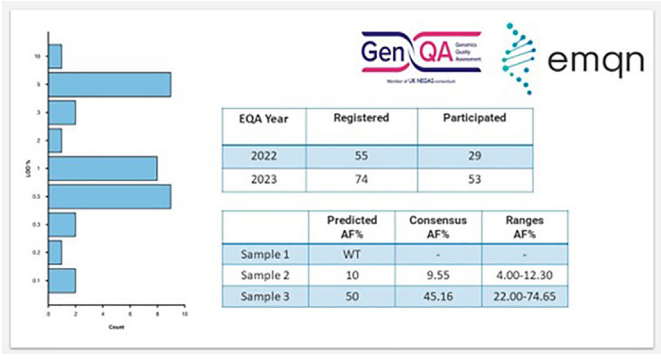
restrict integration of cfDNA analysis into routine clinical practice as primary screening tests.
Testing for BRCA1/BRCA2 genes in cfDNA presents a variety of challenges as variants can occur across the genes rather than in hotspots, thereby necessitating the use of next generation sequencing (NGS) methodologies. In addition, lower variant allele frequencies (VAF) in cfDNA compared with solid tumour samples requires increased NGS test sensitivity to detect the variants.
Methods: EMQN and GenQA have jointly evaluated the performance of laboratories testing BRCA1/BRCA2 in cfDNA from plasma for prostate cancer patients over a period of two years.
Laboratories were sent three artificial plasma samples for cfDNA extraction and BRCA1/BRCA2 testing. One material had no clinically relevant variants, and two samples had variants at different VAFs,10% (BRCA2) and 50% (BRCA1). Participants were asked to return a clinical report with interpretation of results in the context of eligibility for PARP inhibitor treatment.
Results: Data for 2023 EQA will be presented. In summary, 59 laboratories participated in the 2023 EQA. NGS was the primary methodology used. BRCA1/BRCA2 variants were correctly identified and reported in 83% of the cases assessed (diagnostic error rate 18%). The average VAF reported for the two cases harbouring variants, was 9.45% and 46.43% closely matching the expected VAF. VAF ranges were 4-12.3% and 22-74.65%) respectively. The reported LOD for platforms/methodologies ranged from 0.1-10%.
Conclusions: Many laboratories participating in the EQA used NGS methodologies developed for solid tumours with a LOD inappropriate for cfDNA analysis. To deliver an appropriate ctDNA clinical service, laboratories need to use tests with increased sensitivity for detection of low frequency cfDNA variants. As the EQA progresses, laboratories will be challenged with variants at a much lower VAF which more closely mimic real patient samples.
cfDNA analysis is technically challenging and EQA is a useful tool for laboratories to monitor and benchmark their performance.



EQA Year	Registered	Participated
2022	55	29
2023	74	53

	Predicted AF%	Consensus AF%	Ranges AF%
Sample 1	WT	-	-
Sample 2	10	9.55	4.00-12.30
Sample 3	50	45.16	22.00-74.65

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Methylation based CTDNA serial monitoring correlates with immunotherapy response in non-small cell lung cancer

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Purpose: Circulating tumor DNA (ctDNA) can reflect the genetic and epigenetic composition of malignancies and can serve as a non-invasive biomarker for cancer diagnostics and monitoring. This study aimed to evaluate the utility of a methylation-based ctDNA assay as a predictive tool in non-small cell lung cancer (NSCLC) anti-PD1 based immunotherapy monitoring.
Methods: We evaluated a cohort of 33 patients with NSCLC treated with anti-PD1 based immunotherapy, from which 108 blood specimens were collected at baseline and during treatment follow-up. Tumor Methylation Scores (TMS) were measured with an amplicon-based, multiplexed cfDNA assay that utilizes quantitative counting templates (QCTs) in conjunction with next-generation sequencing to count the number of methylated molecules at more than 500 genomic locations that are hypermethylated in cancer tissue. The association between TMS and real-world progression-free survival (rwPFS) on therapy was conducted using Cox proportional hazards model and plotted using the Kaplan-Meier method.
Results: The change in TMS measured 4-10 weeks post-treatment initiation strongly correlated with immunotherapy response, as measured by rwPFS ($p<0.0001$), compared to a weaker correlation of imaging RECIST v1.1 measurements with rwPFS ($p=0.55$). Furthermore, TMS tracked with tumor burden on therapy in real-world cases.
Conclusions: In this real-world dataset of NSCLC patients treated with anti-PD1 immunotherapy regimens, the TMS score measured within a 4-10 week window after treatment initiation can be predictive of response to therapy. Beyond this window, the TMS score can be associated with rwPFS and tumor dynamics. Early evidence suggests that changes in the methylation profile may be informative for monitoring occurrence of new somatic mutations. The cases presented demonstrate the applicability of using TMS for serial therapeutic response monitoring.

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Characterization of plasma cell free DNA variants as tumor-derived vs. Clonal Hematopoiesis of Indeterminate Potential (CHIP) in 11,457 cancer patients

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Introduction: Clonal hematopoiesis of indeterminate potential (CHIP) mutations are an established phenomenon in aging patients that can be revealed via liquid profiling. We have assessed the landscape of over 11,000 liquid profiles and present findings across 48 unique cancer types.
Methods: The assay workflow prepares libraries and sequences both cfDNA and cfRNA simultaneously in a single run using a custom hybridization/capture methodology. Total FASTQ files were divided into DNA FASTQ and RNA FASTQ using the tag on the modified primer. DNA reads were aligned to the hg38 reference sequence using BWA-Mem and variants were called via Mutect2. Variants are characterized as CHIP if the gDNA variant allele frequency (VAF) is less than 20% and the VAF's 95% confidence interval overlaps or is greater than the plasma VAF.
Results: 50.1% of patients presented at least one CHIP mutation among reportable clinical genes. We found 79.6% of all CHEK2 variants to be of CHIP origin, as well as 42.2% of ATM, 7.5% BRCA2, 6.6% NRAS, 6.1% BRAF, 5.3% BRCA1, 2.4% KIT, 1.5% EGFR, and 1.5% KRAS. Prevalence of CHIP by cancer type ranged from a low of 11.6% in endometrial carcinoma to a high of 50.0% in thyroid carcinoma. We confirm previously published findings where CHIP prevalence increases with age. For patients aged 65-69, the median patient had 17% of variants classified as CHIP. For patients aged 70-74, it was 25%, for patients 75-79 it was 33%, and for patients 80+ it was 44%.
Discussion: To our knowledge, this is the largest CHIP-focused profiling landscape ever conducted and highlights the need for thorough CHIP classification in order to appropriately recommend therapies for a given cancer.