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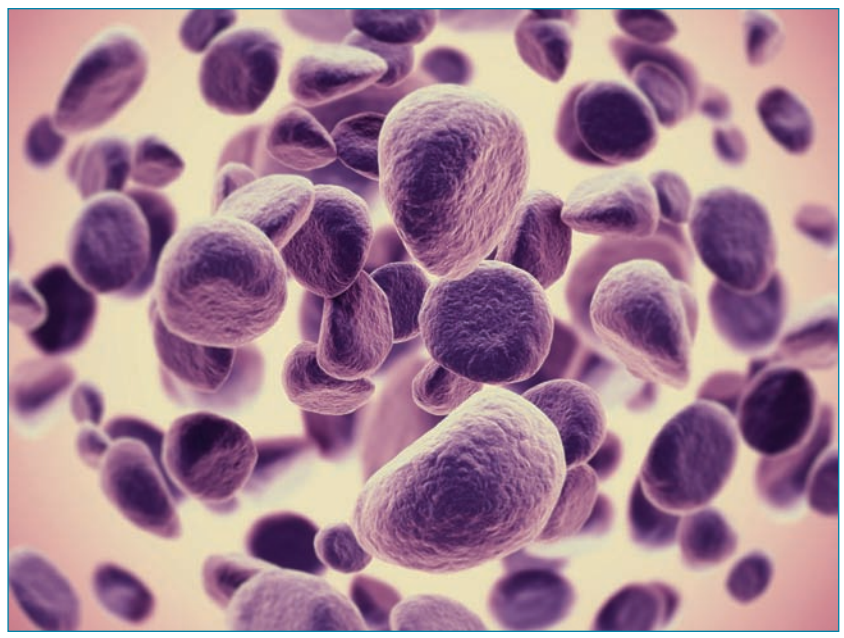
Immunotherapy's Growing Impact on the Treatment of Hematologic Malignancies

BY CATLIN NALLEY

Immunotherapy has revolutionized the field of oncology and continues to gain momentum. With ongoing research and a number of promising therapies, this approach has shifted how oncologists treat a variety of solid tumors.

How has the growth of immunologic therapies impacted the world of hematologic malignancies? Those working in this field have actually been utilizing one of the oldest forms of cancer immunotherapy for decades, allogeneic hematopoietic stem cell transplantation (HCT), which continues to help shape the treatment approaches in this patient population.



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Stem Cell Transplantation

Since its conception, utilization of allogeneic HCT continues to increase with widening clinical indications and the use of alternative

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Unexpected Success for a TKI in Activating Anticancer Innate Immunity

BY RICHARD SIMONEAUX

One of the earliest attempts in the peer review literature to highlight the relationship between infection and cancer remission was provided by William Coley, a New York-based surgical oncologist (*Ann Surg* 1891;14:199-200). The aptly-named Coley's toxins, which consisted of lysates of *Streptococcus pyogenes* and *Serratia marcescens*, were utilized for more than 70 years to treat cancer patients.

Although much clinical success has been noted across several different malignancies for immunotherapies utilizing the adaptive immune system's T-cell based defenses, including monoclonal antibody checkpoint inhibitors that target PD-1, PD-L1, or CTLA4 or, more recently, modified CAR T cells, several patient subpopulations do not experience disease response to these modern treatments. Consequently, therapies that utilize the innate immune system might offer not only the possibility of treating those patients not responding to current T-cell-based therapies, but also of supplementing the responses of those that do.

CME Article

In a recent preclinical study, the promiscuous tyrosine kinase inhibitor (TKI) cabozantinib showed promising activity in an

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Leukemia Hijacks the Microbiome to Glut Itself on Glucose

Cancer needs energy to drive its out-of-control growth. It gets energy in the form of glucose, in fact consuming so much glucose that one method for imaging cancer simply looks for areas of extreme glucose consumption—where there is consumption, there is cancer. But how does cancer get this glucose?

A recently published University of Colorado Cancer Center study shows that leukemia undercuts the ability of normal cells to consume glucose, thus leaving more glucose available to feed its own growth (*Cancer Cell* 2018; doi:10.1016/j.ccell.2018.08.016).

