Editorial

LACES and bootstraps: the hunt for prognostic and predictive markers for adjuvant therapy in NSCLC

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The benefit from adjuvant chemotherapy in resected non-small cell lung cancer (NSCLC) is modest with an overall survival (OS) benefit of only ~5% demonstrated in the Lung Adjuvant Cisplatin Evaluation (LACE) meta-analysis (1). Defining subgroups of patients more likely to derive benefit from chemotherapy promised to provide improved outcomes to those most likely to benefit, while sparing the toxicity and minimal survival advantage in others. Given that lung cancer is the leading cause of cancer-related mortality globally (2) and that even early stage resectable disease confers a dismal prognosis with 5-year survival rates of between 24–59% (3), predictive and prognostic biomarkers are urgently needed. Towards this, efforts to identify individual molecular markers using different platforms in resected NSCLC have included mutations in *TP53* and *KRAS* (4) as well as molecules involved in pathways abrogated by chemotherapy such as *ERCC1*, *RRM1*, *TOP2A* and *BRCA1* (5,6). In addition, several RNA based gene signatures, while appearing predictive and prognostic have not been taken forward due to logistical hurdles such as the need for snap frozen tissues and issues in consistency of data analysis (7). Subsequently, despite extensive investigation, no reproducible predictive or prognostic biomarker(s) for resected NSCLC have emerged.

NSCLC is characterized by genomic instability, with a high frequency of somatic mutations and extensive gene copy number variations (CNV) unique to an individual patient's tumour genome. Interestingly recent data using whole exome sequencing on resected samples suggested that increasing chromosomal instability and subsequent intratumoral heterogeneity was a poor prognostic factor (8). Thus, intratumor molecular heterogeneity can significantly impact different clinical outcomes and responses to treatment even in patients with the same TNM staging (9).

LACE-Bio is an international collaboration that utilised FFPE tumour samples from four major adjuvant chemotherapy trials (IALT, CALGB9633, ANITA and JBR.10) to investigate predictive and prognostic biomarkers including *ERCC1*, tumour infiltrating lymphocytes (TILs), mucin, beta tubulin, *KRAS*, *EGFR* and *TP53* (5,10). To date, these studies have not identified specific markers that can be used clinically but have provided important prognostic information nonetheless.

In the LACE-Bio2 study Rotolo *et al*. evaluated genome-wide CNV as prognostic and predictive biomarkers in early stage NSCLC. From an initial 1,013 available samples, following quality control checks, 976 samples from three major randomised phase 3 trials (CALGB, IALT and JBR.10) were profiled using the OncoScan CNV Plus Assay (ThermoFisher, Carlsbad, California, USA) (11). There were 414 (42%) adenocarcinoma (ADC) and 430 (44%) squamous cell carcinoma (SCC) samples as well as 132 (14%) other subtypes. The most frequent copy number (CN) gains were found on 1p13, 1q21, 3q22–26, 5p13–15, 6p24, and 22q11, the most frequent losses on 3p21.31, 8p23, and 9p21.3, consistent with other studies (12). The global CNV differences that exists among the different histologies of NSCLCs (13) were also confirmed in this analysis with more gains in 3q (including genes *PIK3CA*, *MECOM*, *CCNL1*), 22q (*NF2*, *PDGFB*) and 12p (*KRAS*) and more losses in 3p (*RASSF1*), 4p (*PTTG2*, *NKX2-1*) and 5q in SCC compared to ADC. The region 3q contains

three genes (*TP63*, *SOX2*, and *PIK3CA*) that are often implicated in SCC and several other oncogenes involved in the pathogenesis of SCC (13,14).

The authors have generated a large volume of data given the dataset and the platform used. The dataset enables not only prognostic markers to be interrogated, but also putative predictive markers based on the 491 samples from patients treated with adjuvant chemotherapy. Analyses of 976 samples identified few prognostic genes potentially impacting disease-free survival (DFS), OS and lung cancer specific survival (LCSS).

Losses in 9p21.3, 19p13, 18q12.1 and 19p11–13 and gains in 7p11–12 resulted in shorter DFS ($P \leq 0.005$, $Q \leq 0.142$) in univariate analysis. In multivariate analysis, only 9p21.3 (HRloss =1.5, $P<0.001$, $Q=0.02$) and 7p12.3 (HRgain =2.1, $P=0.002$) were found to be prognostic. Multivariate analysis also confirmed association between higher DFS and losses in 1p31–36 (HRloss =0.32, $P<0.001$). CN losses in 9p21, 18q12, 19p13 were also prognostic of shorter OS ($P \leq 0.005$, $Q \leq 0.092$) but only 9p21.3 was found to be relevant in multivariate analysis (HRloss =2.1, $P<0.001$). Amplification in 3q26 resulted in better OS (HRgain =0.55, $P=0.001$). One of the novel findings was the divergent genomic pattern seen in 14q23.1 with gains in short-term (HRgain =0.46) and losses in long-term survivors (HRloss =2.2). Other studies have also shown a prognostic impact of CN gains on 14q23.1–24.3 and losses on 14q31.1–32.33 in SCC (15). Preplanned sub group analysis of different histologies revealed significant different prognostic profiles of genomic regions including CN gains in region 7p11, 7q11, 11p14 and 20q11 as indicators of worse prognosis in ADC compared to SCC. Whereas gains in 1p13, 4p12–15 and 4q27 resulted in favourable prognosis in ADC and vice versa in SCC. Of these, only 1 region 11p14 was found to have statistically significant impact on OS in ADC ($Q=0.056$).

