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Concurrent *RB1* and *TP53* alterations define a subset of *EGFR*-mutant lung cancers at risk for histologic transformation and inferior clinical outcomes

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Abstract

Introduction: *EGFR*-mutant lung cancers are clinically and genomically heterogeneous with concurrent *RB1/TP53* alterations identifying a subset at increased risk for small cell transformation. The genomic alterations that induce lineage plasticity are unknown.

Methods: Patients with *EGFR/RB1/TP53*-mutant lung cancers, identified by NGS from 2014–2018, were compared to patients with untreated, metastatic *EGFR*-mutant lung cancers without

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both *RB1*- and *TP53*-alterations. Time to EGFR-tyrosine kinase inhibitor (EGFR-TKI) discontinuation (TTD), overall survival, SCLC transformation rate, and genomic alterations were evaluated.

Results: Patients with *EGFR/RB1/TP53*-mutant lung cancers represented 5% (43/863) of *EGFR*-mutant lung cancers but were uniquely at risk for transformation (18%, 7/39), with no transformations in *EGFR*-mutant lung cancers without baseline *TP53* and *RB1* alterations. Irrespective of transformation, patients with *EGFR/TP53/RB1*-mutant lung cancers had a shorter TTD than *EGFR/TP53* and *EGFR*-mutant only cancers (9.5 vs 12.3 vs 36.6 months respectively, $p = 2\times10^{-9}$). The triple-mutant population had a higher incidence of whole genome doubling (WGD) compared to NSCLC and SCLC at large (80% vs 34%, $p < 5\times10^{-9}$; vs 51%, $p < 0.002$ respectively) and further enrichment in triple-mutant cancers with eventual small cell histology (7/7 pre-transformed plus 4/4 baseline SCLC vs 23/32 never transformed respectively, $p = 0.05$). AID/APOBEC mutation signature was also enriched in triple-mutant lung cancers that transformed (FDR = 0.03).

Conclusions: *EGFR/TP53/RB1*-mutant lung cancers are at unique risk of histologic transformation, with 25% presenting with *de novo* SCLC or eventual small cell transformation. Triple-mutant lung cancers are enriched in WGD and AID/APOBEC hypermutation which may represent early genomic determinants of lineage plasticity.

Keywords

EGFR-mutation; *TP53*; *RB1*; Whole genome doubling; Small cell histologic transformation

Introduction

Twenty percent of lung adenocarcinomas harbor a sensitizing epidermal growth factor receptor (*EGFR*) mutation.[1] Patients with *EGFR*-mutant lung cancers have robust responses to EGFR tyrosine kinase inhibitors (EGFR-TKIs) but inevitably their tumors acquire resistance.[2] Multiple on-target and off-target mechanisms of acquired resistance to EGFR-TKIs have been described, including secondary mutations in *EGFR* and activation of other mitogenic signaling pathways.[3–6] One particularly aggressive off-target resistance mechanism is transformation of *EGFR*-mutant lung adenocarcinoma to a small cell lung cancer.[4, 7, 8] Small cell histologic transformation occurs in 3–14% of patients with sensitizing *EGFR*-mutant lung cancers after EGFR-TKI therapy.[3, 4] The molecular determinants of lineage plasticity that underlie this histologic transformation are largely unknown.

Small cell lung cancers universally have bi-allelic loss of TP53 and RB1, among a complex genomic landscape that also includes alterations in *SOX*, *NOTCH*, *PTEN*, *MYC*, and *PIK3CA*[9]; alterations in receptor tyrosine kinases such as *EGFR* are exceedingly rare. Post-transformation, *EGFR*-mutant small cell lung cancers (SCLCs) continue to harbor the original *EGFR*-mutation indicating direct evolution from the original non-small cell lung cancer (NSCLC).[4, 10, 11] After small cell transformation, the clinical outcomes mimic primary SCLC, with a rapid disease course and a transient response to SCLC-directed chemotherapies. Upon transformation and despite retention of the *EGFR*-mutation, EGFR

protein expression decreases[12] and patients have limited benefit from EGFR-TKIs.[13] A parallel event occurs in prostate adenocarcinomas receiving androgen receptor-targeted therapy with functional loss of *RB1* and *TP53* facilitating small cell transformation and reducing sensitivity to anti-androgen therapy.[14]

Concurrent alterations within *EGFR*-mutant lung cancers may contribute to heterogeneous outcomes seen and influence the mechanism of resistance that emerges to EGFR-TKI treatment.[15, 16] Two thirds of *EGFR*-mutant lung cancers have concurrent *TP53*-mutations which are associated with shorter time on EGFR-TKI and shorter overall survival (OS).[3, 15, 16] *RB1* alterations in *EGFR*-mutant lung cancers almost always occur concurrently with *TP53*. *EGFR*-mutant lung cancers with transformation mimic classical SCLC with *RB1* and *TP53* biallelic loss. It has not been fully determined whether *RB1* and TP53 loss are early events within *EGFR*-mutant lung cancers, or alternatively, are acquired late in the process of histologic shift. RB1 and TP53 loss appear necessary, but not sufficient, to induce lineage plasticity.[10]

Single-gene alterations and mutation patterns have been reported in the context of lineage plasticity in other tumor types with alterations in *PIK3CA*, *MYC*, *MDM*, *AURKA*, *FGFR*, *NOTCH*, and *TERT* implicated in bladder and prostate cancers.[14, 17] Beyond single genetic lesions, higher order patterns of mutations may drive or be associated with histological transformation. AID/APOBEC hypermutation signature, previously observed in lung adenocarcinoma,[18] was noted to be further enriched in a cohort of lung cancer patients following SCLC transformation.[10] Based on methods leveraging Memorial Sloan Kettering (MSK) Integrated Mutation Profiling of Actionable Cancer Targets (IMPACT) targeted next-generation sequencing, whole genome doubling (WGD), seen in nearly 30% of metastatic solid tumors including NSCLC (34%) and SCLC (51%), was found to be even more highly recurrent (72%) in small cell cancers of the bladder that have presumably transformed from urothelial carcinomas.[17, 19]

