

Molecular ‘bucket list’ for renal cancer

Karen Titus

September 2018—Leo Tolstoy is not listed as a coauthor on the most recent iteration of The Cancer Genome Atlas on renal cell carcinoma, which focuses on molecular characterization of RCC. But the topic is as rich and complex as a Russian novel, and the authors’ approach is so comprehensive, it’s tempting to picture them at least holding forth at a certain soirée in Saint Petersburg (minus the after-party drunkenness and the bit with a bear, of course).

The project may not be as sprawling as *War and Peace*, which marches 559 characters, speaking two languages, over four volumes, 15 parts, and 333 chapters. It’s a heroic effort nonetheless. There is much to keep track of in renal cell carcinoma, both generally and in this latest document (Ricketts CJ, et al. *Cell Rep.* 2018;23[1]:313–326.e5), which evaluates 843 RCCs from three major histologic subtypes, including 488 clear cell RCC, 274 papillary RCC, and 81 chromophobe RCC. “And remember,” says actual coauthor Victor Reuter, MD, “this is our fourth publication on kidney cancer.” The previous three each focused solely on one of the subtypes.

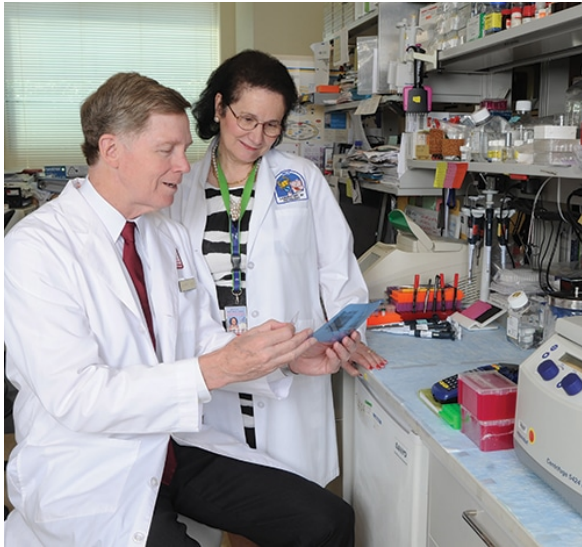
The most recent publication expands matters, looking at larger numbers of cases in each category and eyeing them a bit differently, says Dr. Reuter, vice chairman of the Department of Pathology, Memorial Sloan Kettering Cancer Center, and professor of pathology and laboratory medicine, Weill Cornell Medical College. “It’s a natural progression to the other three stories,” he says. “And it shows some novel information as well.” Molecular perspectives—mutations, copy numbers, RNA, microRNA expression studies, methylation, etc.—enabled researchers to look at each group and show differences as well as similarities within each “basket,” he says.

The TCGA, says coauthor Maria Merino, MD, confirms that the spectrum of kidney cancers is indeed quite ample. “And as pathologists, we need to classify these tumors as far as we can.”

Physicians don’t necessarily have to do a deep dive into the paper to realize the implications of its contents. As part of their big data dive, the researchers also looked at patient survival. Moreover, therapies will be targeted to specific tumor types, plain and simple. “If we don’t do this classification and subclassification and confirm them with the genetics, it is possible that patients may not be treated appropriately,” warns Dr. Merino, chief of translational surgical pathology and a principal investigator, National Cancer Institute. This latest installment is yet another chapter in the bigger, ongoing story of how pathologists and others are unmasking the true nature of these tumors.

The work “has been humbling,” says coauthor W. Marston Linehan, MD, chief of the Urologic Oncology Branch, NCI, who has worked on all four TCGA projects. “It’s so complex.”

That complexity has been mirrored in clinical practice. “There have been a lot of changes going on in this field in general,” says Donna Hansel, MD, PhD, professor of pathology and chief of anatomic pathology, University of California, San Diego. The biggest, she says, has been the swing toward molecular diagnostics.



