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Chairman's Letter

Michael J. Hennessy, Sr

This month's issue of *The American Journal of Hematology/Oncology®* covers a range of tumor types including acute myeloid leukemia, breast cancer, head and neck squamous cell cancer, and colorectal cancer.

In "Emerging Molecular and Immune Therapies in Acute Myeloid Leukemia," Drs Boddu, Kantarjian, Ravandi, and Daver explore the rapid advancements in immune and targeted therapeutics, the genomic landscape, and clonal evolution in acute myeloid leukemia (AML). The result is the emergence of numerous exciting therapies for AML in the last decade. How each of these therapies affect the management of AML will be a matter for ongoing debate.

Cell-cycle inhibition is a new standard-of-care therapy in estrogen-receptor–positive (ER+) metastatic breast cancer (MBC). Drs Vasan and Dickler in their paper, "State-of-the-Art Update: CDK4/6 Inhibitors in ER+ Metastatic Breast Cancer," discuss the current state of palbociclib, ribociclib, and abemaciclib as CDK4/6 inhibitors in MBC, with an emphasis on ongoing clinical trials.

How positron emission tomography scans play a role in identifying those patients who could be followed by surveillance without additional surgery after chemoradiation is the focus of Drs Nagasaka and Sukari in "Can Positron Emission Tomography Scans Post Chemoradiation in Head and Neck Squamous Cell Cancer Spare Patients From Undergoing Salvage Surgery?"

The growth of therapies in the metastatic colorectal cancer arena is encouraging, with 10 new drug approvals, including targeted biologics and tyrosine kinase inhibitors in the past 20 years. In "Optimizing Sequencing Beyond Disease Progression After Second-Line Therapy in Metastatic Colorectal Cancer," Drs Mody and Bekaii-Saab review the current evidence on optimizing sequencing, particularly as it relates to regorafenib and trifluridine-tipiracil.

In this month's CME article, "Optimizing Sequencing in Patients With NSCLC and Actionable Mutations," Dr Karen Reckamp explains the key unmet needs in the treatment of advanced non–small cell lung cancer (NSCLC) for patients who have actionable mutations. She also discusses the advantages and disadvantages of molecular testing to identify patients with actionable mutations, along with the most common genomic alterations in NSCLC.

Thanks for reading.

Michael J. Hennessy, Sr
Chairman and Chief Executive Officer

From the Editor

An important priority for patients and treating physicians, as well as for the National Cancer Institute and the National Clinical Trials Network, is to minimize overtreatments and their short- and long-term consequences. This mission is somewhat complicated and fraught with challenges—after all, we have invested considerable resources to advance treatments and improve outcomes. But each step leaves open questions as to which population and specific subgroups should be included in the new treatment recommendations. The default criteria for treatment are typically the eligibility criteria used in the pivotal study, or if applicable, the FDA approval label. However, these may be overly inclusive (or not sufficiently so), and follow-up studies to refine these are felt to be less urgent than moving in altogether new directions. At the same time, our appreciation of real-world adverse effects that patients experience while on treatment, or many years later, is limited because of patient selection and the fact that most trials do not collect detailed long-term safety data.

Debu Tripathy, MD
Editor-in-Chief

In this issue of *The American Journal of Hematology/Oncology®*, Dr Nagasaka and colleagues provide a refreshing review and perspective on the use of positron emission tomography (PET) scanning after combined modality therapy for head and neck cancer to determine the need for follow-up neck dissection. Key clinical trials exploring this approach for higher stage tumors (N2, N3) are nicely laid out. It is remarkable that residual disease seen with planned neck dissection following chemoradiation is common, around 40%.[1] However, in patients with radiographic responses that can be seen using modern imaging techniques, recurrence rates are under 10%.[2] This review covers trials that have examined imaging-guided decision making or outcomes, and one of the most important trials presented is a randomized trial designed to specifically evaluate, in patients with residual or equivocal residual nodal disease, image-guided decision making with those randomized to neck dissection versus PET/computed tomography scanning and neck dissection only in those with residual or equivocal residual nodal disease.[3] In this trial, neck dissection rates were lower in the imaged group with no inferiority demonstrated in 2-year survival rates. Of course, longer-term follow-up is needed to evaluate both survival and late adverse effects, but we are now seeing more and more examples of "more is less"—a trend in the personalization of cancer care that can improve both quality of life and cost effectiveness.

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Emerging Molecular and Immune Therapies in Acute Myeloid Leukemia

Prajwal Boddu, MD; Hagop Kantarjian, MD; Farhad Ravandi, MD; and Naval Daver, MD

Abstract

Rapid advancements in immune and targeted therapeutics coupled with improved understanding of the genomic landscape coupled with an improved understanding and clonal evolution in acute myeloid leukemia (AML) have resulted in the emergence of numerous exciting therapies for AML in the last decade. In many cases the response rates and tolerability of such targeted or immune-based approaches are superior to those achieved with standard cytotoxic therapy. The response and survival benefit may frequently be further improved by rationally combining targeted, monoclonal, or immune-activating approaches with epigenetic or cytotoxic therapies. The targeted and monoclonal-based strategies may be particularly useful in efforts to improve outcomes in traditionally poor-risk AML, including AML with poor-risk cytogenetics, *TP53* mutation, secondary AML, or AML in elderly patients (aged >60 or >65 years). While the final approval status and clinical roles of a number of these molecular and immune-targeted agents in AML will be determined by ongoing phase II/III trials, it is valuable for practicing oncologists to be aware of the scope, indications, and toxicities of these therapies, and of the significant progress in AML in the last few years. In this review, we discuss emerging molecular and immune-based therapies in AML, and how these may impact the management of AML in the near future.

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Introduction

Acute myeloid leukemia (AML) remains among the few hematological malignancies with no major drugs approved to market in the United States in the last decade. In fact, the only drug approved in the United States for AML in the past 4 decades was gemtuzumab ozogamicin (GO; Mylotarg); it was approved in 2001 and later voluntarily withdrawn from the market due to a concern for increased toxicity in a phase III study.[1] Cytarabine and anthracycline (idarubicin or daunorubicin) regimens remain the standard frontline approach in most patients with AML.[2] However, the last decade has seen major advances in the understanding of molecular leukemogenesis, of immune pathways, and of conjugated and bispecific antibody technology. These advances have provided crucial insights into disease pathophysiology and platforms leading to the development of novel therapies for AML.

The advent of high-throughput sequencing methods has enabled characterization of recurring, prognostically informative mutations that may serve as suitable targets for small-molecule and metabolic therapies. This is exemplified by the successful targeting by novel small-molecule inhibitors of recurrent mutations and mutation-associated pathways that play a role in leukemogenesis (eg, FMS-like tyrosine kinase-3 [*FLT3*] and isocitrate dehydrogenase [*IDH*] 1/2).[3] Additional small-molecule inhibitors targeting overexpressed or aberrantly regulated pathways in AML are also showing encouraging results as single agents or in combinatorial approaches (eg, inhibition of B-cell lymphoma 2 protein [BCL2], of mouse double minute 2 homolog [MDM2], and of chromosomal maintenance 1 [CRM1]) (see **Figure**).

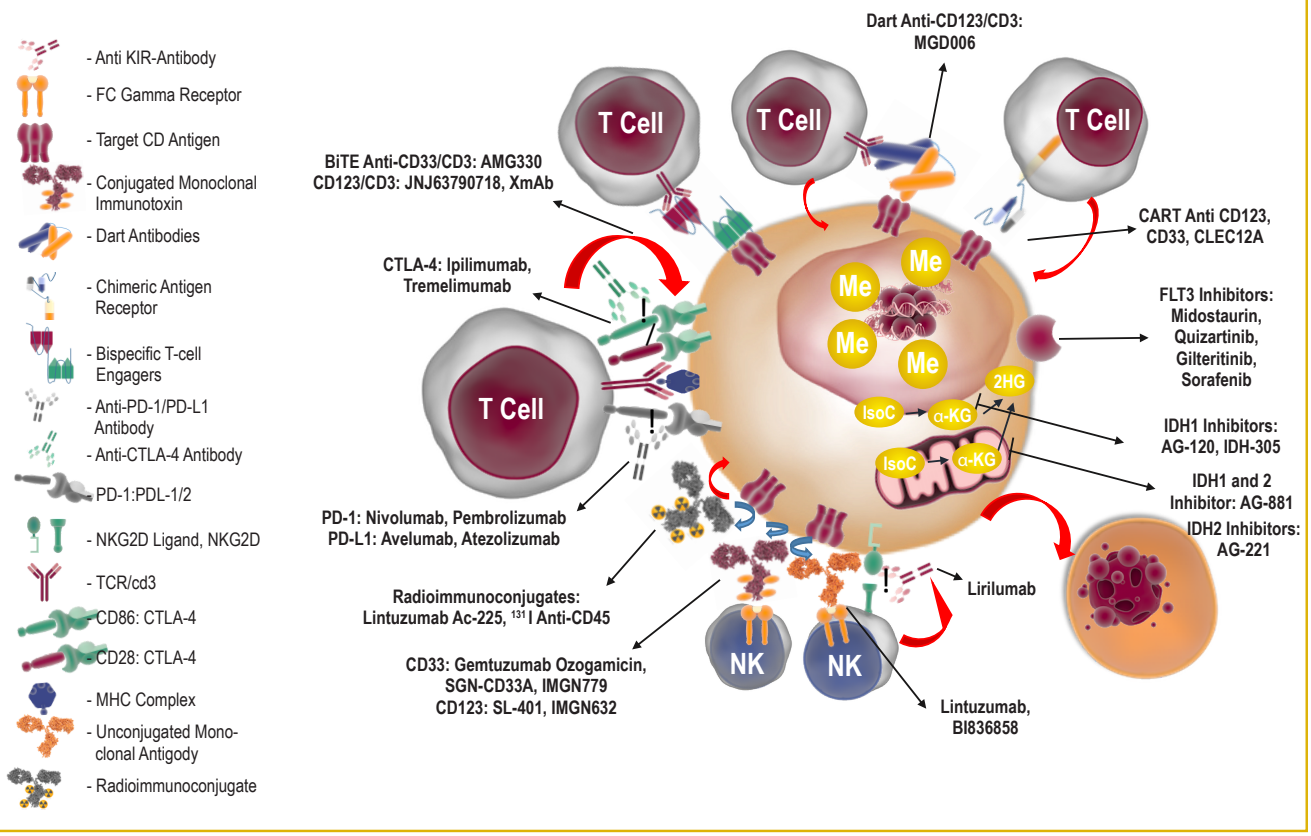
Identification and targeting of leukemia-specific antigens compose a second major area of active research in AML. Different approaches to target these antigens, with the intent of inducing preferential cytotoxicity to leukemia blasts and potentially to leukemia stem cells, are being explored. They include monoclonal antibodies; naked or antibody drug conjugates; radioimmunoconjugates; dual-affinity retargeting antibodies; and T-cell adoptive therapy, such as chimeric antigen receptor-T (CAR-T) cells.

