ESMO *Open*
Cancer Horizons

In the literature: December 2019

Valentina Gambardella,[1,2] Amelia Insa,[1] Juan-Miguel Cejalvo,[1,2]
Andrés Cervantes [1,2]

PAN-CANCER LANDSCAPE AND ANALYSIS OF ERBB2 MUTATIONS IDENTIFIES POZIOTINIB AS A CLINICALLY ACTIVE INHIBITOR AND ENHANCER OF T-DM1 ACTIVITY

The introduction of new high-throughput technologies in oncology and the need to apply precision medicine for cancer patients has led to the detection of several molecular alterations. Among them, activating mutations of ERBB2 have been reported in many solid tumours. In the last years, several clinical trials with covalent tyrosine kinase inhibitors (TKIs) for ERBB2 mutant cancers have been conducted, with different results among several cancer types. In the SUMMIT trial, neratinib was most effective in breast cancer patients, with the majority of responders having tumours with L755S, V777L, or L869R ERBB2 mutations.[1] In an elegant article published in *Cancer Cell* by Robichaux *et al*,[2] different TKI sensitivities between malignancies might be explained by cancer-specific mutational hotspots. In this experiment, specific exon 20 insertions were associated with neratinib sensitivity in breast cancer patients, nevertheless, these were associated with resistance in other cancer types, demonstrating that there may be other mechanisms underlying these tumour-type-specific differences.

The molecular dynamic simulations showed that exon 20 insertion mutations and the exon 19 L755P mutation induced conformational changes that affected the overall size and shape of the drug-binding pocket, justifying the lack of response with the majority of TKIs. To further corroborate these data, in non-small cell lung cancer (NSCLC), where exon 20 insertions frequently occur, patients harbouring ERBB2 exon 20 insertion mutations had response rates of 0%, 11.5% and 18.2% to 18.8% to neratinib, dacomitinib and afatinib, respectively. Moreover, while L755S mutations have been shown to respond to neratinib, L755P mutations are profoundly resistant to both TKIs and antibodies drug-conjugated. Interestingly, when a panel of covalent and non-covalent EGFR and HER2

TKIs was tested against the most common HER2 mutants, it was possible to observe that poziotinib has activity against the most common HER2 variants, including exon 19 and 20 mutants that are resistant to other HER2 TKIs.

Poziotinib is an anilino-quinazoline scaffold and inhibits the epidermal growth factor receptors EGFR, HER2 and HER four and binds covalently to its targets. This drug can bind deeply within the hydrophobic cleft created by A751, K753, L796 and T798 in HER2 exon 20 mutant. Previous preclinical data in lung cancer and current preclinical models of breast and colon cancer demonstrate that poziotinib has broad antitumour effects in multiple ERBB2 mutant cancer types. In a clinical trial, enrolling patients with ERBB2 exon 20 mutant tumours, an overall response rate of 42% was observed with median progression free survival (PFS) of 5.6 months. Moreover, poziotinib was also active in patients with L755P mutations.

As it was known, TKIs could cause accumulation of HER2 on the cell surface, which enhanced trastuzumab binding and could therefore increase its antitumour effects. The combination of poziotinib and T-DM1 was also tested and resulted in a decrease of cell viability in vitro and caused complete regression of HER2 exon 20 mutant NSCLC tumours in mice. A high affinity and specificity of poziotinib for HER2 mutants was observed, making poziotinib a good candidate for combining with T-DM1 by enhancing mutant HER2 on the cell surface for appropriate targeting with T-DM1. Further investigation about the potential role of the combination of poziotinib and T-DM1 is awaited.

CLONAL EXPRESSION BIOMARKER ASSOCIATES WITH LUNG CANCER MORTALITY

Multiple attempts have been made to develop gene expression-based prognostic signatures for lung adenocarcinoma to stratify patients into different predictive subgroups beyond clinical variables. However, none

[1]Department of Medical Oncology, Biomedical Research institute INCLIVA, University of Valencia, Valencia, Spain
[2]CIBERONC, Instituto de Salud Carlos III, Madrid, Spain

Correspondence to
Professor Andrés Cervantes; andres.cervantes@uv.es

of the, so far, tested signatures are ready for clinical application. Transcriptomic intratumour heterogeneity (RNA-ITH) has been reported in many cancer types and has been shown to limit the application of novel molecular biomarkers in diagnostic tumour samples.[3] In a recently published article in *Nature Medicine*, Biswas and colleagues[4] assessed the impact of RNA-ITH in NSCLC and were able to identify clonal transcriptomic biomarkers that may overcome tumour sampling biases and associate with overall survival (OS). For this purpose, they utilise three RNA sequencing (RNA-Seq)-based expression datasets from patients with early-stage lung cancer.