Dr. W. Marston Linehan and Dr. Maria Merino at the National Cancer Institute. “If we don’t do this classification and subclassification [for RCC] and confirm them with the genetics, it is possible that patients may not be treated appropriately,” says Dr. Merino. [Photo: Robert Williams]

The most recent TCGA, she says, helps explain how the various tumor types are classified and how they might be related to one another. Equally important is its emphasis on subclassification. Thirdly, she says, it offers insight into tumor biology and, broadly, the molecular changes that occur. Finally, and perhaps most importantly, in her view, it points to previously unexplored areas that could spur the development of targeted therapies, which are much needed, given that the survival rate for advanced kidney cancer has been, until recently, very dismal.

Dr. Linehan agrees. “Knowledge is power. And the first step in developing effective forms of therapy is to understand what your targets are.”

Though many of the morphological classifications and subclassifications aren’t necessarily new to pathologists, Dr. Hansel says, the paper highlights some overlapping molecular features of many of them, which haven’t been widely appreciated. That, in turn, has launched countless questions as pathologists try to connect the dots between morphologies and molecular alterations. Are there morphological clues that point to a specific mutation, translocation, or chromosomal abnormality, which would trigger further testing? And if such testing is done, what, if anything, do the results mean?

Dr. Hansel sees a large role for molecular testing. “But how far will that go?” she asks. For her, the most pressing question is whether molecular testing will become the modality for subclassifying tumors. “Or is there still a role for morphology in triaging?” she asks. “I’d like to say there’s a role for both—or else you’re going to get a lot of angry letters,” she tells CAP TODAY.

Perhaps only a fool (fun fact: the chief fool in *War and Peace* was Napoleon Bonaparte) would argue against *any* role for molecular, given the changes it has wrought in the field already.



Dr. Hansel

For years, Dr. Hansel says, renal tumors generally were classified into four types of lesions. “And if you didn’t know, you’d just wave your hands and say it was unclassified,” she says, only somewhat jokingly. Over the past 15 years or so, through molecular work primarily but also improved morphology classification, “We’ve gotten much, much better at being able to subdivide these. When you take a look at what we used to call unclassified, a lot of that has shrunk away. We can now put them into very specific baskets.” And new knowledge has upended some baskets entirely.

The “unclassified” basket was “an easy out, in a way,” says Dr. Hansel. It still exists, but only after tumors have been more thoroughly worked up with molecular tools to look for either chromosomal abnormalities or translocation—two of the more common findings in renal cell carcinoma.

Knowing it’s possible to tip some tumors out of the unclassified basket, however, doesn’t mean dropping them in the right basket is a slam dunk. Immunohistochemistry isn’t foolproof. It can lack specificity. Some antibodies have been studied only in small series of tumors; others are quite finicky.

Even when IHC is firing on all cylinders, “The truth is, I continue to see renal tumors that defy classification,” Dr. Hansel says. These cancers exhibit complex and diverse histologies and comprise what Dr. Hansel calls “a maze of tumors, with a whole spectrum of immunohistochemical and molecular changes that accompany them. And trying to put them in the right bucket, and even knowing the bucket exists, has been challenging.”

Complicating matters, renal cell carcinomas seem to be prone to name changes. In some cases, the same tumor is called by different names—not unlike the malleably named characters in *The Brothers Karamazov* (to borrow from another Russian author).

Ever since the new WHO classification came out in 2016, Dr. Hansel says, it has been apparent to her that urologists aren’t always certain of what the changes entail. “So you could be reporting on something they haven’t really heard about before,” she says. They might not know what the classification means; they might also misinterpret what it means.

Dr. Hansel has a simple strategy for this, too: Whenever she makes a diagnosis involving an uncommon category or a new classification, she picks up the phone and calls the clinician. It’s not unusual, she says, for clinicians to hear a word in the name and confuse it with something else (a concept familiar to readers trying to keep up with those Karamazov siblings, all of whom share a middle name—Fyodorovich—and are prone to nicknames as well). There is clear cell renal cell carcinoma, which is not the same as clear cell papillary renal cell carcinoma, which, in turn, is not the same as papillary renal cell carcinoma. “If you have one of those cases, you just want to have a dialogue with the urologists to make sure you’re all on the same page,” Dr. Hansel says.

