Revise Manuscript CROH_2017_159R1

Complexity of the genomic landscape of renal cell carcinoma: Implications for targeted therapy and precision immuno-oncology

Joseph M. Sanfrancesco MD[1] & Liang Cheng MD[1,2]

From the Departments of Pathology and Laboratory Medicine[1] and Urology[2], Indiana University School of Medicine, Indianapolis, Indiana, USA.

Running Head: Genomic Landscape of Renal Cell Carcinoma

Keywords: Kidney, tumor heterogeneity, renal cell carcinoma, clonal evolution, targeted therapy, precision immuno-oncology

Total number of text pages: 17; Number of tables: 0; Number of figures: 3

Address correspondence and reprint requests to Liang Cheng, M.D., Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, 350 West 11th Street, IUHPL Room 4010, Indianapolis, IN 46202, USA. Telephone: 317-491-6442; Fax: 317-491-6419; E-mail: liang_cheng@yahoo.com

---

Key Points/Highlights:

1. Genetic heterogeneity within renal cell carcinoma is of growing interest due to its potential effects on therapeutic options and drug efficacy.
2. While several hallmark truncal molecular aberrations are well known, recent studies have identified spatially heterogenous, subclonal mutations which may contribute to tumor progression and the relatively poor efficacy of current treatment options.
3. The importance of multifocal tumor sampling to appropriately assess the extent of transcriptomic and genomic profile is significant, as single biopsy sampling is likely insufficient.
4. While pathways associated with traditional truncal molecular aberrations (e.g. VHL) remain fundamental targets for treatment in renal cell carcinoma, a growing understanding of subclonal mutations and intratumoral heterogeneity may offer new options for treating clinicians and their patients.

Table of Contents

Abstract

The topic of tumoral heterogeneity at the genetic level has become relevant in various solid origin tumors, particularly in an age of targeted treatment. Renal cell carcinoma is known for a sizable subset of tumors presenting at advanced clinical stage, further highlighting the importance and timeliness of this topic and its potential impact on adjuvant therapy. Recent studies have shown that molecular aberrations in renal cell carcinoma go beyond known truncal mutations and that downstream, subclonal aberrations are spatially heterogenous. Intratumoral heterogeneity as well as the differences in the molecular landscape between primary and metastatic lesions remains underappreciated, often due to inadequate sampling of tumors. The overall effect of these factors on the efficacy of current treatment options in renal cell carcinoma remains unknown; however, several recent studies have attempted to elucidate the extent and impact genetic heterogeneity in renal cell neoplasia may have on patient treatment and prognosis.

1. Introduction

The topic of tumoral heterogeneity is not unique to renal cell carcinoma (RCC). Various studies have evaluated intratumoral heterogeneity in malignancies involving the pancreas, breast, lung, brain, and gynecologic tract by different molecular modalities including chromosomal mutational and copy number analyses[1-5]. RCC continues to be associated with a relatively poor prognosis given that 20 to 30% of cases present with metastatic disease and subsequent poor five-year survival (5 to 10%)[6]. In the setting of adjuvant therapy in RCC, an overwhelming majority of agents show modest to poor results in their effects limiting disease progression and improving overall survival[7-9]. Acquisition of intratumoral heterogeneity as a tumor evolves has been postulated as a contributing factor[10].

2. Morphologic and Molecular Intratumoral Heterogeneity

Even prior to analysis at the molecular level, morphologic heterogeneity within RCCs has been extensively studied[11]. While it may have been reasonable to presume differentiation of tumors by routine light microscopy should correlate with molecular heterogeneity, this may not be the case. For example, in studies comparing conventional carcinoma morphologies with sarcomatoid differentiation, preservation of truncal mutations as well as concordant allelic loss and X-chromosome inactivation have been shown despite obvious morphologic differences[12]. Conversely, a study by Ito el al. identified more complex genetic profiles and increased chromosomal aberrations in sarcomatoid RCC when compared to conventional clear cell, papillary, or chromophobe RCCs[13]. Various methods have been used to assess for intratumoral variability at the molecular level, including assessment of copy number aberrations[14]. While a majority of studies investigating tumoral heterogeneity have focused on clear cell RCC, studies

evaluating papillary and chromophobe RCCs have also been performed. In a study by Brunelli et al, chromophobe RCC with sarcomatoid features demonstrated unique and increased chromosomal changes that differed from those identified in conventional chromophobe RCC. Interestingly, the same study found that primary and metastatic lesions shared the same genetic pattern[15,16].

