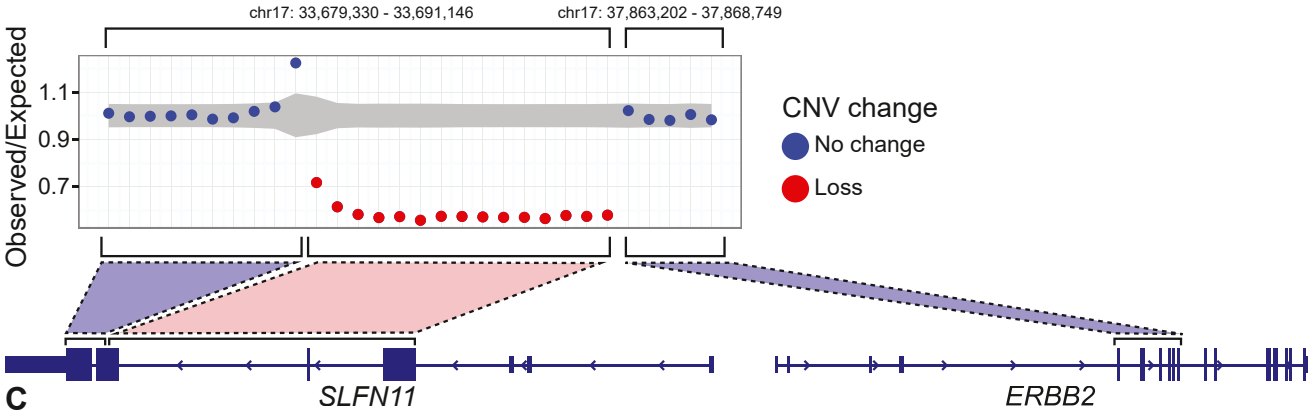


Figure 1. Identification of novel germline alterations in SCLC using a custom gene panel. (*A*) Overview of study and germline variant calling pipeline used. (*B*) Clinical information and germline alteration categories of the seven pathogenic or likely pathogenic alterations identified in the cohort. (*C*) Copy number deletion identified in exons 4 to 6 in the *SLFN11* gene with a neighboring gene, *ERBB2*, for comparison. The y axis evaluates the observed versus expected reads ratio for each hybrid-capture probe denoted by the blue and red circle symbols. Each probe targets a 120-base pair region of exons. Red circles indicate deletion of regions, whereas blue circles indicate no CNV change. ctDNA, circulating tumour DNA; CHIP, clonal hematopoiesis of indeterminate potential; CNV, copy number variation; CTC, circulating tumor cell; indel, insertion/deletion; SNV, single-nucleotide variant; Pop. MAF, population mean allele frequency; VAF, variant allele fraction; VUS, variant of uncertain significance. Panel (*A*) created using Biorender.

Table 2. Breakdown of Pathogenic or Likely Pathogenic Alterations

Alteration Types	Gene	Transcript	cDNA	Protein	VAF	Reference
Novel SNVs	*ATR*	NM_001184.4	c.4915_4918dupGATA	p.Thr1640fs	Heterozygous	This study
	BBC3	NM_001127240.3	c.619C>T	p.Gln207*	Heterozygous	
	BCORL1	NM_001184772.2	c.4079_4081delATT	p.Asp1360_Leu1361delinsVal	Hemizygous	
	FANCC	NM_000136.3	c.553C>T	p.Arg185*	Heterozygous	
Novel CNV	*SLFN11*	NM_001376007.1	c.(?_1)_(1922_?)del	Exon 4-6 deletion	Heterozygous	
Previously described germline	*BRIP1*	NM_032043.3	c.93+1G>T	p.? (c.93+1G>T)	Heterozygous	Tlemsani et al.[4]
	CHEK1	NM_001274.5	c.829C>T	p.Arg277*	Heterozygous	

CNV, copy number variation; SNV, single-nucleotide variant.

ratio = 0.50) and PFS (hazard ratio = 0.45) for patients with pathogenic or likely pathogenic alterations (Supplementary Figs. 2 and 3).