A third approach focuses on unleashing the patient's own immune system to fight against leukemic cells using immune checkpoint antibodies; bispecific T-cell engager (BiTE) antibodies; and adoptively transferred natural killer (NK) cells. Clinical trial experiences with these above-mentioned therapies (**Table**) suggest marginal therapeutic benefit when used as single agents, but suggest additive benefit, and in many instances, synergistic benefit, when implemented in rational combinations.

Molecular Targeted Therapies in Acute Myeloid Leukemia

Genome-wide sequencing on large numbers of AML patients has identified recurrent mutations in genes encoding epigenetic regulators, signaling receptors, and splicing factors, as well as

FIGURE. Immune and Molecular Targeted Approaches in Acute Myeloid Leukemia



other genes that regulate key cellular processes.[3] Notably, there are few obvious associations between a particular baseline mutational status and particular drug sensitivity, as exemplified by the sensitivity of mixed lineage leukemia (MLL)-rearranged leukemias to bromodomain inhibitors and to CDK6 inhibitors; *NPM1*-mutated leukemias to arsenic trioxide; and *IDH2*-mutated leukemias to BCL-2 inhibitors in preclinical and clinical testing.[4-6] While drugs targeting AML molecular mutations—including *EZH, MLL, DNMT3A, ASXL1*, and *TET2*—are currently in preclinical or early clinical development, FLT3 and IDH inhibitors are already in an advanced phase of clinical development (**Table**).[7]

FLT3

Phase II studies have demonstrated that sorafenib, an FLT3 inhibitor, may improve response rates and event-free survival when used in combination with hypomethylating agents or cytotoxic chemotherapy.[8,9] In addition, a number of other FLT3 inhibitors (quizartinib, gilteritinib, midostaurin) are currently being evaluated in phase III trials. In a large retrospective analysis, quizartinib appeared to be superior to standard-of-care regimens, both in response rates (43% vs 11%, $P = .002$) and median survival (in relapsed patients: 128 vs 53 days) when used in FLT3-internal tandem duplication–positive (ITD+) patients with AML who relapsed after salvage chemotherapy (SCT).[10] An interim analysis of 52 patients, showed the combination of quizartinib with low-dose cytarabine (LDAC) or azacitidine to be effective, with overall response rate (ORR) of 73% and a median survival of 14.8 months.[11] Gilteritinib (ASP2215) was evaluated in a phase I/II trial involving relapsed/refractory (R/R) AML (FLT3mut+ = 169) and was effective with an ORR of 52%, median duration of response of 20 weeks, and median survival of 7.8 months.[12] Most recently, midostaurin received breakthrough therapy designation from the FDA for newly diagnosed *FLT3*-mutated AML, after demonstrating significantly improved overall survival (OS) in a randomized phase III study of induction and consolidation chemotherapy combined with midostaurin or placebo.[13] Crenolanib is another orally bioavailable pan-FLT3 inhibitor with added activity against D835H and D835Y mutants. Clinical activity has been seen even in patients who have failed prior FLT3 tyrosine kinase inhibitor (TKI) therapy. A phase II single-center study evaluating crenolanib in *FLT3*-mutated AML suggested modest activity with complete response (CR) rates of 23% in FLT3-TKI–naïve patients and of 5% in patients who had failed prior anti-FLT3 therapy.[14] The combination of crenolanib with standard cytarabine and anthracycline

induction in newly diagnosed *FLT3*-mutant AML provided high CR rates of 88% while being well tolerated with a low incidence of AEs.[15] This agent is currently undergoing evaluation in combination with other standard therapies in the treatment-naïve and salvage setting in *FLT3*-mutated AML (NCT02298166, NCT02626338, NCT02400281, and NCT02283177). Lestaurtinib was among the first FLT3 inhibitors to be extensively evaluated as a monotherapy and in combination with SCT. Clinical activity as a single-agent therapy was shown to be modest.[16] Furthermore, its addition to SCT did not improve response rates, while it was associated with a higher frequency of severe AEs and deaths (NCT00079482).[17] This agent is currently not undergoing active evaluation in *FLT3*-mutated AML.

IDH 1 and 2

Two first-generation IDH inhibitors—AG-221 (IDH2 inhibitor) and AG-120 (IDH1 inhibitor)—and a second-generation pan-IDH inhibitor, AG-881, are currently undergoing active study in ongoing clinical trials. Another agent, IDH-305, targeting *IDH1* R132 mutation, is being evaluated in a phase I trial involving R/R AML (n = 24) and other malignancies. Preliminary data with the IDH1 and IDH2 inhibitors are encouraging, with an ORR in the 35% to 38% range and an acceptable safety profile.[18] The drugs are oral, have been very well tolerated overall, and are now being evaluated in combination with 7 + 3 in younger patients with AML and with azacitidine in older patients with AML harboring *IDH1* or *IDH2* mutations, respectively. Differentiation is frequently seen with IDH inhibitors and often presents as increased white blood cell count, increased blasts, pulmonary infiltrates, and dyspnea. The occurrence of differentiation syndrome with IDH inhibitors does not correlate closely with the degree of leukocytosis, unlike the differentiation syndrome seen with all-trans retionic acid (ATRA) or with arsenic trioxide in acute promyelocytic leukemia (APL). The differentiation syndrome responds rapidly to steroids, and a majority of patients are able to continue therapy with the IDH inhibitors.

IDH inhibitors are differentiation-inducers and do not frequently eliminate the malignant clone, as is the case with AG-221. In contrast, most recent data suggest that AG-120 is able to affect *IDH1* mutational clearance. In 1 study, among 63 patients with R/R AML (of 78 with hematological malignancy), 21 (33%) had objective responses: (CR = 10; complete response with incomplete hematologic recovery (CRi)/complete response with incomplete platelet recovery (CRp) = 8; marrow CR = 2; partial response (PR) = 1. Importantly, m*IDH1* clearance by next-generation sequencing was observed in 36% of CRs and 4% of non-CRs.[19] Patients with an m*IDH1* clearance had improved clinical benefit from IDH inhibitors as compared with those who achieved a clinical response per International Working Group criteria but did not achieve molecular remission. Nevertheless, clonal persistence, and its therapeutic and prognostic implications for the need for continued IDH inhibitor therapy, remain unknown.

BCL2

Among the BCL-2 inhibitors, venetoclax is particularly effective and is being tested in combination with other agents, including hypomethylating agents and LDAC. Venetoclax in combination with azacitidine/decitabine produced an ORR (CR/CRi/PR) of 75% in frontline older patients with AML.[20] The expected response rate with azacitidine or decitabine alone is 18% to 25%, based on published data, suggesting striking synergism when the agents are combined. A phase III, randomized, registrational trial of azacitidine plus venetoclax versus azacitidine alone has begun enrollment. In a separate phase Ib study, Wei and colleagues reported on the safety and efficacy of venetoclax plus LDAC in 61 treatment-naïve patients >65 years with AML . The combination was well tolerated and produced high response rates (CR/CRi of 54%), with median survival not reached among the responders (CR/CRi/PR). Historic response rates with LDAC alone in a similar patient population have been 5% to 10%, highlighting the significant improvement when venetoclax is added to LDAC. The regimen was well tolerated. A future phase III randomized trial of LDAC with venetoclax is planned.[21]

Monoclonal Antibodies in Acute Myeloid Leukemia

CD33

It has been realized that leukemic stem cells exhibit phenotypic characteristics distinct from those of normal hematopoietic stem cells.[22] Of the various differentially expressed cluster differentiation (CD) antigens on leukemic blasts, CD123, CD33, and CD56 have thus far been exploited clinically as targets for monoclonal antibody-based therapies in AML.[23-26] These monoclonal antibodies may exert their anti-AML tumor effect by varied mechanisms, including antibody-mediated neutralization, delivery of toxic payload in the case of conjugated antibodies, antibody-dependent cellular cytotoxicity, complement-mediated cytotoxicity, antbody-dependent cell-mediated phagocytosis, and enhanced T-cell antitumor efficacy by increasing T cell and tumor interaction with bispecific T-cell engaging antibodies.