"Leukemia cells create a diabetic-like condition that reduces glucose going to normal cells, and as a consequence, there is more glucose available for the leukemia cells. Literally, they are stealing glucose from normal cells to drive growth of the tumor," said Craig Jordan, PhD, investigator at University of Colorado Cancer Center, Division Chief of the Division of Hematology and the Nancy Carroll Allen Professor of Hematology at the University of Colorado School of Medicine.

Key Findings

Like diabetes, cancer's strategies depend on insulin. In diabetes, either the pancreas under-produces insulin or tissues cannot not respond to insulin and so cells are left starved for energy while glucose builds up in the blood. The current study shows that leukemia goes about creating similar conditions of glucose buildup in two ways.

First, tumor cells trick fat cells into over-producing a protein called IGFBP1. This protein makes healthy cells less sensitive to insulin, meaning that when IGFBP1 is high, it takes more insulin to use

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Unexpected Success for a TKI in Activating Anticancer Innate Immunity

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invasive *PTEN*/*p53*-deficient prostate cancer mouse model that mimicked castrate-resistant prostate cancer (CRPC) in humans (*Cancer Discov* 2017;7(7):750-765). That investigation, which was performed by a team of researchers led by Akash Patnaik, MD, PhD, MMSc, a faculty member within the Section of Hematology/Oncology, Department of Medicine, University of Chicago, found that the tumor clearance caused by cabozantinib arose from a neutrophil-mediated immune response.

"Cabozantinib eradicated the poorly differentiated, invasive adenocarcinoma in these mice within 48 hours, with concomitant neutrophil infiltration into the tumor bed," Patnaik noted. "Strikingly, neutrophil depletion, or chemotaxis blockade with plerixafor (a CXCR4 inhibitor), or an HMGB1 neutralizing monoclonal antibody reversed the tumor clearance elicited by cabozantinib."

Neutrophils

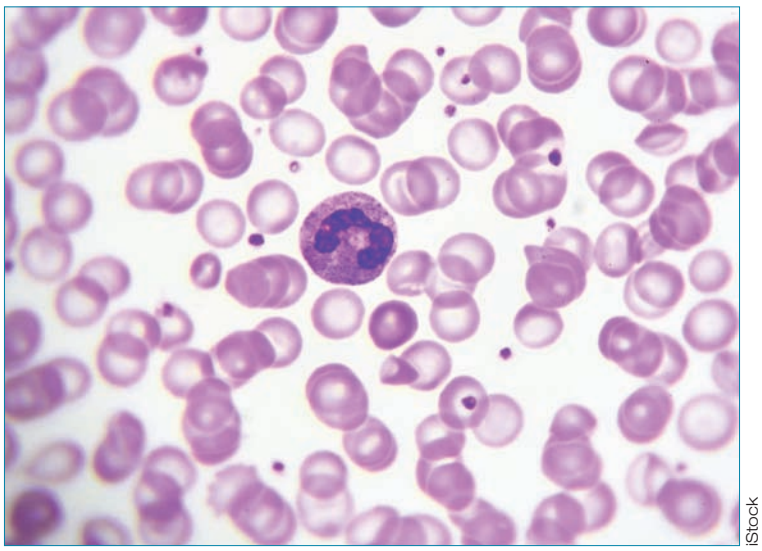
The phagocytes, which are an important component of the innate immune system, consist of the following cell subtypes: dendritic cells, macrophages, and neutrophils. Of these, neutrophils are the most abundant, making up to 50-60 percent of these cells.

Neutrophils are the first responders of inflammatory cells at the onset of inflammation, particularly that arising from bacterial infection, some cancers, or tissue injury. These cells are guided to the site of interest via a process called chemotaxis, by signaling chemicals such as interferon-gamma, interleukin-8, or leukotriene B4.

Recently, neutrophils have been implicated as having different roles in cancer. "The roles that these cells play in cancer are somewhat complex and not without controversy, as different neutrophil subpopulations can exert either pro- or anti-tumor effects, depending on the chemokine context within the tumor microenvironment," Patnaik explained.

Recent research showed that circulating neutrophils, both in cancer patients and murine cancer models, are heterogeneous and consist of distinct subpopulations (*Cell Rep* 2015;10(4):562-573).