Most clinical variables including age ($p = 0.886$), sex ($p = 0.407$), ethnicity ($p = 0.485$), smoking status ($p = 0.613$), or family history of cancer ($p = 1.000$) were not significantly different between patients with or without a detected pathogenic variant (Table 1). For family history of cancer, we further restricted the analysis to only first-degree relatives; however, this was still not significant ($p = 1.000$). Notably, we observed a significant difference between smoking pack-years ($p = 0.013$). Nevertheless, the biological relevance is unclear as smoking pack-years are highly unlikely to affect germline alterations and all patients had a generally high number of pack-years overall (median = 40, IQR: 30–59). Finally, we cannot discount the possibility that this observation was simply owing to chance.

To understand the potential clinical importance of the 191 VUS, we used a compendium of in silico tools (see Materials and Methods section). Of the VUS, 14% (27 of 191) had consensus on predicted damaging/pathogenic effects, whereas 13% (25 of 191) had consensus on predictions to be benign. We separately applied the 2023 Deepmind AlphaMissense AI model catalogue, which unlike conventional in silico tools, combines additional data including structural context, population mutation frequency data, and predicted protein-folding context. AlphaMissense predicted 9% of VUS (17 of 191) to be pathogenic. In addition, 5% (nine of 191) of all VUS variants were predicted to be pathogenic using both AlphaMissense and the other in silico tools.

Other findings included a germline CNV duplication in *MAP4K3*; however, as the entire gene region is duplicated, it was unclear what the clinical genetic significance is. We also identified 20 variants detected at low variant allele fractions, which may be the result of somatic mutations (Supplementary Table 3). Specifically, these were variants detected at fractions from 0.05 to 0.24 and not observed in other cohort samples to rule out possible artifactual errors. Some variants are frequently reported in clonal hematopoiesis of indeterminate potential (CHIP), such as *TP53*, *GNAS*, *STAG*, *TET2*, and *MGA*. Remaining genes, such as *BCLAF1*, *MED12*, *BRCA1*, *PCLG2*, *PRKDC*, and *FLT1*, are likely from somatic tissue, because the variant allele fraction was higher than CHIP genes and was possibly contributed by circulating tumor cells/cell-free DNA present in the buffy coat.

Discussion

We confirmed several germline variants in cancer-predisposition genes in patients with SCLC. We identified that approximately 10% of patients harbored an ACMG pathogenic or likely pathogenic germline variant.

To best of our knowledge, our study is the first to evaluate germline CNVs in SCLC, adding to previously reported CNV analysis of tumor DNA but not germline DNA.[4] Specifically, we identified a novel germline CNV with an exon 4 to 6 deletion of *SLFN11*. This deletion has been reported in the Database of Genomic Variants in healthy/noncancer individuals but at low frequency (0.55%). *SLFN11* has been implicated in DNA replication stress response, and we speculate whether germline CNV changes in *SLFN11* may increase the risk of individuals to developing SCLC. Additional studies to assess the functional impact of this loss are needed, such as immunohistochemistry for SLFN11 protein in tumor tissue. Unfortunately, there was insufficient material remaining from this patient's tumor sample to perform further testing.

Seven patients with pathogenic or likely pathogenic variants had numerically longer OS and PFS. Nevertheless, owing to our sample size, our statistical analyses are underpowered and should be cautiously interpreted. Most patients with pathogenic alterations were smokers, and two patients had maternal (breast cancer, lung cancer) and paternal (leukemia, bone cancer) cancer histories. These patients had mutations in *FANCC*, implicated in hereditary cancers,[12,13] and *BBC3*.

Interestingly, *BBC3* is important in apoptosis and has been implicated in head and neck squamous cell carcinoma where its expression is correlated with tumor size and nodal involvement.[14] In larger tumor sizes, *BBC3* expression is down-regulated, suggesting a role in tumor suppression.[14] Whether these tumor-associated findings have germline implications would be worth future investigation.

