Research Highlights

Highlights from the latest articles in translating genomics to clinical practice in lung cancer

Detection of *ALK* mutations in crizotinib-resistant non-small-cell lung cancer: moving the field into the next generation

Evaluation of: Huang D, Kim DW, Kotsakis A *et al.* Multiplexed deep sequencing analysis of *ALK* kinase domain identifies resistance mutations in relapsed patients following crizotinib treatment. *Genomics* pii: S0888-7543(13)00034-7 (2013).

Non-small-cell lung cancer (NSCLC) is a devastating malignancy, with most patients eventually succumbing to metastatic disease. However, a significant revolution is occurring in the field as we increase our understanding of NSCLC carcinogenesis and discover specific tumor-initiating molecular alterations – frequently known as drivers. In 2007, a translocation between the genes *EML4* and *ALK* was first described, affecting patients with lung adenocarcinomas [1]. Subsequently, *KIF5B* was also reported as a partner in *ALK* rearrangements [2]. These alterations tend to occur in less than 5% of lung adenocarcinomas, especially among patients without a past smoking history. Importantly, these rearrangements generate tumors highly dependent on the *Alk* pathway, which are therefore usually responsive to *ALK*-directed targeted therapy. Indeed, crizotinib – a first-in-class inhibitor of *ALK* – proved dramatically effective among patients with *ALK*-translocated NSCLC, causing tumor shrinkage in more than 50% of cases [3].

Despite the remarkable activity of crizotinib in *ALK*-translocated lung adenocarcinoma, resistance mechanisms eventually emerge, generally within less than 1 year. In fact, point mutations in the *ALK* kinase domain have been implicated

in a third of cases and their detection could potentially guide further therapies by using novel *ALK* inhibitors. In the current paper by Huang *et al.*, the authors describe the design and validation of a next-generation sequencing (NGS) platform to quickly detect these mutations in a clinical setting. The assay sensitivity was initially evaluated using different gDNA titrations from a resistant cell line harboring a *G1269A* mutation. Using this approach, the authors could call the *G1269A* mutation in allele frequencies as low as 0.8% with 99% confidence. Subsequently, the NGS platform was tested in samples from 13 patients with *ALK*-translocated NSCLC presenting with disease progression after crizotinib. Of note, an excess of 500-times coverage was obtained in exons 21–27 in all samples. Previously known *ALK* mutations were detected in three out of 13 patients, including *G1269A*, *C1156Y* and *L1196M* in one case each, with allele frequencies varying from 12 to 15%. The results in four cases were confirmed using standard Sanger sequencing and allele-specific PCR, including a case with the *G1269A* mutation. Two patients also had their baseline tumors tested using the NGS, but no abnormalities were detected.

In summary, Huang *et al.* put forward a NGS platform to detect *ALK* mutations involved in secondary resistance to crizotinib. This platform could be clinically useful in the near future, as novel *ALK* inhibitors prove active in crizotinib-resistant tumors harboring such mutations. NGS technology is progressively supplanting other currently used sequencing approaches [4], since the former tends to be faster, cheaper and more comprehensive. In

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addition, its utility can be further improved as other genes of interest are demonstrated in crizotinib-resistant cases.

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A 'genetic hub' signature as a prognostic and predictive marker in early-stage non-small-cell lung cancer

Evaluation of: Tang H, Xiao G, Behrens C *et al.* A 12-gene set predicts survival benefits from adjuvant chemotherapy in non-small-cell lung cancer patients. *Clin. Cancer Res.* 19(6), 1577–1586 (2013).

Adjuvant chemotherapy (ACT) is an established strategy in early-stage non-small-cell lung cancer, with a proven survival gain in numerous Phase III trials [1–3]. However, the absolute benefit at 5 years is only modest – approximately 5% – and therefore most patients will not derive any advantage from it while eventually experiencing mid- and long-term side effects. Hence, there is a need to develop *bona fide* genetic signatures that will identify patients with good or poor prognosis, or even more importantly, those deriving the benefit from ACT or not. In the current paper, Tang *et al.* present a novel signature based on selected, highly informative genes, useful both as a prognostic and a predictive marker. As opposed to other

signatures based on individual features, the authors applied a systems biology approach to identify candidate 'hub' genes. Each of these genes are connected to at least seven others in a survival-related network, minimizing the information redundancy.

