Commentary

Do mRNA profiles of lung adenocarcinomas provide information that will help individual patients?

Mitchell S. von Itzstein\textsuperscript{a,b}, David E. Gerber\textsuperscript{a,b}, John D. Minna\textsuperscript{a,b,*}

\textsuperscript{a} Department of Internal Medicine, Division of Hematology and Oncology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX, 75390, USA
\textsuperscript{b} Human Center for Therapeutic Oncology Research and the Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX, 75390, USA

Currently every new non-small cell lung cancer (NSCLC) patient being evaluated for specific treatment has a history, physical exam, a series of radiologic procedures (computed tomographic and often PET scans), standard blood work, and evaluation of their lung cancer tumour biopsy to establish a histologic diagnosis of lung cancer, to help determine the individual patient’s prognosis, and to select an appropriate treatment regimen. Key clinical elements going into these decisions are the type of lung cancer (e.g. non-small cell vs. small cell, adenocarcinoma vs. squamous cancer), the stage of the tumour (e.g. localized or metastatic), patient performance status, and co-morbidities. In the past decade patients also receive an evaluation of tumour molecular characteristics (by DNA sequencing of a tumour biopsy for mutations) that could lead to targeted therapy (e.g. presence of an EGFR mutation leading to EGFR tyrosine kinase inhibitor therapy). In addition, with the demonstration of significant survival benefit from immune checkpoint inhibitor (ICI) therapy in a subset (15 – 20%) of non-small cell lung cancers (NSCLCs) there is a concerted effort to determine which molecular and immunologic characteristics of the patient and the tumour or its microenvironment predict response to immunotherapy [1]. Finally, there has been a decades long search for tumour molecular characteristics (e.g. mRNA or protein expression) that provide prognostic information over and above that provided by standard clinical data. While such prognostic information is important, it does not provide much solace to tell patients that provided by standard clinical data. While such prognostic information is important, it does not provide much solace to tell patients that

In this article of \textit{EBioMedicine}, Zhang et al. try to advance this effort by showing that a five-gene (TNFRSF - 6B, 13C, 14, 1A, 27/EDA2R) tumour necrosis factor (TNF) family tumour transcriptomic signature can stratify patients with lung adenocarcinoma (LUAD) into distinct prognostic cohorts [2]. Importantly, they also find these TNF family gene expression profiles associate with distinct tumour microenvironment (TME) immune cell composition. The TME information on individual LUADs their signature generates, potentially could also provide information related to selecting immune therapy for individual patients. Their study in general represents a computational biology analyses of large deposited mRNA datasets to find which LUAD TNF family member expression differences are associated with “high” (worse prognosis) vs. “low” (better prognosis) risk survival. They also provide an independent validation that their 5-gene signature provides prognostic information in their own wet lab evaluation of 102 LUADs. Using a series of computational tools developed by others to take bulk RNA expression information and discern the tumour composition for tumour and different immune microenvironment cells, they found that the 5-gene TNF signature associated with worse prognosis also was associated with an immune suppressive (non-immunogenic) tumour phenotype. Their high-risk cohort had increased populations of activated natural killer cells, neutrophils, activated dendritic cells, and M2 (immunosuppressive) macrophages in the tumour microenvironment, while the low-risk cohort had increased populations of memory B cells, inactivated dendritic cells, CD4+ T cells, gamma delta T cells, and M1 (immune-stimulatory) macrophages. These findings suggest that well-known immune pathways may have prognostic importance in LUAD. Of course, the real issue is whether information from their 5-gene signature would provide information on which LUADs would or would not respond to immune checkpoint blockade or other types of immunotherapy. Since their “high-risk” cohort had increased tumour PD-L1 expression and mutational burden, features associated in other studies with response to ICI, it raises the possibility that their high-risk cohort, although having an immunosuppressive TME might actually be candidates for ICI therapy. It is this obvious contradiction in what one would expect, that the Zhang et al. study brings to light. Namely, at first glance, one would think that the immunologically active “low-

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* Corresponding author: John D. Minna, MD.
E-mail address: John.Minna@utsouthwestern.edu (J.D. Minna).