Dr. Merino concurs. Practicing pathologists (and their clinical colleagues) may not need to absorb every last detail of the molecular analyses presented in the TCGA, “but they should at least see how we classify them,” she says, so they can use the same nomenclature.



Dr. Reuter

Then there are the names that vanished as subclassifications improved. Dr. Reuter alludes to the earlier RCC classifications, when the WHO defined four entities of kidney cancer: clear cell renal cell carcinoma, papillary renal cell carcinoma, granular cell-type renal cell carcinoma, and sarcomatoid renal cell carcinoma.

By the 1990s, pathologists realized neither sarcomatoid carcinoma nor granular cell-type renal cell carcinoma were true entities, and that papillary tumors were rather heterogeneous. By combining good pathology, good IHC, and good molecular biology, Dr. Reuter says, “we were able to tease out a lot of those groups, and understand the morphologic diversity that would be acceptable within each tumor type, as well as its genotype.”

But morphology alone is no longer sufficient to classify a tumor, a point driven home multiple times in the TCGA paper. “If pathologists read it with an open mind, they will see there is a good correlation between pathology and molecular,” Dr. Merino says.

Papillary renal cell carcinoma offers a good example of how matters are evolving. Since the 1990s, says Dr. Reuter, these tumors had been subdivided into Type 1 and Type 2. As it turns out, Type 1s are morphologically indistinct from papillary tumors arising in familial papillary renal cell carcinoma. The hereditary ones, however, characteristically have mutation of *MET* oncogene present on chromosome 7. (They also are usually diagnosed in patients at a younger age and more likely to be multifocal or bilateral.) Sporadic papillary Type 1 cancers do not have mutations of *MET* nearly as frequently but are likely to harbor amplifications of the same gene.

Type 2 tumors, Dr. Reuter continues, are morphologically distinct in that they’re more likely to have eosinophilic cytoplasm, high nuclear grade, and prominent nucleoli. But these tumors share an overlapping morphology with other types of tumors, including high-grade Type 1 papillary renal cell carcinomas. Others with overlapping morphology include tumors associated with fumarate hydratase deficiency, as well as those with translocations of either the *TFE-3* or *TFE-B* gene.

(At this point, a wandering mind in search of lighter fare might find itself thinking that RCC classifications mimic the morphing partnerships behind the Great American Songbook. Who wrote what—Rodgers and Hart? Rodgers and Hammerstein? Kern and Hammerstein?)

In simpler terms, says Dr. Reuter, “Type 2 papillary renal carcinoma is a less-than-pure entity. In fact, it’s not an entity.” Given the encompassing nature of this category, “When confronted with this morphology, a pathologist must consider the differential diagnosis.” The answer can help guide therapy, especially for tumors that might qualify for checkpoint inhibitors, for example. And if a familial cancer is implicated, genetic counseling for family members is in order. “These are lethal tumors,” he warns.

The ongoing confusion is understandable—and widespread. “These tumors,” Dr. Reuter says, “comprise a very large percentage of the cases that people like myself and others get in consultation.”

He credits academic pathologists for educating colleagues in the last five to 10 years, spreading the word about modern classifications and molecular correlates “every chance they get.” Nonetheless, he says, that doesn’t obviate the fact that some of these tumors are rare, nor that the many subclassifications can be confusing. “For that reason it’s not unusual for these tumors that are not absolutely the classic examples to be submitted for second opinions by practicing pathologists.”

