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Improvements in CRISPR May Lead to Greater Precision in DNA Replacement

THE ADOPTION OF NEW technology requires a learning curve, as explained by Tremblay et al in "Integrating Mutational Analysis Into the Clinical Management of Patients With Myelofibrosis." Despite the commercially available assay for high throughput next-generation sequencing in myelofibrosis, adoption by clinicians continues to lag.

Sometimes, though, advances occur even before that technology can reach the masses.

Such is the case of a more precise gene-editing process that could eventually supplant the current technology, clustered regularly interspaced short palindromic repeat, known more commonly as CRISPR/Cas9. The new tool, MAGESTIC, which takes its name from "multiplexed accurate genome editing with short, trackable, integrated cellular barcodes," refines CRISPR, making it less like a blunt DNA cutting instrument, and more like a word processor that uses an efficient search-and-replace function on DNA material.[1]

In laboratories, CRISPR/Cas9 is used to target specific DNA sequences in cancer cells and replace those sequences with cancer-killing genes instead. However, for all its potential, CRISPR allows for random mutations to occur at cut sites in a cell's DNA. This uncertainty is problematic. Imagine using a pair of scissors to cut out words in a newspaper story—the words can be cut out, but it's difficult to remove individual letters or instantly know how the cuts affect the meaning of the text. In addition, many cells do not survive the editing process at all.

Building a more accurate method for CRISPR technology was the impetus for conducting further research by investigators at the Joint Institute of Metrology and Biology, a collaboration between Stanford University and the National Institute of Standards and Technology. The scientists accomplished this by providing a cell with a "donor" DNA, which a cell's DNA repair machinery uses as a template to replace the original sequence at the cut site.

The process by which a cell searches for a suitable donor DNA to repair a cut site is an enormous challenge, as the DNA repair machinery must search to find the correct "donor" DNA. MAGESTIC provides a major advance in gene-editing technology, aiding a cell in this search by artificially recruiting the designed donor DNA directly to the cut site in a process called "active donor recruitment." Such recruitment caused a 7-fold increase in cell survival, a change that surprised the investigators with its efficiency and effectiveness.

We look forward to seeing this technology progress and will continue to cover it in the pages of *The Journal of Targeted Therapies in Cancer*™.

Mike Hennessy, Sr
CHAIRMAN AND CEO

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From the Editor

Refinements in the Use of Genomic Rearrangements Signal Exciting Times Ahead

IN THIS ISSUE OF THE *Journal of Targeted Therapies in Cancer*™, Iams and Lovly describe a model system for patients with non–small cell lung cancer (NSCLC) for precision medicine based on identification and specific targeting of fusion partners that trigger activation of the *RET* gene. Currently, we envision the type of patient and the mechanisms that permit us to identify a subset of patients who may benefit from precision oncology. In NSCLC, chromosomal rearrangements involving the *RET* tyrosine kinase gene are known oncogenic drivers in 1% to 2% of patients. These *RET* rearrangements occur with characteristic partners, and this is reviewed in this issue by Iams and Lovly. Once the identification of these chromosomal rearrangements triggers activation of the RET tyrosine kinase, which most commonly occur in young patients with adenocarcinoma histology and minimal smoking history, therapeutic targeting of the RET-fusion driven NSCLCs may take the form of treatment with broad spectrum tyrosine kinase inhibitors with anti-*RET* activity. These include cabozantinib (Cabometyx), lenvatinib (Lenvima), and sunitinib (Sutent). Response rates range from 20% to 50% in largely pretreated patients. Although sunitinib has been used in fewer patients and additional agents are being developed, the main point is the same: Genomic rearrangements triggering an oncogene addiction can be therapeutically targeted. This allows us to identify mechanisms and monitor potential mechanisms of acquired resistance. Such precision oncology may enable us to combine oncogene-targeted therapies to prolong survival and to clarify the specific mechanism of action of these multikinase inhibitors, such as cabozantinib, vandetanib (Caprelsa), and sunitinib.

What is unclear from the article by Iams and Lovly is the extent to which genomic rearrangements create a true oncogene addiction of those cancer cells and to what extent the allelic fraction of that genomic rearrangement is a major component of that individual's cancer because of tumor heterogeneity. The question remains whether resistance is intrinsic to the rearranged cancer cell, somehow extrinsic due to lack of that genomic rearrangement, or some other feature that the rearrangement and RET tyrosine kinase activity may drive and induce.

It is an exciting time in precision medicine. Identification of genomic rearrangements that can be therapeutically targeted is welcome for all patients as a model of precision oncology marrying genomic analysis with cancer-targeted therapeutic agents.

Robert L. Ferris, MD, PhD
EDITOR-IN-CHIEF

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SIERRA Trial Explores Benefit of Iomab-B in Older Patients With AML

By Ariela Katz

RESULTS FROM MULTIPLE STUDIES have shown that older patients with either active, relapsed, or refractory acute myeloid leukemia (AML) have had lower survival rates, poor risk assessments, and limited therapeutic options. The standard care for these patients is salvage chemotherapy. In a currently accruing clinical trial, investigators will be pretreating patients in this high-risk population with Iomab-B, a novel radiolabeled antibody–drug conjugate, as part of a stem cell transplantation regimen, in hopes of improving remission and survival outcomes.

John M. Pagel, MD, PhD

"For a high-risk refractory AML patient, and one who may be older, it is highly unlikely that they will have significant long-term survival. So, this is an opportunity for them to get something that will, hopefully, control their disease, allow them to get a transplant, and give them some long-term survival advantage," said John M. Pagel, MD, PhD, chief of hematologic malignancies and director of stem cell transplantation at the Swedish Cancer Institute in Seattle, Washington, and study chair for the trial assessing Iomab-B.

Currently enrolling, the phase III SIERRA trial (NCT02665065) randomizes patients to Iomab-B or conventional chemotherapy as a preconditioning regimen before allogeneic hematopoietic cell transplantation (HCT). The primary endpoint for the study is durable complete remission; a secondary endpoint will be overall survival (OS). Participants in the control arm who do not achieve a complete response by day 42 will have the option of moving to the Iomab-B arm.

Patients eligible for the trial must be older than 55 years with active relapsed or refractory AML. Participants cannot have received HCT or prior radiation to maximally tolerated levels to any critical normal organ or have any central nervous system involvement. They must also demonstrate a Karnofsky score of 70 or higher and CD45 expression by leukemic cells via flow cytometry.

Iomab-B is a radioimmunoconjugate consisting of BC8, a murine monoclonal antibody, and iodine-131 radioisotope. Its purpose is to target CD45, a pan-leukocytic antigen widely expressed on white blood cells and the hematopoietic stem cell system.[1]

"We give a very high dose of radioiodine that's targeted to the sites of disease, so the antibody will localize to the bone marrow and other hematolymphoid organs but won't deliver radiotherapy to normal organs. The concept is, you're delivering targeted radiation to sites of disease, and you're trying to limit the amount that's going to normal organs. When you do that, you can escalate the dose to a higher level, so that you can, hopefully, eradicate all of the leukemia," Pagel said.

This method leads to the ablation of the patient's bone marrow. This is part of the stem cell transplantation regimen. Through ablation of bone marrow via CD45 targeting, Iomab-B may facilitate hematopoietic cell transplantation by the destruction of leukemia cells and hosting of immune system cells, which also prevents rejection of the donor cells.[1]

According to a study (NCT000008177) published in *Blood* by Pagel and colleagues, clinicians treating older patients with AML are limited in their ability to use the high-dose preconditioning myeloablative regimens that have proven effective in candidates for HCT, primarily due to the risks of nonrelapse mortality and graft-versus-host disease.[2]

"[NCT000008177] showed that survival rates in these high-risk patients could be about 40%, and that would be a major improvement over what we would expect with the standard stem cell transplant or, certainly, with standard cytoreductive chemotherapy," added Pagel.

Iomab-B was generally well tolerated. Most adverse events discovered were manageable. Of a total of 58 patients, 17% had chills, with 20% requiring treatment with meperidine; 12% experienced nausea and vomiting; and 26% developed respiratory symptoms, such as throat or chest tightness. In addition, 2% of patients developed grade 2 hypotension that required treatment with parenteral fluids.[2] The drug also can cause mucositis but is otherwise well tolerated, Pagel said.

> "The concept is, you're delivering targeted radiation to sites of disease, and you're trying to limit the amount that's going to normal organs."
>
> —JOHN M. PAGEL, MD, PHD
> SWEDISH CANCER INSTITUTE

"All patients will have reduction in their blood counts, and that could lead to subsequent risk for infection," Pagel said.

The study estimated OS and disease-free survival, according to the Kaplan-Meier method. A 1-year survival estimate of 41% (95% CI, 28%-54%) among all 58 patients was found in the assessment of the study. The 1-year survival estimate was 46% (95% CI, 20%-71%) in patients with AML in remission, 46% (95% CI, 20%-71%) in patients with AML in relapse, 38% (95% CI, 12%-65%) in patients with refractory disease, and 33% (95% CI, 9%-57%) in patients with high-risk myelodysplastic syndrome.[2]

Serving now as the basis for the SIERRA trial, this study demonstrated that the drug warranted further testing.

