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Targeted sequencing of plasma cell-free DNA to predict response to PD1 inhibitors in advanced non-small cell lung cancer☆

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ABSTRACT

Objectives: Tumor mutational burden is an emerging biomarker of response to immune checkpoint inhibitors (ICI), whose clinical adoption is challenging. We hypothesized that targeting limited but relevant genetic alterations in plasma cell-free DNA along with early monitoring may non-invasively predict response to ICI in advanced non-small cell lung cancer (NSCLC).

Material and methods: Plasma samples from patients with progressive NSCLC collected before ICI initiation and at 1 month were profiled from responders (R: PFS > 6 months) and non-responders (NR: progressive disease at first evaluation) using amplicon sequencing of hotspots and coding regions from 36 genes. The molecular profile of ctDNA, and its early kinetics were analyzed.

Results: 97 patients were analyzed, of which 86 (39 R, 47 NR) were evaluable. Alterations in ctDNA were detectable in 67/86 baseline samples (78%). The detection of a targetable oncogenic driver was associated with a 2 months PFS. The presence of a *PTEN* or *STK11* mutation was correlated with early progression (HR 8.9, p = 0.09 for *PTEN*, HR 4.7, p = 0.003 for *STK11*), while transversion mutations (Tv) in *KRAS* and *TP53* predicted better outcomes (HR 0.36, p = 0.011 for *TP53* Tv; HR 0.46, p = 0.11 for *KRAS* Tv). Patients with a low "immune score" (driver and/or *PTEN* or *STK11* mutation and/or without *KRAS* or *TP53* Tv) derived poor outcomes (median PFS 2 months), compared with patients with a high immune score (no driver, no *PTEN* or *STK11* and with *KRAS* or *TP53* Tv (median PFS 14 months, p = 0.0001, HR 2.96). Early changes in the ctDNA allele fraction (AF) of 65 specimens were correlated with clinical outcomes (14 months PFS if AF decreases vs. 2 months if AF increases, p < 0.0001).

Conclusion: Targeted sequencing of plasma ctDNA and monitoring its early variations can predict response to ICI.

1. Introduction

The treatment landscape for NSCLC is rapidly changing with the introduction of immune checkpoints inhibitors targeting PD-1/PD-L1 (ICI), but durable clinical benefit to these immune modulating agents is limited to a subset of patients. Furthermore, treatment discontinuation in non-responders is often delayed because of difficult imaging interpretation. Patient selection is therefore crucial and identifying reliable markers that can quickly predict sustained response is a major challenge. PD-L1 expression, the most validated predictive biomarker, is far from being perfect, due notably to operator interpretation variability [1] or its temporal [2] and spatial [3] heterogeneity.

Genomic profiling represents another emerging strong determinant of response to immunotherapy. Exome analysis of tumors from patients

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treated with pembrolizumab, demonstrated that the best responses to PD-1 blockade were observed in tumors harboring high tumor mutation burden (TMB), and thus tumor-specific antigen load [4]. Since then, TMB has been shown to be a strong marker of response to front-line treatment with nivolumab in combination with ipilimumab in advanced NSCLC [5].

However, whole-exome sequencing (WES) or the use of broad NGS panels might be challenging to translate into routine clinical practice due to cost, the lack of a standardized panel and cut-off, the limited availability of tissue and high-quality DNA and required bioinformatics analysis. There is thus a need to move beyond TMB and identify specific genetic determinants of response to PD-1 inhibitors, especially since not all point mutations will result in the genesis of highly immunogenic peptides [6].

Correlations between *KRAS* mutations and durable response to checkpoint inhibitors have been systematically observed [4,7]. On the contrary, oncogenic drivers found in non-smokers (*EGFR*, *ALK, BRAF* V600E) are associated with low tumor burden, resulting in a non-inflamed ("excluded") tumor microenvironment, and low activity of ICI [8,9].

More interestingly, some molecular alterations have been shown to have a direct impact on the tumor microenvironment. In particular, *STK11* mutations, inactivated in ~15% of adenocarcinomas, and often associated with *KRAS* mutations [10], have been shown to be associated with non-inflamed tumor microenvironment and reduced PD-L1 expression [11]. In contrast, tumors mutated for both *KRAS* and *TP53* demonstrate increased PD-L1 expression along with prominently increased mutation burden that is specifically enriched in a transversion-high phenotype and show stronger inflammatory responses than tumors harboring *KRAS* and *STK11* mutations [10,12,13]. These preclinical results have considerable clinical relevance, with *KRAS/STK11* tumors being associated with primary resistance to PD-1 inhibitors (0–7.4% ORR), compared to *KRAS/TP53* tumors (35.7%–57% ORR) [13]. Similarly, loss of *PTEN* results in decreased T-cell infiltration and increased immunosuppressive cytokines expression and poor outcomes under ICI [14].