Lineage plasticity is an off-target adaptive mechanism to decrease dependence on EGFR signaling. We hypothesize that *RB1* and *TP53* alterations represent early events in oncogenesis, and that *EGFR*-mutant lung cancers with *RB1/TP53* alterations are at particularly high risk for SCLC transformation. Patients with *EGFR/RB1/TP53* mutant lung cancers are an ideal population in which to identify early genomic determinants of small cell histologic transformation.

Materials and Methods

We identified patients with somatic sensitizing *EGFR*-mutations with concurrent *TP53* and *RB1* alterations using MSK-IMPACT[20] from January 2014 – August 2018. All cases with concomitant alterations in *RB1* and *TP53* were analyzed given the functional significance of some aberrations remain unknown. This study was undertaken at MSK with the approval of the Institutional Review Board. All patients provided written informed consent.

Time to treatment discontinuation (TTD) was used as a surrogate of progression-free survival and was defined as the time from start of EGFR-TKI to last administered dose

before a treatment change. OS was defined as date of diagnosis of metastatic disease to date of death or last follow-up as of August 2018; we utilized the left truncation method to adjust for survival bias.[21] For comparison, the clinical data were reviewed for all patients identified over the same time-period with *EGFR*-mutant metastatic lung cancers without concurrent *RB1* and *TP53* alterations that were EGFR-TKI naïve at the time of NGS. Differences in outcomes were analyzed using the Mann-Whitney test and the Fisher's exact test was used to compare the proportions. Tumor mutation burden (TMB) was defined as the total number of missense mutations and indels divided by the total coding region captured and was reported as mutations/megabase (mutations/Mb). For survival and TTD analyses, Kaplan-Meier curves were compared using the Mantel-Cox log-rank test with hazard ratios calculated using a Mantel-Haenszel method. Relative risk was calculated using the Koopman asymptotic score. All patients with archival tissue underwent immunohistochemistry (IHC) for RB1 and TP53.

Single nucleotide variants (SNVs) and copy number variants (CNVs) identified by MSK IMPACT were schematized for analysis on the cBioPortal[22]. Analysis of gene set enrichment was performed using the DAVID pathway[23] database with Fisher's exact *p*-values adjusted for multiplicity by the Benjamini-Hochberg method. We used the FACETS algorithm (version 0.5.6)[24] to perform allelic copy number analysis, including detection of loss of heterozygosity (LOH). Following previous methodology, we used allelic copy number estimates to define WGD as any instance where > 50% of the autosome contains major copy number (MCN) ⩾ 2 and compared the distribution of WGD to corresponding published distributions of NSCLC and SCLC samples.[19] Mutation signature analysis was performed using deconstructSigs, with enrichment of mutation signature assessed by permuting sample labels (see Supplemental Methods).[25]

Results

Patient characteristics

We evaluated 4,112 patients with lung cancer, identifying 21% (n = 863) with *EGFR*-mutant lung cancer of whom 43 had concurrent sensitizing *EGFR* mutations along with both *RB1* and *TP53*-alterations (Supplementary Fig. S1). All patients with the *EGFR/RB1/TP53*-mutant genotype had metastatic disease. In patients with *EGFR*-mutant lung cancers with concurrent *RB1*-alterations, only 11 (11/54) had a wild-type *TP53* (Fisher's exact $p < 0.0001$). None of these 11 patients had small cell transformation or *PTEN* loss. Clinical and molecular characteristics of the 43 patients with *EGFR/RB1/TP53*-mutant lung cancers are shown in Table 1 and Supplementary Table S1. Nine percent (4/43) had small cell histology at initial diagnosis; all were never-smokers (100%) and were comparatively younger than other patient's whose tumors harbored an *EGFR/RB1/TP53* alterations (55 vs 68 years old; Mann Whitney $p = 0.009$).

Of the patients with lung adenocarcinomas, 18% (7/39) had SCLC transformation during their disease course, and 82% have not had transformation (median follow up 3.2 years). The median time to transformation from start of initial EGFR-TKI was 1.1 years (interquartile range of 9 months to 3.6 years). There was no difference in smoking history in patients with or without histologic transformation to SCLC. Nineteen patients with *EGFR/RB1/TP53*-

mutant lung cancers had available tissue for IHC analysis (7 transformed SCLC and 12 never transformed cases) demonstrating immunoprobing consistent with adenocarcinoma prior to transformation and consistent with SCLC post-transformation (Supplementary Fig. S2). Sixty five percent (28/43) had brain metastasis during their disease course: 18 at diagnosis and 10 developed on treatment. The median time to the development of brain metastases was 2.0 years (range 4.6 months - 6.4 years) from initial metastatic diagnosis.

For comparison, we identified 142 consecutively identified patients with *EGFR*-mutant metastatic lung cancer during the same period without concurrent *RB1* and *TP53* alterations (Supplementary Table S2). There were no differences in baseline clinical features between the *EGFR/RB1/TP53*-mutant group and control groups. There was enrichment for SCLC transformation in patients whose tumors harbored an *EGFR/RB1/*TP53-mutant genotype compared to *EGFR/TP53-mutant RB1*-wiltype (relative risk 3.5, 95% CI 2.1–7.7) and *EGFR*-mutant *RB1/TP53*-wildtype (relative risk 2.9, 95% CI 1.8–3.8); $p = 0.001$ (Supplementary Table S3).