First, they investigated the effect of RNA-ITH and sampling bias on previously published prognostic signatures using patients from the TRACERx cohort[5] and reported a discordance rate that range from 18% to 82% at the level of individual patients, regardless of the expression profiling technique used (RNA-Seq or microarrays). These data suggested that tumour sample bias limits as expected the clinical utility of gene expression signatures in NSCLC. As next step, to improve biomarker design and to reduce sampling bias due to RNA-ITH, they defined a core set of clonally expressed genes exhibiting high inter-tumour heterogeneity and low ITH. This set of genes was enriched for genes with a reproducible survival association in multiple patient cohorts and exhibits higher pan-cancer prognostic ability than other groups of genes. These genes are linked to biological features of tumour aggressiveness and enriched in pathways involved in cell proliferation.

The authors designed a new strategy and used some of these genes to generate a 23-gene-prognostic signature called the outcome risk-associated clonal lung expression (ORACLE) biomarker. ORACLE compares favourably with the discordance rates for existing prognostic signatures and only 11% of TRACERx-enrolled patients with lung adenocarcinoma showed discordant classification using ORACLE. In a small cohort of patients with stage I disease, ORACLE could separate patients into high-risk and low-risk groups with significantly different survival times. In addition, they showed that ORACLE signature positively correlates with histological markers of cancer cell proliferation and with the tumour copy-number state at the corresponding gene locus.

Finally, the authors explored the mechanisms underpinning RNA-ITH. They assessed the effect of varying the number of samples per tumour and found that RNA-ITH scores saturated with increasing sample number. They evaluated the relation to immune infiltration and showed that RNA-ITH does not correlate with any of the immune cell subsets. In contrast, there is a significant correlation between the median RNA-ITH score per tumour and somatic copy-number alteration (SCNA)-ITH per tumour. It seems clear that SCNA-ITH may contribute to transcriptomic heterogeneity and chromosomal instability might be a major driver of RNA-ITH. Moreover, this work addresses RNA-ITH as a

potential confounding factor for biomarker design and how clonal transcriptomic biomarkers may overcome sampling bias arising from ITH.

PTEN LOSS MEDIATES CLINICAL CROSS-RESISTANCE TO CDK4/6 AND PI3KΑ INHIBITORS IN BREAST CANCER

Cyclin dependent kinase 4 and 6 (CDK4 and CDK6) inhibitors have become the standard of care for advanced hormone receptor positive breast cancer. Several phase III studies have demonstrated that CDK4/6 inhibitors significantly improve PFS and even OS in combination with endocrine therapy in this setting. The PALOMA-3 study was the first randomised, placebo-controlled, phase III trial to compare fulvestrant plus palbociclib/placebo in advanced luminal breast cancer who had disease progression after previous endocrine therapy.[6] Recently, the phase III MONALEESA-3 trial, showed a statistically significant OS prolongation with ribocilib over placebo.[7]

With CDK4/6 inhibitors as a standard of care, it is essential to identify their mechanisms of resistance and to develop novel strategies after clinical progression. Different resistance biomarkers identified in preclinical models are RB1 loss, cyclin E1, cyclin E2 and CDK6 amplification.[8] In fact, RB1 loss does appear to drive resistance in the clinic. Costa *et al* recently published an article in *Cancer Discovery* that proposes *PTEN* loss as mechanism of resistance to CDK4/6 and PI3KΑ inhibitors in breast cancer.[9] They analysed paired pretreatment and at progression biopsies from some postmenopausal patients treated with ribociclib plus letrozol with luminal metastatic breast cancer. They suggest that acquired loss of either RB or PTEN expression confer resistance to CDK4/6 inhibitors.

To confirm their hypothesis, they used CRISPR-based *PTEN* knock-out T47D and MCF7 cells. All *PTEN*-null clones showed decreased sensitivity to palbociclib and ribociclib by maintaining RB phosphorylation. In addition, ectopic expression of the wild-type form of PTEN restored the sensitivity to CDK4/6 inhibitors. These data were confirmed in an in vivo model with *PTEN*-deficient T47D xenograft. As expected, *PTEN*-deficient cells had increased levels of phosphorylated AKT. Therefore, the combination of CDK4/6 plus AKT inhibitors reversed the effect of *PTEN* loss on resistance to CDK4/6 inhibitors and restored their capacity to suppress RB phosphorylation and cell cycle progression. This work finally explored the mechanism of how increased AKT activation leads to resistance to CDK4/6 inhibitors. The study reported that loss of *PTEN*, through increased AKT activity, induces delocalisation of p27 outside the nucleus and therefore, causes an increased activity of both CDK4/6 and CDK2, which together contribute to overcome the blockade in G1 induced by CDK4/6 inhibitors. Overall, this manuscript suggests that *PTEN* loss causes resistance to CDK4/6 inhibitors by initiating signalling cascades that induce hyperactivation of cyclins/CDKs. Moreover, this work underlines a proof of concept indicating how a

single genetic event can cause cross-resistance to multiple targeted therapies and may affect subsequent lines of treatment.

ORCID iD

Andrés Cervantes http://orcid.org/0000-0003-3806-3691

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