3. Truncal versus Subclonal Molecular Aberrations in Intratumoral Heterogeneity

Tumor progression following truncal-type events (e.g. chromosome 3p loss in clear cell RCC) is comprised of the acquisition of subclones[14]. von Hippel-Lindau (VHL) serves as tumor suppressor gene (predominantly through the hypoxia-inducible pathway[HIF]) is located within chromosome 3p[17]. Other tumor suppressor genes, such as SETD2, PBRM1, and BAP1 are notably located on the short arm of chromosome 3p (all within a 50-Mb region)[18-20]. Mutations in the mammalian target of rapamycin (mTOR) pathway have been reported to occur downstream from VHL mutations/3p loss[21,22]. It is also postulated that these mTOR alterations have some effect on the HIF pathway as well[23-25]. VHL function serves an important role in cellular proliferation, angiogenesis, and preservation of epithelial differentiation[17]. In PRCC, besides polysomies (chromosome 7/17), mutations have been identified including MET (on chromosome 7), NF2 (in the Hippo signaling pathway), and PNKD (paroxysmal nonkinesigenic dyskinesia)[26]. While PRCC types 1 and 2 have been shown to be genetically and clinically distinct, a substantial majority show chromosomal gains of 7 and 17[27,28]. Chromophobe renal cell carcinoma (chRCC) is most notably associated with loss of chromosomes 1, 2, 6, 10, 13, 17, and 21[29]. Lesser known chRCC mutations, including Tp53, PTEN, and FAAH2 have also been identified[13,15,29-31].

In the distinction between linear progression of tumor evolution and branched and/or braided evolution patterns, the acquisition of mTOR pathway mutations downstream from truncal mutations likely explains the success with tyrosine kinase-targeting therapeutic agents[32] **(Figure 1)**. The persistence of preserved truncal mutations (e.g. VHL, trisomy 7/17, etc.) despite the intratumoral heterogeneity among subclonal mutations has supported the hypothesis of a "braided progression" of molecular heterogeneity. In this hypothesis, the truncal mutation serves as a possible source for all subsequent mutations, which can be unique, rather than the previously thought linear or parallel-type progression of tumor molecular aberrations[33]. The "braided" model also hypothesizes that spatially heterogenous mutations may happen at different points in time but the overall genomic profile inevitably becomes similar[34].

One key challenge when assessing the role of intratumoral heterogeneity is the balance between acquired heterogeneity, typically through a subclonal population, while also assessing for the preservation of truncal events in all areas of the tumor[35]. Intratumoral heterogeneity likely explains how despite "classic" truncal mutations being seen in various RCC subtypes, individual tumors may behave very differently in spite of these common molecular aberrations[10]. While Gerlinger et al. found previously reported VHL mutations as well as chromosome 3p deletions in all clear cell RCCs in their study, mutations in pathways such as mTOR, BAP1, PTEN, p53, and SETD2 were identified solely in subclonal populations and were spatially heterogenous[36]. Despite acquired subclonal populations in varying tumor regions, Kouba et al. have demonstrated the concordance of truncal mutational status in primary and metastatic sites, even in the setting of sarcomatoid differentiation[6]. It remains unclear if the preservation of truncal mutations in residual disease or metastatic sites serves as an indication for

targeted treatment or if this aggressive behavior is unrelated to the tumor still baring these truncal mutations[36,37]. Additionally, it raises further questions whether assessing the molecular landscape of the primary tumor serves any benefit in the setting of metastatic disease. Since the development of metastases is associated with a poor prognosis, some have postulated whether molecular analysis for treatment options should focus entirely on the genetic landscape of the metastatic disease[36].

The spatial separation of subclones and particularly apparent isolation of various subclonal driver mutations supports the argument that RCCs can undergo unique evolutionary pathways simultaneously, despite arising from the same truncal mutation[36] **(Figure 2)**. Gulati et al. have demonstrated significant intratumoral heterogeneity, particularly in subclones harboring distinct somatic copy number aberrations and driver mutations, within different tumor foci as well as with matched metastatic sites[38]. The apparent ability of tumors to develop diverse and spatially unique subclonal changes could explain how RCCs adapt to external pressures, such as immune responses, hypoxia, or even therapeutic intervention[39].

