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Clinical Utility of Plasma-Based Comprehensive Molecular Profiling in Advanced Non–Small-Cell Lung Cancer

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PURPOSE Comprehensive molecular profiling (CMP) plays an essential role in clinical decision making in metastatic non–small-cell lung cancer (mNSCLC). Circulating tumor DNA (ctDNA) analysis provides possibilities for molecular tumor profiling. In this study, we aim to explore the additional value of centralized ctDNA profiling next to current standard-of-care protocolled tissue-based molecular profiling (SoC-TMP) in the primary diagnostic setting of mNSCLC in the Netherlands.

METHODS Pretreatment plasma samples from 209 patients with confirmed mNSCLC were analyzed retrospectively using the NGS AVENIO ctDNA Targeted Kit (Roche Diagnostics, Basel, Switzerland) and compared with paired prospective pretreatment tissue-based molecular profiling from patient records. The AVENIO panel is designed to detect single-nucleotide variants, copy-number variations, insertions or deletions, and tyrosine kinase fusion in 17 genes.

RESULTS Potentially targetable drivers were detected with SoC-TMP alone in 34.4% of patients. Addition of clonal hematopoiesis of indeterminate potential–corrected, plasma-based CMP increased this to 39.7% ($P < .001$). Concordance between SoC-TMP and plasma-CMP was 86.6% for potentially targetable drivers. Clinical sensitivity of plasma-CMP was 75.2% for any oncogenic driver. Specificity and positive predictive value were more than 90% for all oncogenic drivers.

CONCLUSION Plasma-CMP is a reliable tool in the primary diagnostic setting, although it cannot fully replace SoC-TMP. Complementary profiling by combined SoC-TMP and plasma-CMP increased the proportion of patients who are eligible for targeted treatment.

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INTRODUCTION

Comprehensive molecular profiling (CMP) has become a cornerstone in clinical decision making in metastatic non–small-cell lung cancer (mNSCLC). Identifying genetic biomarkers in tumor tissue allows optimal personalized treatment in the first-line setting. A distinct set of biomarkers is recommended for diagnostic testing in all patients with stage IV NSCLC.[1-3]

In lung cancer, the standard diagnostic procedures are often hampered by a lack of available tumor tissue or tissue being unsuitable for molecular analysis.[4-6] One of the reasons for this can be that no tumor sampling can take place because of a poor clinical condition at the time of diagnosis, and biopsies can be considered too risky. As a result, many patients will not receive optimal personalized treatment.

Analysis of circulating tumor DNA (ctDNA) from a patient's blood has provided minimally invasive possibilities for molecular tumor profiling. Various studies have shown that next-generation sequencing (NGS) of plasma ctDNA can be useful for detecting genetic biomarkers. Plasma NGS has shown high sensitivity and high concordance with standard-of-care tissue-based molecular profiling (SoC-TMP).[7-10] Here, we compared plasma-based CMP plus SoC-TMP to SoC-TMP alone. This was performed in the Dutch diagnostic landscape with a relatively high proportion of tissue profiled patients, in contrast to the diagnostic landscape in earlier studies. Additionally, plasma-CMP in those studies was often outsourced, and here we investigate the performance of in house plasma-CMP on the AVENIO platform.

Therefore, in this study, we explore the additional value of centralized, in-house plasma-CMP next to modern SoC-TMP in the Dutch diagnostic landscape. Secondary aims are to determine the concordance of plasma-CMP and SoC-TMP, and the number of targetable mutations identified by plasma-CMP only.

ASSOCIATED CONTENT

Appendix

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Patients with treatment-naive metastatic non–small-cell lung cancer benefit from molecular profiling of their tumors, to inform targeted treatment options. However, obtaining biopsies of tumor tissue is invasive, can take valuable time, and is not always feasible. Here, we investigate the added value of complete molecular profiling of blood plasma–based liquid biopsies using the AVENIO platform next to standard-of-care tissue biopsies among 209 patients with metastatic non–small-cell lung cancer in the Netherlands.

Knowledge Generated

Even compared with high-class tissue molecular profiling, we find that centralized, in-house plasma complete molecular profiling improved the proportion of patients for whom a clinically targetable alteration was detected from 34.4% to 39.7% ($P < .001$).

Relevance

By identifying more clinically targetable alterations, more patients will be eligible for targeted personalized treatment. How best to combine tissue and plasma molecular profiling, from a cost perspective, is the subject of further research.

METHODS

Patients

All patients in this study consented with the use of plasma and tissue samples by providing written informed consent for participation in a larger project, namely the Lung cancer Early Molecular Assessment trial: ClinicalTrials.gov identifier: NCT02894853. This multicenter diagnostic study was reviewed and approved by the medical ethics committee of the Netherlands Cancer Institute in Amsterdam, the Netherlands. The ctDNA substudy reported here was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and the guidelines for Good Clinical Practice.

Nine hospitals in the Netherlands contributed to patient enrollment. Patients were eligible if they had confirmed stage IV NSCLC, were fully treatment-naive, and had a pretreatment plasma sample taken. To exclude the risk of selection bias, the first consecutive cohort of 224 patients was included.