In that study, neutrophils that had been previously divided into high-density neutrophils (HDNs) and low-density neutrophils (LDNs), were further separated into three different classes. The first



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class was the mature circulating HDNs, designated as N_{C1} (N1-like phenotype), which had previously been referred to as tumor-entrained neutrophils. This neutrophil subpopulation showed antitumor properties. The LDNs present were subdivided into two different groups: 1) mature circulating LDNs (N_{C2}, N2-like phenotype) and 2) immature circulating neutrophils that were previously described as granulocytic myeloid-derived suppressor cells. Both LDNs showed immunosuppressive and pro-tumor properties.

Moreover, their studies also showed there was considerable plasticity within the neutrophils, as adoptive transfer experiments showed that mature HDNs in tumor-bearing mice can switch to the low-density fraction. This transformation was accompanied by an increase in size and, importantly, a gain of immunosuppressive tumor-supporting properties.

It is of interest to note that this change appeared to be driven by transforming growth factor-beta (TGF-β). In prior work, that research group showed that TGF-β both blocked neutrophils' antitumor properties as well as induced the pro-tumor N2 phenotype. Although TGF-β showed the ability to drive the transformation of HDNs to LDNs in tumor-bearing mice, no significant effect was noted for HDNs from cancer-free mice, which implied that additional activation of HDNs is required for TGF-β to exert its effect.

The researchers' findings led them to conclude that, in addition to blocking neutrophil cytotoxicity, TGF-β also mediated a shift in the equilibrium between neutrophil subtypes that have opposing roles.

Cabozantinib

Cabozantinib, which was previously known as XL-184, is a promiscuous TKI with potent activity against several different enzymes that have been implicated in tumor growth and survival, including c-MET, VEGFR2, RET, KIT, AXL, and FLT3.

In November 2012, the FDA granted approval for the use of cabozantinib for the treatment of medullary thyroid cancer, which is a *RET*-driven disease. This approval was largely based on the results obtained in the phase III EXAM trial (NCT00704730), where those receiving cabozantinib had a median progression-free survival (PFS) that was more than twice the value obtained for those in the placebo group (11.2 months vs. 4.0 months). Notably, the results obtained in the cabozantinib patients appeared to be independent of their *RET* mutational status.

In April 2016, cabozantinib received approval from the FDA for the treatment of advanced renal cell carcinoma (RCC) in those patients who had prior anti-angiogenic therapy. In their approval letter, the FDA cited results obtained in the phase III METEOR trial (NCT01865747), where patients receiving cabozantinib had a median PFS of 7.4 months while those receiving everolimus had a median PFS of 3.8 months.

"In a phase II randomized discontinuation trial (NCT01428219) in CRPC patients with bone metastases, 72 percent of patients exhibited regression in soft tissue lesions, while 68 percent of patients had improvement in technetium-99m bone scan response, including complete resolution in 12 percent," Patnaik noted. "This bone scan response was dramatic and unprecedented in bone metastatic CRPC treated with current standard-of-care therapies." The median PFS

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Learning Objectives for This Month's CME Activity: After participating in this CME activity, readers should be better able to: 1. Distinguish the evidence supporting the role of the innate immune system in antitumor response. 2. Summarize study results identifying cabozantinib as a modulator of neutrophil-mediated antitumor innate immunity.

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values obtained were 23.9 weeks and 5.9 weeks for the cabozantinib and placebo-treated cohorts, respectively.

"Despite the promising phase II clinical trial results," he explained, "a recent phase III trial [COMET-1, NCT01605227] of cabozantinib in heavily pre-treated metastatic CRPC patients failed to demonstrate a statistically significant increase in overall survival with cabozantinib versus prednisone alone."

However, as with the previous phase II results, the median radiographic PFS was 5.6 months and 2.8 months for the cabozantinib and prednisone arms, respectively. "For the randomized phase III METEOR trial of cabozantinib versus everolimus in VEGFR inhibitor-resistant RCC, a 42 percent decrease in disease progression was noted," Patnaik stated. "Therefore, a deeper understanding of cabozantinib's anti-tumor mechanism in different disease contexts is critical for the biomarker-based stratification of patients most likely to respond to the drug."