GO, a humanized anti-CD33 monoclonal antibody conjugated to a DNA-damaging toxin (calicheamicin), is among the most extensively studied monoclonal antibodies in AML. It was first approved in May 2000 based on a phase II trial showing a 30% response rate in patients with AML in first relapse. However, GO was subsequently voluntarily withdrawn from the market 10 years later,[27-29] based on a Southwest Oncology Group (SWOG) phase III trial that demonstrated a lack of benefit and potentially increased mortality in patients who received GO with standard chemotherapy.[1] However, multiple criticisms have since been raised against the design and dosing schema of this SWOG study. Since the SWOG study, 4 randomized trials conducted in Europe have demonstrated efficacy of GO in patients with AML with good- and intermediate-risk cytogenetics.[30-34] Additionally, the higher incidence of veno-occlusive disease with the single

high dose of 9 mg/m^2 was significantly reduced by administering multiple split doses of 3 to 6 mg/m^2 in these trials. Based on these data, the use of GO is being reassessed by the European and US drug agencies and is simultaneously being further evaluated in de novo, relapsed, and pretransplant settings in clinical trials (NCT01869803, NCT02473146, NCT02221310).

SGN-CD33A (vadastuximab), an anti-CD33 antibody conjugated to pyrrolobenzodiazepine, is a newer CD33-targeted monoclonal that demonstrated cytotoxicity in AML cell lines irrespective of multidrug-resistant status or karyotype.[35] This agent was evaluated

TABLE. Overview of Trials with Molecular and Immune Agents in Acute Myeloid Leukemia

Class / Agent	Study Design	Trial Regimen	Study Population, n (%)	ORR, n (%)	Treatment-Related Mortality	Median Survival	Comments
FLT3-inhibition							
Quizartinib[85]	Phase II, open label	Single arm monotherapy in FLT3-ITD positive & FLT3-ITD negative R/R AML	N = 92 (FLT3–ITD positive); N = 41 (FLT3-ITD negative)	66 (72%) (FLT3-ITD+); 17 (41%) (FLT3-ITD negative)	Not reported	25.3 weeks	High degree of activity as monotherapy, especially in *FLT3*–ITD positive patients in R/R setting. 13% experienced AEs requiring discontinuation
Gilteritinib[12]	Phase III	Single arm monotherapy in R/R AML	N = 252; FLT3–ITD positive, n = 159; FLT3 D835, n = 13; FLT3–ITD & D835 positive, n = 16; other, n = 64	FLT3^{mut+} (49%); wild-type FLT3 (12%)	7 of 252	31 weeks	Well tolerated and efficacious in FLT3mut+ patients. Phase III testing in FLT3mut+ R/R AML after first-line failure ongoing (NCT02421939)
AZA + sorafenib[9]	Phase II	Single arm in R/R AML	N = 43; FLT3–ITD positive = 40	46%	8-week mortality = 16%	6.2 months	Combination effective for patients with R/R AML and *FLT3*–ITD. Well tolerated with very few grade 4 AEs
(7+3) + sorafenib[8]	Phase II, multicenter, randomized, controlled	Sorafenib + (7+3) vs placebo + (7+3) in AML pts aged <60 years	Placebo, n = 133; sorafenib, n = 134	CR = 81 (60%) in the sorafenib group and CR = 78 (59%) in placebo group	30-day mortality 2% in sorafenib group vs 1% in placebo group; 60-day mortality 4% in both groups	9 months in placebo vs 21 months in sorafenib (*P* = .013)	AML aged ≤60 years; the addition of sorafenib to 7+3 increased efficacy but also increased toxicity; most common AEs: fever, diarrhea, bleeding, cardiac events, hand-foot-skin reactions, rash
(7+3) + sorafenib[86]	Multicenter, randomized, controlled	Sorafenib + (7+3) vs placebo + (7+3) in AML pts aged >60 years	Placebo, n = 95; sorafenib, n = 102	CR + CRi = 64 in placebo; CR + CRi = 57 in sorafenib	60-day mortality higher in the sorafenib arm vs the placebo arm (*P* = .035)	Median OS: 15 months for placebo vs 13 months for sorafenib	Sorafenib combined with intensive chemotherapy not effective in elderly AML pts, and also more toxic than 7+3 alone
Midostaurin + (7+3)[87]	Prospective, randomized, controlled	Newly diagnosed AML pts aged 18-60 yrs with *FLT3* mutation	Midostaurin, n = 360; placebo, n = 357	CR = 59% midostaurin; CR = 54% placebo	NR	Midostaurin OS, 74.7 months vs placebo OS, 26 months (*P* <.01)	Very efficacious when used as a component of therapy in younger adults with mutant *FLT3* AML. Also, not associated with additional toxicity
IDH Mutation							
IDH-mutation AG221[88]	Phase I/II	mIDH2–AML/MDS	R/R-AML/MDS, n = 138 (70%); untreated AML/MDS, n = 60 (30%)	74 (41%) in overall (n = 181 evaluable); 52 (41%) in R/R AML (n = 128 evaluable)	NR	NR	Safe with few AEs, including indirect hyperbilirubinemia and nausea. Good response rates in *IDH2*+ AML.
AG120[89]	Phase I	IDH1-mutant positive advanced hematologic malignancies	N = 57	ORR = 31%; CR = 15%	No drug-related deaths reported; 13 total deaths	NR	Safe with minimal toxicities. Durable response for up to 11 months
IDH305[90]	Phase I	Advanced malignancies including R/R AML/MDS with *IDH1*R132 mutation	N = 81; AML/MDS = 24	ORR = 7 (33%); CR = 2 (9.5%)	NR	NR	Very favorable safety profile. Durable response in complete responders

7+3 indicates 7 days of cytarabine +3 days of daunorubicin; ABT, venetoclax; AE, adverse event; AG120, IDH1 inhibitor; AG221, IDH2 inhibitor; AML, acute myeloid leukemia; ; AZA, azacitidine; BCL-2, B-cell lymphoma-2 protein; CR, complete response; CRi, complete response with incomplete hematologic recovery; DAC, dacogen; FLT-3, FMS-like tyrosine kinase-3; IDH, isocitrate dehydrogenase; ITD, internal tandem duplication; LDAC, low-dose cytarabine; MDM2, mouse double minute 2 homolog; MDS, myelodysplastic syndrome; mIDH2, mutant isocitrate dehydrogenase-2; MRD, minimal residual disease; NR, no response; pts, patients; ORR, overall response rate; OS, overall survival; R/R, relapsed/refractory; and SGN 33A, vadastuximab.

TABLE. Overview of Completed Trials with Molecular and Immune-Based Agents in AML (continued)

Class / Agent	Study Design	Trial Regimen	Study Population, n (%)	ORR n (%)	Treatment-Related Mortality	Median Survival	Comments
BCL-2 inhibition							
AZA/DAC + ABT[20]	Phase Ib, open label	Treatment-naïve AML pts aged >65 years not eligible for intensive chemotherapy	N = 22	ORR = 75% (venetoclax 400 mg); 70% (venetoclax 800 mg)	4 deaths: 3 after treatment discontinuation, 1 from disease progression	NR	Tolerable combination. Rapid responses can be expected with this combination with high CR rates
LDAC + ABT[21]	Phase I	Treatment-naïve AML pts aged >65 years	N = 61	CR/CRi = 54%	60-day mortality rate = 15% (9 of 61 patients)	Median not reached in responders	Highly synergistic combination. Activity in high-risk pts such as aged >75 yrs, secondary AML, adverse karyotypes
MDM2 inhibition							
RG7112[91]	Phase I	Refractory acute and chronic leukemias, including R/R AML	N = 30 evaluable AML pts	CR/CRi = 10%; PR = 13%	31 deaths in entire study, with none related to study drug (n = 116). 17 of 31 due to disease progression	NR	Clinically active in R/R leukemias. RG7388 is a more potent agent in this class and is in phase I clinical development in AML
Monoclonal antibodies							
SGN-CD33A[36]	Phase I	Treatment-naïve CD33+ AML	N = 27 pts	CR/CRi = 60%	60-day mortality = 15%	NR	Double the response rates expected with nonintensive therapies such as hypomethylating agents or LDAC. High MRD-negative remissions, and low early mortality rates
AZA/DAC + SGN-CD33A[39]	Phase I/II	Treatment-naïve CD33+ AML	N = 24 pts	CR/CRi = 65%	60-day mortality = 4%	Median OS not reached for a median of 13.5+ weeks from treatment	The combination is effective and has a favorable toxicity profile. Activity favorable over historical outcomes with single-agent hypomethylating agents
(7+3) + SGN-CD33A[92]	Phase Ib	Newly diagnosed acute AML	N = 42 pts	CR/CRi = 76%	30-day mortality = 2%	MRD-negative CR/CRi: not reached; MRD-positive CR/CRi = 7.46 months	Low 30-day mortality rate; good response rate; no added nonhematological toxicity with SGN-CD33A combined with (7+3)