4. Tumor Sampling

Numerous studies have identified that single biopsy assessment for molecular aberrations is insufficient, particularly in assessment for use of certain targeted therapeutic agents as well as assessment for known aberrations with prognostic significance [6,10,12,14,36,37]. Furthermore, the importance of multifocal sampling to appropriately assess the extent of transcriptomic and overall genomic profile of tumors cannot be understated, as single biopsy sampling is insufficient[38] **(Figure 3A)**. Gerlinger et al. notably applied exome sequencing, analysis for chromosome aberrations, and DNA ploidy profiling to a cohort of RCC primary and metastatic

lesions to assess the genetic landscape of these tumors[10] **(Figure 3B)**. Interestingly, the authors embarked on a multifocal approach, to not only compare tumors between patients, but to in fact, compare different regions of the same tumor. This multifocal approach using various molecular analysis modalities translated to a seminal paper in assessing tumor heterogeneity in RCC[10]. While focal sampling (e.g. single biopsy) is suboptimal in assessment of tumor heterogeneity, even multifocal exome-sequencing may be suboptimal as differences in noncoding regions may be missed[40]. Early findings in many of these studies are promising, but the molecular landscape in RCC appears to be vastly underappreciated even with multifocal sampling.

Advances in sampling have entertained the possibility of non-invasive assessment of tumoral genetic landscape via circulating tumor DNA (ctDNA)[41,42] . Previously, ctDNA assessment by simple peripheral blood sampling has shown promise in assessing molecular aberrations in other solid tumors organ and hematopoietic malignancies. Pal et. al have recently published data on ctDNA findings prior to and then following treatment with systemic therapeutic agents in the setting of metastatic RCC[43]. While the data is limited, it does offer a potentially novel alternative to conventional core biopsy/needle aspiration sampling, which has proved to be suboptimal in many cases.

5. Treatment Implications due to Intra- and Intertumoral Molecular Heterogeneity

While variant histology within RCC may be derived from the same cell of origin, acquisition of unique genetic aberrations within subclonal cell populations may still harbor obstacles to therapeutic treatment despite preservation of truncal mutations[12]. Adjuvant therapy in RCC has typically been reserved for metastatic disease; however, identification of

molecular aberrations that may predict metastatic potential could have significant influence on treatment plans[9,35]. Tyrosine kinase inhibitors that target the VEGF pathways include sunitinib, sorafenib, pazopanib, axitinib, levatinib, and cabozantinib and remain first line therapy in metastatic RCC[8,34,44-49]. Bevacizumab (Avastin) is a pure anti-VEGF therapy which is different from tyrosine kinase inhibitors that indirectly target the VEGF pathway and related proteins[50,51]. Currently, mTOR inhibitors, including everolimus and temsirolimus, are second line therapy; however, have been used as 1st line therapy in poor risk patients[34]. With the advent of new immunotherapeutic options and the persistence of anti-angiogenic agents as first choice, mTOR inhibitors have trended towards becoming third line therapy[52].Some evidence has been shown to suggest therapeutic agents used to treat CCRCC, including sunitinib, sorafenib, and mTOR inhibitors such as everolimus and temsirolimus, could also show efficacy in other RCC subtypes including PRCC and chRCC. Of note, PRCC Type 2 has shown enrichment of CCRCC-associated pathways including, mTOR, VEGF, and HIF-alpha[28]. In patients who initially showed response with mTOR inhibitors (e.g. everolimus and temsirolimus), retrospective tumor analysis highlighted that mutations in the mTOR pathway were spatially heterogenous, which supported the need for multifocal sampling of tumors[35].

Foretinib, which is a mutikinase agent targeting MET, AXL, and other receptors have been used in patients with PRCC in lieu of CCRCC therapies[29]. Foretinib appears to benefit patients with germline MET mutations compared to all PRCC patients[53]. Resistance to first line VEGF inhibitors has been attributed to mutations in tyrosine kinase pathways (e.g. MET or AXL). Combination therapy or use of drugs that target both VEFG and MET pathways (e.g. cabozantinib) would in theory address this issue, but toxicity with agents remains a serious issue in RCC treatment[54]. Currently, there are no readily accepted chemotherapeutic or anti-

angiogenic drugs specific for treating RCC with sarcomatoid differentiation; however, theories behind the efficacy of anti-angiogenic agents, particularly anti-VEGF drugs, in sarcomatoid RCC include the increased proliferation index and persistent HIF-pathway expression in these tumors[25,55].