Study Procedures

Decentralized tissue analysis was performed according to the local standard of care in the hospital of enrollment during routine clinical diagnostic workup. SoC-TMP consisted of NGS (panels shown in Appendix Table A1) and single gene analyses for rearrangements. The results from SoC-TMP were obtained from the clinical pathology reports, or was requested from either the treating pulmonologist, the involved pathologist, or the involved clinical molecular biologist. According to national and international guidelines, molecular profiling should cover known NSCLC oncodriver genes such as *KRAS*, *EGFR*, *BRAF*, *ERBB2*, *ALK*, *ROS1*, *RET*, and *MET*.[1-3]

Plasma-CMP was centrally performed retrospectively and did not affect clinical decision making. Blood samples were centrally stored and processed. Samples from patients at the site of the central laboratory were collected in K_2-EDTA tubes, whereas those from patients in other hospitals were collected in cell-stabilizing tubes (STRECK, Omaha, NE). All whole-blood samples were sent to the central laboratory by regular mailing services. Local sampling, central processing, and central storage of all blood samples were completed within the 5-day stabilizing period.

Blood samples were centrifuged for 10 minutes at 1,700*g* at room temperature. Cells were stored at –80°C and plasma was centrifuged for 10 minutes at 20,000*g* before storage at –80°C. Median 5 mL cell-free plasma—interquartile range 4-6 mL—was used per sample for isolation of cell-free DNA (cfDNA) using the QIAsymphony Circulating DNA Kit (article number 1091063, Qiagen, Düsseldorf, Germany) with the QIAsymphony (Qiagen). A median of 39 ng cfDNA (interquartile range 28-50 ng) was used as input for plasma-based NGS. With the exception of the cfDNA isolation methods used, all sample handlings were performed according to manufacturer guidelines.

Plasma-CMP was performed using the AVENIO ctDNA Targeted kit[11,12] (Roche Diagnostics, Basel, Switzerland), which covers hotspot regions of the aforementioned eight oncodriver genes (*KRAS*, *EGFR*, *BRAF*, *ERBB2*, *ALK*, *ROS1*, *RET*, and *MET*) and in an additional nine other genes: *APC*, *BRCA1*, *BRCA2*, *DPYD*, *KIT*, *NRAS*, *PDGFRA*, *TP53*, and *UGT1A1*. Single-nucleotide variants with a variant allele frequency (VAF) of 0.10% or higher have reported sensitivity and positive predictive value (PPV) of $> 99\%$,[12] and were considered in the analysis. Copy-number variations (CNV) with a test-specific CNV score lower than 5.0 are considered borderline, according to the kit manual. However, we found high variability in CNV score, and no correlation between CNV score and detection rate in tissue was seen. Additionally, a large proportion of CNVs (11 out of 30; 36.7%) that were detected in plasma were not covered in the matched tissue analysis. We

considered that we could not make a reliable statement about CNV testing in this setting, and therefore we excluded all CNVs that were reported by plasma-CMP from our final analysis.

All variants were classified per level of pathogenicity using online databases at OncoKB (update September 17, 2020),[13] ClinVar,[14] IARC TP53 Database,[15] COSMIC,[16] JaxCKB,[17] and Franklin Genoox.[18] The system published at OncoKB (version V2, published on December 20, 2019)[19] was used as the basis for classification of drivers. In this report, level 1 drivers are US Food and Drug Administration (FDA)-recognized biomarkers predictive of response to an FDA-approved drug in NSCLC. Level 2 or 3A drivers are biomarkers predictive of response to a drug that may be available off-label or in the setting of a clinical trial. Level 3B or 4 drivers are biomarkers for which there is an FDA-approved or investigational drug available in another indication, or for which there is compelling biologic evidence of response to a drug.[13]

Genetic variants that are detected in cfDNA may not always be associated with cancer. Other studies have shown that many cfDNA mutations may be consistent with clonal hematopoiesis of indeterminate potential (CHIP).[20,21] Samples containing driver mutations in plasma but not in tissue were verified on the blood cell pellet to exclude CHIP. DNA was isolated from the cells using the QIAsymphony DSP DNA Midi Kit (article number 937255, Qiagen) with the QIAsymphony. The DNA was fragmented sonically using a Covaris ME220 Focused-ultrasonicator (Covaris Inc, Woburn, MA) in microTUBE AFA Fiber Pre-Slit Snap-Cap (PN 520045) vessels, with the following settings: duration 100 seconds, peak power 75 W, duty factor 25%, and 1,000 cycles per burst. DNA input for AVENIO ctDNA Targeted Kit (Roche) was 50 ng. Sequencing depth was identical to the plasma samples to avoid false-negative results.

Index hopping, or index cross-talk, is a possible cause of false positives and is inherent to massively parallel sequencing methods where multiple samples are pooled.[22,23] The plasma-CMP pipeline automatically flags samples that are potentially the result of index hopping. All suspect samples in our cohort were retested.

