

Advanced detection of targetable gene-drivers: tracking ctDNA changes using digital PCR in liquid biopsies from patients with advanced non-small cell lung cancer (NSCLC)

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INTRODUCTION

In the UK, there are almost 50,000 lung cancer diagnoses per year. The most common form of lung cancer is non-small cell lung cancer (NSCLC), with adenocarcinoma histopathology accounting for 40% of new cases. There is a diverse mutational landscape within lung adenocarcinomas, whereby 75% are estimated to harbour oncogene driver mutations[1]. Treatments for patients diagnosed with advanced-stage NSCLC have evolved significantly over the past 20 years. Compared to platinum-based chemotherapy doublets, molecularly targeted and immune-checkpoint inhibitors have prolonged survival and improved quality of life for many patients[2]. Despite advances in targeted therapies for mutation-driven NSCLC, acquired resistance remains a major challenge, limiting their long-term efficacy. As the number of available targeted treatments increases, the ability to dynamically monitor tumour response kinetics is critical for optimising patient management.

Liquid plasma biopsies provide a minimally invasive approach to assess treatment response and detect markers of resistance, in real-time, when combined with rapid-turn around technologies such as digital PCR (dPCR). Circulating tumour DNA (ctDNA) originates from dead or dying tumour cells. Tumour size, stage and histology influence both the rate and abundance of ctDNA identified in patient's plasma. Highly sensitive dPCR-based assessment of ctDNA enables real-time monitoring of DNA mutations and tumour burden, offering dynamic insights into the treatment response and disease status of cancer patients undergoing targeted therapy

Project Aims

- To use the QIAcuityDx dPCR platform in a retrospective cohort analysis of ctDNA to:
- 1 - Evaluate dPCR as a tool for real-time monitoring of treatment response dynamics in NSCLC.
 - 2 - Compare oncogene driver variant allele frequency (VAF) at baseline and disease progression to gain insight into tumour heterogeneity .

TECHNOLOGY

The QIAcuityDx® platform was chosen due to its high specificity and ability to detect rare variants, choosing to prioritise precision over throughput, we used 24-well plates which split the dPCR reaction mixture into 26,000 partitions, maximising our limit for detection. The system also offers a straightforward qPCR-like set-up (below in Fig.1a), delivering easily interpretable results in ~2hrs, making it a great choice for this investigation.

