Inactivation of *RB1* and histological transformation in *EGFR*-mutant lung adenocarcinoma

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The majority of lung cancer diagnoses are smoking-related; however, 10-20% of diagnoses are made in patients who have never smoked [1, 2]. Although it is known that non-smoking-related lung adenocarcinomas are predominantly driven by genetic alterations involving *EGFR, ALK, RET* and *ROS1* [1, 3], there remains an unmet need to understand the aetiology of these cancers and to further define the genomic landscape, in particular, the genetic drivers of treatment resistance and tumour evolution.

Pros and colleagues performed whole-exome and RNA sequencing on patient-derived cancer cell lines (PDCs) from malignant pleural effusions in 11 patients with non-smoking-related lung adenocarcinoma (NSK-LuADs) (Ann Oncol 2019; 30: doi.org/10.1093/annonc/mdXXXX). Whole-exome sequencing data from an independent cohort of 65 patients with primary LuAD was used to validate their findings, and additionally specific analysis of complex rearrangements and mutations in the *RB1* gene was performed.

In the PDCs, Pros and colleagues were able to demonstrate activating alterations in several oncogenes, including amplifications involving *MDM2*, as well as inactivating alterations in tumour suppressor genes (TSGs), such as *TP53* and *RB1*. In addition, three novel in-frame fusions including genes such as *TFG* and *ADGRG7*, were identified. In an independent cohort of patients with primary LuADs, Pros and colleagues identified inactivating mutations in *ATF7IP, GBP3, SETD1B and SETD2*, and proposed *ATF7IP* as a novel and recurrent TSG in LuAD, although not limited to NSK-LuADs.

In the PDCs identified to present alterations in *RB1,* there were specifically homozygous deletions found in *EGFR*-mutant LuADs. The presence of mutations in the *RB1* gene in *EGFR*-mutant LuADs has been previously reported [4, 5]. However, intragenic complex rearrangements (ICRs) in *RB1* have not been studied before. In an independent cohort of patients with primary LuADs, Pros and colleagues found that inactivation in *RB1* was prevalent in *EGFR*-mutant (41%) as opposed to wild type (2%) LuADs, and that this was predominantly in the form of ICRs. In three patients with *EGFR*-mutant LuAD treated with EGFR tyrosine kinase inhibitors (TKIs) who subsequently developed TKI resistant disease, biopsy of tissue at relapse showed histological transformation from LuAD to either small-cell lung cancer (SCLC) alone (1/3) or combined SCLC and lung squamous cell carcinoma (LuSCC) (2/3). All three patients had relapsed disease that harboured inactivating alterations in *RB1*, although it is unclear whether these alterations were present at diagnosis or acquired at relapse, except in one patient where the primary LuAD harboured an ICR in *RB1*.

Whilst this study tries to address the uncertainties relating to the definitive genetic drivers of non-smoking-related lung cancer, and the mechanisms involved in histological transformation and drug resistance, there are several considerations one should take into account.

Firstly, the definition of NSK-LuADs was such that at least one of the following criteria were met: 1) a never-smoker or >25-year ex-smoker; or 2) *EGFR-* or *ALK*-mutant; or 3) < 48-years of age. Whilst 8/11 patients were

never-smokers, whether this definition truly represents non-smoking-related lung cancer may be debatable, given the possibility of lung cancer occurring at a young age and the existence of EGFR-mutant disease in smokers, for example. Given the lower incidence of non-smoking-related lung cancer, there are of course difficulties in establishing a large enough patient cohort from which meaningful results can be obtained, but nonetheless the way in which a cohort is defined can have significant impacts on the conclusions one can draw. In this study, stringent classifications for smoking status are required if the results are to truly reflect non-smoking-related diseases.

Secondly, the PDCs were derived from patients with stage IV metastatic disease and the comparative validation analyses were performed in patients with early stage surgically resectable disease. These two cohorts differ in their disease stage and prior treatment histories; several of the patients from whom PDCs were derived had received either chemotherapy, immunotherapy or a TKI. Since lung cancers are known to evolve throughout the disease course and in response to therapies [6–9], comparisons between these cohorts may not reliably reflect a discovery and validation cohort.

Thirdly, whilst alterations in *RB1* were found to be present in the three patients with *EGFR*-mutant LuADs that developed TKI resistance and underwent histological transformation to SCLC or combined SCLC/LuSCC, it is unclear whether one can conclude that the underlying mechanism for either of these processes is directly driven by *RB1*. Indeed, clonal alterations in *RB1 and TP53* have previously been demonstrated in both *de novo* SCLC [10] and transformed SCLC in *EGFR*-mutant LuAD treated with TKIs [5]. Histological transformation is a known phenomenon in the context of drug resistance [5, 11, 12], and this alone may be responsible for TKI resistance and therefore disease progression. Notably, one of the three patients developed an *EGFR-T790M* mutation, which is the most common mechanism of EGFR-TKI resistance and likely driver of resistance in this case [13–15]. Besides, whether *RB1* inactivation is a feature of the tumour at diagnosis prior to treatment or whether it is acquired at the point of drug resistance, may give further insight into its potential role in either predicting or facilitating histological transformation.

Consistent with previous efforts [4], Pros and colleagues demonstrate that inactivated *RB1* is associated with histological transformation in *EGFR*-mutant lung adenocarcinoma. The fact that it appears to be in the form of ICRs involving *RB1*, gives us greater insights into the type of *RB1* genetic alteration that can be acquired in adopting the molecular and phenotypic features of a histologically transformed lung cancer and informs future studies exploring the mechanisms underlying this process.

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