These correlations of highly prevalent genetic events with response to ICI are of great interest and pave the way towards the use of more targeted sequencing assays.

The lack of tumor tissue, the heterogeneous pattern of PD-L1 expression and of the dynamic molecular landscape through treatment (with a few acquired mutations (*JAK2*, *B2M*) identified as mechanisms of resistance to these agents [15,16]) render non-invasive approaches valuable to predict and monitor response to ICI. While liquid biopsy applications has been widely studied in the targeted therapy space, its potential to guide and follow response to immune therapy is just beginning to be investigated [17]. Several reports suggest potential of cell-free DNA in assessing TMB [18] and monitoring response [19–21]. This last point is particularly appealing because TMB can only be assessed in approximately 60% of tissue specimens [5], providing clinical utility for blood-based approaches covering situations where no adequate tissue is available. However, large gene panels are required for bTMB and if low tumor cell content in tissue samples is a known confounding factor in assessment of TMB [22], with cell-free DNA, the ctDNA fraction is dependent on tumor shedding and on the amount of normal DNA in the plasma and thus is especially variable. Low levels of ctDNA may not allow for an accurate determination of the TMB status of a tumor.

Focusing on specific molecular events known to be associated with response to ICI, reported above, we investigated the utility of a plasma genotyping to stratify patients between durable responders and non-responders, as well explore if early changes in ctDNA AF can serve as a valid pharmacodynamics biomarker to predict sustained response.

2. Methods

2.1. Patients

Patients with stage IIIB/IV, progressive NSCLC, consented to receive PD1 inhibitors were included in the study. Among 187 patients from the IMMUNOPREDICT trial (NCT02827344, blood collection including plasma, circulating tumor cells and peripheral blood mononuclear cells from patients treated by ICI in second line setting), "responders" (Objective response on first evaluation and PFS > 6 months) and "non-responders" (progressive disease at first CT evaluation) patients were selected. Plasma specimens were collected after informed consent, prior to initiation of ICI and when feasible, at 1 month. Plasma analyses were performed blinded to clinical information such as tumor histology, genotype, smoking history, PD-L1 status, or response to ICI.

2.2. Plasma NGS

Amplicon-based plasma NGS was performed by Inivata (Morrisville, NC), using the InVision First®-Lung technology [23]. 36 cancer-related genes were sequenced using gene specific primers designed to hotspots and entire coding regions of interest (Supp. Fig. 1). NGS libraries are prepared from 2,000–16,000 amplifiable copies of the genome using a two-step PCR amplification process incorporating replicate and patient-specific barcodes and Illumina sequencing adaptors. Samples are quantified and pooled to generate a normalized library of 12 nM. 1.8 pM libraries are sequenced on the Illumina NextSeq 500. Sequencing files were analysed using Inivata's proprietary Somatic Mutation Analysis (ISoMA) and FUSP pipelines.

2.3. Monitoring of response using ctDNA kinetics

An early blood draw (before the second cycle of nivolumab, median 28 days) was studied in 65 patients to monitor the early kinetics of plasma ctDNA burden through treatment. At baseline, a single somatic change (a tumor driver when present, or the variant with the highest allelic frequency) was chosen for subsequent monitoring.

2.4. Tissue PD-L1 expression

PD-L1 expression on tissue was assessed by immunohistochemistry using an anti-PD-L1 rabbit monoclonal antibody (clone E1L3N, Cell signalling Technology).

2.5. Statistical analysis

Statistical analysis was performed with MedCalc Statistical Software ver 18.10. Specific statistical tests are indicated. Sensitivity of plasma NGS for detection of driver and resistance mutations were calculated using clinically performed tumor genotyping as reference standard. The median survival time and its 95% CI is calculated according to Brookmeyer et al. [24]. Results of the logrank test are used in the comparison of survival curves.

3. Results

3.1. Detection of known driver and resistance mutations in ctDNA

Samples from 42 responders and 55 non-responders patients were available and analyzed. Characteristics of the patient cohort are listed in Table 1. 86 specimens were evaluable for a baseline molecular profile (7 NR patients received less than 2 cycles due to toxicity and were excluded from analysis and 4 runs failed). Of these 86 baseline samples, at least one variant was detected in 67/86 samples (78% sensitivity). Using tissue genotyping as a reference standard, sensitivity was 74% (25/34) for the detection of *KRAS* mutations and 100% for *EGFR*

Table 1
Characteristics of the population.