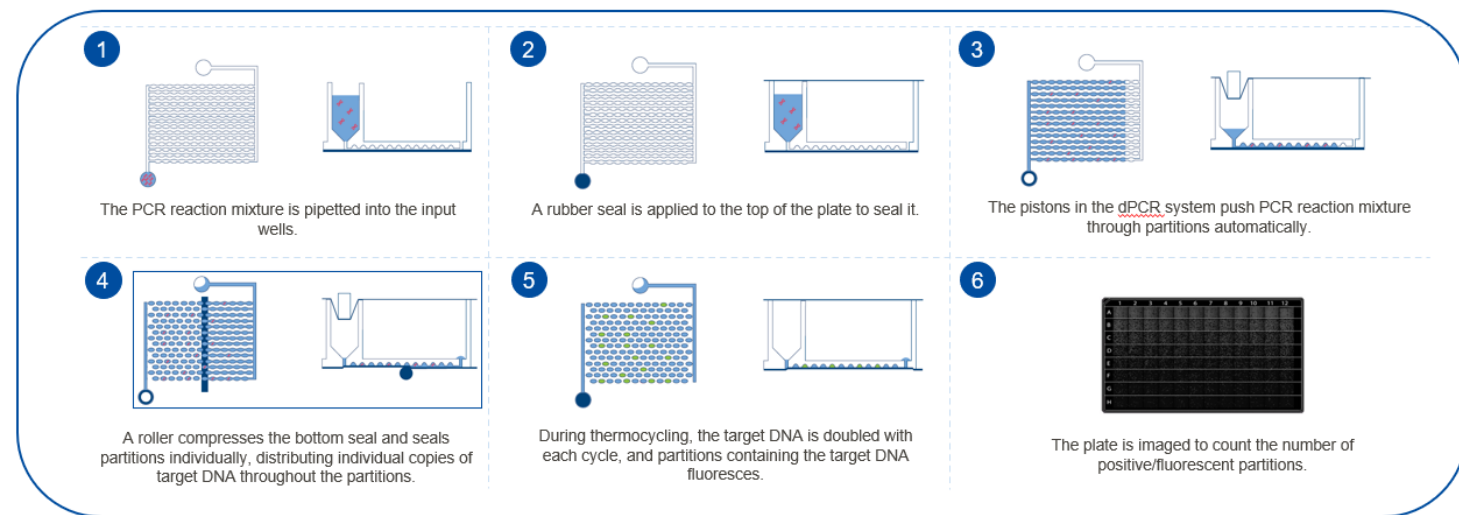


Fig.1a Example workflow for the QIAcuityDx dPCR platform explaining the partitioning technology. Fig.1b Image of the QIAcuityDx dPCR platform.

MATERIALS & METHODS

Liquid Biopsies & Clinical data: collected via the CHEMORES study (Ref: 07/H1014/96) and the RAS-Bio collection within the Manchester Cancer Research Centre Biobank (Ref: 18/NW/0092). Baseline samples were collected from patients, after initial diagnosis, prior to starting first-line advanced or adjuvant treatment for NSCLC. Progression samples were collected on or shortly after date of CT-scan confirming disease progression on first-line treatment. Samples were collected between 2017 and 2024.

Tumour genomic status: determined by UK National Health Service (NHS) molecular testing pathway. Tissue was tested using NGS panel and/or Roche Cobas® EGFR assay. Plasma samples tested via the NHS pathway for EGFR resistance were assessed by droplet digital PCR.

ctDNA extraction: from plasma samples using QIAamp® MinElute® ccfDNA kit, 1.5uL plasma extracted into 50uL H2O. The ctDNA concentration was quantified using Nanodrop, in duplicate.

Digital PCR: performed using QIAcuityDx® Platform and selected dPCR LNA® Mutation Assays, using standard conditions, QIAcuityDx® Nanoplate 26K 24-well plates. The dPCR result assessment threshold setting across samples, and the reporting of wild-type and mutant allele abundance was performed using the QIAcuityDx Suite® software.

Analysis: Mutant and WT copies/uL values were adjusted for starting ctDNA concentration and DNA volume in total reaction volume.

ACKNOWLEDGEMENTS

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RESULTS

We performed a retrospective analysis of 20 advanced NSCLC cases harbouring *EGFR* or *KRAS* mutations. One tumour (QR02) was tested for two distinct *EGFR* mutations. Comparing plasma samples from pre-treatment baseline to those taken at progression, we investigated whether VAF increased or decreased at progression, and how this change correlated with patient's burden of disease.

Baseline Characteristics

	Histology	KRAS (n=5)	EGFR (n=15)
	Adenocarcinoma	5	14
	LCNEC	-	1
Sex			
	Female	4	9
	Male	1	6
Disease Stage			
	IIIA	-	1
	IIIB	-	1
	IV	5	13
Smoking History			
	Current Smoker	-	3
	Ex-Smoker	4	7
	Never Smoker	-	5
	Unknown	1	-
Performance Status			
	ECOG 0	2	6
	ECOG 1	3	8
	ECOG 2	-	1
PDL1 score			
	0%	2	3
	1-49%	1	9
	50-100%	1	1
	Unk	1	5

Table 1. Baseline characteristics for 20 cases used in this retrospective cohort analysis.

Concordance

We reviewed concordance between NGS panel tissue testing to our ctDNA dPCR results. In 2 *EGFR* cases (QR04 and QR27), we were unable to detect the mutant variant see Table 2. Notably, these were some of the older samples tested. Overall concordance was:

- 100% for KRASm cases (5/5)
- 100% for *EGFR* L858R variants (6/6)
- 83% for *EGFR* exon-19 deletions (5/6)
- 80% for other *EGFR* variants (4/5)

Sensitivity

The QIAcuityDx dPCR platform proved effective at detecting variants from low volume (1.5 mL) plasma samples, which had been in long-term storage for 12-99 months.