It can be hard for pathologists, especially in community practice, to keep all these entities straight when the frequency is low, says Michelle Hirsch, MD, PhD, associate professor of pathology, Harvard Medical School, and chief of the Genitourinary Pathology Division and staff pathologist in the Women’s and Perinatal Division at Brigham and Women’s Hospital. There are at least 10 rare renal tumor subtypes that account for only a few percent of all cases. “You’re talking less than one percent for each of these tumor subtypes. So if you’re in a small practice where you’re not seeing a nephrectomy that often, then you’re definitely unlikely to see these unusual subtypes.” (Although none of these are as rare as Aline Kuragina, who apparently shows up only once in *War and*

Peace, and whose husband, naturally, spells his surname Kuragin.)

Dr. Hirsch's advice: Stay caught up with reading and attend CME conferences. "At least if you've heard about or seen these less common tumors in a lecture, you can seek help from somebody who sees these tumors more frequently or in greater volume."

Picking up the phone can also be helpful. "If I'm really struggling with a tumor," says Dr. Hansel, "I call the urologists to get a sense of either their impression of the radiology or how the surgery was—was it difficult to get out? I'm very honest about what I'm struggling with. I feel that sort of communication across the board is very helpful."

These discussions can help Dr. Hansel decide what comes next. "If you get an indeterminate stain, you debate whether you need to take the extra steps to further classify, understanding that that classification may or may not make a difference," she says. Is the added TAT worth it? Is the clinician a little worried, or a lot? "Usually those are the cases I'll call the clinician on. They may know something I don't. Or, it may not change their management. That's something a lot of people struggle with."

Though RCC classifications/sub-classifications can seem at times to be swaying at the rim of a rabbit hole, they are pertinent to patients.

Dr. Hirsch says her clinical colleagues at Dana-Farber Cancer Institute are keen to know the subtypes, "particularly in the metastatic setting," in part because they can determine which clinical trials—some of which they're designing—might help their patients.

Adds Dr. Merino: "Keep in mind, new protocols open all the time."

In some cases, tumors once thought to be aggressive may actually be indolent. In other cases, tumors that have been considered more common in one patient population might occur in other groups more frequently.

As work in this field unfolds, researchers have made intriguing discoveries. *TFE-3* translocation renal cell carcinomas were first identified in children and originally thought to be a pediatric tumor. "Over the years," Dr. Hirsch says, "we've come to recognize this diagnosis in adults, and we now know that we see more cases in adults than in children." It also appears that behavior and outcome of these tumors differ between the two patient groups as well. In children, the tumors are often confined to the kidney, and survival rates are good. In adults, these tumors not infrequently present as a metastasis before they show up as a primary kidney tumor. They also seem to show up much more frequently in middle-aged women. "I've had multiple cases where a *TFE-3* translocation renal cell carcinoma presents in a supraclavicular lymph node," she says. Although Dr. Hirsch can't explain why this tumor type prefers supraclavicular lymph nodes, she does use this information to her advantage. "If I get a case of a middle-aged woman with a supraclavicular lymph node metastasis and a renal mass, in my mind, that's a *TFE-3* translocated renal cell carcinoma until proven otherwise."

"In general, we will continue to struggle in a subset of cases where there is morphologic overlap between renal cell cancer subtypes," Dr. Hirsch says. Without knowing the molecular makeup in all cases, "some of these kidney tumors could very well be misclassified, but this should happen with less frequency as we learn more and more about the genetic and molecular makeup of tumors."

She and Dr. Reuter sing the same chorus: You can't diagnose something if you're not even thinking about it.

Then there's the matter of clear cell papillary renal cell carcinoma. Channeling her inner Dostoyevsky, Dr. Hirsch says, "It goes by two names." The WHO recognizes both. One is clear cell tubulopapillary renal cell carcinoma ("That's the only terminology I use," she says), which is synonymous with the term clear cell papillary renal cell carcinoma.

Likewise, some pathologists prefer to Type 1/Type 2 their papillary renal cell carcinomas; others (including Dr. Hirsch, Dr. Reuter, and Dr. Merino) do not. Says Dr. Merino: "I'm very opposed to saying something is papillary

Type 2. Because that encompasses quite a number of different tumors.”