Statistical Analyses

For this exploratory study, we had a maximum of 224 plasma NGS tests available in the central laboratory. Concordance was defined as the sum of true positives and true negatives as a fraction of all tests. Sensitivity, specificity, PPV, and negative predictive value (NPV) of plasma-CMP were calculated with SoC-TMP as the gold standard. We applied McNemar's chi-square test to assess whether combined SoC-TMP plus plasma-CMP identifies more patients with driver mutations than SoC-TMP alone ($\alpha = .05$). To assess any difference in DNA input between samples in which oncogenic drivers were concordantly detected in tissue and plasma, and samples in which drivers were not detected in plasma, a Mann-Whitney *U* test for unpaired data with no normal distribution was used. Statistical analyses were performed using IBM SPSS Statistics version 27.

RESULTS

Cohort Characteristics

In total, 224 patients with confirmed stage IV NSCLC were included in this study. Fifteen patients were excluded from the analysis. Three patients were not treatment-naive at the time of tissue sampling, and no pretreatment plasma samples were available for 12 patients. In total, 209 patients were included in the analysis (Fig 1). The median time between the collection of tissue for standard diagnostic purposes and the collection of blood for plasma-CMP was 14 days (range, 0-90 days), with 84.3% of paired samples taken within 30 days.

Detection of Oncogenic Variants

In total, 363 oncogenic variants were detected in 209 patients; these are shown in graphic overviews in the Data Supplement. Routine molecular diagnostics in tissue resulted in molecular profiling of 182 patients (87.1%, Data Supplement), centralized in-house plasma-CMP was feasible in 206 patients (98.6%, Data Supplement), and combined feasibility was 85.6% (179/209 patients, Data Supplement). All detected oncogenic drivers are shown in Appendix Tables A2-A4.

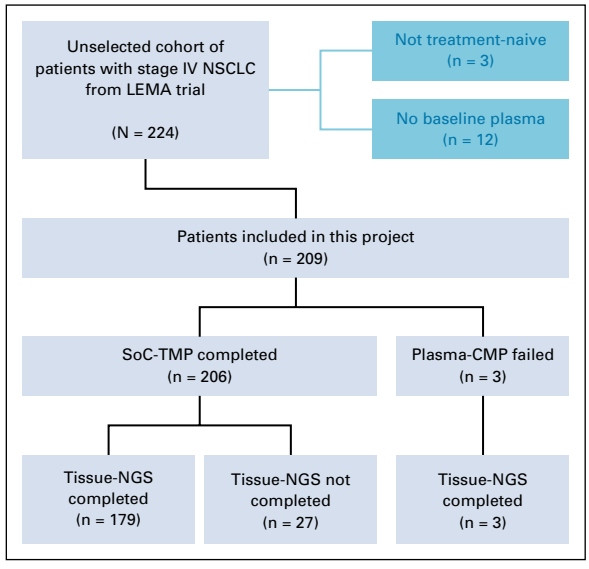

FIG 1. Flow diagram of inclusion. In total, 209 patients had CMP, either tissue-based, plasma-based, or both. Fifteen of the initially selected 224 patients were ineligible. CMP, comprehensive molecular profiling; LEMA, Lung cancer Early Molecular Assessment; NGS, next-generation sequencing; NSCLC, non–small-cell lung cancer; SoC-TMP, standard-of-care protocolled tissue-based molecular profiling.

Out of 182 patients for whom SoC-TMP was feasible, level 1 drivers were identified in 31 patients (17.0%). The number of patients identified with a potentially targetable driver (level 1, 2, or 3A) in tissue was 72 (39.6%). The total number of patients with an oncogenic driver (level 1-4, including most *KRAS* mutations) in tissue was 121 (66.5%). Histologic subtypes in the latter group were 112 adenocarcinomas, four squamous cell carcinomas (SCCs), two large-cell neuroendocrine carcinomas, one sarcomatoid carcinoma, and two not-otherwise-specified NSCLC. The diagnostic yield of SoC-TMP, i.e., the proportion of patients in the total cohort in whom a level 1-4 driver was found, was 57.9% (121 out of 209 patients).

Plasma-CMP identified 24 patients with a Level 1 driver (11.7% of 206), 62 patients with a level 1-3A driver (30.1%), and 103 patients with a level 1-4 driver (50.0%). The diagnostic yield of plasma-CMP was 49.3% (103 out of 209 patients).

Performance of Plasma-CMP Compared With SoC-TMP

Out of 179 patients for whom both SoC-TMP and plasma-CMP were completed, 31 were identified with a level 1 driver in either tissue, plasma, or both. Twenty-one out of 31 patients were identified by both SoC-TMP and plasma-CMP (67.7%). Nine patients were identified by SoC-TMP only, and one patient was identified by plasma-CMP only. Concordance of level 1 driver detection, comprising both negative and positive cases, was 94.4% (169 out of 179 patients).