"If [the SIERRA] trial is successful, the next steps could be to explore [Iomab-B's] use in other patients with perhaps favorable-risk AML, in other hematologic malignancies, as well as other nonhematologic malignancies as an option for curative intent." ■

Iomab-B is developed by Actinium Pharmaceuticals, Inc, based in New York, New York.

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Memory T Cells May Predict Response to CAR T-Cell Therapy in CLL

By Jason Harris

Joseph A. Fraietta, PhD

J. Joseph Melenhorst, PhD

RESULTS FROM A RECENT study conducted at the University of Pennsylvania may demonstrate why some patients with chronic lymphocytic leukemia (CLL) are resistant to tisagenlecleucel (Kymriah) while potentially providing a pathway to enhance patient response.[1]

The findings may also give oncologists a way to identify patients with CLL who are most likely to respond to tisagenlecleucel. Although the chimeric antigen receptor (CAR) T-cell immunotherapy has been shown to induce a complete response (CR) rate of ≥80% in patients with B-cell acute lymphoblastic leukemia (ALL), the CR rate is 26% for patients with CLL.

Results from the study, led by senior author J. Joseph Melenhorst, PhD, and first author Joseph A. Fraietta, PhD, both faculty of the Department of Pathology and Laboratory Medicine at the Center for Cellular Immunotherapies, the University of Pennsylvania, demonstrated that patients with healthier, "early memory" cytotoxic T cells were far more likely to have a complete or partial response to treatment. In a validation study, this early memory T-cell signature predicted patients who would experience CR with 100% accuracy.

The investigators also found a correlation between high levels of the STAT-3 signaling pathway and a positive response to therapy. Previous study results have shown that the pathway was associated with T-cell persistence.

"With a very robust biomarker like this, we can take a blood sample, measure the frequency of this T-cell population, and decide with a degree of confidence whether we can apply this therapy and know the patient would have a response," Fraietta said in a statement. "The ability to select patients most likely to respond would have tremendous clinical impact, as this therapy would be applied only to patients most likely to benefit, allowing patients unlikely to respond to pursue other options."

Researchers sought to identify the reasons why some patients have strong responses to CAR T cells while others do not.

As in previous findings, age, tumor burden, and prior therapies were not predictors for response. Investigators could not identify any patient- or disease-specific predictors.

The research team found that patients who had a CR or partial response (PR) with transformed disease had a "dramatic" expansion of CAR T cells concurrent with B-cell aplasia within 2 weeks of infusion. Nonresponders had little or no T-cell proliferation and displayed minimal B-cell aplasia.

Detectable tisagenlecleucel cells persisted in patients still in CR after 5 years of follow-up.

"It appears that all effective CD19 CAR T cells, regardless of costimulatory domain, specific T-cell subset enriched for, or disease type treated, require in vivo cell expansion and persistence to be effective," investigators wrote.

After comparing the gene expression profiles and phenotypes of T cells in patients with CR, PR, or no response, the investigators concluded that defining features in the infusion product—early memory and nonexhaustion signatures in patients with CR and apoptosis and exhaustion in nonresponding CAR T-cell patients—also defined premanufacturing T cells.

Furthermore, the enhanced glycolysis signature in manufactured T cells from nonresponding patients and STAT3 signature in CR patient CAR T cells proved to be an effective way to enrich for the most potent leukemia killers, according to Melenhorst.

"Preexisting T-cell qualities have previously been associated with poor clinical response to cancer therapy, as well as differentiation in the T cells," Fraietta said. "What is special about what we have done here is finding that critical cell subset and signature."

Investigators conducted a validation study to evaluate the biomarker findings. The early memory T cells from 8 patients were screened before and after CAR T-cell therapy and accurately predicted the complete responders with 100% sensitivity and specificity.

Investigators believe these results could eventually lead to enhancing a patient's immune cells with the fast-expanding CD27-positive CD45RO-negative CD8-positive T cells prior to CAR T-cell therapy.

"What we've seen in these nonresponders is that the frequency of these T cells is low, so it would be very hard to infuse them as starting populations," Melenhorst said in a release. "But one way to potentially boost their efficacy is by adding checkpoint inhibitors with the therapy to block the negative regulation prior to CAR T-cell therapy, which a past, separate study has shown can help elicit responses in these patients."

The FDA approved tisagenlecleucel for pediatric B-cell precursor ALL in August 2017, making it the first approved CAR T-cell therapy in the United States. The agency recently approved a new indication for patients with relapsed/refractory large B-cell lymphoma—including diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma—after 2 or more lines of systemic therapy. ■

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Preclinical Studies of MDMX and MDM2 Lead to Phase I Trials in Patients With AML

By Anthony Berberabe, MPH

IN AN IDEAL WORLD, researchers conduct preclinical studies that generate a targeted therapy, which eventually makes its way through early, middle, and late-stage trial development and FDA approval. That smooth transition does not happen often, but early results involving an agent that affects 2 endogenous inhibitors of p53 look promising.

Ulrich Steidl, MD, PhD

Amit Verma, MD

Results of preclinical research conducted on the endogenous inhibitors MDMX and MDM2 has led to the launch of a phase I study involving the targeted agent ALRN-6924. The tumor suppressor p53 is usually inactivated via its interaction with inhibitors MDMX and MDM2, which are overexpressed in patients with acute myeloid leukemia (AML) and other cancers. Ulrich Steidl, MD, PhD, and colleagues demonstrated that MDMX is "considerably overexpressed in AML, including in leukemia stem cells, compared with age-matched controls."

"We know that p53 is a powerful member of this class of transcription factors—the gene activators," said Steidl, professor of cell biology and medicine at the Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York.

The researchers investigated the effects of dual MDMX/MDM2 inhibition using a stapled alpha-helical peptide in cells from patients with leukemia. Their findings provided a rationale for further development and clinical testing of ALRN-6924 as a therapeutic approach in cancers with wild-type *TP53*.[1]

The researchers found that ALRN-6924 activates p53-dependent transcription at the single-cell and single-molecule levels and displays robust biochemical and molecular biological on-target activity in leukemia cells.

"If you identify a targeted therapy that can reactivate the p53 molecule, then you don't have these tumor-promoting effects on cell cycle and cell death," said Steidl. "MDMX and MDM2 bind to p53 so that it cannot carry out its normal function. This is one of the endogenous ways to inhibit a tumor suppressor."

The new drug is effective at killing leukemia cells from cell lines and primary cells obtained from patients.

The agent is now undergoing investigation in early phase I clinical trials (NCT02264613 and NCT02909972) to determine its safety, as well as its therapeutic potential in AML and high-risk myelodysplastic syndromes. ALRN-6924 has thus far demonstrated efficacy and safety, with no reported grade 3/4 thrombocytopenias, and grade 3/4 neutropenia reported in less than 5% of patients.[2]

"The trials are actively recruiting," said Amit Verma, MD, Steidl's collaborator and a study investigator and director, Division of Hemato-Oncology at the Albert Einstein College of Medicine and Montefiore Medical Center.

NCT02264613 is an open-label, multicenter dose escalation (DEP) and dose expansion (EXP) study evaluating the safety, tolerability, PK (pharmacokinetics), PD (pharmacodynamics) and antitumor effects of ALRN-6924 in patients with advanced solid tumors or lymphomas with wild-type *TP53*.

The DEP portion of the study will enroll adults with histologically or cytologically confirmed malignancies that are metastatic or unresectable and for which standard treatment is not available or is no longer effective. The EXP portion of the study investigates the clinical safety profile and potential efficacy of ALRN-6924 at the maximum-tolerated dose or optimal biological dose. Peripheral T-cell lymphoma has been selected as one of the EXP groups to be further studied.

Treatment of patients in the DEP and EXP phases will continue in the study until disease progression, unacceptable toxicity, or when the patient or physician decides to discontinue therapy.

In NCT02909972, ALRN-6924 is undergoing investigation in patients with relapsed or refractory AML or advanced MDS with wild-type *TP53*.

"With these stapled peptides, which represent a new class of drugs based on novel chemistry that wasn't available 10 years ago, it is now possible, in principle, to target these transcription factors," said Steidl. "The hope is that our study has provided proof of concept, and opens the door for researchers to explore pharmacological targeting of many other of these hard-to-target molecules that play key roles in many cancers including leukemia." ■

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Selinexor Demonstrates Promising Results in Heavily Pretreated Myeloma

By Jason M. Broderick

IN TOP-LINE RESULTS FROM part 2 of the phase IIb STORM trial, selinexor induced an overall response rate (ORR) of 25.4% in patients with penta-refractory multiple myeloma, according to Karyopharm Therapeutics, the manufacturer of the oral selective inhibitor of nuclear export compound.[1]

Patient responses included 2 complete responses and 29 partial responses (PRs) or very good partial responses (VGPRs). The median duration of response was 4.4 months. Previously reported data from part I of the study showed that selinexor achieved an ORR of 20.5% in 78 patients with quad- or penta-refractory myeloma.

Full data from the study will be presented at an upcoming oncology conference, Karyopharm noted in its press release. The company also reported its intent to file an application with the FDA by the end of the year for an accelerated approval for selinexor as a treatment for patients with penta-refractory multiple myeloma.

Paul G. Richardson, MD

"Despite numerous advances in myeloma treatment, currently available therapies are insufficient to address the increasing number of patients with highly resistant penta-refractory myeloma, where the disease has ultimately become nonresponsive to approved therapy," Paul G. Richardson, MD, director of Clinical Research, Jerome Lipper Multiple Myeloma Center at the Dana-Farber Cancer Institute, Boston, Massachusetts, said in a statement.