Immunotherapy has previously been used in treating RCC, most notably interferon-alpha and interleukin-2[56]. These treatments had severe adverse effects and minimal efficacy. Programmed cell death protein 1 (PD-1) and its ligand (PD-L1) being expressed in a majority of RCC has resurfaced interest in using immunotherapy as treatment[54]. Interest in therapeutic options using anti-PD-1 immune checkpoint inhibitors, such as nivolumab, has been noted in RCC, following success in other solid tumors, such as lung adenocarcinoma[57-59]. Initially, treatment with immune checkpoint inhibitors was reserved for refractory patients who had tumor progression after failure with fist- and second-line therapy regimens[60]. With subsequent studies, use of nivolumab in patients with disease progression in metastatic RCC has shown some success in lesion stabilization and even tumor reduction[59]. The introduction of new therapeutic options, particularly with immunotherapies (e.g targeting PD-1 and CTLA-4 pathways) has raised arguments for use and second or third-line options in patients with resistant metastatic RCC[52]. Current trends show that improved data likely supports immunotherapies as second line treatment in metastatic RCC, replacing everolimus [61].

Specific dosing and possibility for combination therapy with therapeutic agents are currently being studied[57]. Combination therapy as first line treatment is not recognized in any current guidelines; however, several options in the setting of treatment resistance have been proposed. Lenvatinib (a multi-target tyrosine kinase inhibitor) has been suggested for use in combination with everolimus in patients with metastatic RCC who have developed resistance to

more common first-line tyrosine kinase inhibitors [62]. Benefits using newer immunotherapeutic agents in combination with first line antiangiogenic agents remain unclear and currently data is lacking to support the vast expense associated with this treatment option [62].

Unfortunately, overall efficacy of immune checkpoint inhibitors in RCC has been mild at best, with some arguing that response rates do not correlate with levels of PD-L1/PD-1 expression[61,63,64]. Combination with another immunotherapy, ipilimumab (a CTLA-4 inhibitor of cytotoxic T cells), is also currently being explored as a treatment option in metastatic RCC[65-67]. Currently, several late stage clinical trials may offer clinicians more information combination treatment options. Newer anti-PD1/PD-L1 agents, such as Atezolizumab and Pembrolizumab, are being tested in combination with anti-VEGF agents to see how patients respond compared to traditional first-line therapy with sunitinib [68,69]. Additionally, in a possible shift from antiangiogenic based treatment, the combination of nivolumab and ipilimumab could provide the first viable immunotherapy-only as first-line therapy in metastatic RCC [54,68,70]. Progressive understanding of the immunotherapeutic landscape, new agents, and potential combination therapies are positive signs in treatment of high-stage RCC. Unfortunately, the limited number of patients showing clinical (and lasting) benefit with treatment and the immense cost has posed difficult challenges for patients and treating clinicians [71]. Potential benefit from combination therapy using tradition first-line agents such as antiangiogenic drugs could bridge treatment regiments until further progress is made with newer targeted agents [72].

The complexities of the immunophenotypic landscape of RCC poses similar challenges in treatment as previously seen with chemotherapeutic/anti-angiogenic agents[66]. Of note, the higher number of mutations for PD-L1 identified in metastases could explain efficacy seen when

using immunotherapeutic agents in this setting, but further studies are required[56,73,74]. Analysis of the genomic landscape in RCC patients status post immunotherapy treatment remains significantly limited. The majority of data currently available comes from the melanoma literature, where immunotherapy has shown high response rate but also high toxicity in patients, particularly in combination therapy with PD-1 and CTLA-4 inhibitors [65,75,76]. The proposed synergistic responses of these two agents, one by activating antigen-specific T cells (e.g anti-CTLA4 pathway) and the other by removing inhibition on T cells to attack cancer cells (e.g. anti-PD1 pathway)shows great promise in RCC[77,78]. Unfortunately, these checkpoint inhibitors are associated with high cost and significant toxicity and currently benefit only a small subset of all patients [79]).

An additional challenge facing patients is the genomic landscape variability between treatment-naive tumor samples, versus metastatic tumor samples, versus tumor post-treatment sampling (either antiangiogenic agents or immunotherapy)[80]. In the short term, identifying patients who will actually benefit from these therapies remains a key priority [72]). A study by Chevrier et al. using mass spectrometry to profile the immunologic microenvironment in ccRCC has provided a strong foundation to guide further development of potential biomarkers and targets [66]. Despite variable modalities for assessing PD-1/PD-L1 expression in RCC, inherently positive expression of PD-1/PD-L1 does not always correlate with treatment efficacy. Resistance to immunotherapeutic agents may, in fact, be secondary to non-immunogenic factors such as metabolic factors which block attacking immune cells or possibly improve cancel cell fitness [81].