Level 1-3A drivers were detected in 75 out of these 179 patients. Fifty-one were identified by both SoC-TMP and plasma-CMP (68.0%), 19 by SoC-TMP alone, and five by plasma-CMP alone. Concordance of level 1-3A driver detection was 86.6% (155 out of 179 patients).

A total of 117 patients were identified with a level 1-4 driver: 88 by both SoC-TMP and plasma-CMP (75.2%), 24 exclusively by SoC-TMP, and five by plasma-CMP only. Concordance of level 1-4 driver detection was 83.8% (150/179).

Compared with current SoC-TMP, sensitivity of plasma-CMP was 70.0% for level 1 drivers, 72.9% for level 1-3A drivers, and 78.6% for level 1-4 drivers. Specificity of plasma-CMP was 99.3%, 95.4%, and 92.5% for level 1, level 1-3A, and level 1-4 drivers, respectively. PPV was 95.5% and NPV was 94.3% for level 1 drivers. PPV and NPV were 91.1% and 84.6% for level 1-3A drivers, respectively. Finally, for level 1-4 drivers, PPV was 94.6% and NPV was 72.1%. Full contingency tables are shown in Appendix Table A5.

Concordance between plasma-CMP and SoC-TMP might have been affected by the DNA input of plasma-CMP. When considering all oncogenic driver variants (level 1-4), the diagnostic yield was correlated with DNA input: median input from concordant samples was 42.95 ng (range, 12.7-50.0 ng), and 28.65 ng (range, 10.3-50.0 ng) in samples in which tissue-identified drivers were not detected in plasma ($P = .038$).

Additional Value of Plasma-CMP

Plasma-CMP identified additional driver mutations in eight patients who reported a completed SoC-TMP. One patient was identified with a *KRAS G12C* in plasma, whereas a *KRAS G12A* was also detected in both tissue and plasma. One patient was identified with a level 1 driver, six patients with a level 2-3A driver, and one with a level 4 driver.

For 27 patients (12.9% of the total cohort), SoC-TMP was not feasible because of insufficient tumor material (n = 13; 48.1%), no tumor material (n = 9; 33.3%), or for unknown reasons (n = 5; 18.5%). This involved 11 patients with adenocarcinoma, nine with SCC, one with large cell neuroendocrine carcinoma, and six with tumors of undetermined histology. A level 1 driver was detected in two of these patients (7.4%), two other patients had a *KRAS G12C* mutation (level 1-3A driver total n = 4; 14.8%), and another three had a level 3B-4 driver (level 1-4 driver total n = 7; 25.9%) (Data Supplement).

In total, plasma-CMP next to SoC-TMP increased the number of patients with a level 1 driver from 31 to 34 in the total cohort; from 14.8% to 16.3% of 209 patients ($P = .250$). For patients with level 1-3A driver, the number significantly increased from 72 to 83 patients (ie, 34.4%-39.7% of the total cohort, $P < .001$). Considering level 1-4 drivers, the number of patients identified also increased significantly from 121 to 135 (ie, 57.9%-64.6% of the total cohort; $P < .001$).

CHIP and Index Hopping

In our cohort, 18 patients (8.6%) were identified in whom a total of 23 level 1-4 driver mutations were detected in plasma that had not been detected by SoC-TMP. For seven of these 18 patients, SoC-TMP was incomplete and did not cover the variant detected in plasma. In the remaining 11 patients, SoC-TMP had not detected the mutation. WBC DNA sequencing detected one of the suspect variants (*KRAS G12S*, patient P177), which was considered to be a CHIP and excluded from further analyses.

The plasma-CMP pipeline flagged two variants that potentially resulted from index hopping. Both were *EGFR L858R* mutations and could not be reproduced by retesting: one sample was negative in the retest, and the other test failed because of technical problems. Moreover, digital droplet PCR did not confirm the *L858R* mutations in these samples. Therefore, both samples were considered negative for *EGFR L858R* in the final analysis and are not shown in figures or tables.

DISCUSSION

We aimed to determine the value of CMP of plasma in a real-world, multicenter, clinical cohort of treatment-naive patients who presented with metastasized NSCLC. Our

results show that plasma-CMP next to SoC-TMP identified significantly more patients with potentially targetable driver mutations (i.e. Level 1-3A, $P < .001$) and other clinically relevant drivers in the Dutch diagnostic landscape. Plasma-CMP produced reliable data in a real-world cohort with PPV and specificity of > 90%. The concordance with SoC-TMP was at least 83.8% and clinical sensitivity at least 67.7% for oncogenic drivers.

The increased number of patients with an oncogenic driver was lower than previously was published.[9,10] This is primarily because the yield of potentially targetable driver mutations from SoC-TMP was higher in our cohort (34.4%) than for others (20.5% [nine] and 21.3% [10]), leaving less room for improvement, given that the total number of patients identified with a potentially targetable driver after addition of plasma-CMP was comparable in our cohort (39.7%) to others (35.8% [nine] and 27.3% [10]).