7+3 indicates 7 days of cytarabine +3 days of daunorubicin; ABT, venetoclax; AE, adverse event; AG120, IDH1 inhibitor; AG221, IDH2 inhibitor; AML, acute myeloid leukemia; AZA, azacitidine; BCL-2, B-cell lymphoma-2 protein; CR, complete response; CRi, complete response with incomplete hematologic recovery; DAC, dacogen; FLT-3, FMS-like tyrosine kinase-3; IDH, isocitrate dehydrogenase; ITD, internal tandem duplication; LDAC, low-dose cytarabine; MDM2, mouse double minute 2 homolog; MDS, myelodysplastic syndrome; mIDH2, mutant isocitrate dehydrogenase-2; MRD, minimal residual disease; NR, no response; pts, patients; ORR, overall response rate; OS, overall survival; PR, partial response; RG7112, small-molecule MDM2 antagonist; R/R, relapsed/refractory; SGN 33A, vadastuximab.

in a phase I study of R/R CD33+ AML and demonstrated blast clearance in 48% of the 85 evaluable patients, with CR/CRi in 27% (23 of 85), of whom 73% (of 23 patients) were negative for minimal residual disease among patients treated at the recommended dose of 40 µg/kg.[36] Efficacy was even higher in the treatment-naïve patients (n = 27) with CR/CRi in 54% (14 of 27 evaluable patients).[37] The most common grade 3 adverse events (AEs), occurring in 20% of patients, were neutropenia, thrombocytopenia, and anemia. Pre-clinical and early clinical-phase studies have shown SGN-CD33A to synergize with hypomethylating agents like azacitidine, and in a recently reported phase II study, the combination produced a CR/CRi rate of >60% with an 8-week mortality of 5% in frontline older patients with AML.[38,39] This agent is currently being studied as monotherapy in maintenance; in the pre- and postallogeneic stem cell transplant (ASCT) settings; and in a phase III randomized study of SGN-CD33A with azacitidine versus azacitidine alone in untreated older patients with AML (NCT02326584, NCT02785900, NCT02706899, NCT02614560).

Another conjugated CD33 antibody that has shown marked

activity both in vitro and in vivo is IMGN779.[40,41] Its activity appears to be more selective to leukemic stem cells while sparing normal hematopoietic stem cells (HSCs), suggesting a potential for reduced myelosuppression. It is currently in a phase Ib trial in patients with relapsed AML (NCT02674763).

CD-123

Overexpression of the interleukin-3 (IL-3) receptor α-chain (IL-3 Rα/CD123) on AML cells is associated with enhanced blast proliferation, disease relapse, and drug resistance in AML.[42,43] Clinical activity was modest with the first-in-class anti-CD123 antibody (CSL360).[44] Two second-generation antibodies (CSL362, SL-401) are being evaluated in phase I/II clinical trials. CSL362, a fully humanized antibody with a modified Fc-domain to enhance NK cell binding, was evaluated as postremission treatment in a phase I study involving 25 patients with CD123+ AML (in first or second CR), and it was able to prolong CRs beyond 6 months (26-52 weeks) in 10 of 20 evaluable patients.[45] SL-401 (DT388IL3), a human IL-3 ligand fused to a truncated diphtheria toxin,[46] was evaluated in a phase I trial in 74 R/R or high-risk de novo AML patients, and it produced an ORR (CR + PR + disease stabilization) of 27% (20 of 74) and CR/CRi rate of 2.8% (2 of 74).The median survival of second-salvage AML patients treated in this trial (n = 33) was 3.2 months (range, 2-8.4), with 22% alive at 1 year.[47] This compound is currently being evaluated in 3 phase II trials in hematological malignancies including 1) in patients with relapsed AML and relapsed/frontline blastic plasmacytoid dendritic cell neoplasm; 2) maintenance after completion of consolidation in patients with high-risk AML patients who are not candidates for or have refused ASCT; and 3) in chronic myeloid disorders (NCT02113982, NCT02270463, and NCT02420873, respectively).

At this time, CD33- and CD123-targeted approaches are the most advanced. Monoclonal antibodies to other AML target antigens including CD25, CD37, and CD38 have recently begun clinical evaluation in phase I studies (NCT02588092, NCT02610062, and NCT01084252, respectively).

Radioimmunoconjugates

The radiosensitive nature of AML and its systemic pattern of involvement provide a rationale for radioimmunotherapy via targeted radionuclide antibody conjugates.[48] Early studies with 2 beta-emitters, ^{131}I-M195 and ^{131}I-HuM195, demonstrated activity in myeloid leukemia, and demonstrated safety when used at myeloablative doses in conjunction with standard chemotherapeutics as a conditioning regimen for HSC transplantation.[49,50] Subsequent clinical studies exploring antibodies against AML targets (CD33, CD45, or CD66) with beta emitters (^{131}iodine, ^{188}rhenium, ^{90}yttrium), alone or as part of a conditioning regimen pre-ASCT in patients with relapsed AML, have demonstrated tolerability and encouraging clinical efficacy.[51,52] The ^{131}I-labeled anti-CD45 antibody BC8 (Iomab B) is currently being tested in phase I (NCT00589316) and phase III (NCT02665065) studies to evaluate its efficacy and safety as a part of myeloablative conditioning regimen prior to ASCT in patients with R/R AML. The properties of high-linear energy transfer and short particle length of decay has been exploited in the development of alpha emitter radioisotope conjugates. A phase I trial with the second-generation actinium-225-lintuzumab (anti-CD33) antibody demonstrated reduction of bone marrow blasts in 65% of 18 evaluable patients with R/R AML.[53] A phase I/II trial to determine the toxicity and efficacy of fractionated dosing of this agent in combination with LDAC in older untreated AML patients is ongoing (NCT02575963).

Immunotherapy in Acute Myeloid Leukemia

Immune Checkpoint Antibodies

The concept of targeting the immune system and not the tumor itself was initially achieved through monoclonal antibody-based inhibition of CTLA-4, a protein receptor on T cells that prevents them from unleashing an immune attack against tumor cells. Another major approach to immune checkpoint blockade involves the inhibition of PD-1/PD-1 ligand, a co-inhibitory receptor-ligand system expressed on activated T cells and B cells.[54] Clinical trials with antibodies targeting these pathways have demonstrated marked efficacy resulting in FDA approvals in a variety of solid tumors.[55-57] Immune checkpoint therapy has more recently been evaluated in hematologic malignancies, with robust activity and approval in Hodgkin lymphoma and modest but clear activity in certain subsets of diffuse large B-cell lymphoma and in mantle cell lymphoma. The rationale to evaluate these agents in leukemia springs from studies demonstrating reduced AML burden and improved survival in murine models on inhibition of PD-1 and CTLA-4.[58-62] Additionally, PD-1 and other clinically targetable stimulatory checkpoint receptors, such as OX40 and ICOS, are overexpressed in the bone marrow of patients with relapsed AML.[63]

A phase I study with a PD-1/PD-L1 inhibitor, pidilizumab, demonstrated a minimal response in the form of stable disease in 1 of 8 salvage AML patients.[64] DNA methyltransferase (DNMT) inhibitors increase expression of PD-1, PD-L1, and PD-L2 in patients with AML/myelodysplastic syndrome (MDS), with higher expression in DNMT inhibitor-resistant as compared with inhibitor-sensitive patients, suggesting that PD-1 upregulation may be involved in resistance to DNMT inhibitors.[65] This has resulted in clinical trials combining PD-1/PD-L1 inhibitors with azacitidine in AML and MDS. A phase Ib/II study of nivolumab in combination with azacitidine in 53 patients with R/R AML (median prior therapies = 2, poor-risk cytogenetics = 45%) showed an ORR (CR, CRi, hematologic improvement) of 34%, and an 8-week mortality rate of 8%. The overall survival with this combination in the first salvage setting was superior to historical outcomes with other hypomethylating agent (HMA)-based salvage protocols at the same institution (9.3 vs 3.9 months, $P = .03$).[66] Furthermore, responses were durable, with 80% of relapsed patients who achieved CR/CRi alive at 1 year. PD-1 inhibitors are also being

evaluated as a maintenance therapy, post induction and consolidation, in patients with high-risk AML who are not candidates for ASCT (NCT02532231) and in combination with standard induction chemotherapy in newly diagnosed younger (aged 18–60 years) patients with AML (NCT02464657). PD-1 inhibitors are also being evaluated in phase 1 trials for patients with AML after ASCT with initial encouraging results, with CR in 5 of 12 patients with post–SCT relapsed AML (median: 3 prior therapies before ASCT), including resolution of extramedullary leukemia in 3 of the patients (NCT01822509). Activation of stimulating checkpoints such as OX40 and ICOS represents another approach to enhance anti-tumor cytotoxicity, and this may prove synergistic in combination with checkpoint inhibition. Clinical trials with such inhibitory and stimulatory combinations are being planned.