Sex (n = 97)			
Male		60	62%
Female		37	38%
Histology (n = 97)			
Adenocarcinoma		72	74,2%
Squamous		21	21,7%
Sarcomatoid		2	2,1%
Mesothelioma		1	1,0%
Unknown		1	NA
Genotype (limited to EGFR, KRAS, ALK, ROS1, BRAF, HER2, cMET, **n = 97)**			
Wild type		34	35,1%
EGFR		5	5,2%
KRAS		31	32,0%
ALK		2	2,1%
cMET		2	2,1%
2 mutations (*KRAS/cMET*)		1	1,0%
Unknown		22	NA
PDL1 expression (n = 97)			
In tissues			
0%		40	41,2%
1–49%		16	16,5%
≥50%		19	19,6%
Unknown		22	NA
In immune cells			
0%		33	34,0%
1–49%		33	34,0%
≥50%		4	4,1%
Unknown		27	NA
Smoking status (n = 97)			
Smokers	*Active smokers*	22	22,7%
	Ancient smokers	63	64,9%
Non smokers		7	7,2%
Unknown		5	NA
Performans status (n = 97)			
0–2		87	89,7%
3–4		4	4,1%
Unknown		6	NA
Stage at ICI initiation (n = 97)			
IIIA		1	1%
IIIB		10	10,3%
IV		86	88,7%
Previous treatment (n = 97)			
Thoracic surgery		16	16,5%
Chemotherapy		97	100,0%
Targeted therapy		18	18,6%
Thoracic radiotherapy		20	20,6%
Number of previous lines of treatment (n = 97)			
1		57	58,8%
≥2		40	41,2%
Immune Checkpoint inhibitor ((n = 97)			
Anti PD-1 (Nivolumab)		90	92,8%
Anti PD-1 (Pembrolizumab)		7	7,2%

mutations (6/6, 2/2 exon 19 deletion, 1/1 T790 M, other 3/3) and *MET* amplifications (2/2). The only *ALK* fusion specimen tested using the fusion assay was not detected. Two *ALK* resistance mutations (G1202R and F1174C) were detected in another *ALK* patient with acquired resistance to crizotinib, in agreement with tissue.

3.2. Targeted profiling of cfDNA to predict outcomes under PD-1 inhibitors: Correlation of an algorithm for prediction of response

Of the 86 evaluable patients, 39 patients exhibited clinical benefit to ICI (PFS > 6 months) while 47 patients exhibited progressive disease. Based on preclinical and clinical observations reported in the introduction, we tested the ability of specific molecular events to stratify responders and non-responders. Median PFS for the overall evaluable population (n = 86) was 7 months. Patients with no detectable ctDNA had a 3.5 months PFS (progression-free survival) and were considered non-evaluable with regards to the predictive algorithm. In agreement with previous reports [8,9], patients with a targetable alteration detected in blood (1/1 *ALK*, 5/5 *EGFR*) progressed on the first clinical evaluation (PFS 2 months). All of them had received multiple targeted therapies before ICI. Patients with a *PTEN* or a *STK11* mutation derived poor outcomes compared with patients without (HR 8.9, p = 0.09 for PTEN; HR 4.7, p = 0.003 for STK11; Fig. 1A). In contrast, patients with a *KRAS* Tv mutation or a *TP53* Tv mutation derived better outcomes compared to patients without (HR 0.36, p = 0.011 for *TP53* Tv; HR 0.46, p = 0.11 for *KRAS* Tv; Fig. 1B). Median PFS for each cohort are detailed in Fig. 1.

An algorithm was built blinded to patients' results combining these molecular alterations known to be associated with good or poor outcomes. A "High immune score" was defined as follow: no targetable driver (*EGFR*, *ROS1*, *ALK*, *BRAF* V600E), no *PTEN* or *STK11* mutations, but a transversion mutation in *KRAS* or *TP53*. A "Low Immune Score" was defined as follow: presence of a targetable alteration, a *PTEN* or *STK11* mutation or in the absence of *KRAS* or *TP53* transversion mutation) (Fig. 1C). Patients with "High Immune Score" had a median PFS of 14 months (HR 2.89, 95%CI 1.63–5.83) compared to 2 months (95%CI 1.5- months, p = 0.0008) in the "Low Immune Score" (Fig. 1D). 6-month PFS was 76% vs 33% for those with high immune score vs low immune score (p = 0.0006).

3.3. Comparison with tissue PD-L1 expression

PD-L1 expression was not statistically associated with response to ICI, using either a cutoff of 1% (HR 1.73, 95%CI 0.93–3.23) or 50% (HR 1.35, 95%CI 0.73–2.49), and was less predictive of response than ctDNA profiling (Sup. Fig. 3).

3.4. Early detection of response through serial plasma NGS

Patients with any decrease of ctDNA AF at one month had longer PFS (median 10 (95%CI 8–18) months) than patients with early increase (median PFS 2 months (95%CI 1.5-ND)). 74% of patients with early molecular response were still responding to treatment after 6 months compared to 16% of patients with plasma increase (Fig. 2A). Using cut-offs of 50% or 30% for AF variations to define molecular response or progression, correlation between early ctDNA kinetics and clinical response was stronger, with only 11% and 6.3% of patients with plasma progressions still responding at 6 months for 30 and 50% thresholds, respectively (Fig. 2B and C).