Dr. Hirsh

Along with everything else, RCC appears to have a branding issue. “Our clinical colleagues do get frustrated,” Dr. Hirsch concedes, “but I think and hope they recognize our good intentions of helping patients get the best possible diagnosis and prognostic information.”

Terminology can eventually change; obviously nothing in this field is static. As noted, sarcomatoid renal cell carcinoma is no longer used; rather, knowledge of genetics and molecular alterations have made clear that tumors with such features arose from one of the recognized RCC subtypes. “We now refer to them as renal cell carcinoma *with* sarcomatoid differentiation,” Dr. Hirsch says. “And we try to give the underlying subtype, whether we get that from morphology or from genetic and molecular findings: clear cell carcinoma with sarcomatoid differentiation, papillary renal cell carcinoma with sarcomatoid differentiation, etc. But sarcomatoid RCC is not its own subclassification of renal tumors.”

In the case of tubulo versus sans tubulo, Dr. Hirsch says using the latter term is confusing, “obviously because of the clear cell and papillary cell subtypes. The word ‘tubulo’ makes it very distinct in my mind and the clinician’s mind that this is a different subtype.”

Since this subtype was first recognized about five years ago, the cards have been reshuffled a bit, Dr. Hirsch says. The tumor can be separated from traditional clear cell and papillary renal cell carcinomas. And where traditional thinking has considered clear cell renal cell carcinoma as being the most common, followed by papillary, then chromophobe, Dr. Hirsch is convinced that the incidence of clear cell tubulopapillary is higher than that of chromophobe and might even approach that of papillary renal cell carcinoma. “We think of papillary renal cell carcinoma being 10 to 15 percent of renal cell carcinomas; I would say the clear cell tubulopapillary renal cell carcinomas [are] definitely five to 10 percent, if not more, of cases.” The majority of renal cell carcinomas—some 70 percent—are conventional/clear cell RCC.

Pathologists have learned more about the morphologic features of the tubulopapillary tumor, for starters. And whereas clear cell renal cell carcinoma has a chromosome 3p loss, and papillary renal cell carcinomas have extra chromosome copies (i.e. trisomes, often with chromosomes 7 and/or 17), none of these chromosomal changes occur in the clear cell tubulopapillary renal cell carcinoma.

“In prior years, we didn’t even realize this tumor existed,” Dr. Hirsch says. “Now I’m finding this a very frequent tumor. As I go back through my files of renal tumors, I see cases of clear cell tubulopapillary carcinoma that were originally misdiagnoses, and now we see this tumor not infrequently on a routine diagnostic basis. It’s growing in incidence, simply based on recognition.”

While these cases are labeled as carcinoma, Dr. Hirsch says, “We think clear cell tubulopapillary renal cell carcinoma is an indolent lesion. It’s a much better behaving renal cell neoplasm. For that reason, teasing it out is really important. These patients have been dubbed with a carcinoma, and that’s scary for them, and they may lose life insurance, and they may get too many CT scans during routine clinical follow-up. But in the end they really have this very low-grade, indolent, probably benign tumor. And they could have had it taken out and just been told that they’re cured. Which is a completely different emotional situation for that patient.”

Clear cell RCC is anything but clear, as it turns out. “It is a very complex disease,” says Dr. Merino. “And it’s clear we don’t know how to subclassify them according to the genetic changes. It’s possible, with clear cell carcinomas,

that we have to do more molecular analysis.” Moreover, she says, “The translocation tumors are tumors that we still don’t know how to recognize very well. Because they have some clear cells, many people would just call them clear cell, when they’re *not* clear cell. They’re translocation tumors.”

Dr. Reuter offers another example from the TCGA study: Some genomic abnormalities present in chromophobe renal cell carcinomas—tumors that normally have a good prognosis—are associated with poor outcome. “So even within tumors that do well, we can identify features that would predict worse outcome,” he says. “But equally, if those are not present, they predict a good outcome.”

Not to be overlooked in any of this is the importance of pathology, a point the TCGA paper drives home multiple times, says Dr. Reuter. While molecular information would appear to be steering the ship (the words “molecular characterization” are in the title, after all), morphologic context adds a much-needed anchor.