Adoptive T-Cell Therapy

The process of adoptive cell transfer involves removal of T cells from a patient and modifying them so that they express receptors specific to the patient's particular cancer. The ex vivo expanded cytotoxic T lymphocytes, now capable of targeting tumor-associated antigens, are reinfused into patients. The genetically engineered receptors consist of an extracellular domain created by the fusion between the variable region of heavy and light chains of an antigen-specific monoclonal antibody and of an intracellular T cell-activating domain, usually CD3-ζ. The tumor specificity of the monoclonal antibodies allows activation of CAR-T cells independent of major histocompatibility complex (MHC). After showing success in acute lymphoblastic leukemia, the approach was recently evaluated in the treatment of patients with AML[67,68] with CAR-T cells targeted to Ley, a carbohydrate antigen that is overexpressed by malignant myeloid cells. The pilot study (n = 5) involved 4 evaluable patients with relapsed AML, 2 of whom had achieved stable disease and 2 who had transient responses.[69] The investigators observed that Ley was only expressed in a proportion of AML blasts, unlike epithelial tumors, in which the expression was more uniform and intense. Therefore, there has been further development of this agent in lung cancer therapy only. Preclinical studies have shown that anti-CD123 CAR-Ts are capable of targeting AML blasts and leukemia stem cells, suggesting these could be promising agents for AML CAR-T cell approaches.[70] A number of phase I clinical trials including anti-CD33, -CD7, and -CD133 CAR-Ts (NCT01864902, NCT02799680, NCT02742727, NCT02541370) and anti-CD123 and anti-NKG2D ligand CAR-T cells (NCT02159495, NCT02623582; NCT02203825) studies are ongoing in patients with R/R AML. The role and future of CAR-T cell therapy in AML remains unknown at this time.

T Cell-Engaging Antibodies

BiTE antibodies are fusion protein constructs consisting of 2 single-chain variable fragments of 2 antibodies; one binds to T cells via the CD3 receptor, and the other binds to a tumor cell via a tumor-specific antigen. BiTE antibodies effectively recruit T cells and link them with tumor cells, thereby effectuating T cell-mediated cytotoxic activity on tumor cells independent of the presence of MHC-I or costimulatory molecules.[71,72] After the promising clinical activity and FDA approval of blinatumomab, an anti-CD19/CD3 first-in-class BiTE antibody in precursor B-cell acute lymphoblastic leukemia, the approach has been adapted to target AML with the development of novel constructs targeting anti-CD3/CD33, such as AMG 330.[73,74] Preclinical studies have demonstrated potent activity of this agent in CD33+ AML[74-76] and a phase I trial of AMG330 is ongoing (NCT02520427). Phase I trials evaluating XmAb CD3/CD123 BiTE (NCT02730312) and JNJ-63709178 CD123/CD3 (NCT02715011) have recently begun enrollment. Early data suggest that close monitoring for cytokine release syndrome will be important with such bispecific approaches in AML.

Next-Generation Hypomethylating Agents

Guadecitabine (SGI-110), a second-generation hypomethylating agent, improves upon the pharmacokinetics of decitabine by incorporating deoxyguanosine dinucleotide into decitabine, thereby increasing in vivo exposure and potentially improving efficacy.[77] The outcomes of a phase II study involving 103 patients with R/R AML treated with SGI-110 were recently presented. CR and median survival rates on the 10-day (n = 53) and 5-day (n = 50) regimens were 30% and 16% at 7.1 and 5.7 months, respectively; the most common grade 3 AEs were anemia, thrombocytopenia, neutropenia, pneumonia, and sepsis.[78] A phase III trial comparing SGI-110 with standard of care in previously treated AML is currently ongoing (NCT02920008).

Other Therapies

DOT1L-like/histone H3K79 methyltransferase/DOT1L is a protein implicated in the development of mixed lineage leukemia (MLL) through aberrant hypermethylation-induced gene expression. A phase I trial evaluating pinometostat (EPZ-5676) in adult R/R MLL (n = 49) determined the agent to have clinical activity (composite CR [n = 2], PR [n = 1], leukemia cutis [n = 3]) with an acceptable safety profile (NCT01684150).[79] Another promising approach involves targeted inhibition of chromatin regulatory proteins such as lysine-specific demethylase-1 (LSD1), an enzyme responsible for histone H3 demethylation apart from other functions. GSK2879552 is an orally administered LSD1 inhibitor that is currently undergoing development in a phase I dose-escalation study involving R/R AML (NCT02177812), and results are eagerly awaited.

The bromodomain (BRD) family is an epigenetic class of histone-modification proteins with an ability to "read" the genome and modulate gene expression through transcriptional regulator recruitment to specific genome locations. BRDs of these reader proteins promote aberrant gene expression and sustain leukemic maintenance, thus providing a rationale for developing inhibitors

against this class.[80] Berthon and colleagues reported on outcomes with OTX105, an oral BRD inhibitor, in a phase I dose-escalation study.[81] Among 36 patients with R/R AML, 3 achieved CR/CRi, and 2 had partial blast clearance. Recommended dose for further phase II studies was 80 mg on a schedule of 14 days on, 7 days off.

Histone deacetylase inhibitors provide another therapeutic approach to exploit the aberrant epigenetic alterations in AML. After showing single-agent activity in a phase I trial in AML, pracinostat was evaluated in combination with azacitidine in a phase II study involving older patients with AML deemed to not be candidates for intensive chemotherapy.[82] The combination arm proved superior, with an estimated median OS of 19.1 months, resulting in the FDA granting a breakthrough designation for the combination's use in elderly patients with AML aged ≥75 years, or ineligible for intensive chemotherapy.

Pevonedistat is a novel NEDD8-activating enzyme inhibitor with single-agent clinical activity in R/R AML.[83] Swords and colleagues presented outcomes of a phase II study evaluating pevonedistat in combination with azacitidine in treatment-naïve older patients with AML.[84] Among 61 patients treated, ORRs were observed in 52 (18 CR, 5 CRi, 8 PR, 21 other) with a median duration of remission of 8.3 months. After a median follow-up of 16.4 months, projected 6-month survival was 52%. Single-agent and hypomethylating combination strategies with pevonedistat and MLN4924, another inhibitor in this class, are ongoing (NCT03009240, NCT02610777, and NCT01814826). NCT00911066 is completed.

Conclusion

Among available molecular targeted therapies, FLT3 inhibitors, as single agents or in combination with standard chemotherapy, in frontline or salvage settings, have already shown benefit (frontline 7+3 with midostaurin in untreated young AML patients in a phase III trial) or are being evaluated in phase III trials. Other inhibitors targeting IDH1/2 and BCL-2 among other small molecule inhibitors have shown promise as single agents and in combination with DNMT inhibitors, and are being evaluated in ongoing expanded phase II trials or soon-to-open phase III trials. Monoclonal antibody conjugates, such as SGN-CD33A and GO, are currently being evaluated in combination strategies with DNMT inhibitors or in cytotoxic induction regimens in phase III trials, while other immune- and antibody-based therapies, discussed above, are still in early phases of clinical development. The integration of informative biomarkers into clinical practice, and trials and implementation of rational combinatorial strategies of targeted, immune, monoclonal, and cytotoxic chemotherapies with each other, all while assessing for tolerability and toxicity, are important steps forward to help define and expand the scope of these novel therapies in AML.

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State-of-the-Art Update: CDK4/6 Inhibitors in ER+ Metastatic Breast Cancer

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Abstract

Cell-cycle inhibition is a new standard-of-care therapy in estrogen-receptor–positive metastatic breast cancer (MBC). The rapid integration of cyclin-dependent kinase (CDK) 4/6 inhibitors into mainstream clinical practice has led to many important investigations into biomarkers of response, mechanisms of resistance, sequencing of therapies, the role of other CDK4/6 inhibitors, and usage in other breast cancer subtypes. Here, we review the current state of palbociclib, ribociclib, and abemaciclib as CDK4/6 inhibitors in MBC, with particular attention to ongoing clinical trials in breast cancer.

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Introduction

Anti-CDK4/6 agents inhibit the phosphorylation of the retinoblastoma (Rb) tumor suppressor, which promotes Rb-E2F binding and prevents E2F-mediated oncogenic transcription. Slamon and colleagues showed compelling preclinical data indicating the efficacy of palbociclib in esrogen receptor positive (ER+) breast cancer cell lines. These experiments established that in the absence of hormonal therapy, palbociclib is cytostatic, and that when combined with estrogen blockade, there is a synergistic decrease in cell proliferation.

The phase II PALOMA-1 trial demonstrated a 10-month improved progression-free survival (PFS) in women with ER+ MBC treated with first-line letrozole plus palbociclib, a CDK4/6 inhibitor, versus letrozole alone (20.2 vs 10.2 months, hazard ratio [HR], 0.488, one-sided $P = .0004$). This led to the larger phase III PALOMA-2 trial, which confirmed a 10-month improved PFS in women with ER+ MBC treated with first-line letrozole plus palbociclib versus letrozole alone (24.8 vs 14.5 months, HR, 0.58; $P < .000001$). Importantly, these trials enrolled women who had not received endocrine therapy for their metastatic disease.