Another factor that helps explain the seemingly small increase of oncogenic driver mutations detected by addition of plasma-CMP is that in our cohort, the group with missing or incomplete SoC-TMP contained relatively more SCCs (nine out of 27 *v* 10 out of 182 in the rest of the cohort), possibly because this histologic subtype is physically harder to reach for biopsy. The prevalence of driver mutations is known to be lower in SCC, meaning that the subset of patients with missing or incomplete SoC-TMP is enriched for a group of patients for whom plasma-CMP is less likely to be of added value.

CHIPs were detected in only one patient (0.5%), contrasting starkly with other studies reporting CHIPs in 53%-62% of patients.[20,24] Most importantly, we showed that CHIPs rarely occur as clinically relevant driver variants. Although other studies report all CHIPs found with sequencing panels up to 2 Mbp in size, we focused exclusively on clinical relevance and only reported variants that might affect treatment decisions. None of these variants was in the top 28 genes most affected by CHIPs. Even among variants found in plasma but not in tissue, the number of CHIPs found was comparatively low, suggesting that these variants may originate from other lesions than the one biopsied for SoC-TMP. Together, these findings indicate that routine testing of blood cell pellets with extensive NGS methods may not be necessary in the setting of treatment selection.

We postulate that plasma-CMP can be used in the clinical setting in two scenarios. First, synchronous combined SoC-TMP and plasma-CMP to increase the proportion of patients in whom a potentially targetable driver is detected. This may increase the number of patients who receives optimal personalized treatment. Our data support the potential utility of plasma-CMP in this scenario. Second, upfront plasma-CMP, followed by SoC-TMP when no targetable driver is detected in plasma, might be a realistic option, given the high specificity and PPV, and lower sensitivity and NPV of plasma-CMP. However, it cannot fully replace tissue-based diagnostics as certain biomarkers (eg, histologic subtype or programmed death-ligand 1) can currently only be assessed on tumor tissue. Until alternative methods for such companion diagnostics are developed,[25] the need for obtaining tumor tissue remains.

We conclude that in-house plasma-CMP improves the detection of clinically relevant oncodriver mutations in patients with mNSCLC. With an expanding palette of treatable mutations, rapid advances in molecular diagnostics, and increasing affordability and performance of plasma-CMP, this relatively new technique is establishing its role in the diagnostic workup of mNSCLC. However, analysis of the cost effectiveness is warranted to determine the optimal implementation in routine clinical care.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Novello S, Barlesi F, Califano R, et al: Metastatic non-small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 27:v1-v27, 2016 (suppl 5)
2. Planchard D, Popat S, Kerr K, et al: Metastatic non-small cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 29:iv192-iv237, 2018 (suppl 4)
3. IKNL IKN: Landelijke richtlijn niet kleincellig longcarcinoom. https://www.oncoline.nl/niet-kleincellig-longcarcinoom
4. Rangachari D, VanderLaan PA, Le X, et al: Experience with targeted next generation sequencing for the care of lung cancer: Insights into promises and limitations of genomic oncology in day-to-day practice. Cancer Treat Commun 4:174-181, 2015
5. Lim C, Tsao MS, Le LW, et al: Biomarker testing and time to treatment decision in patients with advanced nonsmall-cell lung cancer. Ann Oncol 26:1415-1421, 2015
6. Kuijpers C, Heuvel MMvd, Overbeek LIH, et al: Landelijke variatie in moleculaire diagnostiek bij gemetastaseerde longkanker. Ned Tijdschr Geneeskd 163:1-10, 2019
7. Lovejoy A, Pati A, Muñoz A, et al In-depth assessment reveals powerful performance and flexibility of the AVENIO ctDNA Analysis Kits. https://sequencing.roche.com/content/dam/rochesequence/worldwide/resources/SEQ100108_AVENIO%20ctDNA_Performance_White_Paper.pdf2017
8. Balaji SA, Shanmugam A, Chougule A, et al: Analysis of solid tumor mutation profiles in liquid biopsy. Cancer Med 7:5439-5447, 2018
9. Aggarwal C, Thompson JC, Black TA, et al: Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. JAMA Oncol 5:173-180, 2019
10. Leighl NB, Page RD, Raymond VM, et al: Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non–small cell lung cancer. Clin Cancer Res 25:4691-4700, 2019
11. Newman AM, Lovejoy AF, Klass DM, et al: Integrated digital error suppression for improved detection of circulating tumor DNA. Nat Biotechnol 34:547-555, 2016
12. Solutions RS: AVENIO ctDNA Targeted Kit. https://sequencing.roche.com/content/dam/rochesequence/worldwide/resources/brochure-avenio-ctdna-targeted-kit-SEQ100046.pdf
13. Chakravarty D, Gao J, Phillips SM, et al: OncoKB: A Precision Oncology Knowledge Base. JCO Precis Oncol 2017:PO.17.00011, 2017
14. National Library of Medicine: Variation Viewer. https://www.ncbi.nlm.nih.gov/variation/view/
15. Bouaoun L, Sonkin D, Ardin M, et al: TP53 variations in human cancers: New lessons from the IARC TP53 database and genomics data. Hum Mutat 37:865-876, 2016
16. Forbes SA, Beare D, Boutselakis H, et al: COSMIC: Somatic cancer genetics at high-resolution. Nucleic Acids Res 45:D777-d83, 2017
17. JaxCKB: https://ckb.jax.org/gene/grid
18. Franklin Genoox: https://franklin.genoox.com/home
19. OncoKB: OncoKB Therapeutic Levels of Evidence V2. https://www.oncokb.org/levels
20. Razavi P, Li BT, Brown DN, et al: High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. Nat Med 25:1928-1937, 2019
21. Hu Y, Ulrich BC, Supplee J, et al: False-positive plasma genotyping due to clonal hematopoiesis. Clin Cancer Res 24:4437-4443, 2018
22. MacConaill LE, Burns RT, Nag A, et al: Unique, dual-indexed sequencing adapters with UMIs effectively eliminate index cross-talk and significantly improve sensitivity of massively parallel sequencing. BMC Genomics 19:30, 2018
23. Kircher M, Sawyer S, Meyer M: Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. Nucleic Acids Res 40:e3, 2012
24. Leal A, van Grieken NCT, Palsgrove DN, et al: White blood cell and cell-free DNA analyses for detection of residual disease in gastric cancer. Nat Commun 11:525, 2020
25. Kloten V, Lampignano R, Krahn T, et al: Circulating tumor cell PD-L1 expression as biomarker for therapeutic efficacy of immune checkpoint inhibition in NSCLC. Cells 8:809, 2019