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risk” cohort would be more likely than the “high-risk” immunosuppressed cohort to respond to ICI. This contradiction is an eloquent demonstration that such computational analyses lead not to answers but to hypotheses that must be tested in clinical trials. The reason this is stressed, is that it is important to note that the authors also recently published a very similar study exploring the same LUAD deposited datasets where they identified four genes (ARNTL2, ECT2, PP1A, TUBA4A) whose expression they felt gave them prognostic information related to a tumour’s immune status and which they labelled an “immune-related prognostic signature” [3]. In this regard, an obvious question in deciding how to deploy scarce clinical trial resources, is how did the current 5-gene TNF family signature perform compared to their recent 4-gene signature since the authors felt both were moving in the same direction with regard to the TME? While the authors do not make this comparison in the current paper, from looking at the receiver operator curves in the two papers, it seems that their prior 4-gene signature may actually perform better in terms of patient prognosis than their current TNF family 5-gene signature. In addition, it would have been of interest to see if integrating the two signatures together performed better than either signature alone, both for prognosis and for delineating the TME. At the end of the day, the most important issue is if one uses these signatures to characterise LUADs, does that provide predictive information on how the individual patients respond to immune checkpoint blockade? If either do, they represent a significant clinical-translation step towards helping patients. Equally important, from a mechanistic point of view — are any of the 9 genes (the 5 TNF members and 4 gene set) involved in how the tumour initially escaped immune surveillance, and how patient responds to immunotherapy? Thus, could the protein products of these genes be therapeutic targets in themselves?

The prognostic importance of TME composition in patients with LUAD using similar transcriptomic approaches has been described by several studies [4]. Similar gene expression studies discovered a favourable prognosis signature that includes KLRB1 and correlated with the leucocyte composition of the tumour immune microenvironment. By contrast, increased tumour associated neutrophils associated with worse outcomes for patients with LUAD [4]. Recently, an “immuno-predictive” gene expression model (“IMPRESS”) was found to associate with improved ICI efficacy in patients with melanoma and performed better (area under the curve of 0.83) than existing predictors [5]. Thus, any new signature, such as the Zhang TNF signature, will need to be compared in future analyses using deposited datasets with other predictive signatures. While some of these may be lineage specific (e.g. LUAD compared to melanoma) it is likely that several of these will predict ICI responses across tumour types — all things that need to be evaluated going forward. It is important to note from a clinical perspective, that patients receiving ICI can have a wide range of therapy related adverse events ranging from very mild to severe autoimmune disease effects. Patient biomarkers (e.g. cytokine expression in blood samples) predicting adverse events from ICI are thus equally important to establish [6]. For example, amongst patients receiving ICI therapy, decreased baseline and increased post treatment levels of the T cell activating interferon gamma inducible cytokines associated with increased risk of immune-related adverse events [7].

Do their TNF family signature point the way to mechanistic insights and perhaps new therapy targets? The TNF axis plays complex roles in a multitude of signalling pathways, and has been implicated in both tumour promotion and suppression [8]. TNF signalling plays a central role in immune cell regulation, including T cell activation by co-stimulation, modulation of the tumour immune microenvironment, and increasing tissue inflammation [8]. Inhibition of anti-tumour immune responses can occur with TNF signalling by altering the tumour immune microenvironment to a non-immunogenic phenotype [9]. In addition, TNF expression is particularly relevant to LUADs harbouring EGFR activating mutations (which are often refractory to ICI), since resistance to EGFR inhibitor therapy is mediated by increased TNF expression in preclinical models [10]. In this regard, TNF pathway modulators are currently under evaluation as therapeutic agents in cancer clinical trials [8].

Overall, Zhang and colleagues have identified two prognostic transcriptomic signatures for LUAD that also reflect composition of the tumour immune microenvironment. Now the challenge is to clinically translate these and similar prognostic signatures to see if they are predictive of ICI responses in a manner that can improve clinical selection of patients for immunotherapy.

Contributors

All authors (M.S.vI, D.E.G, and J.D.M) reviewed the relevant literature, created the concepts within, contributed to the ideas present, and wrote and edited the Commentary.

Declaration of interests

M.S.vI has nothing to disclose. D.E.G reports financial activities related to involvement in Bristol-Myers Squibb trial steering committee, and patent pending “Prediction and Treatment of Immunotherapeutic Toxicity” (provisional, application number 62/461,455). J.D. M. has a patent pending (US-2015–0,017,210-A1, “Gene signature predicting adenocarcinoma prognosis and therapeutic response”) and he receives cell line licensing fees from the NCI, USA and UT Southwestern Medical centre. There are no other relevant interests including in work under consideration for publication, intellectual property, or relevant financial activities.

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