The phase III PALOMA-3 trial demonstrated a 5-month improved PFS in women with ER+ MBC who had progressed despite endocrine therapy for their metastatic disease, and were treated with palbociclib plus fulvestrant, versus fulvestrant alone (9.5 vs 4.6 months; HR, 0.46; $P < .0001$). Together, these studies have elevated palbociclib plus letrozole as the preferred first-line therapy in women with ER+ MBC, and palbociclib plus fulvestrant as an effective therapy in patients with ER+ MBC not previously treated with palbociclib who have progressed on a nonsteroidal aromatase inhibitor.

The success of palbociclib has spurred the development of other CDK4/6 inhibitors including ribociclib, which is now FDA approved in combination with fulvestrant, and abemaciclib, which has been granted an FDA breakthrough therapy designation. Numerous clinical trials are investigating these CDK4/6 inhibitors in settings beyond metastatic disease (**Table**), including adjuvant and neoadjuvant trials and novel combinations with other targeted therapies and immunotherapies. Therefore, it will be of great interest to see where these drugs show efficacy and if there may be differential activities among the inhibitors.

CDK4/6 Inhibitors and Clinical Profiles

Palbociclib has a half-maximal inhibitory concentration (IC50) for CDK4/6 of 9 to 15 μM.[8] The most frequent adverse events (AEs)of palbociclib are neutropenia, thrombocytopenia, and fatigue. The most frequent grade 3/4 AEs are pulmonary embolism (4%) and diarrhea (2%). Palbociclib is dosed at 125 mg twice daily, 3 weeks on and 1 week off. Importantly in clinical trials, few patients had febrile neutropenia.

Ribociclib has an IC50 for CDK4/6 of 11 to 39 μM.[8] The most frequent AEs with ribociclib are neutropenia, nausea, and thrombocytopenia. The most frequent grade 3/4 AEs are neutropenia and thrombocytopenia. Of note, aspartate aminotransferase/alanine aminotransferase increases (15%) and corrected QT interval prolongation were observed (8%); therefore, serial liver-function test monitoring and electrocardiograms are recommended when prescribing ribociclib. Ribociclib is dosed at 600 mg daily, 3 weeks on and 1 week off.

Abemaciclib has an IC50 for CDK4/6 of 2 to 5 μM,[8] and it can penetrate the blood-brain barrier. The most frequent AEs of abemaciclib are neutropenia and diarrhea. Almost all patients will also have an asymptomatic creatinine increase, which is an on-target AE of abemaciclib because it inhibits renal efflux transporters in the proximal tubule of the kidney. The most frequent grade 3/4 AEs are neutropenia and diarrhea. Some clinical trials now integrate prophylactic loperamide, an antidiarrheal medication, with abemaciclib. Abemaciclib as a single agent is dosed continuously at 200 mg twice a day. In combination with endocrine therapy, abemaciclib is currently under

investigation at 150 mg twice a day. Of note, abemaciclib is effective as monotherapy without the need for hormonal blockade,[7] and this may be due to its increased affinity for CDK4, which is important for breast cancer oncogenesis, as compared with CDK6.

Biomarkers of Response and Resistance, Mechanisms of Sensitivity, and Mechanisms of Resistance

While palbociclib is a targeted therapy, we do not understand the characteristics of ER+ breast cancers that predict for clinical response. Palbociclib owes its development to decades of work on the cell cycle,[9] which culminated in the 2001 Nobel Prize in Physiology or Medicine for Hartwell, Hunt, and Nurse.

Based on these seminal studies, one may predict that amplification of cyclin D1 (which binds to CDK4/6 and is required for its enzyme activity) or loss of p16 (which is a negative regulator of the CDK4/6-cyclin D1 complex) would enhance sensitivity to CDK4/6 inhibitors. PALOMA-1,[3] which tested letrozole with and without palbociclib in patients with ER+ MBC, enrolled molecularly defined

TABLE. CDK4/6 Inhibitor Trials

Trial Name	NCT#	Phase	Drug combinations	Breast cancer subtype	Setting	Status
PALOMA-1	NCT00721409	Phase I/II	P + L vs L	ER+ MBC	Endocrine therapy-naïve for MBC	Completed
PALOMA-2	NCT01740427	Phase III	P + L vs L	ER+ MBC	Endocrine therapy-naïve for MBC	Completed
PALOMA-3	NCT01942135	Phase III	P + F vs F	ER+ MBC	Failed endocrine therapy	Completed
PALOMA-4	NCT02297438	Phase III	P + L vs L	ER+ MBC	Endocrine therapy-naïve for MBC, Asian patients	Recruiting
PATRICIA	NCT02448420	Phase II	P + T + L; P + T	ER+ HER2+ and ER– HER2+ MBC	Failed 2-4 lines of anti-HER2 therapy	Recruiting
PEARL	NCT02028507	Phase III	P + E vs P + F vs C	ER+ MBC	Failed AI	Recruiting
PENELOPE-B	NCT01864746	Phase III	P vs placebo	ER+ locally advanced BC	s/p NACT with taxane-containing regimen, without pCR	Recruiting
PALLAS	NCT02513394	Phase III	P + standard endocrine therapy vs standard endocrine therapy	ER+ early stage BC	Adjuvant or neoadjuvant study, may have received NACT	Recruiting
PATINA						
NCT02947685		Phase III	P + any endocrine therapy + T + pertuzumab vs any endocrine therapy + T + pertuzumab	ER+ HER2+ MBC	Failed trastuzumab or other anti-HER2 therapies	Not yet open
NCT02605486		Phase I/II	P + bicalutamide	AR+ MBC	No limit to number of prior therapies	Recruiting
NCT01823835		Phase I/II	P + GDC-0810 +/- OS	ER+ MBC	Inclusion criteria vary per arm	Recruiting
NCT01320592		Phase I	P + paclitaxel	MBC, all subtypes	Rb wildtype	Ongoing
NCT01976169		Phase Ib	P + T-DM1	HER2+ MBC	Failed trastuzumab or other anti-HER2 therapies	Recruiting
NCT02871791		Phase I/II	P + E + evero	ER+ MBC	Progression on prior CDK4/6i and AI	Recruiting
NCT02778685		Phase II	P + L + pembrolizumab	ER+ MBC	Stable disease on P + L	Recruiting
NCT02760030		Phase II	P + F	ER+ early-stage unresectable BC	Newly diagnosed and untreated, age >70	Recruiting
NCT01723774		Phase II	P + AN	ER+ early-stage BC	Neoadjuvant chemotherapy-sparing trial	Recruiting
NCT02684032		Phase I	P + L + gedatolisib; P + F + gedatolisib	ER+ MBC	Inclusion criteria vary per arm	Recruiting
NCT03007979		Phase II	P + L; P + F; with P given 5 days on, 2 days off	ER+ MBC	One prior therapy for metastatic disease allowed	Not yet open
NCT03006172		Phase I	P + L + GDC-0077 (in arm B)	ER+ PIK3CA-mutated MBC	Must have *PIK3CA* mutation	Recruiting
NCT01037790		Phase II	P	MBC, all subtypes	Multiple inclusion criteria	Recruiting

A indicates abemaciclib; AI, aromatase inhibitor; AN, anastrozole; AR, androgen receptor; BC, breast cancer; C, capecitabine; CDK, cyclin-dependent kinase; CRC, colorectal cancer; E, exemestane; ER, estrogen receptor; evero, everolimus; F, fulvestrant; GBM, glioblastoma multiforme; HER2, human epidermal growth factor receptor 2; L, letrozole; MBC, metastatic breast cancer; NACT, neoadjuvant chemotherapy; NSAI, nonsteroidal aromatase inhibitor; NSCLC, non–small cell lung cancer; OS, ovarian suppression; P, palbociclib; pCR, pathologic complete response; R, ribociclib; Rb, retinoblastoma; T, trastuzumab; Tam, tamoxifen; T-DM1, ado-trastuzumab emtansine; TNBC, triple-negative breast cancer.

TABLE. CDK4/6 Inhibitor Trials (contd.)