4. Discussion

TMB, measured whether in tissue or blood, is correlated with response to ICI but integration into routine clinical practice is challenging due to lack of tissue availability, high costs associated with WES or broad panels exhibit high and time-consuming approaches. Furthermore, less than 10% of non-synonymous mutations will harbor MHC class I bindings motifs capable of recognition by T cells as neoantigens [6,25].

In parallel of efforts to develop TMB analysis platforms, an increasing number of specific alterations that correlates to ICI outcomes have been investigated, suggesting an opportunity to use more restrictive sequencing assays. A good concordance between targeted NGS covering more than 200 genes and WES for TMB evaluation was demonstrated [26], demonstrating the ability of NGS panels to stratify patients between responders and non-responders with high accuracy. We herein show that more limited, carefully selected genes panel can accurately predict response to ICI, using a simple algorithm. Moreover, this molecular profiling was done in plasma, offering a rapid, tissue-sparing and repeatable access to the tumor genomics. If previous studies have demonstrated that TMB can be accurately estimated in cfDNA

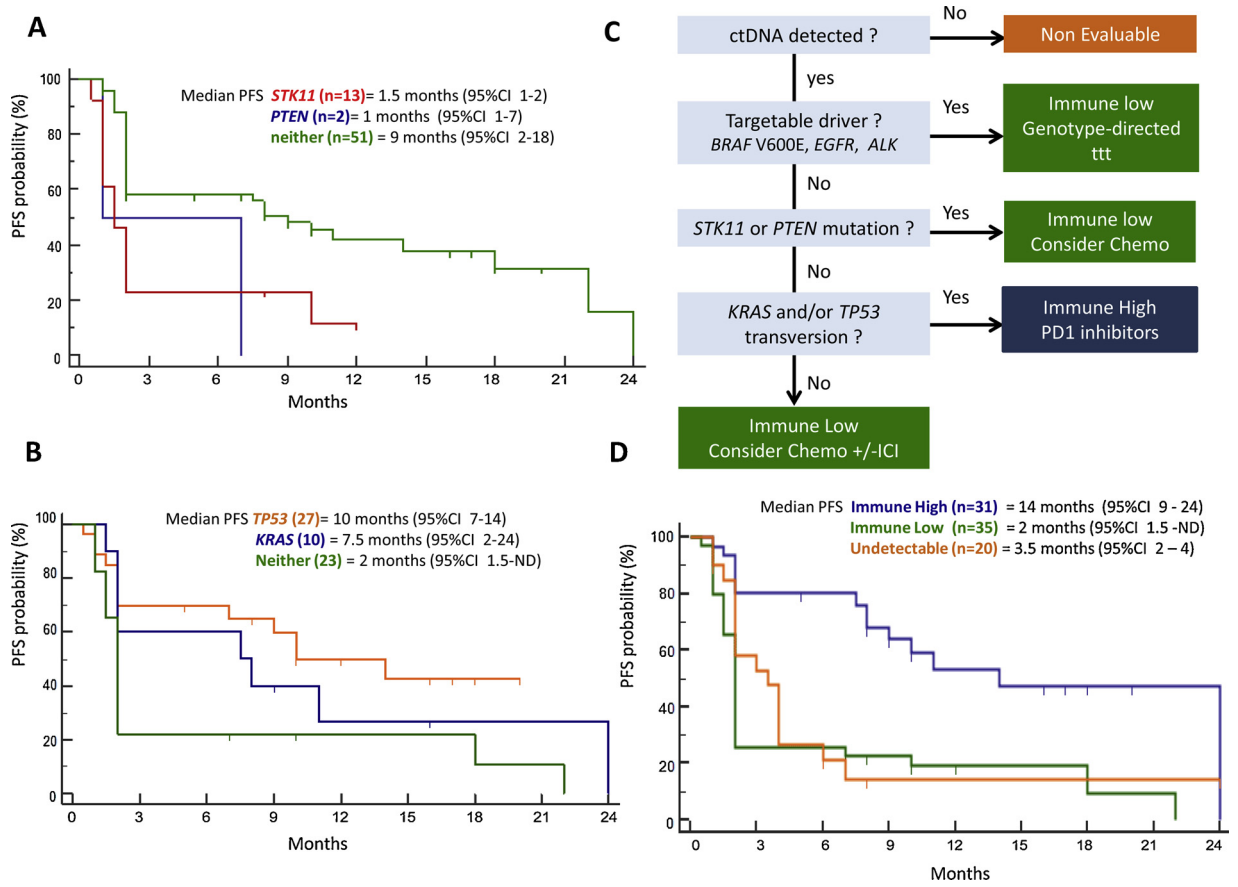

Fig. 1. Proposed algorithm of interpretation of cfDNA molecular profile in order to guide ICI initiation. **1A**: Patients harboring *STK11* or *PTEN* mutations derive poor benefit from PD-1 inhibitors compared with patients who don't (HR 4.7, p = 0.003 for *STK11*; HR 8.9, p = 0.09 for *PTEN*). **1B**: Patients with a *KRAS* or *TP53* transversion mutation showed better responses compared to patients without (HR 0.36, p = 0.011 for *TP53* Tv; HR 0.46, p = 0.11 for *KRAS* Tv). **1C:** Algorithm genesis: If a targetable oncogenic driver is detected, the probability of response is low and targeted therapies (or chemotherapy if already received) must be favored over immunotherapy. The presence of *PTEN* or *STK11* mutations also predicts primary resistance to ICI, while transversion mutations in *KRAS*, *TP53* or other genes ("immune high" somatic signature) is associated with good outcomes. **1D:** Correlation of the algorithm with outcomes: An "Immune High Score", defined by the absence of targetable driver, *PTEN* or *STK11* mutation but the presence of *KRAS* and/or *TP53* mutations, strongly predict favorable outcomes under PD-1 inhibitors (p = 0.0008).

(blood TMB, bTMB) with good concordance with tissue and response to ICI [18]; a more targeted analysis of cfDNA focusing on specific alterations known to alter or increase response to ICI has never been investigated.