"There is, therefore, a real urgency for new therapies with novel mechanisms of action for these patients, who have a critical unmet medical need. Selinexor's targeted inhibition of nuclear export could potentially expand the armamentarium of treatment options significantly in this important population for which no other established treatment is readily available," added Richardson.

The 122 penta-refractory myeloma patients in part 2 of the STORM trial were treated with 80 mg of selinexor twice weekly combined with 20 mg of low-dose dexamethasone. According to Karyopharm, the study defined penta-refractory as "patients who have previously received at least 1 alkylating agent, glucocorticoids, 2 immunomodulatory drugs (IMiDs; lenalidomide [Revlimid] and pomalidomide [Pomalyst]), 2 proteasome inhibitors (PIs; bortezomib [Velcade] and carfilzomib [Kyprolis]), and daratumumab (Darzalex), and whose disease is refractory to glucocorticoids, at least 1 PI, at least 1 IMiD, and daratumumab, and whose disease has progressed following their most recent therapy."

Sundar Jagannath, MD

"The 25.4% response rate and 4.4-month duration of response observed in the STORM study are highly compelling," Sundar Jagannath, MD, director of the Multiple Myeloma Program and professor of medicine (hematology and medical oncology) at Tisch Cancer Institute at Mount Sinai School of Medicine, said in a statement. "For an orally administered therapy, these new data underscore selinexor's potential to be an exciting new treatment option for these difficult-to-treat patients who have exhausted approved therapies."

Results from part 1 of the STORM study were presented at the 2016 ASH Annual Meeting. In 48 patients with quad-refractory disease, the ORR was 20.8% (n = 10), and in 30 patients with penta-refractory disease, the ORR was 20% (n = 6). In the overall population, the median progression-free survival (PFS) and overall survival (OS) were 2.3 and 9.3 months, respectively.

In the multicenter single-arm phase IIb STORM trial, 79 patients with heavily pretreated relapsed/refractory multiple myeloma (median of 7 prior treatment regimens) received 80 mg of oral selinexor plus 20 mg of oral dexamethasone twice weekly. Over each 4-week cycle, patients were dosed continuously (8 doses/cycle) or for 3 weeks on and 1 week off (6 doses/cycle). The primary endpoint was ORR.

Sixty-one percent of patients (n = 48) were quad-refractory, meaning they had received the PIs

> "There is... a real urgency for new therapies with novel mechanisms of action for these patients [with penta-refractory myeloma]."
>
> —PAUL G. RICHARDSON, MD
> DANA-FARBER CANCER INSTITUTE

bortezomib and carfilzomib and the IMiDs lenalidomide and pomalidomide. Thirty-nine percent of patients (n = 31) were penta-refractory, meaning they were also refractory to an anti-CD38 agent, such as daratumumab or isatuximab.

In the quad-refractory group, the median age was 62 years (range, 41-78), the median number of prior regimens was 7 (range, 3-16), and the median duration from diagnosis was 4 years (range, 1-6). Eighty-three percent of patients received the 6-dose regimen, and 17% of patients received the 8-dose regimen.

Among the penta-refractory cohort, the median age was 68 years (range, 34-78), the median number of prior regimens was 7 (range, 5-17), and the median duration from diagnosis was 4 years (range, <1-35). Thirty-five percent of patients received the 6-dose regimen, and 65% of patients received the 8-dose regimen.

The clinical benefit rate (CBR; defined as VGPR + PR + minor response [MR]) was 33% in the overall population. The VGPR, PR, and MR rates were 5%, 15%, and 13%, respectively. The stable disease (SD) and progressive disease (PD) rates were 35% and 12%, respectively.

In quad-refractory patients, the CBR was 29%, comprising VGPR, PR, and MR rates of 4%, 17%, and 8%, respectively. The SD and PD rates were 44% and 8%, respectively. In penta-refractory patients, the CBR was 40%, comprising VGPR, PR, and MR rates of 7%, 13%, and 20%, respectively. The SD and PD rates were 20% and 17%, respectively.

Among patients receiving the 6-dose regimen, the ORR was 20%. The CBR was about 29%, comprising VGPR, PR, and MR rates of 6%, 14%, and 10%, respectively. The SD and PD rates were 41% and 8%, respectively. In those receiving the 8-dose regimen, the ORR was 22% and the CBR was about 41%, comprising VGPR, PR, and MR rates of 4%, 19%, and 19%, respectively. The SD and PD rates were 22% and 19%, respectively.

In patients with del(17p), the ORR was 38%. The CBR was 63%, comprising VGPR, PR, and MR rates of 13%, 25%, and 25%, respectively. The SD and PD rates were 25% and 13%, respectively.

The median time to response was 1 month, and the median duration of response was 5 months. Among patients who achieved at least an MR, the median OS was not reached and the median PFS was 5.5 months.

The most common all-grade adverse events (AEs) included nausea (73%), thrombocytopenia (73%), fatigue (63%), anorexia (49%), anemia (49%), vomiting (44%), diarrhea (43%), hyponatremia (42%), weight loss (33%), leukopenia (32%), neutropenia (24%), lymphopenia (14%), dehydration (11%), and dysgeusia (11%).

Grade 3 AEs occurring at the highest rates were anemia (27%), thrombocytopenia (25%), hyponatremia (22%), fatigue (15%), and leukopenia (13%). Grade 4 AEs included thrombocytopenia (34%), neutropenia (6%), anemia (1%), leukopenia (1%), and lymphopenia (1%).

Selinexor dose modifications included interruptions, reductions, and discontinuations in 52%, 37%, and 18% of patients, respectively.

On March 14, 2018, the FDA placed a partial clinical hold on trials of selinexor. Although the hold stopped additional enrollment in the trials, patients who had achieved SD or better could continue treatment.

"The FDA has indicated that the partial clinical hold is due to incomplete information in the existing version of the investigator's brochure [IB], including an incomplete list of serious adverse events associated with selinexor. At the FDA's request, Karyopharm has amended the IB and updated the informed consent documents accordingly and has submitted such documents to the FDA as requested," Karyopharm reported in a statement at the time the hold was placed.

The company noted the hold was not related to any new safety concerns. On March 30, 2017, Karyopharm announced that the hold had been lifted and new patient enrollment and dosing recommenced for the selinexor clinical trial program. ■

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Reardon Addresses Tumor-Specific Immune Response in GBM

By Danielle Ternyila

THE USE OF PERSONALIZED neoantigen vaccines led to promising results in a recent phase I/Ib trial for patients with glioblastoma multiforme (GBM). In this small study, presented at the 2018 AACR Annual Meeting, researchers at Dana-Farber Cancer Institute created personalized vaccines for 8 patients based on their individual tumor types following standard-of-care treatment.

Although a small number of patients were enrolled in this trial, investigators found evidence of neoepitope-specific T-cell responses to the vaccine in a subset of the patients, according to David A. Reardon, MD. There was also a correlation between response to the vaccine and corticosteroids. Patients who received a corticosteroid to treat inflammation did not respond, although patients who did not require corticosteroid therapy showed promising responses to the vaccine. Overall survival was 16.8 months.

In a disease that is both deadly and difficult to treat, Reardon says these findings provide hope for the feasibility of immunotherapy approaches in this patient population. In an interview with *The Journal of Targeted Therapies in Cancer*™ (*JTT*), Reardon, clinical director, Center for Neuro-Oncology at Dana-Farber Cancer Institute, discussed the results of this trial in further detail and explained the impact these findings may have on future research for GBM.

JTT: Can you provide an overview of your recent trial with personalized vaccines in GBM?

David A. Reardon, MD

Reardon: Our trial involved administration of a personalized, individualized vaccine for patients with newly diagnosed glioblastoma, the most common and deadliest cancer arising in the central nervous system in adult patients. This is a tumor that has proven refractory to therapy, and our currently available best treatments are essentially palliative, unfortunately. We took a novel approach where we took advantage of next-generation sequencing technology to characterize the mutational burden, or landscape, in each individual patient's tumor. And then, using established algorithms, we were able to predict the coding mutations that gave rise to [the] mutant peptides that were most likely to be immunogenic for each individual patient, based on their individual human leukocyte antigen status. We then synthesized the top 20 of those coding neoepitope peptides predicted to be most immunogenic for that patient and administered those peptides back to the patient as an individualized personalized vaccine for their tumor.

The standard of care for newly diagnosed patients is surgical resection. Patients have to heal for 3 to 4 weeks [before undergoing] radiation therapy, typically with chemotherapy, and that phase takes about 6 weeks. Then patients have a 4-week break typically built into the standard-of-care therapy. This standard of care really lent itself to this approach. While they were recovering from their initial surgery, undergoing radiation therapy for 6 weeks, and then having a few weeks recovery, we took advantage of that time period to engineer their individual vaccine, because, as you can imagine, this is a labor-intensive effort.

After they finished their radiation therapy, their vaccine would be prepared. We would have it ready, and then they would undergo a series of priming vaccine injections of up to 20 tumor-specific neoepitope peptides. We gave 5 doses over 4 weeks as the timing phase, which was then followed by separate booster doses of these same 20 neoepitope peptides, spaced out at 8-week intervals. That was the treatment approach: 5 priming vaccines followed by 2 booster intervals at separate 8-week intervals.

