Supplementary Data

Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer

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Supplementary Figure S1. Computed Tomographic (CT) findings in patient CGLU116. Pre-treatment axial IV contrast enhanced CT image of the chest, coned down to left hemithorax, demonstrates a large, central necrotic, subcarinal nodal mass, measuring 5.3 x 5.3 cm (flanking orange arrows). Additional pleural implants are present with representative pleural implant denoted by red arrow, measuring 1.2 cm. First follow up CT, 8 months later, demonstrates resolution of subcarinal mass (asterix) and pleural implants. Second and third follow up CTs, 12 and 16 months from treatment, demonstrate progression of pleural implant, increasing to 2.7 cm and 3.5 cm respectively.

Supplementary Figure S2. CT findings in patient CGLU127. Pre-treatment axial IV contrast enhanced CT images of the right lower lobe in lung window and hilar region in soft tissue window demonstrates numerous soft tissue pulmonary nodules, compatible with metastatic disease (top panel, multiple red arrows). A right upper lobe segmental lymph node is seen measuring 0.9 x 0.9 cm, suspicious for nodal metastatic disease (bottom panel, single red arrow). First post-treatment follow CT demonstrates near complete resolution of pulmonary nodules and hilar lymphadenopathy. Subsequent follow up CT demonstrates stability of post-treatment changes throughout the right lower lobe, although hilar lymph node enlarges to 2.2 x 2.3 cm. Third follow up CT demonstrates progression of pulmonary metastatic disease with new pulmonary nodule (red arrow). Hilar Lymph node further enlarges, measuring 2.8 x 2.4 cm.

Supplementary Figure S3. CT findings in patient CGLU161. Axial IV contrast enhanced CT images of the upper chest in soft tissue window performed on two separate follow up intervals: 3 months and 8 months after treatment initiation. Pretreatment images demonstrate a right upper lobe paramediastinal (red arrow) 2.9 x 2.5 cm heterogeneous low density mass. First follow up interval demonstrates reduction in the paramediastinal lesion whereas second follow up interval demonstrates marked progression. IV contrast enhanced MR imaging of the brain 10 months after treatment initiation demonstrates interval appearance of a 3.2 cm homogenously enhancing right cerebellar mass (red arrow).

Supplementary Figure S4. Tumor burden kinetics. Tumor burden was measured as the sum of longest diameters of target lesions (RECIST 1.1) and baseline tumor measurements were standardized to zero. Stars indicates first occurrence of progression in non-target lesions and triangle indicates first occurrence of new lesion.

Supplementary Figure S5. Neoantigen-specific TCR expansion in stimulated T cell cultures for patient CGLU127. Peptides generated from the eliminated mutation associated neoantigen candidates were synthesized and used to stimulate autologous peripheral T cells. T cells were stimulated with respective mutant and wild type peptides and cultured for 10 days, followed by next generation TCR sequencing of expanded T cell cultures. Reactive TCR clonotypes were matched to clones found in infiltrating tumor lymphocytes. Neoantigen-specific TCR reactivity was observed for the mutant peptides associated for both mutant (KSCKHTEK) and wild type (SVKSCKHKEK) peptides associated with a single base substitution in ANKRD12. Adjusted p values are given for pairwise comparisons between productive frequencies in peptide stimulated versus unstimulated T cells. Solid bars represent mutant and bars with diagonal pattern denote wild type peptides.

Supplementary Figure S6. Neoantigen-specific TCR expansion in stimulated T cell cultures for patient CGLU161. Peptides generated from the eliminated mutation associated neoantigen candidates were synthesized and used to stimulate autologous peripheral T cells. T cells were stimulated with mutant peptides and cultured for 10 days, followed by next generation TCR sequencing of expanded T cell cultures. Reactive TCR clonotypes were matched to clones found in infiltrating tumor lymphocytes. Neoantigen-specific TCR reactivity was observed for the mutant peptide associated with the mutant peptide associated with a single base substitution in EP300. Adjusted p values are given for pairwise comparisons between productive frequencies in peptide stimulated versus unstimulated T cells.

Supplementary Figure S7. Loss of heterozygosity analyses for patient CGLU116. The graphs represent B allele frequencies (BAFs) for the indicated chromosomes. A value of 0.5 indicates a heterozygous genotype (AB) whereas allelic imbalances in tumor samples are observed as a deviation from 0.5. BAF values of 0 typically indicate loss of heterozygosity, although normal contaminating tissue may limit the minimum observed value. Stars indicate the genomic positions of mutations lost at the time of resistance, either through loss of heterozygosity (red) or subclonal elimination (beige).

Supplementary Figure S8. Loss of heterozygosity analyses for patient CGLU117. The graphs represent B allele frequencies (BAFs) for the indicated chromosomes for responsive and resistance tumors. A value of 0.5 indicates a heterozygous genotype (AB) whereas allelic imbalances in tumor samples are observed as a deviation from 0.5. BAF values of 0 typically indicate loss of heterozygosity, although normal contaminating tissue may limit the minimum observed value. Stars indicate the genomic positions of mutations lost at the time of resistance, either through loss of heterozygosity (red) or subclonal elimination (beige).