With regards to LCSS, multivariate analysis confirmed prognostic role for chromosomes 3q26, 9p21, 6p24, 8p23, 19p1 and 20q11.21 while penalized regression designated 17 prognostic regions on chr1, chr9, chr12, chr19 and chr20. The authors also found an association between elevated tumour CN heterogeneity (a marker of dynamic chromosome instability) and increased risk for disease recurrence (HR =1.5, $P=0.015$) and short LCSS (HR =1.7, $P=0.003$) but no impact on OS and treatment effect.

The validity of the copy number alterations (CNAs) as potential predictive biomarkers was not proven in this study. Regions in 14q32.33, 8p23 and 20q11 although potentially predictive of better survival following adjuvant chemotherapy were also associated with very high Q values and none significant in penalised regression.

Based on the results of LACE-Bio2 project, potential novel genes identified were 9p21 and chr19 encoding *STK11* and *FSTL*. Region 9p21 along with *CDKN2A/B* also codes for *p16* and *p14* which are tumour suppressor genes. *CDKN2/p16/MTS1* encodes *CDK4*, which is frequently abnormal in NSCLC (16% to 100%) (14). Other studies have also demonstrated that promoter methylation of *p16* is associated with recurrence after resection in stage 1 lung cancer, poor 5-year survival rate in resectable NSCLC and in the development of squamous-cell lung cancer (14,16). 9p21 had impact on DFS, OS and LCSS which was statistically significant in this study.

The *STK11* gene (chromosome 19) which is also a tumour-suppressor gene is frequently mutated in non-small cell lung tumours. Several studies have also found it to be associated with risk of metastases. In other studies, *STK11* mutations (including point mutations and deletions) were found to be more common in ADC than SCC (14). In the current study, it was associated with shorter DFS and OS.

Another novel prognostic gene *FSTL3* was identified with its amplification significantly increasing the risk of death. *FSTL3*, encodes a secreted glycoprotein, and transcriptional factors *MLLT1*, *SH3GL1*, and *TCF3*, and the guanine nucleotide exchange factor (GEF) gene *VAV1*. *FST* can weaken the Activin A-induced A549 cell apoptosis. Hoda and colleagues (17) showed high expression of Activin A in lung adenocarcinoma correlated with poor prognosis and tumour progression.

Even though regions in 14q32 and 20q11 had high false discovery rate as predictive regions for chemotherapy effect, they should still be considered for future validation. *MOAP1* (or MAP-1) is an interesting gene present in the survival region on chromosome 14q32.12 associated with BAX induces apoptosis. *MOAP1*-deficient cells show anchorage-independent cell growth (15). The 20q11.21 region is rich in genes such as *HCK* (tyrosine kinase), *BCL2L1* (apoptotic regulator), *MAPRE1* and *TPX2* (microtubule associated factors), *DNMT3B* (epigenetic modifier) and transcriptional regulators which may have potential role in cancer.

The LACE-Bio dataset is an impressive collaborative effort and the investigators should be congratulated for utilising available technology to better investigate their samples. However, it is notable that no independent validation of novel genes was performed and while deletions identified are likely to be real, CN gains can be difficult to interpret as the assay and analyses cannot account for

polysomy. Two important components missing from CNA evaluation were clustering based analyses and percentage genome alteration (PGA). Clustering of CNAs has often led to resolution of heterogeneity of the expression-only subgroups and successful identification of clinically important subgroups (18,19). Incorporation of clustering may have identified relevant CNAs as predictive biomarkers to chemotherapy. PGA is a global measure of CNA burden across tumour genomes which has proven to be of prognostic value in numerous cancers (20).

While the OncoScan platform has the ability to interrogate prespecified genomic regions, in contrast, whole genome (WGS) or whole exome sequencing of tumours using next generation sequencing (NGS) (21) is an unbiased approach that provides extensive genomic mapping. WGS can provide information both at the single nucleotide level, as well as detect structural variations such as large rearrangements, gross deletions and duplications. While at the time this study was conceived, WGS had limited applicability in FFPE tissues, more recent studies have been demonstrated that robust results can be generated (22,23).

In the era of immunotherapy, the hunt for biomarkers is proceeding at a rapid pace. As immunotherapies potentially move into adjuvant and neoadjuvant NSCLC paradigms, better genomic information will be required. However, despite important collaborative ventures such as LACE-Bio, our ability to both predict patients more likely to benefit and importantly identify patients who do not need adjuvant therapies remains elusive. The need to prospectively collect tissues and validate biomarkers is of utmost importance therefore in order to better personalise therapies. As genomic technologies rapidly evolve, it is feasible that better predictive and prognostic information will be available to better guide treatment decisions for patients undergoing adjuvant therapies.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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