We treated 8 patients on this initial experience. In 5 of the patients, we were able to collect blood for in-depth immunologic analyses, comparing prior-to-vaccination to post vaccination, and we were able to interrogate how well the vaccine worked in generating effective immune responses.

Unfortunately, 3 of the patients enrolled progressed before we could really get to the point where we could collect blood after their priming to do the analysis of the vaccine's effects. I think that underscores the difficulty of the indication we are dealing with here. Nonetheless, in 5 patients, we were able to analyze the pre- and postvaccination responses, and interestingly, in those 5 patients, in addition to peripheral blood, we also had tumor samples at the time of radiographic progression so that we could not only compare the peripheral blood and the systemic immune responses pre- and postvaccination, but the tumor samples. Of course, we had tumor samples from prior to therapy as well as at the time of progression, so we were able to compare those in order to determine if the vaccine had worked.

JTT: What were the results of this study?

We saw pretty striking evidence of multineoepitope responses to the vaccine that we generated in a subset of patients, including remarkable multiple neoepitope peptide responses and fully functional T-cells specific for the mutant peptide

and not the wild-type. Even though there is typically only 1 amino acid difference between the wild-type and the mutant neoepitope peptide, it was sufficient enough to generate specific immune responses to that mutant neoepitope peptide. We saw patients who didn't respond, and we saw a very important correlation of whether patients responded or not, based on whether they were receiving concurrent corticosteroids therapy.

With [patients with brain cancer], there's often a lot of swelling in the brain associated with the tumor, which can be exacerbated by surgery and radiation therapy. If that swelling causes symptoms, like headaches, functional deficits, or seizures, we have to treat that swelling with anti-inflammatory medicines, and the only anti-inflammatory medication that effectively improves symptoms related to cerebral edema is dexamethasone [Decadron]. Decadron is a highly potent corticosteroid. It's 5 to 10 times more potent than prednisone or Solu-Medrol [methylprednisolone], and it does decrease the inflammatory reaction in the brain. Patients who developed symptomatic cerebral edema requiring corticosteroid dosing while they were getting their vaccine priming, unfortunately, didn't respond. So that potent anti-inflammatory effect also negated their immunological response to the vaccine. The patients who did not require corticosteroids, those are the ones who responded beautifully. Although we are dealing with small numbers of patients, it was a striking correlation. Any corticosteroids done in priming, no immunological responses. No corticosteroids, nice responses.

JTT: Were there any other significant discoveries you came across with these findings?

Another important observation we made is that the glioblastoma type of tumor is characteristically and immunologically identified as a cold tumor with a microenvironment that has very few infiltrating immune-effector cells. We could look at the prevaccine immune landscape from the patient tumors, and because we had tumor collected postvaccination, we could look to see how the immune infiltrate in the microenvironment changed post vaccination. In the patients who weren't getting the steroids, we also saw a striking, statistically significant increase in various immune effector cells into the tumor microenvironment. With this personalized vaccine approach, we were able to convert a cold tumor microenvironment to at least a warm or inflamed one with a significant immune infiltrate.

The final important discovery observed was that we were able to conduct T-cell receptor [TCR] clonality analysis, which identified specific TCRs for the neoepitope peptides that we vaccinated against in the reactive T cells in the peripheral blood. We were able to separate out the immune effector cells from those tumor samples and identify identical TCR clones in the brain, in the tumor, as opposed to the same ones that were identified in the blood. So we had identical TCR clones in the peripheral blood and in the brain after vaccination.

This is the first documentation that a systemically induced tumor-specific immune response can effectively traffic into the brain and infiltrate into the tumor in the GBM tumors. Although we would like to see the vaccine-generating tumor-specific immune responses systemically, we also really have to confirm that they are getting to where they need to go to have their antitumor effect. This is the first evidence in this disease and indication where this has been able to be accomplished by an immunotherapy treatment approach. It's a small step, but it is an important one to highlight the ability of a personalized vaccine approach utilizing tumor-specific mutations to generate tumor-specific antitumor T cells, for them to potentially impact this disease in the tumor with a cold microenvironment.

Importantly, glioblastoma is also a tumor that has a relatively low mutational burden. This neoantigen vaccine approach is possible and successful in melanoma, but we know that's a tumor with a high mutational load. Being able to take advantage of those mutations and identify a personalized vaccine is important, but it's one that is more relevant in that setting. When you have a tumor with a 10-fold lower tumor mutational burden, can you still pick out and identify the appropriate neoepitope, or coding, mutations and utilize this approach? Indeed, in our experience, we were able to confirm and demonstrate that this type of approach is feasible in a tumor with a cold microenvironment and characterized by a relatively low mutational burden.

JTT: Are there any follow-up steps you plan to take with these results?

Yes, our next step is ready to go. We are going to combine this neoantigen vaccine with checkpoint blockade. We have a study that we are going to initiate with approximately 30 new patients who enrolled for this summer. We are anxious to see if the addition of a checkpoint blockade can potentially expand the number of responding neoepitope peptides–T cells responding to the number of vaccinated neoepitope peptide–and hopefully we can block some of these suppressive factors in the microenvironment, particularly those mediated by checkpoint blockade, to allow the T cells to have a more significant impact.

JTT: What do you think is the take-home message from this study for community oncologists?

I think the take home message is that for glioblastoma, a very deadly and challenging disease, this study provides some hope that immunotherapy approaches are feasible and may be able to be successfully exploited for this disease. It's still very early, with a long way to go, but what we've been able to demonstrate here is that we can successfully stimulate tumor-specific immune cells that could have a potentially meaningful impact. The take-home message for community oncologists is that our study helps to provide hope that immunotherapy can have a benefit in this very challenging group of patients. ■

Integrating Mutational Analysis Into the Clinical Management of Patients With Myelofibrosis

Douglas Tremblay, MD; Lauren Schwartz, MD; Bridget Marcellino, MD, PhD; and John Mascarenhas, MD

ABSTRACT

Myelofibrosis is a myeloproliferative neoplasm characterized by splenomegaly, progressive cytopenias, and transformation to acute myeloid leukemia. Several somatic mutations are pathogenetically responsible for this phenotype, the most important of which are *JAK2, CALR,* and *MPL.* However, the advent of high-throughput next-generation sequencing has identified multiple other molecular alterations that hold prognostic and possibly therapeutic potential. This tool is now commercially available, yet clinicians are frequently unfamiliar with how to interpret the results and incorporate them into the care of an individual patient. This review will describe mutations detected in myelofibrosis and discuss how to incorporate mutation information into risk stratification and therapeutic decision making for patients with myelofibrosis.

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Introduction

Myelofibrosis (MF) is a myeloproliferative neoplasm (MPN) characterized by progressive cytopenias, splenomegaly, bone marrow fibrosis, and clonal proliferation of myeloid cells. MF can be either primary (PMF) or secondary, arising from antecedent essential thrombocythemia (ET) or polycythemia vera (PV), termed PET/PPV-MF. The natural progression of MF is to bone marrow failure and then evolution to acute myeloid leukemia (AML), which portends a bleak prognosis.[1] Overall survival (OS) is influenced by a number of clinical variables, including advanced age, anemia, red blood cell transfusion dependence, thrombocytopenia, presence of peripheral blood blasts, and karyotypic abnormalities. These risk factors are encapsulated in currently utilized risk stratification tools, such as the Dynamic International Prognostic Scoring System (DIPSS) and DIPSS Plus.[2]

High-throughput next-generation sequencing (NGS) to detect the presence of acquired somatic mutations holds the potential to not only enhance predictive abilities of clinical scoring systems, but also to personalize therapeutic approaches with mutation-directed targeted therapy.[3] Technologic advancements in DNA sequencing have decreased the turnaround time while reducing costs, allowing for widespread commercial availability and expanding the application in routine clinical care.[4] Therefore, it is important for clinicians to be familiar with the clinical applications of NGS in prognostication and therapeutic decision making when caring for patients with MF.

We will first describe the most commonly mutated genes in MF and their prevalence. A thorough review of their prognostic potential including the integration of genomic data into current risk stratification tools will then be presented. Then we will describe the current utilization of mutational analysis in determining the treatment plan and discuss the development of molecularly based targeted therapy. Finally, we advocate for routine integration of mutational profiling into routine clinical practice.