Preclinical Mouse Studies

In describing the mice utilized in this study, Patnaik commented, "Mice with probasin Cre-driven conditional prostate-specific knockout of *PTEN* and *p53* genes [Pb-Cre; $PTEN^{fl/fl}$ $p53^{fl/fl}$] develop invasive CRPC as early as 9 weeks of age with locally aggressive tumors by 3 months of age, that are invariably lethal to the host by 7 months of age." When asked about the relevance to human CRPC, he replied, "The loss of *PTEN* and *p53* function are genetic events that are frequently observed in human metastatic CRPC."

Since cabozantinib was developed as a c-MET/VEGF2R inhibitor, and prior research found that 67 percent of the tumors obtained in these mice exhibited c-MET amplification, a logical starting point for mechanistic studies was to evaluate whether tumor eradication was c-MET-dependent. To accomplish this, aggressive prostate cancer tumors in 5/6-month-old Pb-Cre; $PTEN^{fl/fl}$ $p53^{fl/fl}$ mice were treated with vehicle, cabozantinib, or PF-04217903 (a specific c-MET inhibitor). Tumor treatment was initiated after the solid tumor had reached a long-axis diameter of ≥5 mm by ultrasound and MRI analysis.

"Mice treated with cabozantinib showed an approximately 70-percent reduction in tumor volume, with nearly-complete clearance of the poorly differentiated, invasive prostate carcinoma in 4 days, which was sustained over 3 weeks of cabozantinib treatment," Patnaik noted.

In direct contrast, uninhibited tumor growth was observed in mice receiving c-MET inhibitor PF-04217903 over 3 weeks of treatment, despite there being similar intratumoral phospho-MET inhibition with both cabozantinib and PF-04217903 treatment. Additionally, prostate tumors isolated from PF-04217903-treated mice showed persistent, poorly differentiated, and invasive prostate carcinoma after 3 weeks of treatment.

"Collectively, these data demonstrated that c-MET inhibition alone was insufficient to explain cabozantinib's anti-tumor mechanism of action," Patnaik clarified.

To gain further insight as to the mechanism by which cabozantinib elicited acute tumor clearance *in vivo*, transcriptional profiling and gene set enrichment analyses were performed on prostate tumors recovered from both mice that had received 48 hours of cabozantinib treatment and those receiving vehicle for the same period of time.

"These analyses revealed a statistically significant upregulation of immune response transcripts following cabozantinib treatment *in vivo*, while subsequent quantitative PCR-based RNA profiling of cabozantinib-treated tumors showed a spike in gene expression of the chemokine CXCL12 and its receptor CXCR4 within the tumor microenvironment (TME)," Patnaik stated. "CXCR4 is implicated in both lymphocyte and neutrophil chemotaxis; additionally, CXCR4 can engage CXCL12 as a homodimer or a 2:1 heterocomplex of CXCL12 and HMGB1 [a danger signal during immunogenic cell death (ICD)].

"Since tumor cells secrete a number of chemokines that can engage in crosstalk with different TME-based stromal cells, which may alter innate and adaptive immune function, it was necessary to determine the predominant cell type within the tumor that is responsible for cabozantinib-induced CXCL12 production," Patnaik explained.

RNA *in situ* hybridization (RISH) for CXCL12 and subsequent PTEN immunohistochemical analyses were performed on prostate tumors from mice treated with vehicle or cabozantinib for 24 hours.

"We observed an increase in intratumoral CXCL12 staining by RISH following cabozantinib treatment, specifically in *PTEN*-deficient cells within the microenvironment, which implied that the increased CXCL12 expression was predominantly occurring within the tumor cells," Patnaik stated.

Support for these *in vivo* results was obtained in *ex vivo* studies that revealed CXCL12 release from cabozantinib-treated *PTEN*/*p53*-deficient tumor-derived SC1 cells. "These data clearly show that cabozantinib treatment drives CXCL12 expression within the cancer cells in the TME," he observed.

> "This therapeutic strategy of activating innate immunity could be used in combination to enhance immune-responsiveness in cancers with very low response rates to immune checkpoint blockade."