The 26K partition technology proved highly sensitive at detecting low frequency mutant variants. Positive detection ranged from 0.13-28.5 copies/uL and 2.03 – 430.2 copies/uL for mutant and wild-type alleles, respectively.

Conclusions

We have demonstrated that pairing minimally invasive liquid biopsies with highly sensitive dPCR detection of mutant alleles, using the QIAcuityDx platform offers a rapid, minimally-invasive option for mutation testing - complementary to NHS molecular tests. We evaluated dPCR as a tool for assessing treatment response in a retrospective cohort and found high concordance with NGS testing, demonstrating high sensitivity to detect and quantify the mutant allele abundance. When comparing the VAF between baseline and progression samples, it was essential to consider this within the context of the patient's response to treatment, as treatment response impacts tumour burden/volume, which in-turn affects ctDNA concentrations. In a minority of cases, we observed an inverse relation between VAF and tumour burden - likely a result of tumour heterogeneity and genetic adaptation resulting in treatment resistance. This study highlights the knowledge that can be gained from tracking VAF in response to targeted treatment and its potential to detect progression early from a simple blood test. When paired with broader molecular testing to identify drivers of resistance, further clinical impact could be offered.

FUTURE DIRECTIONS

Due to the fast turn-around time of the QIAcuityDx we plan to evaluate clinical utility for real-time monitoring, in combination with standard molecular testing. We are currently recruiting prospective cohorts of patients with locally advanced or metastatic NSCLC for longitudinal monitoring of ctDNA; 1 First-Line KRASm all variants, receiving chemo-immunotherapy or immunotherapy, 2 Second-Line KRAS G12C receiving Sotorasib, 3 First-line *EGFR* patients receiving Osimertinib, 4 First-line *EGFR* patient receiving Osimertinib monotherapy or combined Osimertinib + chemotherapy. Sampling - schedule shown in Fig 4.