Indeed, the *Cell Reports* paper features, on the opening page, a graphical abstract illustrating RCC subtypes (at right). That was intentional, says Dr. Linehan. “People might say we are entering an era where genomics will make pathology less relevant. That is not the case. The role of the pathologist, armed with new genomic information, is expanding.”

Experienced pathologists, he says, will be invaluable in evaluating the genomic data as well as interpreting histology. “The more we combine those two, the better off we’ll be.” In his view, “The role of the pathologist is even more critical today than it was 15 years ago.”

Dr. Merino agrees, noting that in her quarter of a century of working with Dr. Linehan and others, “It’s been a team effort that has led to successful stories.”

As the complexity of RCC pathology grows, much of the workup is surprisingly within reach of most laboratories.

“You can get pretty far with morphology and immunostains,” says Dr. Hirsch. “But you definitely can’t get to all diagnoses in 100 percent of cases without genetic or molecular information. Even then we still use the ‘unclassified’ category in a small subset of cases. But in this day and age, immunostains are a relatively quick and inexpensive ancillary study that can get to the diagnosis in many of the cases the majority of the time.”

She suggests a “bare bones” group of stains that pathologists could have on hand: PAX8, CK7, CD10, AMACR, CA9, HNF-1beta, S-100A1, CK20, FH (for FH-deficient renal cell carcinoma), and SDHB (for SDH-deficient renal cell carcinomas). “And then I would have a TFE-3 antibody on board, with the caveat that the TFE-3 antibody is very finicky. So I use it with caution. If it’s weak or focal, it’s not contributory and I turn to fluorescence in situ hybridization.” Anything shy of strong and diffuse staining in every single tumor cell is not useful, she says.

“I wouldn’t use these for every case,” she says, “but this group of antibodies would get me to a diagnosis the majority of the time.”

She also recommends that pathologists “go with their gut. If they see a tumor that doesn’t fit into a typical category, they should seek help.”

Despite the intrigue in Anna Pavlovna’s (or is it Annette Scherer’s?) salon, Tolstoy’s story continued to unfold, its characters succumbing to love and loss, disillusionment, revolution and ruin, winter, and many chapters of battle.

Dr. Linehan is familiar with the concept of an ongoing saga. Surveying the field of renal cell carcinoma and his 35-plus years of treating patients, he says, “We have an enormous amount of work to do.” What about those four TCGA renal cell carcinoma projects? “It’s a great start,” he says.

Physicians are only beginning to grasp the complexity of hereditary tumors, for example, and pathologists need to be aware that they might be seeing an index case.

Familial tumors, it turns out, are much more common than previously thought, says Dr. Reuter. “It is fair to say that pathologists in the community will confront these cases as specimens without the clinical information that this

is a patient with a hereditary syndrome.”

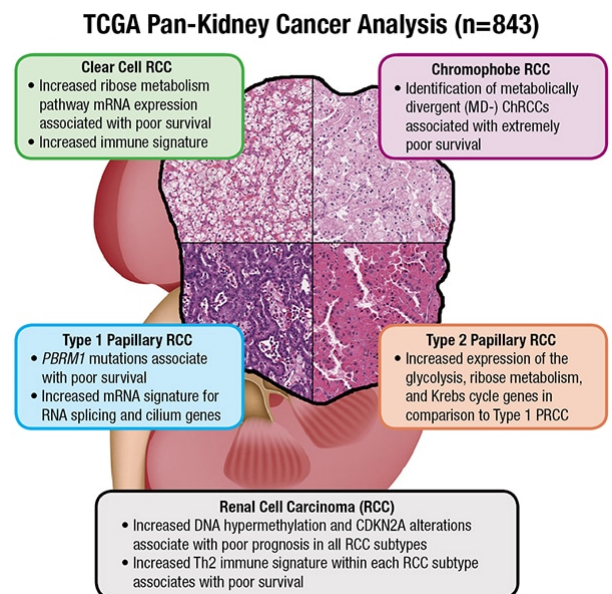
Some morphologies, he continues, are more likely to be associated with hereditary syndromes. There are also findings in the adjacent, supposedly normal renal parenchyma that can point pathologists toward the possibility of a tumor being associated with a hereditary syndrome.

