Therapeutic Guide for mTOuRing through the Braided Kidney Cancer Genomic River

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SUMMARY

mTORC1 inhibitors were first approved for the use in metastatic kidney cancer. However, observed treatment benefit was highly heterogeneous among patients. Through case-based cancer genomic sequencing of therapeutic outliers, we can begin to appreciate the convergent evolution of given cancer pathways/phenotypes beyond genes in kidney cancer, like a braided river.

In this issue of *Clinical Cancer Research*, Kwiatkowski and colleagues (1) report findings correlating mutations in *TSC1*, *TSC2*, and *MTOR* with response to rapalogs in patients with metastatic renal cell carcinoma (mRCC). The authors analyzed 560 cancer genes from tumor tissues of 79 mRCC patients treated with mTOR inhibitors who displayed distinct clinical outcomes. Response was defined as either partial response, or stable disease with any tumor shrinkage for at least 6 months, and non-response was progressive disease within the first 3 months of therapy. Based on such definitions, 43 responders and 36 non-responders were included. By focusing on somatic mutations of the 5 core mTOR pathway genes (*MTOR*, *TSC1*, *TSC2*, *PTEN*, and *PIK3CA*), these authors found that each of these five gene mutations were more frequently detected in responders than non-responders. This study added significant support to the available next generation sequencing (NGS) literature suggesting that *TSC1* and *TSC2* loss-of-function, or *MTOR* activating mutations could predict therapeutic benefits to Everolimus or Temsirolimus in various cancer types (2). Intriguingly, this study also demonstrated that most (56%) responders did not and some (22%) non-responders did carry these mutations, respectively, explaining why only marginal statistical significance was observed.

What can we learn from the cancer genomics of these outliers in terms of deciphering individual cancer pathobiology and possibly making treatment decision? We cancer doctors/ researchers need to better leverage NSG data with a few caveats in mind. The first remark is "Number Matters", i.e. sequencing coverage, copy number, and how many regions sequenced are important determinants for the value of individual mutations. High-coverage

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and multi-regional sequencing better capture the genomic landscape of analyzed tumors (3, 4). The second remark is "Frequency Matters", i.e. varied allelic frequencies of mutations connote important therapeutic significance. Reported mutation frequencies of a given gene are heavily influenced by stromal contribution and clonal evolution. For example, clear cell renal cell carcinoma (ccRCC) is in principle a *VHL* mutated disease (5). *VHL* mutations represent the truncal mutation event. Hence, the allelic frequency of *VHL* mutation can be used to gauge tumor purity. By assessing allelic frequencies of co-detected mutations within the same tumor sample one could assess the clonal composition within the tumor. The third remark is "Position Matters", i.e. not all missense mutations are the same. The position of detected missense mutations and corresponding amino acid changes could either represent a real gain-of-function or simply a passenger mutation. For example, missense mutations clustered within the FAT or Kinase domains are likely activating mutations whereas those scattered thorough the HEAT domains are likely passenger mutations (5–7). The fourth remark is "Site Matters", i.e. sequencing obtaining from primary or metastatic tumors of the same patient carries different therapeutic significance. For example, the primary tumors of ccRCC tend to be fairly large, and encompass tens/hundreds or more subclones (3). Which clone(s) eventually metastasize and take life of the afflicted patient is likely miss- or under-represented if only a small piece of the primary tumors was sequenced. The fifth remark is "Time Matters", i.e. the chronology of samples acquired for sequencing is important. For example, cancer genomics before treatment could offer prognostic/predictive values, whereas genomics after treatment likely offer clues concerning adaptive resistance mechanism. Incorporating these NGS "Matters" with corresponding therapeutic outcomes, we can now attempt reconciling these seemingly contradictory results about mTOR pathway mutations detected in both responders and non-responders.

One key lesson learned from studying targeted therapeutic outliers of a given cancer type is the recurrent theme about convergent evolution on select sets of oncogenic pathways (2, 6). More importantly, such pathway or phenotype convergences take places within the same tumor, among tumors of the same patient, and probably shared by the same histopathological subtype of given cancer types despite intra- or inter-tumor heterogeneity (2). For example, mTOR pathway activation due to either *MTOR* activation mutations or *TSC1* loss-of-function mutations occurred at high frequencies in ccRCC where *VHL* mutation serves as the universal tumor-initiating event. This could explain why the two main categories of targeted therapeutic agents approved for the treatment of metastatic ccRCC are inhibitors of vascular endothelial growth factor (VEGF) or mTOR signaling pathways (8). It also supports the notion that cancer metabolism plays key roles in ccRCC pathogenesis (9). As VHL-loss and the resulting HIF hyperactivation are nearly universal in the pathogenesis of ccRCC, it would predict VEGF inhibitors such as Sunitinib, Pazopanib, and Axitinib to be more efficacious than mTOR inhibitors such as Everolimus and Temsirolimus in ccRCC (10). This was indeed supported by multiple randomized clinical trials comparing these two kinds of agents in mRCC. For example, Record-3 (11) a randomized trial comparing Sunitinib with Everolimus in previously untreated mRCC patients demonstrated median progression-free survival (PFS) with Sunitinib at 10.7 months and Everolimus at 7.9 months.

