**Supplementary Data for**

**Dynamics of tumor and immune responses during immune checkpoint blockade in non-small cell lung cancer**

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**Supplementary Figure S1**

**ctDNA clonal dynamics during anti-PD1 for patients with a clinical response.** For patients with a molecular response, ctDNA levels decreased to undetectable levels early after anti-PD1 therapy initiation **(A-H)**, followed by a rise in levels of tumor specific variants at the time of molecular acquired resistance **(A, C, F, G)**. For CGLU135, where baseline blood was not available for analysis, ctDNA levels rose at the time of acquired resistance compared to the time of response **(B)**. Changes in RECIST tumor burden, shown on the secondary vertical axis of each graph, indicated stable disease for the majority of molecular responders and were therefore not reflective of each patient’s clinical benefit from immune checkpoint blockade. These tumors harbored a wide range of sequence alterations (TMB; 50-846) indicating that TMB alone may be not sufficient to predict response. MAF; mutant allele fraction, SLD; sum of longest diameters, TMB; tumor mutation burden, PFS; progression-free survival, OS; overall survival.

**Supplementary Figure S2**

**ctDNA clonal dynamics during anti-PD1 for patients with molecular primary resistance.** ctDNA levels continued to rise or showed no significant changes from baseline for molecular non-responders **(A-I)**. Detection of ctDNA molecular resistance preceded disease progression determined by RECIST response assessments by an average of 5.5 weeks. Tumor mutation burden did not accurately predict response as a high mutation burden was observed for patients CGLU117, CGLU115, CGLU162 and CGLU348 **(A, C, G, I)**. MAF; mutant allele fraction, SLD; sum of longest diameters, TMB; tumor mutation burden, PFS; progression-free survival, OS; overall survival.

**Supplementary Figure S3**



**ctDNA molecular responses precede radiologic responses.** Detection of molecular responses was achieved within 6.7 weeks, on average 8.7 weeks earlier than conventional RECIST1.1 response assessments (6.7 vs 15.4 weeks, p=0.004). Patient CGLU337 was excluded from this graph, as we could not determine time to best response by radiologic imaging.

**Supplementary Figure S4**

**Molecular responses correlate with a favorable prognosis.** Patients with a molecular response had a longer progression-free **(A)** overall survival **(B)** compared to molecular non-responders (Mann Whitney P=0.002 and P=0.008 respectively).

**Supplementary Figure S5**

**Duration of molecular response correlates with progression-free and overall survival.** Minimum duration of molecular response was retrieved for 7 patients who achieved a molecular response but ultimately developed acquired resistance. Duration of molecular response highly correlated with progression-free **(A)** and overall survival **(B)** for these patients (Pearson’s coefficient R2=0.86 and R2=0.92 respectively).

**Supplementary Figure S6**



**Disease prognostication by radiologic imaging at first assessment.** Patients with partial response had a longer overall survival compared to patients with stable disease and progressive disease (log rank P=0.04). The disease prognostication by imaging was inferior to molecular response assessments. PR; partial response, SD; stable disease, PD; disease progression.

**Supplementary Figure S7**



**Differential clinical outcome by molecular responses in patients with radiologically stable disease.** Patients with stable disease (n=12) were more accurately classified in terms of clinical outcome based on their molecular response pattern. Within this group, patients with a molecular response (n=5) had a significantly longer progression-free survival compared to molecular non-responders (n=7; log rank P=0.01).

**Supplementary Figure S8**

**Disease prognostication by tumor mutation burden.** Patients with higher tumor mutation burden (TMB) had a marginally longer PFS (**A**, log rank p=0.063) and significantly longer overall survival compared to tumors with low TMB (**B**, log rank p=0.027).