Example dPCR output

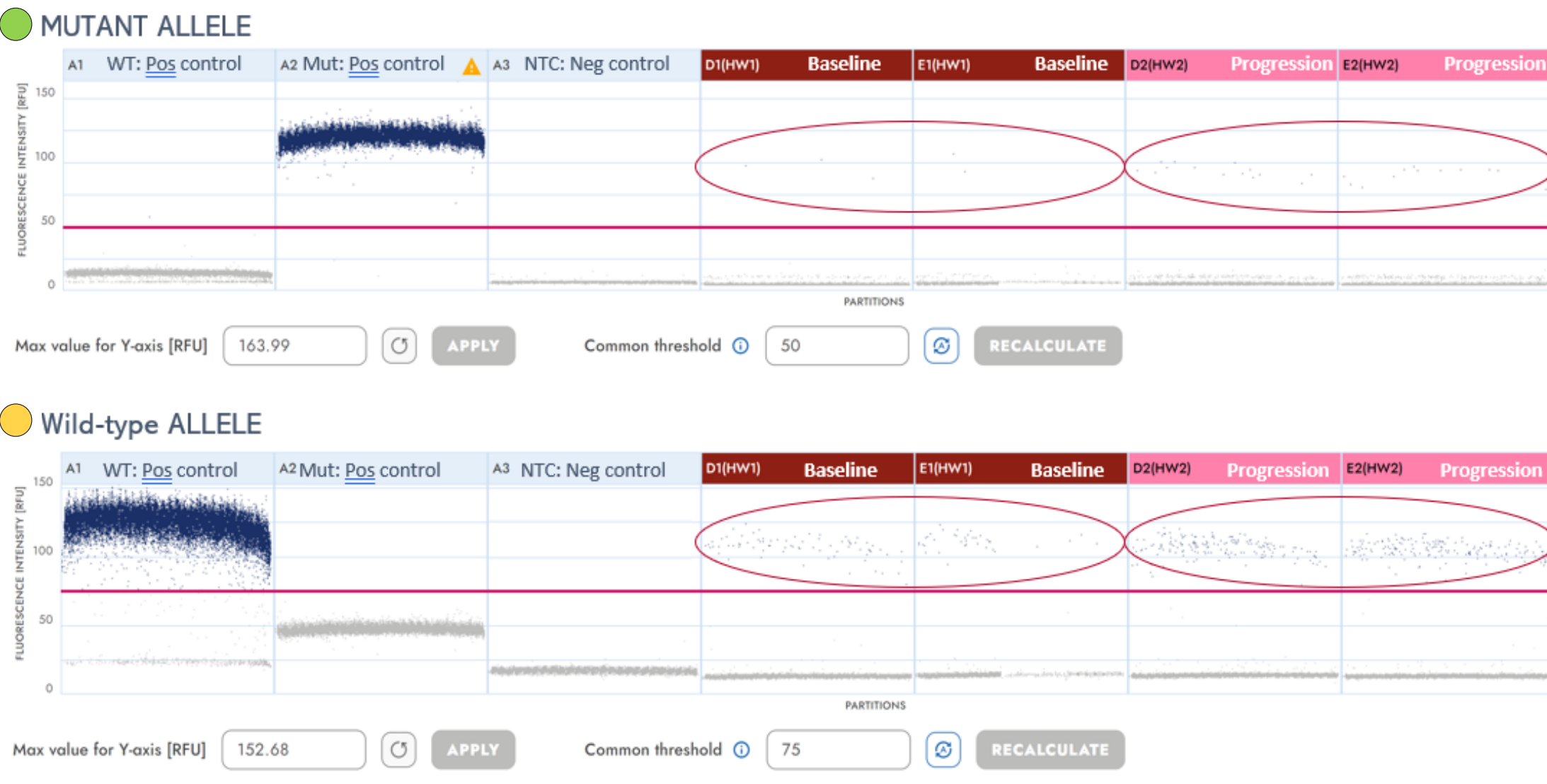


Fig2. Actual read-out from QIAcuityDx Suite software demonstrating the 1D scatterplot of fluorescence for both mutant (FAM green) and wild-type (HEX yellow) alleles from the same reaction well. A1 used a positive control for the WT-allele, A2 used a mutant allele positive control, A3 was a water control, D1 and E1 used baseline sample (duplicate), and D2 and E2 used progression sample (duplicate) from the same patient.

ID	VARIANT	Treatment	Best Response	PFS (days)	BASELINE			PROGRESSION		VAF Change	
					Mutant copies/μL	VAF %	Tumour Burden	Mutant copies/μL	VAF%		Tumour Burden
QR15	G12D	Carboplatin + pemetrexed	SD	319	1.6	0.12	High	10.91	1.62	DOWN 21%	INCREASE
QR16	G12V	Cisplatin + pemetrexed	PD	72	656.48	38.96	High	6.54	1.85	DOWN* +	DECREASE
QR17	G12C	Pembrolizumab	PD	46	10.06	0.88	High	160.63	5.43	UP 18%	INCREASE
QR18	G12C	**Clinical trial	CR	320	28.76	11.34	Low	3.93	1.33	DOWN 54%	DECREASE
QR20	G12C	Gemzar + carboplatin	SD	217	1.6	0.18	High	3.2	0.35	UP 17% +	STABLE
QR01	L858R	Osimertinib	SD	22	6.2	0.20	High	9.5	0.06	UP 17%	STABLE
QR03	L858R	Erlotinib	CR	981	26.36	5.53	Low	0	0	UP 40%	DECREASE
QR08	L858R	Gefitinib	PR	329	7.76	2.89	High	16.29	8.81	DOWN 46%	INCREASE
QR09	L858R	Gefitinib	SD	105	9.53	0.66	High	3.09	0.13	UP* +	STABLE
QR11	L858R	Gefitinib	SD	143	1.6	0.18	Low	0.78	0.07	DOWN 11% +	STABLE
QR24	L858R	Osimertinib	SD	19	0	0	High	3.59	0.19	UP 17%	INCREASE
QR02	E709A	Osimertinib	PD	155	20.06	18.25	High	53.99	29.49	UP 60%	INCREASE
QR02	G719S	Osimertinib	PD	155	23.23	16.38	High	69.04	37.62	UP 60%	INCREASE
QR04	G719S	Afatinib	PR	574	0	0	Low	0	0	UP 104%	ND
QR07	L861Q	Gefitinib	PR	213	5.85	0.33	Low	4.93	0.24	UP* +	STABLE
QR05	Q746_A750del	ccChemo+Rad (III)	SD	445	0	0	Low	1.57	0.49	DOWN 25%	INCREASE
QR06	L747_P573S	Gefitinib	SD	289	0	0	Low	11.55	1.84	UP 40%	INCREASE
QR10	Q746_A750del	Gefitinib	PR	402	947.87	49.97	Low	226.83	78.28	UP 48% +	INCREASE
QR19	Q746_A750del	Gefitinib	SD	189	0.49	0.08	Low	0.58	0.05	UP 13%	STABLE
QR23	Q746_A750del	Gefitinib	PR	260	955.9	40.24	Low	353.82	46.56	DOWN 55% +	INCREASE
QR27	Q746_A750del	Osimertinib	PD	91	0	0	High	0	0	NA*	ND