"In order to ascertain whether cabozantinib could elicit an immunogenic cell death (ICD)-like response via HMGB1 release and heterocomplex formation with CXCL12 to engage the CXCR4 receptor, we asked whether treatment of *PTEN*/*p53*-deficient tumor-derived SC1 cells with cabozantinib *ex vivo* resulted in the release of HMGB1." Increased release of HMGB1 into the supernatant was shown by ELISA following *ex vivo* treatment of SC1 cells with cabozantinib for 32 hours.

"A similar response was observed with the known ICD-inducer, doxorubicin," Patnaik said. "In contrast, *PTEN* wild-type, *p53*-mutant human prostate cancer cells, VCaP, DU145 and 22Rv1, did not exhibit increased HMGB1 release following cabozantinib treatment."

To rule out the involvement of T-cell or natural killer (NK) cells in cabozantinib-induced tumor response, quantitative real-time PCR was performed on prostate tumors from mice treated with cabozantinib for 24 hours.

"Interestingly, we observed no increase in gene expression of T-cell markers (CD3, CD4, CD8) or NK and activated CD8 T-cell marker (NKG2D) following cabozantinib treatment," he stated. "If intratumoral resident NK and T cells play a dominant role in the anti-tumor immune response, then one would expect that removal of these cell types, singly and/or in combination, should attenuate the tumor clearance effects observed for cabozantinib treatment."

To test this hypothesis, Pb-Cre; $PTEN^{fl/fl}$/$p53^{fl/fl}$ mice were pretreated with antibodies that targeted conventional T-cell subsets (CD4/CD8) and NK cells (anti-sialo GM1) to deplete those respective cell populations, followed by cabozantinib co-administration. "We found that neither the CD4/CD8 nor NK depleting antibodies, singly or in combination, attenuated the tumor clearance observed for cabozantinib, which suggested that its anti-tumor immunologic mechanism is independent of both conventional T and NK cells," Patnaik stated.

Discussion

When asked to summarize their findings, Patnaik explained, "To our knowledge, this is the first time a tyrosine kinase inhibitor has been shown to modulate neutrophil-mediated anti-tumor innate immunity, resulting in the eradication of a poorly differentiated invasive tumor in a treatment-refractory *PTEN*/*p53*-deficient mouse model of prostate cancer.

"Mechanistically, this outcome was found to be independent of both T-cell and NK-cell function, and could be interrupted by concomitant treatment with granulocyte depletion (dexamethasone), an anti-HMGB1 monoclonal antibody (3E8), or a CXCR4 inhibitor (plerixafor), thus affirming neutrophils as the key effectors."

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HEAD & NECK CANCERS

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investigators also hope to find out if treatment can be de-intensified for the HPV-positive patients who tend to have more successful outcomes by virtue of their cancer type, thus allowing them to avoid some of the severe side effects.

"Of course, even in HPV-positive cancers, not every patient is cured," cautioned Mowery, "so we want to see if we can identify, early on, who is going to do well and who, in contrast, still needs that full 7-week intensive course of radiation therapy and chemotherapy."

Another clinical trial ongoing at Duke in which Mowery is involved is testing a drug called BMX-001 given to patients through a subcutaneous injection during radiation. "We hope the drug will reduce the mucositis—the inflammation and irritation of the lining of the mouth and throat during radiation—and dry mouth," she said.

Mowery is also busy in lab with intensive work in developing new mouse models of both HPV-related and HPV-unrelated squamous cell carcinoma of the head and neck. "My objective is to develop a platform in which I can develop radiation with immunotherapy, as well as with chemotherapy and various novel systemic agents, to try to improve outcomes particularly for HPV-negative disease," noted Mowery, also the winner of a 2017 Conquer Cancer Young Investigator Award. "I want to discover if there are ways that we can make our bodies and our immune system realize that these cells are not 'self' and activate the immune system to attack and eliminate them."

Tobacco-related cancer is induced in mice by giving them a carcinogen present in tobacco, "… causing them to become like a tobacco chewer or smoker," Mowery explained. "Having that exposure causes mutations in cells in the lining of their mouth."

Mowery further said her research is taking advantage of large sequencing projects in which various head and neck tumors have been sequenced. These data are publicly available and published primarily by The Cancer Genome Atlas organization. "I have been able to see which genes are most commonly mutated and then can genetically engineer mice to have those mutations. In other words, I can specifically knock out certain genes in the head and neck to model the cancer in mice."