Additionally, subclones that may be responsible for therapeutic resistance could possibly be clustered in certain un- or under-sampled tumor regions or of low enough frequency to evade

detection with single or possibly even multi-site sampling[14]. Determining the effectiveness of targeting ubiquitous truncal driver mutations (e.g. VHL, trisomy 7/17) relies heavily on the tumor's dependence on them for survival; however, if subclonal drivers in fact are the cause of tumor progression, the relative ineffectiveness of many drugs could be explained[36]. Furthermore, this would make the relative preservation of truncal mutations in metastatic sites irrelevant, as they are no longer the factors driving disease advancement.

Increased intratumoral heterogeneity accompanied with increased copy number variations (genomic instability) has been hypothesized to possibly favor improved overall outcome. Andor et al. have argued this could be secondary increased immune response or the possibility of generating defective daughter cells[40]. It may be that the same genomic instability driving tumor progression and poor outcomes could also have a negative effect if proliferating tumor cells are nonviable[36]. Despite the obvious morphologic, immunophenotypic, and now molecular differences in various RCC subtypes, the treatment approach is essentially the same regardless. Regardless of spatial heterogeneity seen in RCC, the relative preservation of truncal driver mutations will likely continue to serve as optimal targets for treatment therapeutic agents until the role of subclonal populations in tumor progression is better understood[7-10,36,37].

6. Conclusion

The concept of intra- and intertumoral genetic heterogeneity remains a timely yet underappreciated topic in renal cell carcinoma. While traditional truncal molecular aberrations have been well-known for some time, particularly in clear cell and papillary RCCs, recent findings have shown that these mutations may not be optimal targets for therapeutic treatment in advanced disease. The most significant challenge remains that despite these frequent and

apparently ubiquitous alterations in VHL-VEGF and mTOR pathways, overall efficacy of targeted drugs does not seem to correlate with mutational status. Further study of the molecular landscape in renal cell carcinoma, particularly in acquired subclonal aberrations and driver mutations, may provide invaluable information for future treatment options and overall prognosis.

Conflict of interest: The author declares that there are no conflicts of interest.

Acknowledgements: The authors would like to thank Natasha Gibson for excellent editorial assistance.

References:

1. Campbell PJ, Yachida S, Mudie LJ, et al: The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature. 2010;467,1109-13.

2. Navin N, Kendall J, Troge J, et al: Tumour evolution inferred by single-cell sequencing. Nature. 2011;472,90-4.

3. Bashashati A, Ha G, Tone A, et al: Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. J Pathol. 2013;231,21-34.

4. Venkatesan S, Swanton C: Tumor Evolutionary Principles: How Intratumor Heterogeneity Influences Cancer Treatment and Outcome. Am Soc Clin Oncol Educ Book. 2016;35,e141-9.

5. Morrissy AS, Cavalli FMG, Remke M, et al: Spatial heterogeneity in medulloblastoma. Nat Genet. 2017;49,780-788.

6. Kouba EJ, Eble JN, Simper N, et al: High fidelity of driver chromosomal alterations among primary and metastatic renal cell carcinomas: implications for tumor clonal evolution and treatment. Mod Pathol. 2016;29,1347-1357.

7. Buchler T, Bortlicek Z, Poprach A, et al: Outcomes for Patients with Metastatic Renal Cell Carcinoma Achieving a Complete Response on Targeted Therapy: A Registry-based Analysis. Eur Urol. 2016;70,469-75.

8. Choueiri TK, Escudier B, Powles T, et al: Cabozantinib versus everolimus in advanced renal cell carcinoma (METEOR): final results from a randomised, open-label, phase 3 trial. Lancet Oncol. 2016;17,917-27.

9. Voss MH, Molina AM, Chen YB, et al: Phase II Trial and Correlative Genomic Analysis of Everolimus Plus Bevacizumab in Advanced Non-Clear Cell Renal Cell Carcinoma. J Clin Oncol. 2016.

10. Gerlinger M, Rowan AJ, Horswell S, et al: Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366,883-92.

11. H Moch PH, TM Ulbright, VE Reuter: WHO Classification of Tumours of the Urinary System and Male Genital Organs (ed 4th), International Agency for Research on Cancer, 2016

12. Jones TD, Eble JN, Wang M, et al: Clonal divergence and genetic heterogeneity in clear cell renal cell carcinomas with sarcomatoid transformation. Cancer. 2005;104,1195-203.

13. Ito T, Pei J, Dulaimi E, et al: Genomic Copy Number Alterations in Renal Cell Carcinoma with Sarcomatoid Features. J Urol. 2016;195,852-8.