In MF, mutations involving 3 genes–*JAK2*, *CALR*,

and *MPL*–are known to be directly related to the MPN phenotype. Janus-associated kinase 2 (*JAK2)* encodes a tyrosine kinase integral to hematopoietic cell function. The gain-of-function *JAK2* V617F results in the constitutive activation of the JAK-signal transduction and activator of transcription (STAT) signaling pathway and culminates in upregulation of downstream targets.[5] Additionally, *JAK2* V716F has been shown to directly phosphorylate histone H3, resulting in epigenetic transcriptional changes including upregulation expression of the hematopoietic oncogene *LMO2*.[6] Calreticulin (*CALR)* encodes for a calcium-binding protein, which localizes to the endoplasmic reticulum and has a multitude of intracellular, extracellular, and cell-surface functions. This protein was first discovered to play a role in protein homeostasis but subsequently has been found to be involved in immune regulation, calcium metabolism, phagocytosis, cell adhesion, and migration.[7-9] *CALR* frameshift mutations within exon 9 result in activation of the JAK/STAT signaling pathway,[10] and interact with the thrombopoietin receptor, encoded by *MPL*, causing overactivation of the JAK/STAT pathway.[11] Several recurrent mutations exist in *MPL*, the most common being *MPL*W515L/K in which the tryptophan between the cytosolic and transmembrane domains is altered, leading to dimerization of the MPL transmembrane helix and constitutive activation of JAK signaling.[12] The commonality among these mutations is that they all result in JAK/STAT pathway activation, the central pathobiologic mechanism of MF.[13]

Additionally, other acquired subclonal mutations are present in MF. The prevalence of these mutations is noted in **Table 1**. Many of these mutations occur in genes that are important for epigenetic regulation of gene expression. Some examples are *ASXL1*, *EZH2*, *TET2*, and *DNMT3A*, with *ASXL1* mutations being the most prevalent and most associated with adverse outcomes across the myeloid malignancies.[14,15] *ASXL1* encodes a scaffolding protein involved in epigenetic regulation,[16] and its mutations promote myeloid transformation via loss of polycomb repressive complex 2–mediated histone H3 lysine 27 methylation.[17] Another subset of genes found mutated in MF are those involved in mRNA splicing, including *SRSF2* and *SF3B1*.[18] Although less prevalent than the aforementioned mutations, *IDH1/IDH2* mutations are of particular interest in light of the development of isocitrate dehydrogenase (IDH) inhibitors currently in trial and, in the case of *IDH2*, now approved. *IDH1/IDH2* encode IDH, which catalyzes oxidative decarboxylation of isocitrate to alpha-ketoglutarate. The resultant decrease in alpha-ketoglutarate and/or accumulation of 2-hydroxyglutarate is believed to be oncogenic.[19] Notably, *IDH1/IDH2* mutations are found in a significant percentage of patients with MPN, especially those whose disease transforms to AML; however, these mutations are very rare in patients with MPN in chronic phase.[20]

TABLE 1. Frequency of Somatic Mutations in Myelofibrosis

Mutation	Prevalence
JAK2 V617F	57.0%[45]
CALR exon 9	35.0%[10]
ASXL1	22.0%[46]
U2AF1	15.0%[47]
TET2	9.7%[46]
SRSF2	8.5%-22.0%[46,47]
DNMT3A	7.0%-15.0%[48,49]
SF3B1	6.5%[50]
EZH2	5.1%-13.0%[46,51]
*MPL*515L/*MPL*515K	5.0%[52]
CBL	4.4%[46]
SETBP1	2.5%[53]
IDH1/IDH2	2.0%[46]
KIT	>1%[54]

Risk Stratification

Several prognostic scoring systems have been developed and employed to risk-stratify patients with PMF. The International Working Group for Myelofibrosis Research and Treatment devised the International Prognosis Scoring System (IPSS) based on a retrospective cohort study of 1054 patients with PMF from 7 centers. Five factors were noted in multivariate analysis to hold independent prognostic significance: presence of constitutional symptoms (ie, weight loss >10%, night sweats, fever), age >65 years, hemoglobin <10 g/dL, leukocyte count >25,000/microL, and circulating blast cells ≥1%.[21] DIPSS includes the same 5 factors but assigns 2 points for anemia.[22] Both the IPSS and DIPSS are calculated by adding points weighted by their corresponding hazard ratio to calculate 4 discrete risk categories of low, intermediate-1, intermediate-2, »

TABLE 2. Prognosis Associated With Somatic Mutations in Myelofibrosis

Mutated Genes Associated With:		Mutations Associated With Progression to AML
Favorable Prognosis	Poor Prognosis	
CALR	*ASXL1*, *SRSF2*, *EZH2*, and *TP53*	*IDH1/IDH2*, *SRSF2*, *ASXL1*, *TP53*

and high risk. The DIPSS is routinely used in clinical practice to determine a prognostic category at any time during the MF clinical course. Given a growing understanding of clinical features that also influence outcomes independent of the established DIPSS, this scoring system was again updated to include red blood cell transfusion dependence, thrombocytopenia, and unfavorable karyotype, each receiving an additional point. This enhanced prognostication score, DIPSS Plus, was validated in 793 patients with PMF at Mayo Clinic, where the median overall survival with 0 points (low risk), 1 point (intermediate-1), 2 to 3 points (intermediate-2), or 4 to 6 points (high risk) was 15.4, 6.5, 2.9, and 1.3 years, respectively.[2]

These prediction models may be enhanced by incorporating data from mutational profiling, as this technology can delineate prognostically distinct molecular subtypes. In particular, knowledge about the driver mutational status in patients with MF is essential when counseling patients on their prognosis and in guiding treatment decisions. This is best demonstrated by a study of 428 patients with MF in which the median overall survival in *JAK2*-, *MPL*-, and *CALR*-mutated patients was 5.9, 9.9, and 15.9 years, respectively. Importantly, in the absence of all 3 driver mutations–designated as "triple negative" status (TN)–the median survival was only 2.3 years; TN status portends the worst prognosis. In terms of leukemic transformation, TN carries the highest risk, and *CALR*-mutated in the absence of other mutations carries the most favorable risk.[23] Interestingly, type 1 (52 base-pair deletions) *CALR* mutations may be associated with a longer survival versus type 2 (5 base-pair insertions), according to results of a study of 358 patients with PMF. However, in multivariable analysis, which included DIPSS Plus score and *ASXL1* mutational status, this survival difference disappeared.[24]

Aside from the main driver mutations, other somatic mutations also hold significant prognostic information. In a study of 879 patients with MF initiated in a European cohort and validated in a Mayo Clinic cohort, mutations involving *ASXL1*, *SRSF2*, and *EZH2* were found to be associated with a shorter OS. However, only mutated *ASXL1* remained independent of DIPSS, effectively identifying this mutation as a negative prognostic factor with potential to enhance established risk stratification systems. There was discrepancy between cohorts on the mutations found to be associated with leukemic transformation and shortened leukemia-free survival (LFS); however, the results of the study indicated that *IDH1/IDH2*, *SRSF2*, *EZH2*, and *ASXLI* mutations portend a poor prognosis. A separate study of 254 patients with MF shed further light on molecular-based risk stratification. Specifically, TN status was associated with a median overall survival of 2.5 years; however, patients harboring an *ASXL1* mutation without a *CALR* mutation carried the worst prognosis, with a median overall survival of 2.3 years.[25] Together, this identifies TN status and *ASXL1*-mutated/*CALR* wild-type as high-risk molecular subtypes of MF.

The number of high-risk mutations (ie, *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/IDH2*) may also be important, as demonstrated in a study of 797 patients with PMF. The cohort with 2 or more high-risk mutations had a median OS of 2.6 years, compared with 7.0 years and 12.3 years, respectively, in the groups with 1 and 0 high-risk mutations. The presence of 2 or more mutations was also associated with a shorter LFS when compared with patients with no prognostically detrimental mutations.[26]

Other mutations may also share prognostic importance in MF. In a series including 197 patients with PV, ET, or PMF, the presence of mutated *TP53* and *TET2* were independently associated with shorter OS and shorter LFS.[27] However, other studies have noted a neutral effect of *TET2* on survival and leukemic transformation.[28] **Table 2** summarizes the current prognostic knowledge of these mutations in MF.

Recently, several risk stratification tools have been developed that incorporate mutational information to refine prognostication and therefore improve treatment decision making. One such scoring system is the mutation-enhanced IPSS (MIPSS), which takes into account the mutational status of *CALR*, *JAK2*, and *MPL*; TN status; each single variable included in the IPSS; and additional key detrimental mutations (*ASXL1*, *SRSF2*, *EZH2*, and *IDH1/IDH2*) to create 4 distinct risk categories (**Table 3**). MIPSS performed better than IPSS in predicting survival (1611.6

vs 1649.0) based on Akaike information criterion, which estimates the quality of a statistical model.[29] A second novel prognostic model, the Genetic-based Prognostic Scoring System, takes into account age, karyotyping, and mutational information.[30] However, the utility of these scoring systems in routine clinical practice remains unknown as they have not been prospectively validated.

Impact on Treatment

Treatment with ruxolitinib (Jakafi), a JAK1/JAK2 inhibitor, is the sole FDA-approved treatment for patients with MF. Ruxolitinib significantly reduces splenomegaly and improves symptom burden in patients with MF.[31] Importantly, this clinical benefit accrues regardless of *JAK2* mutational status or the presence of prognostically detrimental mutations (*ASXL1*, *EZH2*, *SRSF2*, *IDH1/IDH2*).[32] However, the number of mutations present may predict spleen response with ruxolitinib treatment. This finding was noted in a posthoc analysis of 95 patients in a phase I/II trial of ruxolitinib. The number of mutations present was inversely correlated with spleen response; patients with ≤2 mutations had 9-fold higher odds of spleen response compared with those with ≥3 mutations. Additionally, patients with 3 or more of these high-risk mutations (*ASXL1*, *EZH2*, *IDH1*, or *IDH2*) had a shorter time to treatment discontinuation and shorter OS.[33] Thus, the absence of *JAK2* V617F does not preclude a patient from benefit with ruxolitinib treatment. However, high mutational burden (ie, more than 3 mutations) likely represents more aggressive disease and portends a worse outcome even with the use of ruxolitinib. NGS may therefore be a useful tool for prognostication as well as in the context of discussing the role of ruxolitinib versus consideration of clinical trial or hematopoietic stem cell transplantation (HSCT).