The prototypic hereditary type is VHL, or the von Hippel-Lindau syndrome-related renal cell carcinoma, Dr. Hirsch says. Patients with VHL often develop clear cell renal cell carcinoma, but not everyone with clear cell renal cell carcinoma has VHL—in other words, the *VHL* gene mutations seen in the kidney tumors can be either germline or somatic.

But VHL is only one of several inheritable syndromes that affect the kidneys. Others include but aren’t limited to *MET* mutation with hereditary papillary renal cell carcinoma (HPRC); tuberous sclerosis (TSC); the fumarate hydratase-deficient renal cell carcinomas, which are associated with hereditary leiomyomatosis and renal cell carcinoma; and Birt-Hogg-Dubé, which involves an *FLCN* gene mutation.

These are a frequent topic of discussion with her clinical colleagues, Dr. Hirsch says, and labs are starting to take note. The first step, she says, is to think about the possibility of the diagnosis. If she sees a tumor that she thinks might be an FH-deficient renal cell carcinoma, for example, her next step would be to do a screen with a fumarate hydratase immunostain. “In this case we’re looking for loss of staining of the FH antibody.” If this is indeed the case, “in a note we will tell our clinical colleagues that this patient needs genetic testing to determine if this is a somatic or a germline mutation. And if it’s somatic, they just get treated for their kidney tumor. But if it’s germline, then they need to have their children and family members tested as well.”

Dr. Linehan seconds that. In the case of HLRCC, “Many times the clinician doesn’t know that’s what the patient is affected with.” This cancer spreads early, when the tumor is still quite small. Knowing the alteration status will change his surgical approach. “It’s more than invaluable; it’s essential,” says Dr. Linehan. “It really is a matter of life or death.”



In the meantime, the questions continue. Will specific genetic abnormalities or a better understanding of the immunologic landscape lead to better therapeutic interventions? Will new molecular assays help subclassify tumors even further? Will more RCC categories be helpful? Or will they become so numerous they merely create chaos, like the parade of visitors to the Marschallin in “Der Rosenkavalier”?

A common consult involves tumors with oncocytic cytoplasm, that is, tumors that enter the differential diagnosis of oncocytomas, which by definition are benign tumors. One of the morphologic and molecular limits to identifying this tumor is cytoplasmic eosinophilia. Asks Dr. Reuter: “Have we defined them too strictly in the past, and for that reason not made the diagnosis enough? Or have we used the term too liberally?” Addressing that question will require a combination of better outcomes data and improved molecular findings. “Because what happens now is, if I cannot classify a tumor as an oncocytoma, not put it into any one of those other categories that have eosinophilic cytoplasm, I end up putting it into the ‘unclassified’ category.” This is unpalatable to everyone, he says—clinician,

pathologist, and patient.

And so the work continues. Echoing Dr. Linehan, Dr. Reuter takes note both of the ongoing accomplishments and the daunting task ahead. "Putting everything together is very, very difficult," he says (possibly echoing the words of Tolstoy's agent after hearing the writer's *War and Peace* pitch).

And if it's not clear by now, the TCGA paper—and related work in the field—is a step, but only a step, toward a better understanding of renal cancer, says Dr. Reuter. "It's certainly not the end game."

As readers—both actual and the merely well intentioned—of Russian novels know, the end game is a long one. (This might be a good place to point out that even when *War and Peace* was over, it wasn't really over. It concludes with not one, but two—yes, two—epilogues, which add 28 more chapters to the book.)

But somehow even Dr. Linehan can manage to write a simple coda. "I'm very optimistic about the future."

Karen Titus is CAP TODAY contributing editor and co-managing editor.