Interestingly, patients with no detectable ctDNA tended to experience poor outcomes (PFS 3.5 months), even though the presence of ctDNA is usually of detrimental prognostic impact. The limited number of patients does not allow for definitive conclusion, but the absence of ctDNA could be an indirect indication of low TMB. This is consistent with what was observed using bTMB, the "biomarker-evaluable population" (patients with MAF > 1%) experiencing the best outcomes [18].

We here confirm the significant impact of the subtype of mutations (transversion vs transitions) on ICI outcomes, a phenomenon already clearly demonstrated in tissue [4,25] but not used routinely in clinical practice nor on a specific group of genes. This work also shows that the detrimental effect of *STK11* and *PTEN* mutations on ICI outcomes reported in tissue is relevant in liquid biopsy. The combination of all these specific markers, along with known targetable oncogenic drivers, allows guiding patient treatment towards either targeted therapy or ICI (depending on the molecular event(s) detected) with one and only, non-invasive and rapid assay.

We also show that early kinetics of ctDNA burden strongly predicts durable responses, as previously demonstrated in 2 reports where a complete [21] or partial [20] ctDNA clearance at 8 weeks was strongly predictive of sustained response. These observations are of great value in the space of immune therapy, early radiological changes being challenging to interpret. Plasma genotyping has the potential to early distinguish pseudo from true progression during ICI treatment, as reported in limited series [19,27]. Other applications of cfDNA are likely to emerge, such as the detection of MRD for adjuvant immunotherapy, or detection of mechanisms of resistance, like acquired *JAK*1/2 or *B2M* mutations [17].

The first major limitation of this study is its retrospective nature. Even though the ctDNA analysis was performed blinded to clinical results, the study was enriched in responders' patients (45% of the population), partially explaining the unusual PFS in the overall population (7 months) and in the "High immune score" cohort (14 months). These promising data will need to be validated prospectively in larger and unselected populations. A second limitation is the lack of a non-ICI treatment arm to discriminate a true predictive value from a prognostic effect of the algorithm. However, *KRAS* [28] and *TP53* [29] are known to be of detrimental effect in NSCLC, whereas oncogenic drivers like *EGFR* mutations [30] or *ALK* fusion [31] are of better prognosis. The opposite results observed in this work are thus very likely due to a predictive value of these alterations more than to a simple prognostic impact.

Another limitation of our study is the incomplete tissue genotyping data available. Although we could test and demonstrate a good concordance with tissue for major driver alterations, no tissue was left to