APPENDIX

TABLE A1. Standard-of-Care Tissue-Based Molecular Profiling Techniques

Ion Ampliseq Cancer Hotspot Panel
Ion Ampliseq Colon and Lung Cancer Research Panel
TruSeq Amplicon Cancer Panel
Single-Molecule Molecular Inversion Probes Panel

NOTE. Molecular profiling of tumor tissue was performed decentralized, according to the local standard of care. The participating centers used various versions of the panels shown here, including customized versions. In addition to these next-generation sequencing panels, the molecular diagnostics included single gene analyses for rearrangements and copy-number variations (eg, immunohistochemistry and fluorescent in situ hybridization).

TABLE A2. Level 1 Driver Mutations Detected in Tissue, Plasma, or Both

Detected in Both Tissue and Plasma				Detected in Tissue Only				Detected in Plasma Only			
Patient	**Gene**	**Variant**	**VAF Plasma (%)**	**Patient**	**Gene**	**Variant**	**VAF Plasma**	**Patient**	**Gene**	**Variant**	**VAF Plasma (%)**
P082	*ALK*	Fusion EML4	No VAF	P068	*BRAF*	V600E	Not detected	P091	*ALK*	L1196M	0.1
P085	*ALK*	Fusion EML4	No VAF	P113	*EGFR*	E746_A750del	Not detected	P131[a]	*BRAF*	V600E	2.0
P089	*ALK*	Fusion EML4	No VAF	P152	*EGFR*	E746_S752delinsV	Not detected	P176[b]	*EGFR*	E746_A750del	6.2
P100	*BRAF*	V600E	49.6	P119	*EGFR*	L858R	Not detected	P019[b]	*MET*	c.3082+3 A>G	0.1
P097	*BRAF*	V600E	16.9	P034	*EGFR*	L858R	Not detected				
P053	*BRAF*	V600E	16.8	P191	*EGFR*	S752_I759del	Not detected				
P135	*BRAF*	V600E	1.0	P117	*EGFR*	T790M	Not detected				
P008	*BRAF*	V600E	0.9	P075[c]	*MET*	Y1003F	Not detected				
P150	*EGFR*	E746_A750del	13.1	P063	*ROS1*	fusion	Not detected				
P067	*EGFR*	E746_A750del	0.3	P009	*ROS1*	Fusion	Not detected				
P029	*EGFR*	E746_S752delinsV	3.0								
P001	*EGFR*	L747_E749del	1.2								
P139	*EGFR*	L858R	11.4								
P157	*EGFR*	L858R	3.9								
P131	*EGFR*	L858R	3.7								
P093	*EGFR*	L858R	1.2								
P126	*EGFR*	L861Q	4.2								
P136	*MET*	c.3028+3A>G	5.2								
P087	*MET*	c.3082+1G>A	14.1								
O16	*RET*	Fusion KIF5B	No VAF								
P057	*ROS1*	Fusion SLC34A2	No VAF								

Abbreviations: CMP, comprehensive molecular profiling; SoC-TMP, standard-of-care protocolled tissue-based molecular profiling; VAF, variant allele frequency.

[a]*EGFR L858R* mutation was detected in both tissue and plasma from this patient. In retrospect, this patient had a synchronous colorectal carcinoma that might have harbored the *BRAF V600E* mutation. However, the patient died before this could be confirmed.

[b]SoC-TMP could not be performed in these patients.

[c]Plasma-CMP was not successful in this patient.