**Supplementary Figure S9**

**ctDNA molecular response more accurately distinguish clinical responders from non-responders compared to clonal tumor mutation burden.** Patients with a molecular response (shown in green) achieved durable clinical benefit from immune checkpoint blockade compared to molecular progressors (shown in blue) independent of the clonal tumor mutation burden **(A)**. Representative examples of patients with a high mutation burden with a molecular progression signature and dismal prognosis are highlighted with black arrows and vice versa, a patient with molecular response achieving durable clinical benefit despite a low tumor mutation burden is highlighted with a red arrow. Survival analysis revealed that patients with a ctDNA molecular response had a longer overall survival independent of the tumor mutation burden **(B)**. cTMB; clonal tumor mutation burden, PR; partial response, SD; stable disease, PD; disease progression.

**Supplementary Figure S10** 

**Molecular responses mirror pathologic responses to anti-PD1 therapy in early stage operable NSCLC.** Patients with major pathologic response (<10% residual tumor at the time of resection) and tumors with partial pathologic response (>30% reduction in tumor burden at the time of resection) were also identified as molecular responders by ctDNA dynamics **(A-D)**. In contrast, for a patient with 75% residual tumor at the time of resection **(E)**, ctDNA dynamics revealed an increase in levels of tumor specific mutations, consistent with a molecular and pathologic nonresponse. Radiologic imaging failed to accurately determine the therapeutic effect as an assessment of stable disease was established for all patients. RECIST tumor burden dynamics are plotted on the secondary vertical axis of each graph. Timing of anti-PD1 therapy, CT imaging and surgery is shown for each patient at the bottom of each graph.

**Supplementary Figure S11**



**TCR clonal dynamics during response and acquired resistance to anti-PD1.** Intratumoral TCR clonotypic amplifications were observed in peripheral blood at the time of radiographic response compared to baseline **(A, C, E)** followed by clone contractions at the time of acquired resistance **(A, E)**. For patients CGLU161 and CGLU135, where baseline blood was not available, a significant decrease in productive frequencies of intratumoral clones was observed in the peripheral blood at the time of acquired resistance compared to radiographic response **(B, C)**. Average productive frequencies of the statistically significant differentially abundant TCR clones are shown for each individual patient, error bars represent standard error of the mean.

**Supplementary Figure S12**



**TCR clonal dynamics for patients with clinical primary resistance.** There were no clones with statistically significant differential abundance between baseline and week 4 for patient CGLU115 with primary resistance to anti-PD1 therapy **(A)**. For patients CGLU162 **(B)**, CGLU243 **(C)** and CGLU203 **(E)** clonotypic expansions were observed in peripheral blood, however this pattern was not consistent with the ctDNA molecular response or the clinical outcome. For patient CGLU159 transient clonotypic TCR amplifications were detected in the peripheral blood followed by clone contraction at the time of clinical primary resistance determined by radiologic imaging **(D)**. Average productive frequencies of the statistically significant differentially abundant TCR clones are shown for each individual patient, error bars represent standard error of the mean.

**Supplementary Figure S13**

**Differential VJ gene usage for a patient with clinical response compared to a patient with clinical primary resistance.** For patient CGLU111, who had a sustained response to anti-PD1 therapy, differential V and J gene usage is shown between baseline and time of radiographic response (week 18). Usage of V and J gene segments increased at the time of radiologic response **(A)**. In contrast, for patient CGLU121, who developed primary resistance to anti-PD1 therapy, we did not observe any changes in V and J gene usage between baseline and week 4 **(B)**. Clones with significant expansions are colored, TCR clonotypes with no significant expansion between the two time points are shown in gray.

**Supplementary Figure S14**



**Dynamic changes in pro-inflammatory and immunosuppressive cytokines in early stage NSCLC patients with differential responses to anti-PD1 therapy.** Cytokines were measured by a multiplexed immunoassay at baseline and 2-6 weeks on anti-PD1 therapy for a patient with a major pathologic response (CGLU206), 2 patients with partial pathologic response (CGLU205, CGLU221) and a patient with pathologic nonresponse (CGLU222). We did not identify any differences in cytokine levels among patients with differential responses to anti-PD1 therapy. Differences in cytokine concentration were evaluated by T test.