Using a publicly available consortium dataset as a training cohort ($n = 442$), the authors initially found 797 genes whose expression correlated with survival. Subsequently, 18 'hub' genes were selected based on the constructed network and a prognostic signature was designed using their expression levels. This signature was validated in five independent datasets, with the high-risk group consistently presenting with worse survival than the low risk. Of note, the prognostic role of this signature was specific to adenocarcinomas. In order to assess the predictive role of the signature, 12 genes were selected based on their previously demonstrated 'synthetic lethality' with chemotherapy or genes known to commonly harbor genetic aberrations in lung cancer. The selected genes were: *DOCK9*, *RRM2*, *AURKA*,

HOPX, *NKX2-1*, *TTC37*, *COL4A3*, *IFT57*, *C1orf116*, *HSD17B6*, *MBIP* and *ATP8A1*. The 12-gene signature was validated as both prognostic and predictive in the testing cohorts, with high-risk patients deriving significant survival benefit from ACT, while low-risk patients had no benefit whatsoever. This signature based on selected 'hub' genes is promising and warrants further investigation in prospective trials.

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Novel utility of miRNA-associated SNPs in early-stage non-small-cell lung cancer clinical assessment

Evaluation of: Pu X, Roth JA, Hildebrandt MA *et al.* MicroRNA-related genetic variants associated with clinical outcomes in early-stage non-small cell lung cancer. *Cancer Res.* 73(6), 1867–1875 (2013).

Major advances have been made in the treatment of early stage cancers, specifically, malignancies of the breast, colon and prostate; however, the 5-year survival rate for lung cancer patients with localized disease remains just above 50%, as opposed to 98, 90 and 100% for breast, colon and prostate tumors, respectively [1].

Improved modalities for risk stratification of patients with early stage lung cancer could lead to improved outcomes. Therapeutic and prognostic biomarkers would allow oncologists the opportunity to administer the most optimal treatment for select patients while avoiding unnecessary regimens of adjuvant therapies following

curative intent surgery in early stage non-small-cell lung cancer (NSCLC) [2–4]. In addition to sensitizing mutations/translocations in NSCLC including EGF receptor and ALK, miRNAs have emerged as biomarkers that may guide decision making in lung cancer [5,6]. Previous studies have shown that these functional 22-nucleotide ncRNAs may function as either oncogenes or tumor suppressor genes. In the current study, Pu and colleagues hypothesized that SNPs in miRNA seed regions of cancer-related genes or genes essential to miRNA bioprocessing could predict clinical outcomes in early stage NSCLC patients.

Pu *et al.* identified miRNA-related genetic variants (240 SNPs) associated with clinical outcomes in early stage NSCLC patients ($n = 535$): survival, time to recurrence and treatment-specific benefit. The investigators identified a FAS:rs2234978 SNP in which all patients with the GA + AA genotype had an improved overall survival (hazard ratio [HR]: 0.59). After dividing patients into treatment-specific subgroups: surgery or surgery + chemotherapy (HR: 0.19 and 0.59, respectively), the FAS:rs2234978 SNP consistently exhibited a positive correlation with clinical outcome. Using luciferase reporter assays containing either wild-type or variant FAS constructs in two representative lung cancer cell lines (H460 and H2444), the investigators demonstrated that FAS:rs2234978 created a functional miR-561 binding site and cotransfection of miR-561 with the variant FAS reporter led to decreased relative

luciferase activity, suggesting functionality. A second set of SNPs, SP1:rs17695156 and DROSHA:rs6886834, correlated with negative clinical outcomes. The SP1 variant had the highest association with risk of death, with a 3.36 HR in the overall patient population. However, *in vitro* studies failed to demonstrate any difference between wild-type and variant alleles. The DROSHA:rs6886834 SNP exhibited a nearly sixfold increase in risk of death, with a greater than tenfold reduction in median recurrence-free time compared with the wild-type genotype among surgery-only patients. In an unexpected finding, the researchers identified SNPs displaying opposite clinical outcomes between treatment subgroups. The FZD4:rs713065 SNP predicted an increase in survival time among surgery-only patients (HR: 0.46; 117 vs 59 months). However, in surgery + chemotherapy patients, the presence of the FZD4:rs713065 SNP was associated with a lower median survival time (84 vs 132 months) and a HR of 1.50.

In conclusion, the authors have identified novel miRNA-associated SNPs in early stage NSCLC that correlate with clinical outcome. The functional and mechanistic implications for these variants remain largely unknown. This represents an important initial step in the clinical application of miRNAs. However, in order for miRNAs to truly guide therapeutic decisions in lung cancer, both a better understanding of underlying mechanisms and larger clinical validation will still be required.

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