With the expansion of mutational profiling utilizing NGS panels, novel targeted therapies are actively being evaluated in MF. Particular attention has been focused on the inhibition of IDH2, as IDH2 mutations are associated with more advanced forms of MF, including MPN blast phase.[34] A specific potent reversible inhibitor of mutant IDH2, enasidenib (Idhifa), has been shown in multiple models to dramatically reduce the level of 2HG in acute myeloid leukemia (AML) cells, supporting enasidenib's clinical development.[35] In vitro use of these inhibitors, in addition to the first-in-human phase I/II study of enasidenib, have shown proof-of-concept and promising data that such targeted therapy can produce cytostatic effects and induce terminal cellular differentiation in patients with mutant-*IDH2* relapsed/refractory AML.[36,37] Based on the favorable clinical response rates, the FDA approved enasidenib for the treatment of relapsed or refractory AML harboring an *IDH2* mutation.[38] Given the prevalence and association of *IDH2* mutations with blast-phase MPN, there is potential for these molecularly targeted novel agents to prevent or treat leukemic transformation, as well as to treat secondary AML arising from MPN. Additionally, preclinical evidence in a *JAK2/IDH2* co-mutated murine model suggests that combined JAK2 and IDH2 inhibition decreases evidence of disease burden and reverses abnormal gene expression in hematopoietic stem cells to a greater extent than JAK2 inhibition alone.[39]

There are a paucity of data on how mutational status affects HSCT outcomes. In patients with low or intermediate-1 risk by DIPSS, the risk of death outweighs the potential benefit of HSCT. However, in eligible patients with intermediate-2 or high-risk disease, »

TABLE 3. Comparison Between MIPSS and GPSS

	MIPSS	GPSS
Age >60 years	1.5	2
Constitutional symptoms	0.5	NA
Hemoglobin <10 g/dL	0.5	NA
Platelets <200 x 109/dL	1	NA
Triple-negative status	1.5	2
JAK2- or *MPL*-negative	0.5	2
ASXL1 mutation	0.5	1
SRSF2 mutation	0.5	1
CALR type 2 or type-2–like	NA	2
Unfavorable cytogenetics[a]	NA	3 for very high risk; 2 for high risk
Low risk:	0-0.5 points	0 points
Intermediate-1 risk:	1.0-1.5 points	1-2 points
Intermediate-2 risk:	2.0-3.5 points	3-4 points
High risk:	4+ points	5+ points

GPSS indicates Genetics-based Prognostic Scoring System; MPSS, Mutation-enhanced International Prognostic Scoring System; NA, not applicable.
[a]Very high-risk cytogenetics include monosomal karyotype, inv(3), i(17q), –7/7q–, and 11q or 12p abnormalities; high-risk cytogenetics include complex non-monosomal, 2 abnormalities not included in very high-risk category, 5q–, +8, other autosomal trisomies except +9, and other sole abnormalities not included in other risk categories.

there is potential benefit.[40] Some experts have also reasoned that patients with high-risk mutations, such as *ASXL1* or *SRSF2*, or TN disease, should proceed to HSCT earlier than patients without these mutations regardless of their clinical risk score.[20,21] The role of driver mutations *JAK2, MPL*, and *CALR* in HSCT remains unclear. There may, however, be a potential benefit of molecular genotyping to predict outcomes after HSCT. Several studies exemplify this potential role in the HSCT setting. One study of 133 patients with PMF receiving HSCT found that patients with mutated *CALR* fared better than patients with wild-type *CALR* post transplant. When compared with their wild-type counterparts, the patients with mutated *CALR* demonstrated better 4-year OS, better nonrelapse mortality (NRM), and a trend toward improved cumulative incidence of relapse. Overall, patients with mutated *CALR* were found to have the best prognosis, patients with *JAK2* or *MPL* were found to have intermediate prognosis, and patients with TN status clearly had the worst prognosis.[41] The association of these mutational statuses with posttransplant prognosis parallels the findings in nontransplant patients, with *CALR* mutants faring best, patients with TN status faring worst, and those harboring mutated *JAK2* and *MPL* having intermediate prognosis.[25]

Another study looked specifically at the role of the *JAK2* V617 in 162 patients with MF undergoing HSCT. Patients with *JAK2* wild-type demonstrated decreased OS after HSCT when compared with patients harboring the *JAK2* V617F mutation. However, achieving *JAK2* V617F negativity by highly sensitive real-time polymerase chain reaction assay after HSCT was associated with a decrease in relapse.[42]

These results have been duplicated and expanded to include nondriver mutations including *ASXL1*, *SRSF2*, *EZH2*, and *IDH1/IDH2*, in a retrospective study of 169 patients with MF. Patients with *CALR* mutations were found to have lower NRM and improved PFS and OS, while *ASXL1* and *IDH2* mutations were associated with lower PFS. TN status and *SRSF2* or *EZH2* mutations were not associated with poorer outcome post HSCT.[35] The small number of patients studied in each group does, however, limit the generalizability of the findings.[43]

Further validation is required in large prospective studies, but the results of these studies exemplify the potential for genomic data to refine risk stratification. It is evident that the presence of a poor prognostic mutation in a patient categorized as intermediate-1 or low-risk by DIPSS category likely warrants more aggressive treatment than the same DIPSS risk patient without such a high-risk mutation. To formalize this logic, it is imperative to adapt risk stratification to include genomic mutations.

There has also been an attempt to investigate the role of early treatment with ruxolitinib in patients with MF with low-risk disease but high-risk mutations, using a clinical scoring system. The ReTHINK trial was a multicenter, double-blind, placebo-controlled, phase III study that set out to accrue 320 patients with MF who had a low symptom burden; nonpalpable or barely palpable spleen; and at least 1 of the following mutations: *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/IDH2*.[44] However, this trial was recently terminated because of poor accrual (NCT02598297).

Conclusions

Mutational profiling has revolutionized oncologic care. In the case of MF, NGS has the potential to aid in prognostication through refined risk stratification and thereby assist in guiding treatment decisions for individual patients. Furthermore, similar to developments in the fields of other related myeloid malignancies and in oncology in general, movement toward molecular-based personalized therapies will become commonplace. This will drastically change the treatment paradigm for a molecularly heterogeneous population of patients. Genomic-based technology is commercially available and should be integrated into the routine care of patients with MF. As biotechnology and molecular insights increase in MF, it is imperative that clinicians continue to stay abreast of these advances to provide state-of-the-art care for their patients. ■

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RET Rearrangements in Non–Small Cell Lung Cancer

Wade T. Iams, MD, and Christine M. Lovly, MD, PhD

ABSTRACT

Chromosomal rearrangements involving the gene that encodes the RET tyrosine kinase are known oncogenic drivers in 1% to 2% of patients with non–small cell lung cancer (NSCLC). These *RET* rearrangements occur with characteristic partners, most commonly *KIF5B*, but also *CCDC6*, *NCOA*, *TRIM33*, *CUX1*, *KIAA1217, FRMD4A,* and *KIAA1468*. They are typically identified in young patients with adenocarcinoma histology and minimal smoking history. Therapeutic targeting of *RET*-fusion–driven NSCLCs has taken the form of treatment with broad-spectrum tyrosine kinase inhibitors with anti-RET activity, such as cabozantinib (Cabometyx; Cometriq), vandetanib (Caprelsa), lenvatinib (Lenvima), RXDX-105, and sunitinib (Sutent). Cabozantinib and vandetanib have been the most heavily studied multi-kinase inhibitors (MKIs), with response rates of 20% to 50% in largely pretreated patients with *RET*-rearranged NSCLC. Sunitinib has been used in fewer patients to date with initial results demonstrating a 22% response rate. RXDX-105 has exhibited uniquely impressive response rates (75%) in patients with non–*KIF5B-RET*-fusion NSCLC, compared with 0% response in patients with *KIF5B-RET*-fusion–positive NSCLC. BLU-667 has demonstrated an objective response rate of 50% in patients with RET-fusion positive NSCLC, and LOXO-292 reported a 74% ORR in patients with RET-fusion positive NSCLC. Notably, RXDX-105, BLU-667, and LOXO-292 have all demonstrated some central nervous system activity in these early phase trials. Future directions of RET inhibition in patients with *RET*-rearranged NSCLC include additional clinical validation of the next generation RET-selective inhibitors RXDX-105, BLU-667, and LOXO-292 and comparing multikinase inhibitors with RET-selective inhibitors to determine the optimal sequencing of RET-targeted therapies.