14. Martinez P, Birkbak NJ, Gerlinger M, et al: Parallel evolution of tumour subclones mimics diversity between tumours. J Pathol. 2013;230,356-64.

15. Brunelli M, Gobbo S, Cossu-Rocca P, et al: Chromosomal gains in the sarcomatoid transformation of chromophobe renal cell carcinoma. Mod Pathol. 2007;20,303-9.

16. Brunelli M, Fiorentino M, Gobbo S, et al: Many facets of chromosome 3p cytogenetic findings in clear cell renal carcinoma: the need for agreement in assessment FISH analysis to avoid diagnostic errors. Histol Histopathol. 2011;26,1207-13.

17. Frew IJ, Moch H: A clearer view of the molecular complexity of clear cell renal cell carcinoma. Annu Rev Pathol. 2015;10,263-89.

18. Bi M, Zhao S, Said JW, et al: Genomic characterization of sarcomatoid transformation in clear cell renal cell carcinoma. Proc Natl Acad Sci U S A. 2016;113,2170-5.

19. Manley BJ, Hsieh JJ: Sarcomatoid renal cell carcinoma: genomic insights from sequencing of matched sarcomatous and carcinomatous components. Transl Cancer Res. 2016S160-S165.

20. Manley BJ, Zabor EC, Casuscelli J, et al: Integration of recurrent somatic mutations with clinical outcomes: A pooled analysis of 1049 patients with clear cell renal cell carcinoma. Eur Urol Focus. 2016.

21. Chen F, Zhang Y, Senbabaoglu Y, et al: Multilevel Genomics-Based Taxonomy of Renal Cell Carcinoma. Cell Rep. 2016;14,2476-89.

22. Brugarolas J: Molecular genetics of clear-cell renal cell carcinoma. J Clin Oncol. 2014;32,1968-76.

23. Sato Y, Yoshizato T, Shiraishi Y, et al: Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet. 2013;45,860-7.

24. Semenza GL: HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest. 2013;123,3664-71.

25. Harlander S, Schonenberger D, Toussaint NC, et al: Combined mutation in Vhl, Trp53 and Rb1 causes clear cell renal cell carcinoma in mice. Nat Med. 2017.

26. Gonzalez Del Alba A, Arranz JA, Puente J, et al: Recent advances in genitourinary tumors: A review focused on biology and systemic treatment. Crit Rev Oncol Hematol. 2017;113,171-190.

27. Linehan WM, Spellman PT, Ricketts CJ, et al: Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. N Engl J Med. 2016;374,135-45.

28. Saleeb RM, Plant P, Tawedrous E, et al: Integrated phenotypic/genotypic analysis of papillary renal cell carcinoma subtypes: identification of prognostic markers, cancer-related pathways, and implications for therapy. Eur Urol Focus. 2016.

29. Abbosh P, Sundararajan S, Millis S, et al: Molecular and genomic profiling to identify actionable targets in chromophobe renal cell cancer. Eur Urol Focus. 2017(in press).

30. Malouf GG, Ali SM, Wang K, et al: Genomic Characterization of Renal Cell Carcinoma with Sarcomatoid Dedifferentiation Pinpoints Recurrent Genomic Alterations. Eur Urol. 2016;70,348-57.

31. Davis CF, Ricketts CJ, Wang M, et al: The somatic genomic landscape of chromophobe renal cell carcinoma. Cancer Cell. 2014;26,319-30.

32. Zhang J, Yang PL, Gray NS: Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer. 2009;9,28-39.

33. Hsieh JJ, Manley BJ, Khan N, et al: Overcome tumor heterogeneity-imposed therapeutic barriers through convergent genomic biomarker discovery: A braided cancer river model of kidney cancer. Semin Cell Dev Biol. 2016.

34. Hsieh JJ, Purdue MP, Signoretti S, et al: Renal cell carcinoma. Nat Rev Dis Primers. 2017;3,17009.

35. Voss MH, Hakimi AA, Pham CG, et al: Tumor genetic analyses of patients with metastatic renal cell carcinoma and extended benefit from mTOR inhibitor therapy. Clin Cancer Res. 2014;20,1955-64.

36. Gerlinger M, Horswell S, Larkin J, et al: Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet. 2014;46,225-33.

37. Gerlinger M, Swanton C: How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. Br J Cancer. 2010;103,1139-43.

38. Gulati S, Martinez P, Joshi T, et al: Systematic evaluation of the prognostic impact and intratumour heterogeneity of clear cell renal cell carcinoma biomarkers. Eur Urol. 2014;66,936-48.