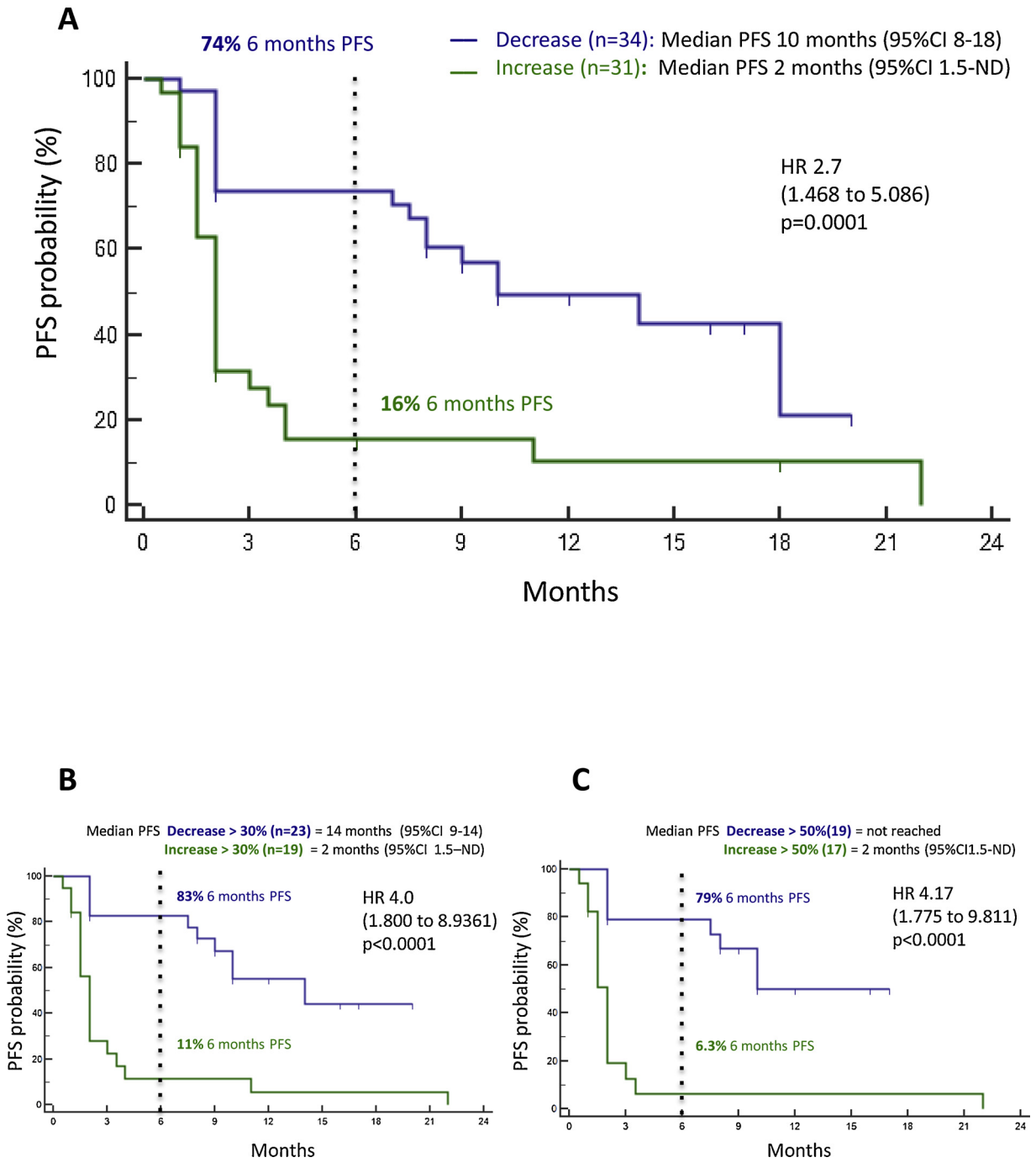

Fig. 2. Early changes in ctDNA level to predict sustained responses under PD-1 inhibitors. 2A: A decrease in ctDNA burden at one month is strongly predictive of durable response (74% PFS at 6 months), while an early increase indicates progression (84% progressive disease at 6 months). Using cut-offs of 30% (**2B**) and 50% (**2C**) for allelic fractions changes enhances this predictive effect.

analyze other alterations such as *PTEN*, *STK11* or *TP53*. However, this is, in our opinion, an interesting point of our study, demonstrating that ctDNA can be a powerful tool to identify biomarkers when no tissue is available (a frequent situation in NSCLC where there is a current paradox between the development of minimally invasive diagnostic tools leading to small samples and the multiplication of markers for targeted therapy and ICI. The concordance of the plasma genotyping platform used in this work with tissue has however been studied in a prospective manner in another study and appeared very high (97.8%; with 82.9% positive predictive value, 98.5% negative predictive value, 70.6% sensitivity, and 99.2% specificity) [32].

In conclusion, plasma NGS using limited panel can accurately predict sustained responses under PD-1 blockade. This approach has the unique ability to select patients for either genotype-directed therapies or ICI using one unique assay, in particular when tissue is not available.

Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

The manuscript doesn't include any individual person's data.

Ethics approval and consent to participate

Plasma specimens and clinical information's were collected after informed consent (IMMUNOPREDICT trial (NCT02827344)).