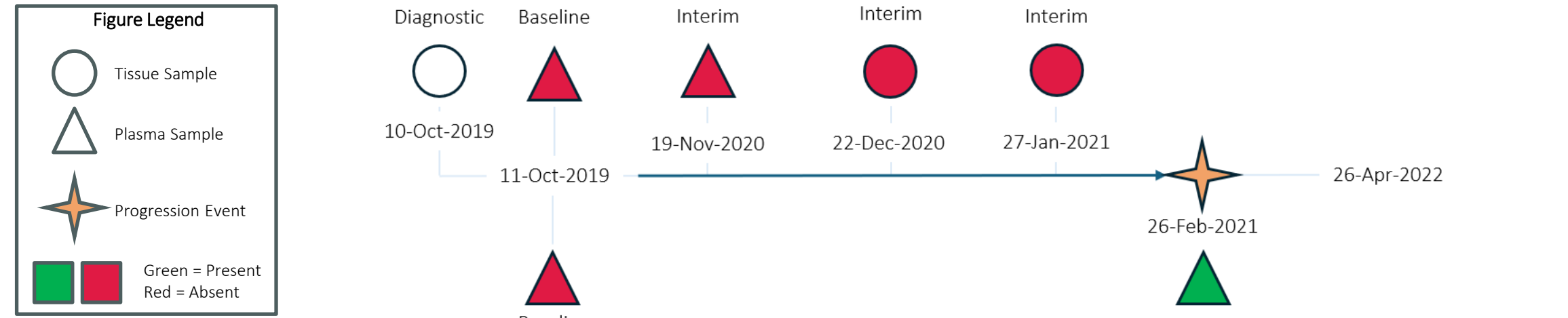
Table 2. Table of clinical response and dPCR results for paired baseline and progression samples. Treatment regimen commenced after baseline sample collected, best response to treatment as per RECIST 1.1, PFS = progression free survival in days. Tumour burden at progression reporting volume change in marker lesions from baseline. Results shown as mutant and allele copies/uL. VAF calculated from values adjusted for starting DNA concentration and volume in reaction mixture, shows relative abundance of mutant allele. VAF change is reported as increase when >1% higher, or when progression is positive after a 0% detection at baseline, decrease if there is >1% decrease and stable if there is less than 1% VAF change from baseline. ND = not detected, *Tumour volume not measurable, + new sites of metastasis, **Clinical trial treatment regimen; Carboplatin + pemetrexed + pembrolizumab + Lenvatinib.