TABLE A3. Level 2-3A Driver Mutations Detected in Tissue, Plasma, or Both

Detected in Both Tissue and Plasma				Detected in Tissue Only				Detected in Plasma Only			
Patient	**Gene**	**Variant**	**VAF Plasma (%)**	**Patient**	**Gene**	**Variant**	**VAF Plasma**	**Patient**	**Gene**	**Variant**	**VAF Plasma (%)**
P141	*ERBB2*	A775_G776insYVMA	37.9	P081	*EGFR*	Exon 20 ins	Not detected	P087[a]	*ERBB2*	R103Q	0.2
P056	*KRAS*	G12C	67.5	O21	*KRAS*	G12C	Not detected	P002	*ERBB2*	R896C[b]	0.1
P163	*KRAS*	G12C	37.9	P003	*KRAS*	G12C	Not detected	P125	*ERBB2*	S310Y[b]	0.3
P180	*KRAS*	G12C	33.9	P048	*KRAS*	G12C	Not detected	P130[c]	*KRAS*	G12C	10.8
P156	*KRAS*	G12C	20.0	P052	*KRAS*	G12C	Not detected	P175[d]	*KRAS*	G12C	3.0
P051	*KRAS*	G12C	14.8	P059	*KRAS*	G12C	Not detected	P148[c]	*KRAS*	G12C	0.9
P041	*KRAS*	G12C	13.9	P195	*KRAS*	G12C	Not detected	P151	*KRAS*	G12C	0.5
O22	*KRAS*	G12C	12.9	P201	*KRAS*	G12C	Not detected	O26	*KRAS*	G12C	0.2
P129	*KRAS*	G12C	11.3	P040	*MAP2K1*	K57N[e]	Not detected	P047	*KRAS*	G12C	0.2
P183	*KRAS*	G12C	10.8	P155	*MET*	CNV	Not detected				
P077	*KRAS*	G12C	7.6	P121	*MET*	CNV	Not detected				
P122	*KRAS*	G12C	5.3								
P174	*KRAS*	G12C	3.3								
P026	*KRAS*	G12C	3.2								
P153	*KRAS*	G12C	3.1								
P078	*KRAS*	G12C	2.6								
P065	*KRAS*	G12C	2.5								
P006	*KRAS*	G12C	2.2								
P011	*KRAS*	G12C	2.0								
P055	*KRAS*	G12C	1.0								
P096	*KRAS*	G12C	0.8								
P023	*KRAS*	G12C	0.7								
P039	*KRAS*	G12C	0.7								
O27	*KRAS*	G12C	0.6								
P098	*KRAS*	G12C	0.4								
P186	*KRAS*	G12C	0.3								
P038	*KRAS*	G12C	0.2								
P074	*KRAS*	G12C	0.2								
P190	*KRAS*	G12C	0.1								
P102	*MET*	CNV	No VAF								
P126[f]	*MET*	CNV	No VAF								
P139[f]	*MET*	CNV	No VAF								

Abbreviations: CMP, comprehensive molecular profiling; CNV, copy-number variation; SoC-TMP, standard-of-care protocolled tissue-based molecular profiling; VAF, variant allele frequency.

[a]*MET* exon 14 skipping mutation also detection in this patient. *ERBB2 R103Q* not covered in the SoC-TMP panel.

[b]*ERBB2 R896C* and *S310Y* variants were not covered in the SoC-TMP panel.

[c]SoC-TMP could not be performed in these patients.

[d]*KRAS G12A* was also detected in both tissue and plasma from this patient.

[e]*MAP2K1 K57N* variant was not covered in the plasma-CMP panel.

[f]Level 1 *EGFR* driver mutations were also detected in these patients.