Time to treatment discontinuation and overall survival

We identified the subset of patients that were EGFR-TKI naïve at the time of molecular testing and used time to EGFR-TKI discontinuation as an indicator of clinical benefit. Time to initial EGFR-TKI discontinuation, which includes treatment beyond radiographic progression, was 9.5 months in the *EGFR/RB1/TP53*-mutant cohort (n = 20), 12.3 months in the *EGFR/TP53*-mutant cohort (n = 79), and 36.6 months in the *EGFR*-mutant only cohort (n = 60; log-rank for trend $p = 2e^{-9}$) (Fig. 1A). The median overall survival was 29.1 months for the *EGFR/RB1/TP53*-mutant cohort, 40.8 months for the *EGFR/TP53* cohort, and 56.4 months for the *EGFR*-mutant *RB1/TP53* wildtype cohort; log-rank for trend $p = 0.16$; Fig. 1B).

Molecular analyses

Defining the genomic landscape of EGFR/RB1/TP53-mutant lung cancers—We performed integrated analysis of single nucleotide variants (SNVs), focal copy number variations (CNVs), and fusions in 43 patients with concomitant *EGFR, RB1*, and *TP53* alterations. All had *EGFR*-sensitizing mutations, including L858R, L861Q, G719C, and in-frame exon 19 deletions (Fig. 2). To determine whether the wild-type allele of *TP53* and *RB1* was present, we estimated allelic copy number variants[24] on samples in which the NGS data allowed (*TP53* evaluable in 32, *RB1* in 37 samples). The wild-type allele of *TP53* was lost in 29 of 32 cases (6 by heterozygous loss and 23 by LOH). Similarly, the majority of *RB1* point mutations occurred in conjunction with loss of wild-type allele (31 of 36; 6 by heterozygous loss and 25 by LOH) (Supplementary Fig. S3). Among the 29 samples that had both *TP53* and *RB1* allelic information available, all 5 samples that retained either *TP53* or *RB1* wild-type allele remained adenocarcinoma, compared to small cell histology in 5 of the 24 samples with biallelic functional loss of both genes. There was no significant difference in the frequency of biallelic wild-type *TP53* or *RB1* loss between samples before and after EGFR-TKI therapy. These data confirm functional inactivation of TP53 and RB1 in nearly all tumors. Pooling somatic mutations and copy number changes across all samples with concurrent *EGFR/TP53/RB1* alterations, the most frequently co-altered genes were

PIK3CA (20%), *NTRK1* (11%), *MCL1* (11%), *NKX2-1* (11%), *ERBB2* (9%), *FOXA1* (9%), *PLCG2* (9%), *PTEN* (9%), *RBM10* (9%), *SDHA* (9%), *SOX17* (9%), and *TERT* (9%) (Fig. 2).

To identify the genomic features enriched in *EGFR/RB1/TP53*-mutant lung cancers, we compared them to our cohort of 142 *EGFR*-mutant lung cancers without concurrent *RB1/TP53*-mutations (Fig. 3A). The most enriched concurrent alterations in the triple mutant cohort included point mutations in *ERBB2* (7% versus 0%), *AKT3* (7% versus 0%), *PLCG2* (7% versus 1%), and SOX17 (7% versus 1%), as well as copy number changes in *PTEN* loss (7% versus 0%), *NTRK1* amplification (7% versus 0%), *MYCL* amplification (7% versus 1%), and *PTPRT* amplification (7% versus 1%); no comparisons were significant after adjusting for multiplicity. On the other hand, *CTNNB1* mutations (2% versus 13%), *CDKN2A* (5% versus 19%) and *CDKN2B* (2% versus 18%) homozygous deletions, were underrepresented in the *EGFR/RB1/TP53*-mutant cohort compared to *EGFR*-mutant lung cancer without *RB1* and *TP53* mutations.

Early genomic determinants of SCLC transformation in EGFR/RB1/TP53-mutant lung cancer—To assess early genomic determinants of SCLC transformation, we compared 7 samples prior to SCLC transformation to 32 samples that never underwent transformation (Fig. 3B). Candidate genes potentially enriched in cancers that eventually transformed compared to never-transformed cancers included SNVs in *SMYD3* (29% versus 0%) and *NOTCH2* (29% versus 3%), as well as amplifications in *ELF3* (29% versus 0%) and *CCNE1* (29% versus 3%) (Fig. 3B). Other recurrent alterations included *PIK3CA* (29% versus 13%), *MYC* (14% versus 0%), *CREBBP* (14% versus 3%), *PTEN* (14% versus 3%). None of these comparisons were significant after adjusting for multiplicity.

Beyond single genetic lesions, we assessed whether there were any biologically relevant gene sets that were enriched. We found that the 50 most enriched co-mutations in pre-transformed samples (Fisher's p-value < 0.2) were overrepresented in a number of pathways, including MAPK signaling cascade (*ARAF, ERBB2, FGFR1, FGFR3, FGFR4, GRIN2A, MAPK3, MYC*) (FDR = 0.002); Jak-STAT signaling (*CRLF2, IFNGR1, IL7R, SOCS1, MYC*) (FDR = 0.04); ERBB signaling (*ARAF, SRC, ERBB2, MAPK3, MYC*) (FDR = 0.008); FGFR signaling (*FGFR1, FGFR3, FGFR4, MAPK*) (FDR = 0.04); MTOR signaling (*BRAF, RRAGC, MAPK3, PIK3CG*) (FDR = 0.01); and PI3K-Akt signaling (*CCNE1, FGFR1/1/3, FLT4, IL7R, MAPK3, RAC1, MYC*) (FDR = 0.003).