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Author's contributions

NG: Principal Investigator of the work: Conception and design of the work. Acquisition and interpretation of data. Drafting manuscript. **GJ, VP, CM** and **JB**: Plasma sequencing. Statistical analysis of data. Approved the final version of the manuscript. **JMo, MD, LK**: Acquisition of data. Approved the final version of the manuscript. **JMa, GF and AP**: Conception and design of the work. Acquisition and interpretation of data. Revising manuscript. Approved the final version of the manuscript.

Declaration of Competing Interest

GJ, VP, CM and JB are employees and share-holders of Inivata Ltd. Inivata Ltd commercializes assays based on the technology described in this paper. All other authors have no potential conflicts of interest to report.

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Appendix A. Supplementary data

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References

[1] W.A. Cooper, P.A. Russell, M. Cherian, E.E. Duhig, D. Godbolt, P.J. Jessup, et al., Intra- and interobserver reproducibility assessment of PD-L1 biomarker in non-small cell lung cancer, Clin. Cancer Res. 23 (August (16)) (2017) 4569–4577.
[2] J.J. Han, D.-W. Kim, J. Koh, B. Keam, T.M. Kim, Y.K. Jeon, et al., Change in PD-L1 expression after acquiring resistance to gefitinib in EGFR-mutant non-small-cell lung cancer, Clin. Lung Cancer 17 (4) (2016) 263–270 e2.
[3] M. Ilie, E. Long-Mira, C. Bence, C. Butori, S. Lassalle, L. Bouhlel, et al., Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies, Ann. Oncol. 27 (January (1)) (2016) 147–153.
[4] N.A. Rizvi, M.D. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, J.J. Havel, et al., Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer, Science 348 (April (6230)) (2015) 124–128.
[5] M.D. Hellmann, T.-E. Ciuleanu, A. Pluzanski, J.S. Lee, G.A. Otterson, C. Audigier-Valette, et al., Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden, N. Engl. J. Med. (April) (2018).
[6] D.S. Chen, I. Mellman, Elements of cancer immunity and the cancer-immune set point, Nature 541 (7637) (2017) 321–330.
[7] A. Calles, X. Liao, L.M. Sholl, S.J. Rodig, G.J. Freeman, M. Butaney, et al., Expression of PD-1 and its ligands, PD-L1 and PD-L2, in smokers and never smokers with KRAS-mutant lung cancer, J. Thorac. Oncol. 10 (December (12)) (2015) 1726–1735.
[8] J.F. Gainor, A.T. Shaw, L.V. Sequist, X. Fu, C.G. Azzoli, Z. Piotrowska, et al., EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: a retrospective analysis, Clin. Cancer Res. 22 (September (18)) (2016) 4585–4593.
[9] J. Mazieres, A.E. Drilon, L. Mhanna, J. Milia, A. Lusque, A.B. Cortot, et al., Efficacy of immune-checkpoint inhibitors (ICI) in non-small cell lung cancer (NSCLC) patients harboring activating molecular alterations (ImmunoTarget), J. Clin. Oncol. 36 (May (15_suppl)) (2018) 9010–9010.
[10] M.B. Schabath, E.A. Welsh, W.J. Fulp, L. Chen, J.K. Teer, Z.J. Thompson, et al., Differential association of STK11 and TP53 with KRAS mutation-associated gene expression, proliferation and immune surveillance in lung adenocarcinoma, Oncogene 35 (24) (2016) 3209–3216.
[11] S. Koyama, E.A. Akbay, Y.Y. Li, A.R. Aref, F. Skoulidis, G.S. Herter-Sprie, et al., STK11/LKB1 deficiency promotes neutrophil recruitment and proinflammatory cytokine production to suppress T-cell activity in the lung tumor microenvironment, Cancer Res. 76 (March (5)) (2016) 999–1008.
[12] Z.-Y. Dong, W.-Z. Zhong, X.-C. Zhang, J. Su, Z. Xie, S.-Y. Liu, et al., Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma, Clin. Cancer Res. 23 (June (12)) (2017) 3012–3024.
[13] F. Skoulidis, M.E. Goldberg, D.M. Greenawalt, M.D. Hellmann, M.M. Awad, J.F. Gainor, et al., STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma, Cancer Discov. 8 (July (7)) (2018) 822–835.
[14] W. Peng, J.Q. Chen, C. Liu, S. Malu, C. Creasy, M.T. Tetzlaff, et al., Loss of PTEN promotes resistance to T cell-mediated immunotherapy, Cancer Discov. 6 (February (2)) (2016) 202–216.