TABLE A4. Level 3B-4 Driver Mutations Detected in Tissue, Plasma, or Both

Detected in Both Tissue and Plasma				Detected in Tissue Only				Detected in Plasma Only			
Patient	Gene	Variant	VAF Plasma (%)	Patient	Gene	Variant	VAF Plasma	Patient	Gene	Variant	VAF Plasma (%)
P047	*BRAF*	G469A	2.1	P133[a]	*AKT1*[b]	E17K	Not detected	P160[c]	*BRAF*	G469V	11.2
P039[d]	*BRAF*	G469V	1.4	P134[e]	*AKT1*[b]	E17K	Not detected		*NRAS*	Q61H	0.1
P108	*BRAF*	K601E	23.3	P023[d]	*ATM*[b]	L2890P	Not detected	P036[a]	*BRCA2*	S3376*	0.2
P149	*ERBB2*	CNV	No VAF	P132[e]	*ATM*[b]	R3008C	Not detected	P131[f]	*KRAS*	A146T	0.4
P131[g]	*ERBB2*	CNV	No VAF	P127	*CDKN2A*[b]	D108Y	Not detected	P174[d]	*KRAS*	G12A	0.1
P199	*KRAS*	G12A	67.5	P166	*CDKN2A*[b]	E88*	Not detected	P178[c]	*KRAS*	G12D	25.6
P004	*KRAS*	G12A	16.8	P016[a]	*CDKN2A*[b]	H83L	Not detected	P104[c,h]	*KRAS*	G12D	0.3
P028	*KRAS*	G12A	6.2	P180[d]	*CDKN2A*[b]	M53I	Not detected		*KRAS*	A146P	0.2
P134	*KRAS*	G12A	5.1	O13	*CDKN2A*[b]	R87G fsTerS9	Not detected		*KRAS*	L19F	0.4
P175[i]	*KRAS*	G12A	0.6	O27[d]	*ERBB2*	CNV	Not detected		*KRAS*	Q61E	0.2
P132	*KRAS*	G12A	0.4	P099	*KRAS*	G12A	Not detected		*KRAS*	Q61H	0.1
P187	*KRAS*	G12A	0.2	P005	*KRAS*	G12F	Not detected	P052[j]	*KRAS*	G12V	0.1
P072	*KRAS*	G12D	3.1	P016	*KRAS*	G12V	Not detected	P074[d]	*KRAS*	G13D	0.5
P194	*KRAS*	G12D	0.8	P120	*KRAS*	G13D	Not detected	P181	*KRAS*	V14I	0.5
P007	*KRAS*	G12D	0.4	P185	*KRAS*	Q61H	Not detected	P199[e]	*NRAS*	G12D	0.1
P070	*KRAS*	G12D	0.4	P075[k]	*NRAS*	Q61L	Not detected	P091[l]	*PDGFRA*	D846Y	0.1
P076	*KRAS*	G12D	0.4	P049	*PIK3CA*[b]	E542K	Not detected				
P171	*KRAS*	G12D	0.2	P037	*PIK3CA*[b]	E545K	Not detected				
P092	*KRAS*	G12F	7.7	P184	*PIK3CA*[b]	E545K	Not detected				
P071	*KRAS*	G12F	5.3	P150[m]	*PIK3CA*[b]	E545K	Not detected				
P192	*KRAS*	G12F	1.1	P146	*PIK3CA*[b]	M1043V	Not detected				
P173	*KRAS*	G12S	15.2								
P110	*KRAS*	G12V	7.5								
P162	*KRAS*	G12V	3.2								
P167	*KRAS*	G12V	1.6								
P182	*KRAS*	G12V	1.5								
P133	*KRAS*	G12V	0.9								
P144	*KRAS*	G12V	0.8								
P036	*KRAS*	G12V	0.4								
P143	*KRAS*	G12V	0.2								
P095	*KRAS*	G13C	2.8								
P179	*KRAS*	G13D	0.7								
P062	*KRAS*	Q61H	31.5								
P022	*KRAS*	Q61H	14.6								
P064	*KRAS*	Q61H	4.4								
P172	*KRAS*	Q61H	0.3								
P203	*KRAS*	Q61H	0.2								
P170	*KRAS*	Q61K	0.7								
O04	*NRAS*	Q61K	13.9								

Abbreviations: CMP, comprehensive molecular profiling; CNV, copy-number variation; SoC-TMP, standard-of-care protocolled tissue-based molecular profiling; VAF, variant allele frequency.

[a]*KRAS G12V* also detected in both tissue and plasma from this patient.

[b]*AKT1, ATM, CDKN2A* and *PIK3CA* genes not covered in the plasma-CMP panel.

[c]SoC-TMP could not be performed in these patients.

[d]*KRAS G12C* also detected in both tissue and plasma from this patient.

[e]*KRAS G12A* also detected in both tissue and plasma from this patient.

[f]*EGFR L858R* also detected in both tissue and plasma from this patient.

[g]*EGFR L858R* also detected in both tissue and plasma from this patient.

[h]Patient had a synchronous hepatocellular carcinoma.

[i]*KRAS G12C* also detected in plasma from this patient.

[j]*KRAS G12C* also detected in tissue from this patient.

[k]*MET* exon 14 skipping mutation also detected in tissue from this patient, and plasma-CMP not successful.

[l]*ALK L1196M* also detected in plasma from this patient.

[m]*EGFR* exon 19 deletion also detected in both tissue and plasma from this patient.

TABLE A5. Contingency Tables

A		SoC-TMP	
		Positive	**Negative**
Plasma-based CMP	Positive	21	1
	Negative	9	148

B		SoC-TMP	
		Positive	**Negative**
Plasma-based CMP	Positive	51	5
	Negative	19	104

C		SoC-TMP	
		Positive	**Negative**
Plasma-based CMP	Positive	88	5
	Negative	24	62

NOTE. Descriptive statistical comparison of plasma-CMP versus SoC-TMP. SoC-TMP is considered the gold standard in these tables. Numbers indicate the number of patients with an oncogenic driver identified by SoC-TMP and/or plasma-CMP. (A) Level 1 drivers. (B) Level 1-3A drivers. (C) Level 1-4 drivers.

Abbreviations: CMP, comprehensive molecular profiling; SoC-TMP, standard-of-care protocolled tissue-based molecular profiling.