Regarding ethnicity, most ACMG pathogenic or VUS mutations were detected in White patients (six of seven) likely because gnomeAD is overrepresented by White reference samples (77%). In contrast, samples from East Asian, South Asian, or Middle Eastern ethnicities are underrepresented (<5%) in gnomeAD. Consequently, there may be undetected pathogenic variants in the 35% of our cohort who are non-White, highlighting the need to increase ethnic diversity in genomic studies.

We validated previously identified pathogenic alterations, including *CHEK1* and *BRIP1*.[4] We also identified 191 VUS, of which 5% to 14% were predicted to be pathogenic using a suite of in silico tools, such as the 2023 DeepMind AlphaMissense AI model. Genes frequently implicated in CHIP and somatic mutations in *TP53* and *BRCA1* were also identified. Interestingly, we detected a somatic mutation in *PLCG2*, which is implicated in poor survival in SCLC and an immunosuppressive phenotype.[15]

Our study has several limitations. Namely, our sample size was limited to 67 individuals, and our panel evaluated protein-coding regions of only 192 genes. On occasion, somatic variants in the peripheral blood can be difficult to distinguish from germline variants. In our study, we addressed this limitation through a stringent filtering criterion including using variant allele fraction to filter out artifacts and qualitatively ruling out CHIP/tumor mutations using known databases. Nevertheless, despite these limitations, these data provide important novel evidence that heritable factors may increase an individuals' risk to developing SCLC beyond well-established environmental factors.

Currently, there is no clinical guidance for germline testing of patients who present with SCLC. Our findings support that a diagnostic yield of 10% for detecting germline mutations in patients with SCLC is evident. Many patients reported a family history of cancer, where five of seven patients (71%) reported a family history of cancer compared with 37 of 60 (63%). Our results suggest germline testing in patients with SCLC with a family history of cancer should be further investigated.

In summary, we have identified several novel germline alterations (mutations and CNVs) in patients with SCLC. In addition, in silico prediction models further categorized potential high-risk VUS. Our findings suggest that nonenvironmental, heritable factors may also modulate the risk of SCLC tumorigenesis. Last, our study has wider implications for the potential management of SCLC. Future directions could include determining whether germline findings in patients with SCLC have utility in screening high-risk family members of patients. Moreover, germline findings in patients with SCLC (i.e., in DNA damage-response genes) might highlight vulnerabilities with substantial implications for DNA repair inhibitors and other DNA-targeting therapies.

CRediT Authorship Contribution Statement

Sami Ul Haq: Conceptualization, Methodology, Software, Investigation, Formal analysis, Data Curation, Writing - original draft, Writing - review & editing, Visualization.

Gregory Downs: Software, Investigation, Formal analysis.

Luna Jia Zhan: Data Curation, Formal analysis, Visualization.

Sabine Schmid: Data curation.

Devalben Patel: Investigation.

Danielle Sacdalan: Data curation.

Janice J.N. Li: Data curation.

Dangxiao Cheng: Resources.

Nicolas Meti: Data curation.

Vivek Philip: Project administration, Writing - original draft, Writing - review & editing.

Raymond H Kim: Writing - review & editing.

Geoffrey Liu: Resources, Supervision, Writing - review & editing.

Scott V. Bratman: Resources, Supervision, Writing - review & editing.

Peter Sabatini: Methodology, Validation, Software, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration.

Benjamin H. Lok: Conceptualization, Funding acquisition, Resources, Project administration, Supervision, Writing - original draft, Writing - review & editing.

Disclosure

Dr. Bratman is an inventor on patents related to cell-free DNA mutation and methylation analysis technologies that have been licensed to Roche and AdelaBio, respectively, and is co-founder of, has ownership in, and serves in a leadership role at AdelaBio. Dr. Liu reports receiving grants and personal fees from AstraZeneca and Takeda; grants from Boehringer Ingelheim; and personal fees from Hoffman-La Roche, Merck, Bristol-Myers Squibb, and Pfizer outside of the submitted work. Dr. Lok reports receiving grants from Pfizer and grants, personal fees, and nonfinancial support from AstraZeneca outside the submitted work. The remaining authors declare no conflict of interest.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100702.

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