

## Spotlight

## The ongoing mystery of renal cell cancer

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Clear cell renal cancer has had a durable reputation for being immunogenic and immuno-responsive. Beginning with rare but well-documented instances of spontaneous regression, continuing with its low but consistent response rate to interleukin-2 (with a 5%–7% long-term cure rate for metastatic disease),<sup>1</sup> and most recently its response to immune checkpoint blockade (ICB), there has always been something special about renal cell cancer (RCC) and the immune system. Studies of another immunogenic cancer, melanoma, have revealed the central role played by the products of tumor-specific mutations, which generate T cell responses and drive the clinical responses to multiple immunotherapies.<sup>2</sup> This “neoantigen” paradigm could be extended to many cancers, best exemplified by the very high responsiveness of tumors with DNA repair defects (generating huge numbers of potential neoantigens) to anti-PD1 antibody therapy. All seems right in the immunotherapy world until one looks at clear cell renal cancer. With a very modest level of tumor-associated mutations (fewer than immune-resistant cancers such as microsatellite-stable colon cancer),<sup>3</sup> large clinical studies also showed that there was no relationship within RCC patients between the tumor mutational burden and likelihood of response to ICB. Explanations are needed.

In a recent *Cancer Cell* paper, Au et al.,<sup>4</sup> with investigators associated with the TRACERx (TRACKing renal cell Cancer Evolution through Therapy) consortium and PEACE (Posthumous Evaluation of Advanced Cancer Environment) study, have published an in-depth sequential look at 15 patients over the course of anti-PD1 therapy using a variety of genomic, transcriptomic, and proteomic

platforms. Using multi-regional sampling, they focused on both tumor and T cells before treatment, upon treatment, and, in three cases, at autopsy. Efforts were made to look at parameters associated with clinical response, but given the intensity of the analyses, there were only five responding patients and ten non-responding patients studied, so these correlations have limited power.

First, the reassuring news—there is circumstantial evidence that T cells are involved. T cell receptor sequencing of biopsies showed that the more abundant (clonally expanded?) T cell clones in the pre-treatment samples persisted in the tumors after anti-PD1 antibody in responders but not in non-responders. In addition, an effector phenotype (characterized by molecules such as granzyme B and enrichment of immune activation gene sets) was more prominent in the on-treatment intratumoral CD8 cells of responders than non-responders. Deeper dives in a few patients proved interesting as well. Multiplex flow showed that tumor-infiltrating CD8 cells from a responding patient had a higher frequency of cells with a stem-like phenotype (TCF1+) combined with an exhausted phenotype (PD-1+, CD39+, TIM-3+) than the tumor-infiltrating lymphocytes (TILs) of a non-responding patient. This apparent paradox has also been seen in melanoma and is in accord with the hypothesis that some T cells are arrested in a reversible “exhausted” state with remaining capacity for proliferation and activation and these are the cells that primarily drive clinical responses.<sup>5</sup> The authors also document through autopsy in one patient the acquisition of a DNA mismatch repair defect (MLH1 loss) concurrent with loss of all MHC class I (through biallelic B2M loss) in sites of progressive tumor but not sites

that were still under control by ICB therapy. This is likely to be a very rare conjunction, but it is perhaps most interesting in anecdotally supporting a role for neoantigen-reactive class I restricted T cells in tumor control in this patient.

On the other hand, analysis of tumor cells sheds no light on the antigens recognized by these T cells. The authors confirmed the findings of others, that tumor mutational burden did not affect response status and that overall T cell and CD8+ T cell infiltrates did not either. RCC is known to have increased numbers of insertions and deletions compared to other cancers, which could generate long aberrant neo-peptides, but ICB responders did not have more indels. Childs et al.<sup>6</sup> reported that T cells recognizing human endogenous retroviral (HERV) sequences could be found in the blood of a patient during regression of RCC, so expression of HERVs was also examined in the current study. First, they found that HERV expression from bulk transcriptomics was not reliable in that HERVs previously associated with immune responses were actually being expressed more by infiltrating immune cells than by tumor cells. Given that there is no clear consensus on how many such integrations are in the human genome, what their structure is, and which ones are actually expressed, the investigators tended to find no differences in tumor-specific HERV expression related to treatment response.

This raises perhaps the crucial question for renal cancer immunologists. Which cells are responding to immunotherapy and what mechanisms cause tumor regressions? Nearly a decade ago, studies on melanoma quickly revealed the presence of T cells showing autologous tumor recognition through targeting neoantigens<sup>7</sup>



(as well as normal tissue differentiation proteins and some tumor-germline antigens), but this has not happened for RCC. In fact, multiple studies have shown that (unlike in melanoma and some other cancers) a higher T cell infiltrate in RCC is associated with a poorer prognosis. Given the presence of PDL-1 on tumor as a biomarker for a better chance of response to anti-PD1 in many tumors, there is also no indication it has any significance in ICB treatment of RCC.

In summary, the study by Au et al. has ambitiously combined in-depth analyses of both tumor and T cells in a longitudinal patient-specific manner, trying to understand the mechanisms of response to ICB. Although some data tangentially point to a T cell response as the driver of RCC rejection, many parameters that associate with response to ICB in other cancers either are of no utility in RCC or predict the opposite outcome. The fact that a molecular identification of tumor-associated antigens recognized by T cells in renal cancer is lacking should open us up to possibilities outside of the

standard paradigms for other cancers. A focused effort is needed to define the antigens for T cells recognizing autologous RCC tumor lines, or for the clonally expanded and retained T cells in ICB responders. Once we know what they are seeing, our own eyes may be opened.

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

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