AID/APOBEC hypermutation in EGFR/RB1/TP53-mutant lung cancer as an early predictor of small cell transformation—Considering *EGFR/RB1/TP53*-mutant tumors as a group, the distribution of substitutions identified through NGS showed a strong preference for C>T (24%) and G>A (18%) transitions consistent with cytidine deamination. (Supplementary Fig. S4). We investigated whether our cohort matched any of the 7 canonical mutation signatures known to be associated with lung cancers (signatures 1, 2, 4, 5, 6, 13, 15, and 17 corresponding to spontaneous deamination, AID/APOBEC hypermutation, smoking, unknown, mismatch repair, AID/APOBEC, mismatch repair, and unknown respectively). Prior to the transformation event, *EGFR/RB1/TP53*-mutant lung

cancers destined for transformation were significantly enriched for AID/APOBEC signature compared to those that never transformed (Fig. 4A, FDR = 0.03).

Whole genome doubling in EGFR/RB1/TP53-mutant lung cancer as an early predictor of small cell transformation—Beyond mutational and sub-chromosomal structural variants, we sought to analyze potential drivers of histologic transformation at the genome level. WGD is a frequent event in oncogenesis, associated with dysregulation of G2/M cell cycle checkpoints. By calculating the proportion of the autosome with major copy number of at least 2, we assessed for WGD in our triple-mutant cohort as well as all NSCLC and SCLC from Bielski, et al. for comparison.[19] In contrast to the bimodal distribution of WGD in all lung cancers (Hartigan's $p < 2.2 \times 10^{-16}$ rejecting unimodality), the *EGFR/RB1/TP53*-mutant cohort had a distinctly skewed unimodal distribution (Hartigan's $p = 0.94$ accepting unimodality). Using 50% as a cutoff, the rate of WGD was elevated at 80% in our cohort compared to 34% of NSCLC (Fisher's p-value $< 5 \times 10^{-9}$) and 51% of SCLC samples (Fisher's p-value < 0.002) (Fig. 4B).

Within our cohort of patients with triple-mutant lung cancers, there was further enrichment of WGD in baseline SCLC or pre-transformation samples compared to never-transformed cancers: 4 out of 4 (100%) of baseline SCLC and 7 out of 7 (100%) pre-transformation cancers had WGD compared to 23 of 32 (72%) patients with never-transformed cancers (Fisher's p-value = 0.05). Using clonality estimates inferred from FACETS analysis, the timing of *TP53* and *RB1* mutations was assessed in relation to WGD. Of 24 samples that underwent WGD and had sufficient data for unambiguous timing, *TP53* mutations preceded WGD in 96% (23/24), and *RB1* mutations preceded WGD in 71% of cases (17/24). Only in one case did both *TP53* and *RB1* mutations follow WGD. Using clonality estimates for allelic copy number variants, we found that 65% of all heterozygous deletions followed a WGD event in our cohort, similar to previously reported rates.[19]

Discussion

Small cell histologic transformation occurs in the subset of patients with *EGFR*-mutant lung cancers with concurrent alterations in *TP53* and *RB1* and is associated with large-scale genomic alterations including both WGD and the APOBEC mutation signature. Patients with co-occurring *EGFR/RB1/TP53* alterations (5% of all *EGFR*-mutant lung cancers) are uniquely at risk for SCLC transformation during their disease course. While not all *EGFR/RB1/*TP53-mutant lung cancer patients will transform to SCLC during their disease, our observation agrees with previously published findings that *RB1/*TP53-mutations are necessary, but not sufficient, for lineage plasticity.[10] All cases of SCLC transformation occurred among patients with pre-existing mutations in *TP53* and *RB1* and the frequency of histologic transformation is 6-fold higher in this cohort compared to the *EGFR*-mutant lung cancer population at large (18% vs 3%).[15] Patients with co-occurring *EGFR/RB1/TP53*-altered NSCLC also have shorter time on EGFR-TKI therapy ($p = 0.0007$) which was similar to published data by Marcoux et al[8] for patients with known histologic transformation. The 18% rate of SCLC transformation (7/39 adenocarcinoma at baseline patients) and shorter time on EGFR-TKI highlight the poorer outcomes seen in this genomic subset of *EGFR*-mutant lung cancers.

Given the unique clinical features of *EGFR/RB1/TP53*-mutant lung cancers, we sought to characterize the genomic features that define this cohort. Fewer *EGFR* T790M mutations were identified among the samples that ultimately transformed (1/7 pre-transformed versus 6/34 never-transformed versus 32/68 relapsed *EGFR*-mutant samples without concomitant *RB1* and *TP53* mutations). Concurrent *EGFR* amplification was also less frequently seen in the pre-transformation and baseline SCLC samples (2/7 pre-transformed versus 15/34 never-transformed versus 42/68 relapsed *EGFR*-mutant samples without concomitant *RB1* and *TP53* mutations). Relative absence of *EGFR* T790M and *EGFR* amplification is consistent with the loss of *EGFR* dependence in transformed SCLC.[12] That we observe this pattern in pre-transformed samples suggests that loss of EGFR dependence may begin prior to overt histologic transformation.