[15] J.M. Zaretsky, A. Garcia-Diaz, D.S. Shin, H. Escuin-Ordinas, W. Hugo, S. Hu-Lieskovan, et al., Mutations associated with acquired resistance to PD-1 blockade in melanoma, N. Engl. J. Med. 375 (September (9)) (2016) 819–829.
[16] D.S. Shin, J.M. Zaretsky, H. Escuin-Ordinas, A. Garcia-Diaz, S. Hu-Lieskovan, A. Kalbasi, et al., Primary resistance to PD-1 blockade mediated by JAK1/2 mutations, Cancer Discov. 7 (February (2)) (2017) 188–201.
[17] L. Cabel, C. Proudhon, E. Romano, N. Girard, O. Lantz, M.-H. Stern, et al., Clinical potential of circulating tumour DNA in patients receiving anticancer immunotherapy, Nat. Rev. Clin. Oncol. (July) (2018).
[18] D.R. Gandara, S.M. Paul, M. Kowanetz, E. Schleifman, W. Zou, Y. Li, et al., Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab, Nat. Med. 24 (September (9)) (2018) 1441–1448.
[19] N. Guibert, J. Mazieres, M. Delaunay, A. Casanova, M. Farella, L. Keller, et al., Monitoring of KRAS-mutated ctDNA to discriminate pseudo-progression from true progression during anti-PD-1 treatment of lung adenocarcinoma, Oncotarget 8 (June (23)) (2017) 38056–38060.
[20] L. Cabel, F. Riva, V. Servois, A. Livartowski, C. Daniel, A. Rampanou, et al., Circulating tumor DNA changes for early monitoring of anti-PD1 immunotherapy: a proof-of-concept study, Ann. Oncol. 28 (August (8)) (2017) 1996–2001.
[21] S.B. Goldberg, A. Narayan, A.J. Kole, R.H. Decker, J. Teysir, N.J. Carriero, et al., Early assessment of lung cancer immunotherapy response via circulating tumor DNA, Clin. Cancer Res. 24 (April (8)) (2018) 1872–1880.
[22] 56PD Analytic validation of tumor mutational burden as a companion diagnostic for combination immunotherapy in non-small cell lung cancer, Ann. Oncol. (November) (2018) Oxford Academic.
[23] N. Guibert, Y. Hu, N. Feeney, Y. Kuang, V. Plagnol, G. Jones, et al., Amplicon-based next-generation sequencing of plasma cell-free DNA for detection of driver and resistance mutations in advanced non-small cell lung cancer, Ann. Oncol. 29 (April (4)) (2018) 1049–1055.
[24] R. Brookmeyer, J. Crowley, A confidence interval for the median survival time, Biometrics 38 (1) (1982) 29–41.
[25] D. Miao, C.A. Margolis, N.I. Vokes, D. Liu, A. Taylor-Weiner, S.M. Wankowicz, et al., Genomic correlates of response to immune checkpoint blockade in micro-satellite-stable solid tumors, Nat. Genet. 50 (September (9)) (2018) 1271–1281.
[26] H. Rizvi, F. Sanchez-Vega, K. La, W. Chatila, P. Jonsson, D. Halpenny, et al., Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing, J. Clin. Oncol. 36 (March (7)) (2018) 633–641.
[27] J.H. Lee, G.V. Long, A.M. Menzies, S. Lo, A. Guminski, K. Whitbourne, et al., Association between circulating tumor DNA and pseudoprogression in patients with metastatic melanoma treated with anti-programmed cell death 1 antibodies, JAMA Oncol. 4 (May (5)) (2018) 717–721.
[28] L.C. Villaruz, M.A. Socinski, D.E. Cunningham, S.I. Chiosea, T.F. Burns, J.M. Siegfried, et al., The prognostic and predictive value of KRAS oncogene substitutions in lung adenocarcinoma, Cancer 119 (June (12)) (2013) 2268–2274.
[29] T. Mitsudomi, N. Hamajima, M. Ogawa, T. Takahashi, Prognostic significance of p53 alterations in patients with non-small cell lung cancer: a meta-analysis, Clin. Cancer Res. 6 (October (10)) (2000) 4055–4063.
[30] B. Izar, L. Sequist, M. Lee, A. Muzikansky, R. Heist, J. Iafrate, et al., The impact of EGFR mutation status on outcomes in patients with resected stage I non-small cell lung cancers, Ann. Thorac. Surg. 96 (September (3)) (2013) 962–968.
[31] F.H. Blackhall, S. Peters, L. Bubendorf, U. Dafni, K.M. Kerr, H. Hager, et al., Prevalence and clinical outcomes for patients with ALK-positive resected stage I to III adenocarcinoma: results from the European Thoracic Oncology Platform Lungscape Project, J. Clin. Oncol. 32 (September (25)) (2014) 2780–2787.
[32] M.A. Pritchett, D.R. Camidge, M. Patel, J. Khatri, S. Boniol, E.K. Friedman, et al., Prospective clinical validation of the InVisionFirst-Lung circulating tumor DNA assay for molecular profiling of patients with advanced nonsquamous non–small-cell lung cancer, JCO Precis. Oncol. (April (3)) (2019) 1–15.