Interpretation

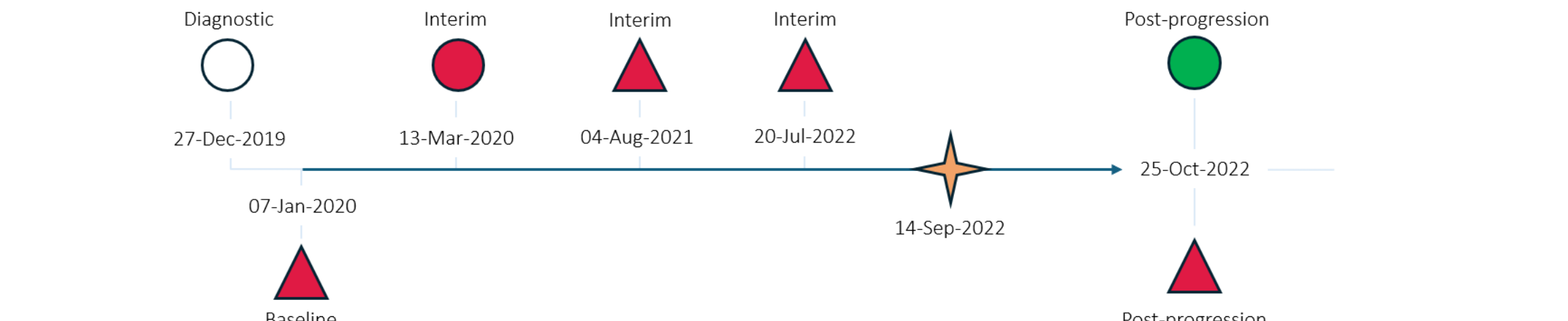
In 2 cases (QR04 and QR27) the mutant allele was not detected by dPCR at either timepoint tested, they are not included in further discussion. In 68% of cases (13/19) showed correlation between tumour burden at progression and relative mutant allele abundance. Of those, 9/13 cases received targeted treatment and 4/6 received non-targeted treatment. Of 6 cases with discordant tumour burden and VAF: 3 showed PR/CR to targeted-treatment thus lowering the tumour burden overall and radiological threshold for progression. 2 showed SD on non-targeted treatment, and 1 on targeted treatment.

Review of clinical application – EGFR T790M case review

We present four retrospective case examples where patients were receiving 1st/2nd generation TKIs for *EGFR*-driven NSCLC. The *EGFR* T790M mutation is a well characterised marker of treatment resistance for earlier generation TKIs[3]. At the time, NHS standard was to request *EGFR* T790M testing on plasma samples if treatment resistance or progression was clinically indicated. If tested positive, patients' treatment could be changed in-line with clinical recommendation. Below, we illustrate timelines of 4 *EGFR* cases that received serial NHS testing for T790M treatment resistance markers. Tests performed by the NHS are shown above the treatment line, and research samples used in this analysis are shown below. NHS diagnostic tissue testing is not assessed for T790M as it's a known treatment resistance marker and patients are treatment naive.



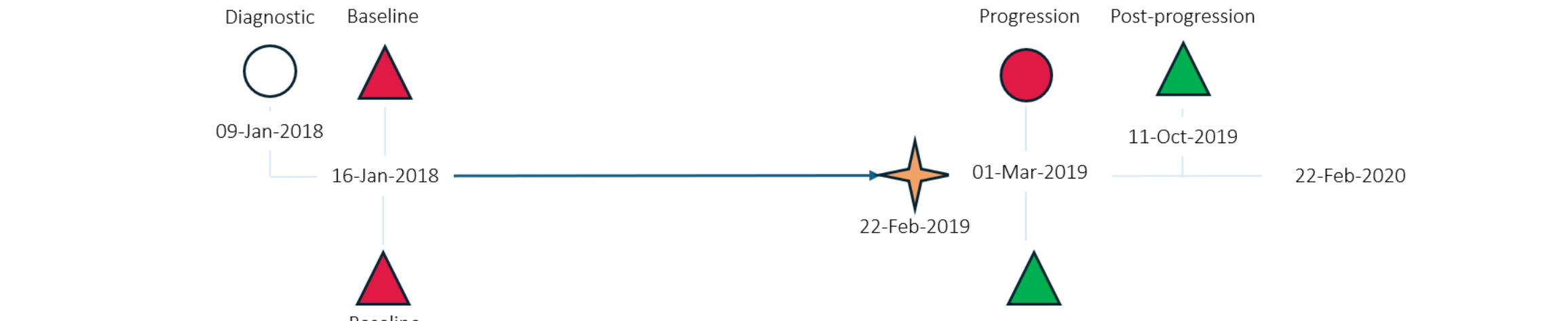
QR06: Patient received multiple interim tests to monitor for progression due to length on treatment and stable treatment response. Tissue testing one month prior to progression event did not identify T790M variant using NHS Roche Cobas® *EGFR* assay or a droplet digital PCR assay. The limit of detection for these technologies is 0.5% and 0.2% VAF respectively. We obtained a positive, identifying a 0.27% VAF from sample taken on day of CT-scan confirming radiological progression.