A number of mutations commonly seen in SCLC are enriched in our cohort compared to *EGFR*-mutant lung cancer without *TP53* and *RB1* loss (*PIK3CA, PTEN, MYCL*), as well as in *EGFR/RB1/TP53*-mutant samples with eventual transformation compared to those that never transformed (*PIK3CA, MYC, CREBBP, PTEN, NOTCH2*).[8, 10] While *SOX17* frequently found in our *EGFR/RB1/TP53*-mutant cohort has not been directly implicated in neuroendocrine transformation, other *SOX* family members, including *SOX2,* play a role in small cell transformation seen in *RB1/TP53*-mutant prostate cancer.[26] In addition, a number of these highlighted alterations are overrepresented in key canonical pathways activated in classical SCLC, including the PI3K-PTEN-AKT pathway.[27] These genetic alterations present prior to SCLC transformation represent potential early biomarkers of lineage plasticity.

We also assessed the mutational signatures of these triple mutant lung cancers and found an enrichment of the AID/APOBEC hypermutation signature in *EGFR/RB1/TP53*-mutant tumors destined for SCLC transformation. This corroborates the increased APOBEC hypermutation seen in post-transformation SCLC cases by Lee et al.[10] The enrichment in the adenocarcinomas that eventually transform suggest this hypermutation occurs even prior to transformation and may facilitate the transformation process. Considering its prevalence in SCLC and NSCLC, we assessed for WGD within our cohort, and found a markedly higher prevalence of WGD when compared to previously published WGD rates of lung cancers.[1928] We note that other publications use alternative WGD calling methods,[28, 29] but for consistency, we use here the method from Bielski, et al. based specifically on the MSK IMPACT targeted next-generation sequencing platform.

Moreover, there was further enrichment of WGD in samples with histologic transformation, seen prior to the transformation event. Given the role of *TP53* and *RB1* loss in genomic instability, we assessed the timing of these mutations relative to WGD and found that in all but one case, *TP53* mutations and *RB1* alterations preceded WGD, suggesting that these lesions may predispose to WGD. While it remains unclear whether WGD itself facilitates transformation, there is a precedent for WGD providing a vehicle for evolutionary change as well as conferring tolerance to further chromosomal instability in other cancers, as evidenced by the higher number of heterozygous deletions following WGD in our cohort.[19] To our knowledge, this is the first report suggesting a role for WGD in histologic transformation.

Our study has several limitations. The relatively small number of patients identified with concurrent *EGFR/RB1/TP53* alterations limits comparative analyses. Most patients in our cohort received first-line erlotinib or afatinib. Now that osimertinib is the standard initial treatment, we need to assess whether the EGFR-TKI used affects the frequency of transformation. As we treat our patients with more potent and selective EGFR inhibitors, we suspect the frequency of off-target resistance mechanisms such as small cell transformation will increase. Due to the exploratory nature of our analyses, co-mutation analysis was not limited by FDR correction and findings will ultimately need to be validated in larger datasets. Although analysis of pre-transformation samples is valuable to identify early-determinants of lineage plasticity, analysis of paired pre- and post-transformation samples is critical to understand what factors may induce transformation in each specific case. All available tissue samples from the *EGFR/RB1/*TP53-mutant cohort were evaluated for RB1 and TP53 loss by IHC, however, given limited tissue availability a comprehensive analysis was not feasible.

After initial EGFR-TKI response, a persister cell population remains that serves as the reservoir for eventual clinical progression. Prior trials have evaluated the role of combinatorial first- and second-generation EGFR-TKIs with cytotoxic chemotherapy (pemetrexed or taxol based) in *EGFR*-mutant lung cancer patients regardless of co-mutation status, showing a potential benefit with the use of the combination.[30] Based on those prior studies, we hypothesize that in patients with *EGFR/RB1/TP53*-mutant lung cancers, the persister cell population might include the subclone that is predisposed to small cell histologic transformation and may benefit from the combination of a potent EGFR-TKI and a neuroendocrine-based chemotherapy regimen. To eradicate this subclone, we have developed a trial of upfront osimertinib and small cell directed chemotherapy (platinum/etoposide) in patients with triple mutant lung cancers ().

In conclusion, patients with *EGFR*/*TP53*/*RB1*-mutant lung cancers demonstrate inferior clinical outcomes and define the population at risk for SCLC transformation. We have identified both small- and large-scale genomic alterations potentially associated with SCLC transformation. This work highlights the importance of understanding genomic heterogeneity to identify high-risk patients that may benefit from treatment intensification and to predict and subsequently prevent mechanisms of resistance to EGFR targeted therapy likely to emerge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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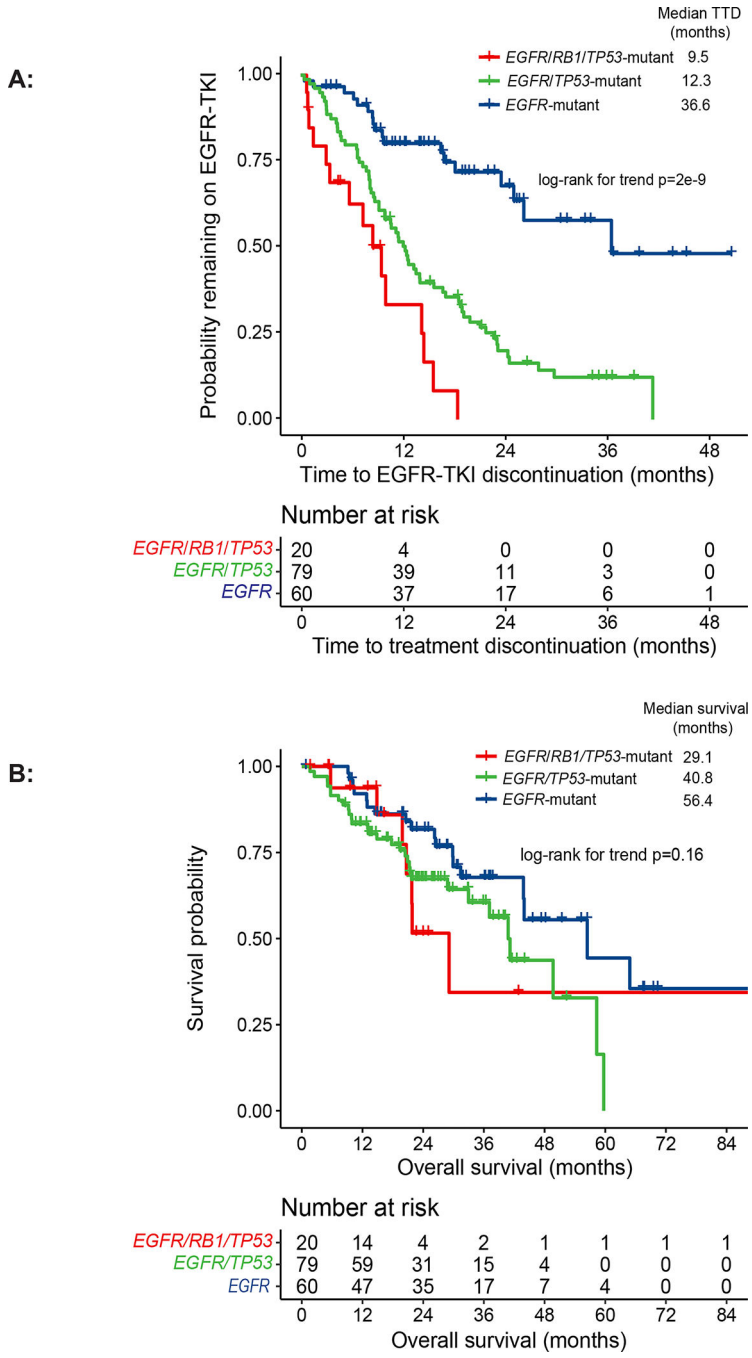