On the other hand, some RCC patients experienced markedly longer survival on mTOR inhibitors. To help improve depiction of cancer evolution and advise therapeutic selection of

targeted agents, we proposed a novel "braided river model" (2). This model illustrates parallel and convergent events occurring throughout tumorigenesis. Starting from initiating driver mutations, it depicts the stepwise acquisition of different driver mutations (early, intermediate, late and speedy drivers) during cancer evolution. In ccRCC, *PBRM1*, a chromatin remodeling SWI/SNF complex gene, is the second most commonly mutated gene (~45%) (5). It was reported that RCC tumors with mutant *PBRM1* associates with longer PFS on Everolimus at 11.1 months than those with wild-type *PBRM1* at 5.3 months (12). It implicated that mTOR pathway activation through various means not limited to mutations in *MTOR/TSC1/TSC2* could be the preferred route for ccRCC pathogenesis after mutations of *VHL* and *PBRM1*.

Here, we employ three different ccRCC evolution scenarios that all start with *VHL* mutations and incorporate mTOR activation to illustrate the importance of knowing the spatiotemporal sequence of mTOR activation during pathogenesis, and surmise how sequences could impact treatment outcome (Fig. 1). Scenario *(1)* starts with *VHL* mutation, followed immediately by mTOR pathway mutation, which renders nearly all ccRCC subclones of this tumor addicted to the mTORC1 hyperactivity. Scenario *(2)* carries *PBRM1* mutation as the early (second) driver event, followed by mTOR pathway activation as the intermediate (third) driver event, which renders major clones of this tumor sensitive to mTOR inhibitors. Scenario *(3)* starts with *VHL* mutation, followed by a few early, intermediate pathway convergences that do not involve *PBRM1* mutations or direct activation of mTOR pathway, and only acquired mTOR pathway mutations late as late or speedy drivers, which renders limited or no benefit to mTOR inhibitors.

Altogether, current report leaves us with the following conclusions. The advent of diagnostic molecular pathology in our clinics provides opportunity to study the RCC biology of individual cancers and can provide background to unusual outcomes. The armamentarium for advanced RCC continues to expand, targeted immunotherapy recently having been added as the third class of agents. With eight targeted agents approved for treating mRCC, it is self-evident that most patients will not receive all 8 drugs, and many patients may not even experience all three classes. Thus, the key question lies in front of us is "Can we sequence mTOR inhibitor therapy with VEGF or PD1 inhibitors in the near future based on cancer genomics?" The answer will be a positive one if we begin to incorporate NGS Matters into both trial-based and case-based studies.

Acknowledgments

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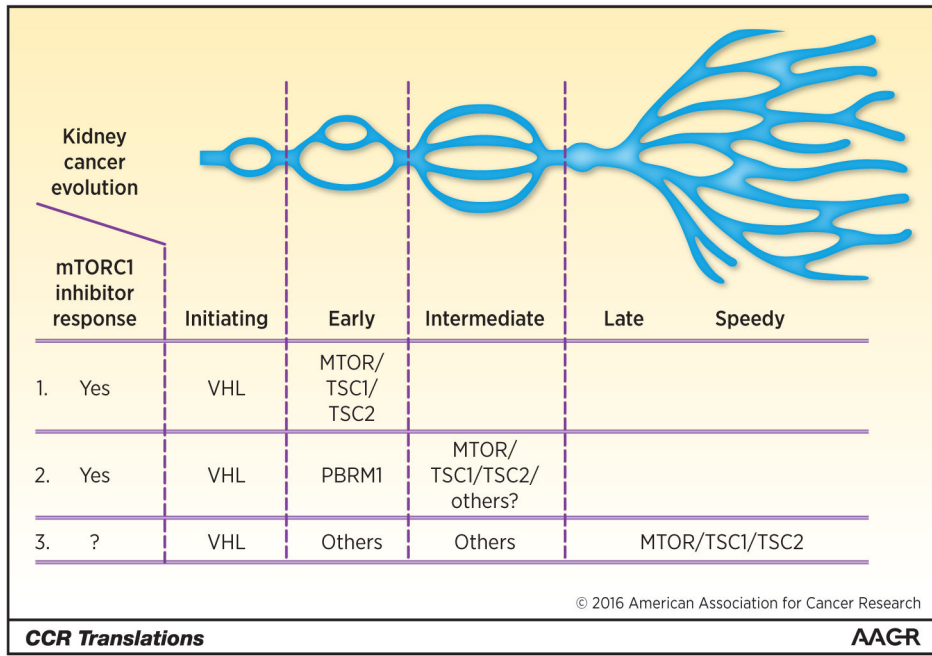
Figure 1.

Diagram exemplifies clear cell kidney cancer evolution initiated with the *VHL* mutation, which was followed by mTOR pathway activation that could take places at unique temporospatial sequence within individual tumors. The early acquisitions of mTOR pathway activation through either genetic or epigenetic means as illustrated scenarios (1) and (2) could render such tumors dependent on continuous mTOR pathway hyperactivation and thereby more susceptible to mTORC1 inhibitors. River branch image adapted from Wei and Hsieh (2).