QR03: There are discordant findings between plasma ctDNA and NGS testing on tissue from different samples collected on the same day. Clinically, this patient had a relatively low burden of disease, limited to the thorax. This is likely to result in low levels of overall ctDNA, which was below average in our dataset.



QR08: Concordant results between the research and standard of care NHS testing, both on plasma samples collected 10 days apart.



QR10: NHS re-biopsy at progression did not identify the presence of T790M variant whereas we did identify this in the research plasma sample, 7 months before it was identified in a post-progression plasma NHS test. Notably, it's possible that the tissue biopsy was unable to detect the T790M variant due to sampling bias of the tumour – whereby the tumour exhibits uneven clonal distribution, which could result in the tissue sample under-representing the tumour heterogeneity.

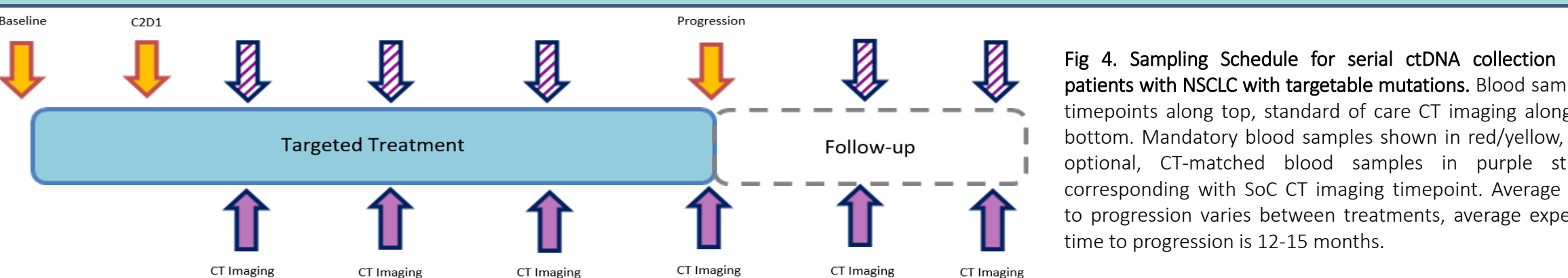


Fig 4. Sampling Schedule for serial ctDNA collection from patients with NSCLC with targetable mutations. Blood sampling timepoints along top, standard of care CT imaging along the bottom. Mandatory blood samples shown in red/yellow, with optional, CT-matched blood samples in purple stripes corresponding with Soc CT imaging timepoint. Average time to progression varies between treatments, average expected time to progression is 12-15 months.

References

- [1] Cancer Genome Atlas Research Network. "Comprehensive molecular profiling of lung adenocarcinoma." *Nature* 511.7511 (2014): 543.
- [2] Olivares-Hernández, Alejandro, et al. "Immune checkpoint inhibitors in non-small cell lung cancer: From current perspectives to future treatments—A systematic review." *Annals of Translational Medicine* 11.10 (2023): 354.
- [3] Oxnard, Geoffrey R., et al. "Assessment of resistance mechanisms and clinical implications in patients with *EGFR* T790M–positive lung cancer and acquired resistance to osimertinib." *JAMA oncology* 4.11 (2018): 1527-1534.



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