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Introduction

The *RET* gene is a receptor tyrosine kinase proto-oncogene that can acquire oncogenic activity through mutation or rearrangement.[1-4] RET is normally expressed on neurons, sympathetic and parasympathetic ganglia, testis germ cells, urogenital tract cells, adrenal medullary cells, and thyroid C cells.[5-7] RET ligands are members of the glial cell line–derived neurotrophic factor family, and ligand binding results in RET autophosphorylation and activation of downstream cellular proliferation, cell migration, and differentiation pathways including RAS/MAPK/ERK, PI3K/AKT, and phospholipase C-gamma.[8] While loss-of-function mutations in *RET* are associated with Hirschsprung disease, gain-of-function mutations are associated with a variety of human malignancies, including non–small cell lung cancer (NSCLC).[9-11] Gain-of-function point mutations in *RET* are associated with medullary thyroid carcinoma,[12] but in NSCLC, oncogenic changes in *RET* take the form of chromosomal rearrangements.[11]

The most common fusion partner for *RET* rearrangements in patients with NSCLC is *KIF5B,*

TABLE. Summary of Clinical Trials in *RET*-Rearranged Tumors

	Phase	Pts (N)[a]	ORR	Fusion Types With Response	Median PFS (months)	Median OS (months)	Most Common Grade ≥3 Toxicity
Cabozantinib							
Drilon 2016[32]	II	25	28% (95% CI, 12%-49%)	*KIF5B-RET* *TRIM33-RET* *CLIP1-RET* Unknown	5.5 (95% CI, 3.8-8.4)	9.9 (95% CI, 8.1-not reached)	Lipase elevation (15%)
Gautschi 2017[28]	NA	21	33% (95% CI, 16.3%-61.6%)	NR	3.6 (95% CI, 1.3-7.0)	4.9 (95% CI, 1.9-14.3)	NA
Vandetanib							
Gautschi 2017[28]	NA	11	18% (95% CI, 2.3%-51.8%)	NR	2.9 (95% CI, 1.0-6.4)	10.2 (95% CI, 2.4-not reached)	NA
Lee 2017[34]	II	17	18% (CI NR)	*CCDC6-RET* Unknown	4.5 (CI NR)	11.6 (CI NR)	Hypertension (18%)
Yoh 2017[30]	II	17	47% (95% CI, 24%-71%)	*CCDC6-RET* *KIF5B-RET* Unknown	4.7 (95% CI, 2.8-8.5)	11.1 (95% CI, 9.4-not reached)	Hypertension (58%)
Lenvatinib							
Velcheti 2016[35]	II	25	16% (CI, NR)	NR	7.3 (95% CI, 3.6-10.2)	NE (95% CI, 5.8-NE)	Hypertension (68%)
Sunitinib							
Gautschi 2017[28]	NA	9	22% (95% CI, 2.8%-60.0%)	NR	2.2 (95% CI, 0.7-5.0)	6.8 (95% CI, 1.1-not reached)	NA
RXDX-105							
Drilon 2017[36]	I/Ib	21 (8 pts with non–*KIF5B-RET*)	75% (of pts with non–*KIF5B-RET*)	Non–*KIF5B RET*	NA	NA	Rash (10%)
BLU-667							
Subbiah 2018[30]	I	14	50%	*KIF5B RET* and Non–*KIF5B RET*	NA	NA	Hypertension (8%)
LOXO-292							
Drilon-2018[31]	I	27	74%	*KIF5B RET* and Non–*KIF5B RET*	NA	NA	None

NA indicates not applicable; NE, not evaluable; NR, not reported; pts, patients; ORR, objective response rate; PFS, progression-free survival; OS, overall survival.

[a]Includes all patients with *RET*-fusion–positive non–small-cell lung cancer in the clinical trial.

although *RET* fusions with *CCDC6*, *NCOA*, *TRIM33*, *CUX1*, *KIAA1217*, *FRMD4A*, and *KIAA1468* have been identified.[11,13-17] Importantly, oncogenic *RET* rearrangements in NSCLC result in constitutive activation of RET and consistently preserve the RET tyrosine kinase domain.[18-21] Since *RET* rearrangements typically do not co-occur with other well-established oncogenic mutations in NSCLC such as *EGFR*, *KRAS*, *ALK*, *HER2*, and *BRAF*, they are believed to harbor independent oncogenic driver potential.[11,18,19,21-23]

RET rearrangements are found in 1% to 2% of patients with NSCLC,[11] typically younger patients with adenocarcinoma histology and minimal smoking history.[23] Although there is no gold-standard method to identify *RET* rearrangements, rearranged *RET* has been detected in NSCLC tumor tissue using a variety of methods including immunohistochemistry (IHC),[21,23] fluorescence in-situ hybridization,[18,20,23,24] real-time polymerase chain reaction (RT-PCR),[14,15,18-21,23-25] and next-generation sequencing.[19-21,25,26] Importantly, despite the observation that RET is minimally expressed in normal pulmonary tissue,[27] RET IHC has not yet proven to be an effective screening tool for detecting *RET* rearrangements in patients with NSCLC.[21]

Therapeutic Targeting

Building on the observations that lung cancer cell lines harboring *RET* rearrangements are sensitive to multikinase tyrosine kinase inhibitors (MKIs) with anti-RET activity, such as sunitinib, sorafenib (Nexavar), and vendatinib, but not to MKIs without RET activity, such as gefitinib (Iressa) or crizotinib (Xalkori), a variety of TKIs with anti-RET activity have been applied in patients with *RET*-rearranged NSCLC.[18,21]

The 5 most heavily studied agents in patients with *RET*-rearranged NSCLC have been cabozantinib, vandetanib, lenvatinib, RXDX-105, and sunitinib. Less-specific MKIs with some anti-RET activity that have been used in patients with *RET*-rearranged NSCLC include sorafenib, alectinib (Alecensa), nintedanib (Ofev; Vargatef), ponatinib (Iclusig), and regorafenib (Stivarga).[28] More recently, early clinical results with RET-selective inhibitors RXDX-105,[29] BLU-667,[30] and LOXO-292,[31] have also been reported.

Multikinase Inhibitors

Cabozantinib

Based on promising antitumor activity observed in murine models of *RET*-rearranged lung cancer, cabozantinib was used in a phase II clinical trial of patients with *RET*-rearranged NSCLC.[32]

From 2012 to 2016, 26 patients with metastatic or unresectable *RET*-rearranged lung cancer were treated with cabozantinib at 60 mg daily. A quarter of the patients were treatment-naïve, half had received 1 line of chemotherapy, and the remaining quarter had received 2 or more lines of therapy prior to study enrollment. Most patients (16 of 26; 62%) had the *KIF5B-RET* fusion, and among the 25 patients evaluated for response, the objective response rate (ORR) was 28% (7 of 25). The responses occurred within 4 weeks of therapy initiation in 5 of the 7 (71%) patients who responded, and the median duration of response was 7 months (95% CI, 3.7-38.9 months). The median progression-free survival (PFS) was 5.5 months (95% CI, 3.8-8.4 months), and the median overall survival (OS) was 9.9 months (95% CI, 8.1-not reached). Toxicity was manageable, with no grade 4 or 5 adverse events (AEs), and the only grade 3 toxicity to occur in greater than 10% of patients was lipase elevation (**Table**).[32]

In order to systematically capture outcomes data for patients with *RET*-rearranged NSCLC treated with RET inhibitors outside the context of a clinical trial, the Global *RET* Registry (GLORY) was launched in 2015. This collective experience has provided additional data regarding response rates to cabozantinib, along with other RET inhibitors that we will discuss later, in patients with *RET*-rearranged NSCLC.[28] The GLORY database includes data from 53 patients with relapsed stage III or stage IV NSCLC treated with RET inhibitors between June 2015 and April 2016 outside a clinical trial at 29 centers: 15 in Europe (51%), 3 in Asia (11%), and 11 in the United States (38%). In 21 patients from this cohort treated with cabozantinib, the ORR was 33% (7/21); median PFS was 3.6 months (95% CI, 1.3-7 months); and median OS was 4.9 months (95% CI, 1.9-14.3 months).[28]

Vandetanib

Based on its in vitro anti-RET activity, including against a lung adenocarcinoma cell line harboring the *CCDC6-RET* fusion, vandetanib has been used in patients with *RET*-rearranged NSCLC.[33-36]

In a phase II multicenter clinical trial in South Korea that enrolled between 2013 and 2015, 18 patients with metastatic or recurrent *RET*-rearranged NSCLC were treated with vandetanib 300 mg daily.[37] All patients had progressed through at least 1 line of systemic therapy, and 72% had received 2 or more lines of systemic therapy. Among 17 patients evaluated, 3 had a partial response (PR; 18%) and an additional 8 had stable disease (overall disease control rate [DCR], 65%). All 3 patients with a PR had disease control for more than 6 months, and 4 of the 8 patients with stable disease (SD; 50%) had disease control for more than 6 months. The most common grade 3 AE was hypertension (18%), and there were no grade 4 or 5 AEs.[37]

An analogous phase II multicenter clinical trial in Japan was reported in 2017 (LURET).[33] Between 2013 and 2015, 19 patients with *RET*-rearranged NSCLC were treated with vandetanib at 300 mg daily. Approximately one-third (37%) of patients had progressed through 1 prior line of systemic therapy, and the remaining 63% had received 2 or more lines of systemic therapy. Ten patients (53%) had the *KIF5B-RET* fusion, and 6 (31%) had the *CCDC6-RET* fusion. Nine (47%) patients had a PR, and 7 patients (37%) had a greater than 50% reduction in tumor size. The median PFS was 4.7 months (95% CI, 2.8-8.5 months) and the median duration of response was 5.6 months (95% CI, 2.1-9.1 months). The most common grade 3 AE was hypertension (58%), and 3 grade 4 AEs occurred (bacterial pneumonia, prolonged QT, and rash).[33]