This is extremely important because it allows Mowery and team to interrogate the biology of the mutations, and determine which genetic changes and pathways lead to the cancer spreading from its site of origin to the lymph nodes or the lungs. "It helps us to develop therapies to block the cancer and keep it at bay, and to determine if there are better ways to sensitize the cancer to radiation and chemotherapy," she detailed. "And we have an opportunity to test drugs that we hope will help with side effects of radiation. We must make sure that drugs protecting normal tissue are not also protecting the tumor. Having great animal models of human cancer is really important to making progress."

As if her work in head and neck cancer were not enough, Mowery is continuing an earlier effort begun in the lab of her research mentor David G. Kirsch, MD, PhD, by acting as radiation oncology principal investigator for a multi-site, international prospective randomized clinical trial investigating the combination of the immune checkpoint inhibitor pembrolizumab (anti-PD-1 antibody) and radiation therapy for patients with high-risk soft tissue sarcoma of the extremities. The researchers are also examining the biology behind the effects of radiation combined with pembrolizumab in a co-clinical trial using primary mouse models of sarcoma.

"We saw promising results combining them in this model. Our hope is by using this combination during the early stage of disease we may be able to eliminate those cells that have escaped the primary tumor before they cause a problem."

Who Has Time for Hobbies?

Asked about her life outside of the clinic and lab, Mowery admitted that little time is left for hobbies. "I used to play tennis, but now I just enjoy watching it," she said through a chuckle. "I splurged on a Labor Day vacation to the U.S. Open in New York. In my off time, I mostly read and spend time with my family. I am married; my wife is a nurse at Duke working in bone marrow transplant. We have no children."

> "I want to discover if there are ways that we can make our bodies and our immune system realize that these cells are not 'self' and activate the immune system to attack and eliminate them."

But the couple does have the patter of little feet in their midst. "We have two small dogs, Heidi and Cassie, a Maltese and a Maltese Shih Tzu mix—both less than 10 lbs.," Mowery offered. "We live in downtown Durham, N.C., which is a burgeoning area. It's kind of cool, and a little bit grungy—but in a good way. I love going for walks and checking out new restaurants. And I love food," she added brightly.

After a brief pause, Mowery turned her thoughts again to patients. "There is one other clinical trial we've recently opened in the head and neck space. We are looking at financial toxicity of patients," she said. "We are very concerned about the bills patients incur for cancer care and how that affects their quality of life.

"Unfortunately, some people just can't afford to fill their whole prescription. Some take their drugs every other day because they are worried about cost. Some patients just do not follow through on therapy. We need to get a better sense of how much of that is going on and if there are early warning signs we can detect allowing us to intervene."

Mowery added that better communications between health care providers and patients are needed to help patients better understand costs they face and identify resources that can help them.

"We just opened this survey-based pilot trial in June. We hope to have data next year and be able to develop a follow-up plan to employ the strategies that we find," said Mowery. "There are a lot of ways we can try to help our patients." OT

Valerie Neff Newitt is a contributing writer.

ANTICANCER INNATE IMMUNITY

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Regarding this important observation, Patnaik noted, "This has significant clinical relevance, since advanced prostate cancer patients frequently receive concomitant steroid therapy, which could interfere with anti-tumor immune responses elicited by hormonal and/or cytotoxic chemotherapy."

The cancer targeted in these studies had PI3K signaling amplification as a result of PTEN loss, which has been shown to cause disease which is resistant to immune checkpoint blockade (ICB). "Consequently, our work not only represents a paradigm shift in the field by showing that innate immune cells can independently provide an antitumor response, but it also suggests that strategies that activate neutrophils to target cancer may be a promising approach to overcome resistance to the recently developed T-cell-based immunotherapies," Patnaik explained.

When asked which malignancies might be good candidates for this TKI-mediated neutrophil-based immunotherapy, he replied, "This therapeutic strategy of activating innate immunity could be used in combination to enhance immune-responsiveness in cancers with very low response rates to ICB, such as CRPC and ER+ breast cancer. Clinical trials are underway to test such approaches." OT

Richard Simoneaux is a contributing writer.