39. Melchor L, Brioli A, Wardell CP, et al: Single-cell genetic analysis reveals the composition of initiating clones and phylogenetic patterns of branching and parallel evolution in myeloma. Leukemia. 2014;28,1705-15.

40. Andor N, Graham TA, Jansen M, et al: Pan-cancer analysis of the extent and consequences of intratumor heterogeneity. Nat Med. 2016;22,105-13.

41. Abbosh C, Birkbak NJ, Wilson GA, et al: Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature. 2017;545,446-451.

42. Wan JC, Massie C, Garcia-Corbacho J, et al: Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017;17,223-238.

43. Pal SK, Sonpavde G, Agarwal N, et al: Evolution of circulating tumor DNA profile from first-line to subsequent therapy in metastatic renal cell carcinoma. Eur Urol. 2017.

44. Ravaud A, Motzer RJ, Pandha HS, et al: Adjuvant Sunitinib in High-Risk Renal-Cell Carcinoma after Nephrectomy. N Engl J Med. 2016;375,2246-2254.

45. Haas NB, Manola J, Uzzo RG, et al: Adjuvant sunitinib or sorafenib for high-risk, non-metastatic renal-cell carcinoma (ECOG-ACRIN E2805): a double-blind, placebo-controlled, randomised, phase 3 trial. Lancet. 2016;387,2008-2016.

46. Beuselinck B, Job S, Becht E, et al: Molecular subtypes of clear cell renal cell carcinoma are associated with sunitinib response in the metastatic setting. Clin Cancer Res. 2015;21,1329-39.

47. Michaelson MD, McKay RR, Werner L, et al: Phase 2 trial of sunitinib and gemcitabine in patients with sarcomatoid and/or poor-risk metastatic renal cell carcinoma. Cancer. 2015;121,3435-43.

48. Motzer RJ, Hutson TE, Cella D, et al: Pazopanib versus sunitinib in metastatic renal-cell carcinoma. N Engl J Med. 2013;369,722-31.

49. Choueiri TK, Escudier B, Powles T, et al: Cabozantinib versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med. 2015;373,1814-23.

50. Barbieri CE, Chinnaiyan AM, Lerner SP, et al: The Emergence of Precision Urologic Oncology: A Collaborative Review on Biomarker-driven Therapeutics. Eur Urol. 2017;71,237-246.

51. Voss MH, Molina AM, Chen YB, et al: Phase II Trial and Correlative Genomic Analysis of Everolimus Plus Bevacizumab in Advanced Non-Clear Cell Renal Cell Carcinoma. J Clin Oncol. 2016;34,3846-3853.

52. Wells JC, Stukalin I, Norton C, et al: Third-line targeted therapy in metastatic renal cell carcinoma: results from the international metastatic renal cell carcinoma database consortium. Eur Urol. 2017;71,204-209.

53. Choueiri TK, Vaishampayan U, Rosenberg JE, et al: Phase II and biomarker study of the dual MET/VEGFR2 inhibitor foretinib in patients with papillary renal cell carcinoma. J Clin Oncol. 2013;31,181-6.

54. Choueiri TK, Motzer RJ: Systemic Therapy for Metastatic Renal-Cell Carcinoma. N Engl J Med. 2017;376,354-366.

55. Sircar K, Yoo SY, Majewski T, et al: Biphasic components of sarcomatoid clear cell renal cell carcinomas are molecularly similar to each other, but distinct from, non-sarcomatoid renal carcinomas. J Pathol Clin Res. 2015;1,212-24.

56. Jilaveanu LB, Shuch B, Zito CR, et al: PD-L1 Expression in Clear Cell Renal Cell Carcinoma: An Analysis of Nephrectomy and Sites of Metastases. J Cancer. 2014;5,166-72.

57. Choueiri TK, Fishman MN, Escudier B, et al: Immunomodulatory Activity of Nivolumab in Metastatic Renal Cell Carcinoma. Clin Cancer Res. 2016;22,5461-5471.

58. Lavin Y, Kobayashi S, Leader A, et al: Innate Immune Landscape in Early Lung Adenocarcinoma by Paired Single-Cell Analyses. Cell. 2017;169,750-765.e17.

59. George S, Motzer RJ, Hammers HJ, et al: Safety and Efficacy of Nivolumab in Patients With Metastatic Renal Cell Carcinoma Treated Beyond Progression: A Subgroup Analysis of a Randomized Clinical Trial. JAMA Oncol. 2016;2,1179-86.