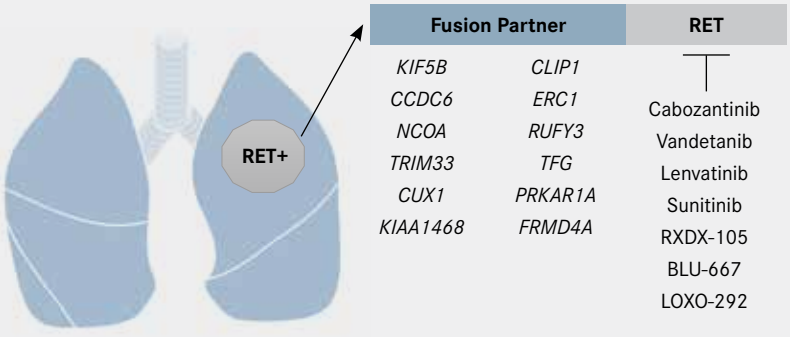
In 11 patients from the GLORY cohort treated with vandetanib, the ORR was 18% (2 of 11), median PFS was 2.9 months (95% CI, 1.0-6.4 months), and median OS was 10.2 months (95% CI, 2.4-not reached) (Table).[28]

Lenvatinib

Based on its in vitro anti-RET activity, lenvatinib has been applied in a multicenter, combined US and Japanese phase II clinical trial. In this trial, 25 patients with RET-rearranged NSCLC were treated with lenvatinib at 24 mg daily.[38] Of these 25 patients, 40% had progressed through 1 line of systemic therapy, while the remaining 60% had received

FIGURE. Therapeutic Targeting of RET fusions in NSCLC

RET rearrangements harbor independent oncogenic driver potential and are found in 1% to 2% of patients with non–small-cell lung cancer. Multiple different fusion partners have been detected for *RET*, as shown in the figure. The most common RET fusion partner in NSCLC is *KIF5B*. Multiple tyrosine kinase inhibitors have proven efficacy against *RET* rearrangements and have been tested in the clinical setting, as described in detail in the body of the text.



2 or more lines of systemic therapy. Importantly, 7 patients (28%) had received previous RET-targeted therapy. Four patients had a partial response (16%), and an additional 15 patients had stable disease, for an overall DCR of 76%. Lenvatinib proved highly toxic in these patients, with 92% experiencing a grade 3 or higher AE; the most common grade 3 or higher AE was hypertension (68%), and there were 3 fatal AEs, 1 of which was pneumonia attributed to lenvatinib (**Table**).[38]

Sunitinib

Based on its in vitro anti-RET activity, 9 patients from the GLORY cohort were treated with sunitinib, and the ORR was 22% (2/9), median PFS was 2.2 months (95% CI, 0.7-5.0 months), and median OS was 6.8 months (95% CI, 1.1-not reached).[28]

Next Generation *RET*-selective Inhibitors

The previously discussed MKIs have biochemical activity against RET but were not designed to selectively target RET. RET-driven NSCLC are not optimally RET-selective. More recently, the results of early clinical trials with RET-selective inhibitors, RXDX-105,[29] BLU-667,[30] and LOXO-292,[31] have been reported.

RXDX-105

As recently reported at the European Society of Medical Oncology 2017 Congress, RXDX-105 is a VEGFR-sparing RET inhibitor with activity against patient-derived xenograft tumor models harboring *RET* rearrangements[39]; it has been applied to patients with varying tumor histologies harboring *RET* rearrangements and *RET* mutations.[29] In a multicenter phase I/Ib clinical trial, an established phase II dose of 275 mg daily was determined. No patients had received previous RET inhibitor therapy. A total of 21 patients with *RET*-rearranged NSCLC were treated in the phase Ib expansion cohort, and among 8 patients with non–*KIF5B-RET* fusions, the ORR was 75% (6/8), including a central nervous system (CNS) response in 1 patient. However, among 13 patients harboring *KIF5B-RET* fusions, none had an objective response. The most common grade 3 AE was rash (10%), and no grade 4 or 5 AEs occurred.[29] Experimental studies have not yet identified a unique basis for *KIF5B-RET* insensitivity to RET inhibition compared with non–*KIF5B-RET* sensitivity (**Figure**).

BLU-667

The results of a phase I, multi-histology basket trial treating patients with RET-driven malignancies (papillary thyroid cancer (PTC), NSCLC, and medullary thyroid cancer (MTC)) were orally presented at the American Association of Cancer Research Annual Meeting in 2018 and simultaneously published. BLU-667 was designed to maximize on-target and minimize off-target effects, and it was validated in both cell-line and patient-derived xenograft models.[30]

In the dose escalation cohort the maximum tolerated dose was determined to be 400 mg daily. The ORR for all 40 response evaluable patients was 45%, and of these 40 patients 53% (n=21) had received prior RET-directed MKI treatment. In patients with RET-mutated PTC the ORR was 40% (10/25), and in patients with RET-fusion NSCLC the ORR was 50% (7/14). While PFS and OS data are not mature, adverse events were reported, and the only grade >2 adverse event that occurred in more than 5% of patients was hypertension (>grade 2 in 8% of patients). Other grade 3 adverse events included neutropenia in 2 patients, leukopenia in 1 patient, ALT increase in 1 patient, fatigue in 1 patient, and diarrhea in 1 patient. There were no grade 4/5 adverse events. Importantly, CNS activity was reported in one patient with KIF5B-RET fusion NSCLC.

LOXO-292

LOXO-292 is another RET-selective TKI that was strategically designed to maximize on-target activity while minimizing activity against other kinases. Results from a multi-histology phase I basket trial of this novel agent in patients with RET-driven malignancies was recently presented at the American Society of Clinical Oncology Annual Meeting in 2018.

In the dose escalation cohort, patients with NSCLC, PTC, MTC, and RET-fusion positive pancreatic cancer (2 patients) were included. In this cohort, 66% (n=55) of patients had previously received a RET-directed MKI. The maximum tolerated dose was not reached, and the maximum dose administered in trial was 240 mg twice daily. The confirmed ORR in 34 evaluable patients was 74% (25/34), including a 74% (20/27) confirmed ORR in patients with RET-fusion NSCLC and 33% (6/18) confirmed ORR in patients with RET-mutant MTC. Responses were seen in both patients with KIF5B-RET fusions and non-KIF5B-RET fusion NSCLC. There were 10 patients with CNS metastases, and 3 of 3 patients with measurable CNS disease had CNS responses, including one patient with CLIP1-RET fusion NSCLC. The only grade >2 adverse events reported were grade 3 dyspnea, grade 3 tumor lysis syndrome, and grade 3 increase ALT.

Central Nervous System Penetration

The central nervous system (CNS) penetration of oncogene-targeted therapies in patients with NSCLC is an active area of research, including in patients with *RET*-rearranged NSCLC. Preclinical models suggest that the CNS penetration of vandetanib is decreased by P-glycoprotein and breast cancer resistance protein 1-mediated efflux,[40] and mTOR inhibition with agents such as everolimus overrides this efflux. Therefore, combination therapy with vandetinib and everolimus has been assessed. A recent case report has shown CNS activity of combination therapy with vandetanib and everolimus in a patient with *KIF5B-RET*–fusion NSCLC.[41] Although alectinib has not been as heavily studied in patients with *RET*-rearranged NSCLC as have other agents described above, it is important to note that it, too, has demonstrated CNS activity in patients with *KIF5B-RET*–fusion NSCLC.[39]

Importantly, all 3 next generation RET inhibitors RXDX-105, BLU-667, and LOXO-292 have demonstrated CNS activity. These data are described above under the relevant sections for each drug. While expected rates of CNS activity have not been defined in early clinical data presented for these agents, this will be an important clinical activity metric to follow in subsequent, larger reports.

Acquired Resistance

Anticipating the inevitable acquired resistance that accompanies oncogene-targeted therapies, in vitro studies have begun to analyze RET inhibitor resistance mechanisms. Several potential mechanisms of resistance to RET inhibition involve EGFR signaling, as it has been shown that EGF ligand binding to *EGFR* can blunt the pharmacologic activity of RET inhibitors on their target fusion kinase; adaptor protein binding can be shifted from the target kinase protein to EGFR; and *EGFR* can act as a bypass pathway to downstream oncogenic signaling through mitogen-activated protein kinase.[42] Additionally, in lung adenocarcinoma cell lines that develop resistance to RET inhibitors, oncogenic mutations and overexpression of *NRAS* have been identified, as have upregulations of *EGFR* and *AXL*.[43]

Conclusions

The 2 most heavily studied RET inhibitors have been cabozantinib and vandetanib, and these agents have demonstrated response rates between 20% and 50% in a majority of patients who have received 2 or more lines of systemic therapy. Tolerability has been manageable with these agents, with lipase elevation the most common grade 3 toxicity observed with cabozantinib and hypertension the most common grade 3 AE seen with vandetanib. While lenvatinib has proven excessively toxic and sunitinib has not been broadly applied in patients with RET-rearranged NSCLC, RXDX-105 has shown notably higher response rates in patients with non-KIF5B-RET–fusion NSCLC, and phase I clinical trials have demonstrated promise for the RET-selective inhibitors, BLU-667 and LOXO-292. Future directions for this exciting avenue of clinical research include further testing of the RET-selective inhibitors in larger patient cohorts, identifying the optimal sequence of RET inhibitors, and defining mechanisms of acquired resistance to both MKIs and RET-selective inhibitors. ■

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