Trial Name	NCT#	Phase	Drug combinations	Breast cancer subtype	Setting	Status
MONALEESA-1	NCT01919229	Phase II	R (400 mg) + L vs R (600 mg) + L vs L	Early stage BC	Pre-surgical	Terminated
MONALEESA-2	NCT01958021	Phase III	R + L vs L	ER+ MBC	Endocrine therapy-naïve for MBC	Ongoing
MONALEESA-3	NCT02422615	Phase III	R + F vs F	ER+ MBC	Newly diagnosed or relapsed, also includes men	Ongoing
MONALEESA-7	NCT02278120	Phase III	R + OS + AI/Tam	ER+ MBC	Endocrine therapy-naïve for MBC	Ongoing
SIGNATURE	NCT02187783	Phase II	R	Metastatic TNBC, other metastatic solid and liquid tumors	CDK4/6, cyclin D, or p16 aberrations	Ongoing
COMPLEEMENT-1	NCT02941926	Phase I/II	R + L vs L	ER+ MBC	De novo metastatic, also men	Ongoing
TRINITI-1	NCT02732119	Phase I/II	R + E + Evero	ER+ locally advanced BC	Progressed CDK4/6, also includes men	Recruiting
LeeBLet						
NCT02154776		Phase I	R + L + buparlisib	ER+ MBC	Therapy-naïve for MBC	Ongoing
NCT01857193		Phase Ib	R + E + Evero; R + E	ER+ MBC	Failed AI, some arms include patients who have failed other CDK4/6	Recruiting
NCT01872260		Phase Ib	R + L; Alp + L; R + Alp + L	ER+ MBC	Multiple inclusion criteria for different arms	Recruiting
NCT02657343		Phase I/II	R + T; R + T-DM1	ER+ HER2+ MBC	Multiple inclusion criteria for different arms	Recruiting
NCT02632045		Phase II	R + F vs F	ER+ MBC	Failed CDK4/6, also includes men	Recruiting
NCT02088684		Phase II	R + F; R + F + Alp; R + F + buparlisib	ER+ MBC	Failed endocrine therapy and 1 or 2 lines of chemotherapy	Ongoing
NCT02599363		Phase I	R + weekly paclitaxel	MBC, any subtype	Failed up to 3 lines of chemotherapy	Recruiting

A indicates abemaciclib; AI, aromatase inhibitor; Alp, alpelisib; AN, anastrozole; BC, breast cancer; C, capecitabine; CDK, cyclin-dependent kinase; CRC, colorectal cancer; E, exemestane; ER, estrogen receptor; evero, everolimus; F, fulvestrant; GBM, glioblastoma multiforme; HER2, human epidermal growth factor receptor 2; L, letrozole; MBC, metastatic breast cancer; NACT, neoadjuvant chemotherapy; NSAI, nonsteroidal aromatase inhibitor; NSCLC, non–small cell lung cancer; OS, ovarian suppression; P, palbociclib; pCR, pathologic complete response; R, ribociclib; Rb, retinoblastoma; T, trastuzumab; Tam, tamoxifen; T-DM1, ado-trastuzumab emtansine; TNBC, triple-negative breast cancer.

cohorts of patients with amplification of cyclin D1, loss of p16, or both. However, these tumor alterations did not predict for response to palbociclib, and ER positivity remains the only validated biomarker of response. In addition, PALOMA-3[5] showed that neither *PIK3CA* mutational status (as detected by circulating tumor DNA) nor quantitative level of ER positivity predicted for response to palbociclib. Additional biomarker analyses from PALOMA-2[10] did not reveal any other cell-cycle–related genes that predicted for response to palbociclib plus letrozole.

Forty-five percent of patients on palbociclib do not derive an objective response and, among the patients who initially respond, 50% of them progress after 2 years of therapy.[1] Currently, the only accepted mechanism of intrinsic resistance to CDK4/6 inhibitors in patients is Rb loss, which is rare (2.4%) in non-triple negative MBC.[11] In vitro experiments have implicated cyclin E amplification,[12] CDK6 amplification,[13] and increased pyruvate dehydrogenase kinase 1[14] as mechanisms of acquired resistance to palbociclib monotherapy; however, these associations with clinical resistance to CDK4/6 blockade have yet to be confirmed.

Biomarkers have also been explored in the neoadjuvant CDK4/6 inhibitor space. The NeoPalAna trial[15] studied neoadjuvant anastrozole for 4 weeks, followed by the addition of palbociclib to anastrozole for four 28-day cycles, with single-agent anastrozole continuing until surgery. Biopsies were collected on starting palbociclib, after 2 weeks of palbociclib, and at surgery. There was a significantly increased rate of complete cell-cycle arrest (defined as Ki67 protein <2.7%) after 2 weeks of palbociclib plus anastrozole as compared with when starting palbociclib (87% vs 26%). How CDK4/6 inhibitors modulate Ki67 and whether or not decreased Ki67 translates into a decreased risk of recurrence and increased survival are open questions. Additional biomarker analysis showed that neither luminal breast cancer subtype nor *PIK3CA* mutational status predicted for response to palbociclib plus anastrozole. Palbociclib-resistant tumors had increased expression of cell-cycle genes *CCND3*, *CCNE1*, and *CDKN2D* on gene-expression analysis. Given that these genes are all downstream targets of the E2F1 transcription factor, it will be interesting to test to see if palbociclib resistance may be characterized by a cell-cycle gene signature.

The phase II neoMONARCH trial,[16] which investigates neoadjuvant abemaciclib plus anastrozole, includes a "window study"

TABLE. CDK4/6 Inhibitor Trials (contd.)

Trial Name	NCT#	Phase	Drug combinations	Breast cancer subtype	Setting	Status
MONARCH 1	NCT02102490	Phase II	A	ER+ MBC	Failed endocrine therapy and 2 lines of chemotherapy	Ongoing
MONARCH 2	NCT02107703	Phase III	A + F vs F	ER+ MBC	Failed endocrine therapy	Ongoing
MONARCH 3	NCT02246621	Phase III	A + AI vs AI	ER+ MBC	Endocrine therapy-naïve for MBC	Ongoing
neoMONARCH	NCT02441946	Phase III	A vs L vs A + L for two weeks (window study); A + L for 14-22 weeks	ER+ locally advanced BC	Neoadjuvant trial	Ongoing
monarcHER	NCT02675231	Phase II	A + T + F vs A + T vs T + Chemo	ER+ HER2+ MBC	Failed 2 lines of anti-HER2 therapy including taxane and T-DM1	Recruiting
JPBH	NCT02057133	Phase I	A + multiple therapies	ER+ MBC, HER2+ MBC	Multiple inclusion criteria for different arms	Recruiting
JPBA	NCT01394016	Phase I	A + F; A in other cohorts	ER+ MBC, MBC; also NSCLC, GBM, Melanoma, CRC	Failed F; or failed standard therapies	Ongoing
JPBO	NCT02308020	Phase II	A	ER+ HER2+ MBC, ER+ MBC; also NSCLC, melanoma	Brain metastases without leptomeningeal disease	Recruiting
nextMONARCH1	NCT02747004	Phase II	A + T vs A vs A + prophylactic loperamide	ER+ MBC	Failed endocrine therapy and no more than 2 lines of chemotherapy	Recruiting
	NCT02779751	Phase II	A + pembrolizumab	ER+ MBC; also squamous NSCLC, KRAS+ PD-L1+ NSCLC	Failed endocrine therapy and no more than 1 line of chemotherapy	Recruiting
	NCT02763566	Phase III	A + NSAI vs NSAI; A + F vs F	ER+ MBC	Therapy-naïve for MBC	Recruiting
	NCT02784795	Phase I	A + LY3039478; multiple other arms in other cancers	MBC with Notch pathway alterations	Multiple inclusion criteria for different arms	Recruiting

A indicates abemaciclib; AI, aromatase inhibitor; AN, anastrozole; BC, breast cancer; C, capecitabine; CDK, cyclin-dependent kinase; CRC, colorectal cancer; E, exemestane; ER, estrogen receptor; evero, everolimus; F, fulvestrant; GBM, glioblastoma multiforme; HER2, human epidermal growth factor receptor 2; L, letrozole; MBC, metastatic breast cancer; NACT, neoadjuvant chemotherapy; NSAI, nonsteroidal aromatase inhibitor; NSCLC, non–small cell lung cancer; OS, ovarian suppression; P, palbociclib; pCR, pathologic complete response; R, ribociclib; Rb, retinoblastoma; T, trastuzumab; Tam, tamoxifen; T-DM1, ado-trastuzumab emtansine; TNBC, triple-negative breast cancer.

in which patients obtain a pretreatment biopsy and are initially randomized to 2 weeks of abemaciclib monotherapy, anastrozole monotherapy, or a combination of the 2, after which they receive a posttreatment biopsy. After these 2 weeks, patients are continued on abemaciclib and anastrozole for 14 to 22 weeks. The primary endpoint is a decrease in Ki67 after 2 weeks of treatment. The study met its primary endpoint and both abemaciclib monotherapy as well as abemaciclib and anastrozole combination caused decreased Ki67 as compared with anastrozole alone.

Another research team[17] has shown that decreases in tumor Ki67 parallel decreases in serum thymidine kinase in patients on neoadjuvant palbociclib plus anastrozole. This may provide preclinical data for a new serum biomarker for response to CDK4/6 inhibitors, at least in the neoadjuvant arena.

Many CDK4/6 inhibitor clinical trials are collecting pre-, on-, and posttreatment biopsies, as well as circulating tumor DNA, for targeted next-generation sequencing, and these studies may reveal biomarkers or determinants of response and resistance to CDK4/6 inhibitors. In summary, other than ER-positivity, we do not understand the mechanisms of sensitivity or resistance to palbociclib and CDK4/6 inhibitors in ER+ MBC, apart from Rb loss predicting for resistance. Elucidating these resistance mechanisms will be crucial to leveraging the efficacy of CDK4/6 inhibitors.