60. Rini BI, McDermott DF, Hammers H, et al: Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of renal cell carcinoma. J Immunother Cancer. 2016;4,81.

61. Escudier B, Motzer RJ, Sharma P, et al: Treatment Beyond Progression in Patients with Advanced Renal Cell Carcinoma Treated with Nivolumab in CheckMate 025. Eur Urol. 2017.

62. Zarrabi K, Fang C, Wu S: New treatment options for metastatic renal cell carcinoma with prior anti-angiogenesis therapy. J Hematol Oncol. 2017;10,38.

63. Gupta K, Tiu DY, Tiu J, et al: The promising role of nivolumab in renal cell cancers. Cancer Biol Ther. 2016;17,123-4.

64. Motzer RJ, Escudier B, McDermott DF, et al: Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med. 2015;373,1803-13.

65. Larkin J, Hodi FS, Wolchok JD: Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med. 2015;373,1270-1.

66. Chevrier S, Levine JH, Zanotelli VRT, et al: An Immune Atlas of Clear Cell Renal Cell Carcinoma. Cell. 2017;169,736-749.e18.

67. Kluger HM, Zito CR, Turcu G, et al: PD-L1 Studies Across Tumor Types, Its Differential Expression and Predictive Value in Patients Treated with Immune Checkpoint Inhibitors. Clin Cancer Res. 2017.

68. Song M: Recent developments in small molecule therapies for renal cell carcinoma. Eur J Med Chem. 2017.

69. Albiges L, Choueiri T, Escudier B, et al: A systematic review of sequencing and combinations of systemic therapy in metastatic renal cancer. Eur Urol. 2015;67,100-10.

70. Hammers HJ, Plimack ER, Infante JR, et al: Safety and Efficacy of Nivolumab in Combination With Ipilimumab in Metastatic Renal Cell Carcinoma: The CheckMate 016 Study. J Clin Oncol. 2017Jco2016721985.

71. Sharma P, Hu-Lieskovan S, Wargo JA, et al: Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168,707-723.

72. Einstein DJ, McDermott DF: Combined blockade of vascular endothelial growth factor and programmed death 1 pathways in advanced kidney cancer. Clin Adv Hematol Oncol. 2017;15,478-488.

73. Mukai S, Yorita K, Kawagoe Y, et al: Matriptase and MET are prominently expressed at the site of bone metastasis in renal cell carcinoma: immunohistochemical analysis. Hum Cell. 2015;28,44-50.

74. Laird A, O'Mahony FC, Nanda J, et al: Differential expression of prognostic proteomic markers in primary tumour, venous tumour thrombus and metastatic renal cell cancer tissue and correlation with patient outcome. PLoS One. 2013;8,e60483.

75. Postow MA, Chesney J, Pavlick AC, et al: Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372,2006-17.

76. Roh W, Chen PL, Reuben A, et al: Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. Sci Transl Med. 2017;9.

77. Chen DS, Mellman I: Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39,1-10.

78. Wolchok JD, Kluger H, Callahan MK, et al: Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369,122-33.

79. Liu KG, Gupta S, Goel S: Immunotherapy: incorporation in the evolving paradigm of renal cancer management and future prospects. Oncotarget. 2017;8,17313-17327.

80. Tumeh PC, Harview CL, Yearley JH, et al: PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515,568-71.

81. Ascierto ML, McMiller TL, Berger AE, et al: The intratumoral balance between metabolic and immunologic gene expression Is associated with anti-PD-1 response in patients with renal cell carcinoma. Cancer Immunol Res. 2016;4,726-33.

Figure Legends

Figure 1: Traditional thinking in terms of tumor evolution has changed in recent years with the linear model **(A)** now being replaced with a "braided" evolution pattern **(B)**. In the "braided" pattern, while mutations such as von Hippel Lindau or loss of chromosome 3p may be truncal events, subsequent mutations likely interact and persistence of truncal mutations is possible.

Figure 2: The spacial heterogeneity of subclones acquired in renal cell neoplasia remains unclear, despite several hypotheses. The interaction of subclones in malignant renal tumors could be complete independence via geographic separation **(A)**, survival of the strongest subclones via competition **(B)**, or an interactive-symbiotic relationship **(C)**.

Figure 3: Various studies have argued that single biopsy sampling of renal tumors is likely inadequate to assess the genetic heterogeneity. Furthermore, concordance and/or discordance of molecular aberrations between metastatic disease and primary tumors may offer insights into efficacy of various therapeutic treatment options.