Figure 1.

Time to treatment discontinuation (TTD) and overall survival (OS) of patients with *EGFR/RB1/TP53*-mutant lung cancers: patients with *EGFR/RB1/TP53*-mutant lung cancer without baseline small cell lung cancer (SCLC) who were EGFR-TKI naïve at the time of next-generation sequencing (NGS) (n = 20) versus patients with *EGFR/TP53*-mutant *RB1*-wildtype (n = 79) and *EGFR*-mutant *RB1/TP53*-wild type lung cancer who were EGFR-TKI naïve at the time of NGS (A) The median TTD for patients with *EGFR/RB1/TP53*-mutant lung cancer was 9.5 months versus 12.3 months for *EGFR/TP53*-mutant *RB1*-wildtype (HR

2.0, 95% CI 1.1 – 3.6) versus 36.6 months in *EGFR*-mutant *RB1/TP53*-wiltype groups (HR 7.7, 95% CI 3.6 – 14.2; log-rank for trend $p = 2e^{-9}$). (B) The median OS of patients with *EGFR/RB1/TP53*-altered lung cancer was 29.1 months as compared to 40.8 months in *EGFR/TP53*-mutant *RB1*-wildtype and 56.4 months in patients with *EGFR*-mutant *RB1/TP53*-wildtype (HR 1.0 95% CI 0.4 – 2.4, HR 1.8 95% CI 0.7 – 4.3, respectively; log-rank for trend $p = 0.16$).