Supplementary Figure S9. Loss of heterozygosity analyses for patient CGLU127. The graphs represent B allele frequencies (BAFs) for the indicated chromosomes for responsive and resistance tumors. A value of 0.5 indicates a heterozygous genotype (AB) whereas allelic imbalances in tumor samples are observed as a deviation from 0.5. BAF values of 0 typically indicate loss of heterozygosity, although normal contaminating tissue may limit the minimum observed value. Stars indicate the genomic positions of mutations lost at the time of resistance either through loss of heterozygosity (red) or subclonal elimination (beige).

Supplementary Figure S10. Loss of heterozygosity analyses for patient CGLU161. The graphs represent B allele frequencies (BAFs) for the indicated chromosomes for the baseline and resistant tumor. A value of 0.5 indicates a heterozygous genotype (AB) whereas allelic imbalances in tumor samples are observed as a deviation from 0.5. BAF values of 0 typically indicate loss of heterozygosity, although normal contaminating tissue may limit the minimum observed value. Stars indicate the genomic positions of mutations lost at the time of resistance either through loss of heterozygosity (red) or subclonal elimination (beige).

Supplementary Figure 11. TCR clonality analyses for patients CGLU127 and CGLU161. Peripheral T cell expansion of a subset of intratumoral clones was noted to peak at the time of response and decrease to baseline levels at the time of resistance for patient CGLU127 (left panel). For patient CGLU161 a decrease in predominant peripheral productive TCR frequencies was observed at the time of emergence of resistance (right panel). The TCR clone that expanded after stimulation with the $EP300^{1250C>Y}$ MANA was one of the top 3 clonotypes observed in peripheral blood at the time of response and its frequency decreased upon emergence of resistance (black arrow). Productive TCR frequency denotes the frequency of a specific rearrangement that can produce a functional protein receptor among all productive rearrangements. TCR: T cell receptor

Supplementary Figure S12. TCR clonality analyses for a responder and a non-responder to PD-1 blockade. Peripheral T cell expansion of a subset of intratumoral clones was noted for patient CGLU111 who derived durable clinical benefit from PD-1 blockade. TCR expansion persisted after initial assessment of response, 6 months after initiation of nivolumab (left panel). In contrast, there was no evidence of peripheral TCR expansion for patient CGLU115 that did not respond to nivolumab (right panel). Productive TCR frequency denotes the frequency of a specific rearrangement that can produce a functional protein receptor among all productive rearrangements. TCR: T cell receptor

Supplementary Figure S13. PD-L1 expression in responsive and resistant tumors. Immunohistochemical analysis of PD-L1 in tumor samples obtained before treatment initiation (C, E, G) and at the time of resistance (D, F, H) did not reveal any differences in expression for CGLU116, CGLU117 and CGLU127. For patient CGLU161 PD-L1 expression was evaluated only in post-progression tumor (I) as baseline tumor was not available for evaluation by IHC. NCI-H226 (A), a lung cancer cell line with known PD-L1 expression was used as a positive control, whereas MCF-7 cells that do express PD-L1 served as a negative control (B).

Supplementary Figure S14. Eliminated mutations for case CGHN2. Somatic mutations in HSPA12B and GLS that are associated with functionally validated neoantigens were detected in the pre-treatment tumors but not in the resistant tumor or matched normal DNA; MAF denotes the mutant allele frequency (panel B). Mutation cellularities at baseline (T1) and upon progression (T2) were estimated with the SCHISM pipeline; a cellularity of 0 was observed for these 2 sequence alterations in the resistant T2 tumor (panel B). Panel C shows B allele frequency graphs for chromosome 20, a value of 0.5 indicates a heterozygous genotype whereas allelic imbalance is observed as a deviation from 0.5. The LOH event involving chromosome 20 in the resistant tumor (orange box) contains the HSPA12B mutation and the associated neoantigen that is thus eliminated.

Supplementary Figure S15. Comparison of five methods for estimation of tumor purity. We compared tumor purity estimates derived by SCHISM (here referred to as CopyLoss) to four additional methods of estimating purity: 1) Pathology review, 2) Mutation allele frequency, 3) PyLOH and 4) Sequenza. Comparison of tumor purity estimates indicated a relatively high level correlation ($r >= 0.78$) with

statistical significance ($p < 0.05$) across all pairs of methods. On average, results from PyLOH had the largest difference (as measured by root mean squared error-rmse) with those of other methods, and the smallest dynamic range. Of the three copy number based methods, our method had the smallest root mean squared error when compared to the mutation-based approach.

Supplementary Figure S16. CD8+ T cell density in resistant tumors. Examples of CD8 expression in tumors for patients CGLU116 (A), CGLU117 (B) and CGLU161 (C) at the time of resistance. Normal tonsil tissue was used as a positive control (D).