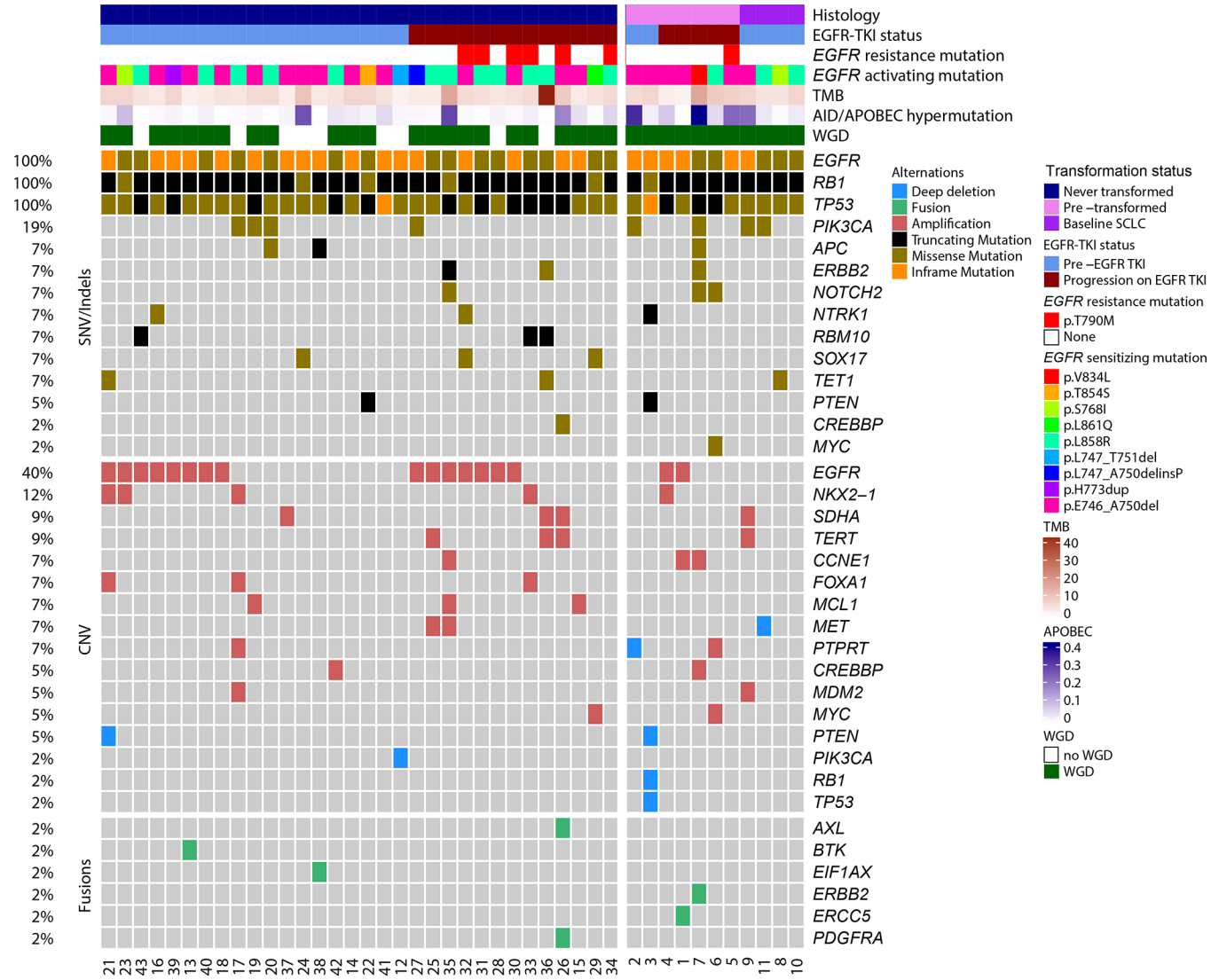

Figure 2.

Genomic landscape of lung cancer with concurrent *EGFR/RB1/TP53* mutations. The type of genetic alteration (missense, in-frame, truncating, amplification, deep (homozygous) deletion, fusion/intragenic alteration) is described in the legend. The frequency of mutations is noted on the right. Mutations present in at least 5% of cases were included in the figure, as well as *PIK3CA*, *MYC*, and *CREBBP* mutations given their known relevance in small cell lung cancer.

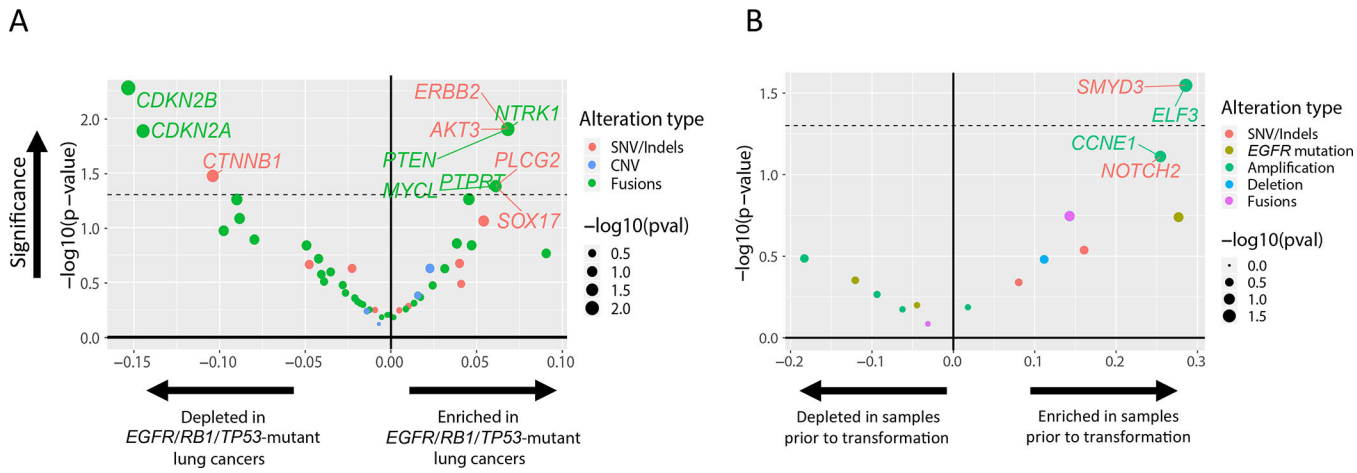

Figure 3.

Enrichment analysis of genomic alterations. (A) Enrichment of mutations in *EGFR*-mutant lung cancer with concurrent *TP53/RB1* mutations versus without concurrent *TP53/RB1* mutations. (B) Within *EGFR/RB1/TP53*-mutant lung cancer, enrichment of mutations in cases with eventual SCLC transformation. Level of enrichment is represented as a volcano plot with the log ratio in frequency between the two states (x-axis) and its significance -log(p-value) (y-axis). The type of alteration is represented by color. The dashed line represents *p*-value = 0.05.

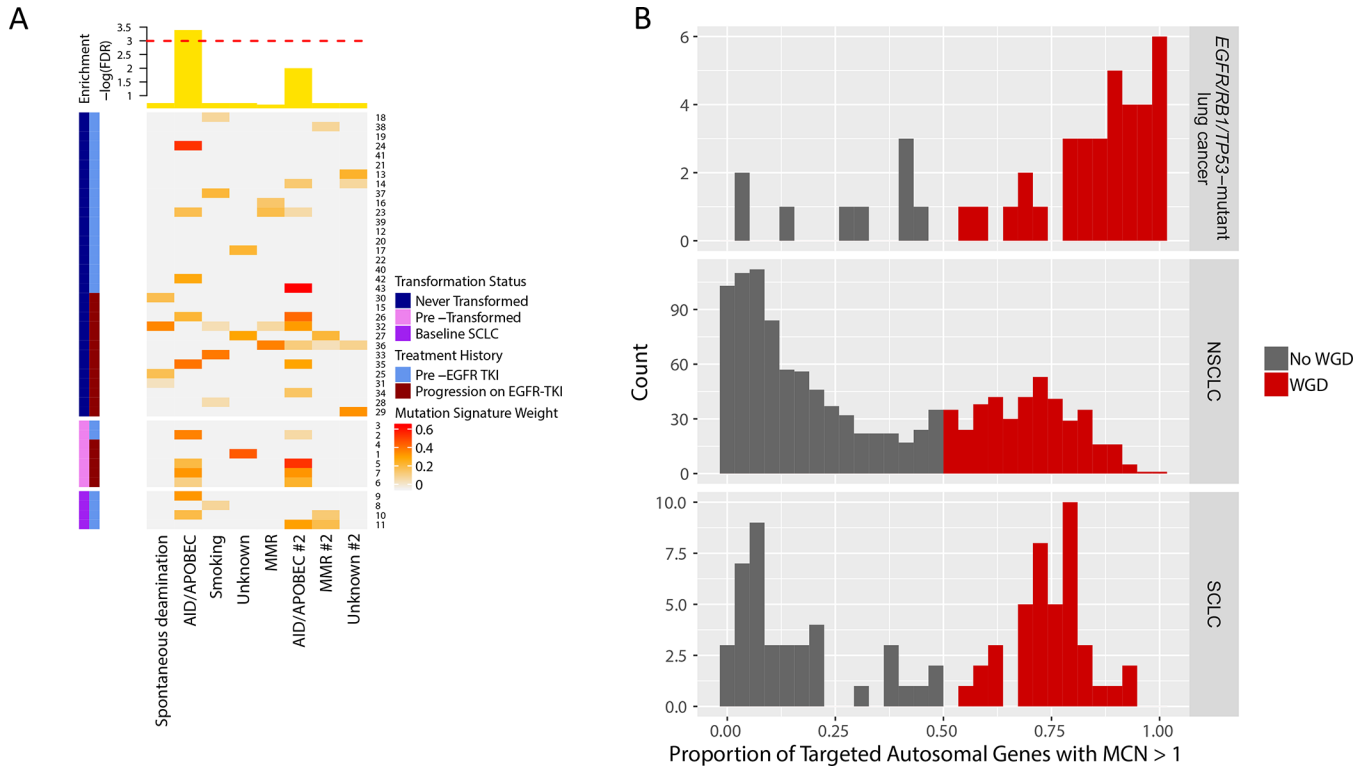

Figure 4.

AID/APOBEC mutation signature and whole genome doubling (WGD) in *EGFR/RB1/TP53*-mutant lung cancers. (A) Mutation signature analysis identified significant enrichment of AID/APOBEC in pre-transformed SCLC compared to never transformed. Heatmap is calculated based on weights [0,1] measuring how strongly a mutation signature is represented in a given sample. Top annotation plots significance as -log(FDR) for enrichment of a mutation signature in pre-transformed SCLC. The dashed red line corresponds to an FDR of 0.05. (B) The frequency of WGD in lung cancer with concurrent *EGFR/RB1/TP53* mutations is higher compared to all NSCLC and SCLC. Plots show the distribution of the proportion of autosomal genes with major copy number of at least 2 in lung cancers with concurrent *EGFR/RB1/TP53* mutations, NSCLC, and SCLC respectively. Using a cutoff of 50%, the proportion of samples with WGD is colored in red, corresponding to a WGD frequency of 80% in lung cancer with concurrent *EGFR/RB1/TP53* vs 34% in NSCLC ($p < 5\times10^{-9}$) vs 51% in SCLC ($p < 0.002$).

Table 1.

Demographics of patients with *EGFR/RB1/TP53*-mutant lung cancers by small cell lung cancer status during the course of their disease.

	Total (*N=43*)	Never Transformed (*N=32*)	Transformed SCLC (*N=7*)	SCLC at Diagnosis (*N=4*)
Median age (range)	67 (25–86)	68 (25–86)	65 (58–73)	55 (27–60)
Sex				
Female	26 (60%)	21 (66%)	3 (43%)	3 (75%)
Male	17	11	4	1
Smoking Status				
Never	26 (60%)	18 (56%)	4 (57%)	4 (100%)
Ever (median; range)	17 (7; 0–30)	14 (8; 0–30)	3 (3; 1–9)	0 (0)
Histology at diagnosis				
Adenocarcinoma	39 (91%)	32 (100%)	7 (100%)	-
Small Cell	4	-	-	4
***EGFR*-Mutation**				
L858R	17 (40%)	13 (41%)	2 (29%)	2 (50%)
Exon 19 Deletion	22 (51%)	16 (50%)	5 (71%)	1 (25%)
S768I/G719C	2	1	0	1
L861Q	2	2	0	0
Never EGFR-TKI	4 (9%)	1** (3%)	0	3 (75%)
Initial EGFR-TKI used	-	-	-	-
Erlotinib	28 (65%)	22 (69%)	6 (86%)	0
Gefitinib	1	1	0	0
Afatinib	5	4	1	0
Osimertinib	5	4	0	1
NGS showing				
EGFR/RB1/TP53	25 (58%)	19 (59%)	2 (29%)	4 (100%)
Pre-EGFR-TKI	18	13	5	0
Progression on EGFR-TKI				
Median TMB* (range)	6.4 (2.6–42.1)	5.6 (2.6–42.1)	7.9 (2.6– 16.7)	6.6 (5.3– 7.9)

SCLC: small cell lung cancer; TKI: tyrosine kinase inhibitor; NGS: next generation sequencing; TMB: tumor mutation burden.

* mutations/megabase

** 1 never-transformed patient was lost to follow up