Sequencing of Therapies and Finding a Place for Ribociclib and Abemaciclib

Currently, palbociclib is approved as first-line therapy in patients with de novo ER+ MBC, and palbociclib and ribociclib are approved for patients with recurrent metastatic disease who have progressed on endocrine therapies. Given the emergence of other CDK4/6 inhibitors, one important question is whether patients who have progressed on or after palbociclib may derive benefit from continued CDK4/6 inhibition, including treatment with palbociclib or another agent such as ribociclib or abemaciclib.

Some preclinical data suggest non–cross-resistance among CDK4/6 inhibitors. One study[18] generated palbociclib- and ribociclib-resistant cell clones and showed that some of these clones were sensitive to abemaciclib. Many ribociclib trials are exploring this question, including NCT01857193[19] (ribociclib plus exemestane plus everolimus, a mechanistic target of rapamycin inhibitor; or ribociclib plus exemestane),

TRINITI-1[20] (ribociclib plus exemestane plus everolimus), and NCT02632045[21] (ribociclib plus fulvestrant vs fulvestrant) in patients who have previously received a CDK4/6 inhibitor.

Palbociclib and ribociclib are similar in that both require hormonal therapy for efficacy in ER+ MBC. However, abemaciclib also has single-agent activity, as shown in the phase II MONARCH-1 trial.[7] MONARCH-1 enrolled a heavily pretreated patient population after prior progression on endocrine therapy and at least 1 prior chemotherapy agent for MBC, and showed about a 20% response rate and about a 40% clinical benefit rate, including patients with stable disease, with a median overall survival of about 22 months on abemaciclib monotherapy.[22] Expanding on these data, and capitalizing on the penetration of abemaciclib into the central nervous system, the JPBO trial[23] is investigating single-agent abemaciclib in patients with ER+ and ER+/human epidermal growth factor receptor–positive (HER2+) brain metastases.

Another outstanding clinical question concerns the optimal therapy after a patient has progressed on first-line palbociclib plus letrozole. Current standard options are hormonal therapy plus everolimus, hormonal therapy alone, or chemotherapy (eg, capecitabine). Hopefully, detailed correlative molecular analyses of patients on CDK4/6 inhibitor clinical trials will be able to answer this critical question, and to determine if genomically defined patient subsets (eg, *ESR1* mutations, *PIK3CA* mutations) may respond differently.

CDK4/6 Inhibitors in Other Breast Cancer Subtypes and Settings, and With Chemotherapy and Immunotherapy

While CDK4/6 inhibitors are effective in ER+ breast cancer, it remains to be seen if they are also effective in patients with ER+/HER2+ breast cancer or triple-negative breast cancer (TNBC).

Some trials exploring the efficacy of CDK4/6 inhibitors in combination with trastuzumab or ado-trastuzumab emtansine (T-DM1) in ER+ HER2+ MBC are PATRICIA[24] (palbociclib plus trastuzumab plus letrozole vs palbociclib plus trastuzumab, also with arms for ER– HER2+ patients), NCT01976169[25](palbociclib plus T-DM1), NCT02657343 (ribociclib plus trastuzumab; ribociclib plus T-DM1), monarcHER[26] (abemaciclib plus trastuzumab plus fulvestrant vs abemaciclib plus trastuzumab vs trastuzumab plus chemotherapy of physician's choice), and JPBO[20] (abemaciclib monotherapy).

While CDK4/6 inhibitors were initially thought to have improved efficacy in TNBC, preclinical work did not support this hypothesis, although there were some TNBC cell lines that did have adequate IC50s for palbociclib. CDK4/6 inhibitors are the subject of multiple trials in the metastatic TNBC space including NCT02605486[27] (palbociclib plus bicalutamide) in androgen-receptor–positive patients, SIGNATURE[28] (ribociclib monotherapy), NCT02599363[29] (ribociclib plus weekly paclitaxel) in *Rb*-wildtype patients of any subtype, JPBA[30] (arm with abemaciclib monotherapy), and NCT02784795[31] (abemaciclib plus Notch inhibitor LY3039478) in patients with Notch pathway alterations of any subtype.

Since many chemotherapeutics (eg, taxanes) require an intact cell cycle, combining these therapies may or may not be synergistic. Preclinical data supporting the combination of CDK4/6 inhibitors with chemotherapy are mixed, suggesting either lack of cytotoxic synergy with CDK4/6 inhibitors[32] or attenuation of CDK4/6 inhibitor-induced cytotoxicity.[33] Some phase I clinical trials are exploring combining CDK4/6 inhibitors with cytotoxic chemotherapy, including NCT02599363[26] (ribociclib plus weekly paclitaxel). Palbociclib plus weekly paclitaxel[34] can be administered safely, and we await trials exploring efficacy of these combinations.

Checkpoint blockade immunotherapy is a clear success in melanoma, non–small cell carcinoma, and other solid tumors; however, its role in breast cancer is not clear. An arm of KEYNOTE 012[35] (pembrolizumab, a monoclonal antibody against PD-1) showed that of 32 patients with heavily pretreated PD-L1+ metastatic TNBC, there was a 19% response rate and a 26% clinical benefit rate. An arm of KEYNOTE 028[36] (pembrolizumab) demonstrated a 12% response rate and 20% clinical benefit rate in heavily pretreated patients with ER+ HER2– PD-L1+ MBC. As in other solid tumors, PD-L1 positivity is predictive but not prognostic of response to checkpoint blockade. Low response rates in MBC have also been observed in the JAVELIN trial[37] (avelumab, a monoclonal antibody against PD-L1).

The modest response of checkpoint blockade in ER+ MBC has spurred clinical trials looking at ways to potentiate immunotherapy with CDK4/6 inhibition. NCT02779751,[38] a phase II clinical trial, is evaluating the safety and preliminary efficacy of abemaciclib plus pembrolizumab. NCT02778685,[39] another phase II clinical trial, is investigating the safety and preliminary efficacy of adding pembrolizumab to palbociclib plus letrozole in patients with stable disease on palbociclib plus letrozole. These studies and their correlative biomarkers may reveal ways to potentiate the modest efficacy of checkpoint blockade in ER+ MBC.

Conclusion

The addition of palbociclib to the armamentarium of therapies in ER+ MBC is of great utility for patients; however, many fundamental questions remain about biomarkers for response and resistance, the role of next-generation CDK4/6 inhibitors, efficacy in other breast cancer subtypes, and combinations with other targeted therapies, chemotherapy, and immunotherapy. Multidisciplinary work integrating basic science, translational science, and clinical trials will be required to leverage fully the potential of CDK4/6 inhibitors in patients.

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Can Positron Emission Tomography Scans Post Chemoradiation in Head and Neck Squamous Cell Cancer Spare Patients From Undergoing Salvage Surgery?

Misako Nagasaka, MD, and Ammar Sukari, MD

Abstract

Positron emission tomography (PET) scans performed after definitive chemoradiation may help differentiate between viable tumor and nonmalignant tissue that has been affected by treatment. What follows is a case presentation and review of the literature. A 58-year-old man with stage IVa base-of-tongue cancer was treated with chemoradiation. Initial magnetic resonance imaging (MRI) 12 weeks post chemoradiation showed a worrisome enhancement, possibly indicating residual disease. A repeat MRI at 20 weeks showed no improvement. However, a PET scan at 25 weeks did not show any fluorodeoxyglucose (FDG) uptake, meaning gross residual disease was unlikely. He continued on surveillance and has successfully been spared from undergoing salvage surgery.

PET scans in patients with head and neck squamous cell carcinoma post chemoradiotherapy have shown high negative predictive values of 96%. This means that if the PET scan was negative, 96% of the time, this was truly negative, meaning no evidence of cancer. Some reports suggest that tumors positive for human papillomavirus may take more time to involute.

PET scans may have a role in identifying those who could be followed by surveillance without undergoing additional surgical post chemoradiation even with what may appear to look like potential residual findings on computed tomographies or MRIs.

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Introduction

The treatment of residual disease after definitive chemoradiation in head and neck squamous cell carcinoma (HNSCC) often involves extensive salvage surgery. Residual disease, however, may be difficult to distinguish from posttreatment changes when computed tomography (CT) or magnetic resonance imaging (MRI) scans are used. The issue becomes even more challenging when suspicious findings on imaging persist, despite a negative biopsy. In such cases, the use of positron emission tomography (PET) scans post treatment may help differentiate between viable tumor and nonmalignant tissue that has been affected by treatment.

Case Report

A 58-year-old man with stage IVa (T4a N0 M0) squamous cell carcinoma (SCC) of the right base of tongue, p16 positive, was treated with definitive chemoradiation utilizing cisplatin 100 mg/m^2 every 3 weeks and a total of 70 Gy of radiation. His initial MRI findings 12 weeks post chemoradiation showed a bulky enhancement at the base of tongue, 1.2 cm × 1.9 cm × 1.2 cm in size **(Figure1A)**, worrisome for possible residual disease. He had a direct laryngoscopy at 13 weeks with bilateral base of tongue biopsies which were negative for disease. A repeat MRI done at 20 weeks did not show improvement **(Figure 1B)**. However, a PET scan at 25 weeks did not show any fluorodeoxyglucose (FDG) uptake **(Figure 2),** meaning it was likely he was disease-free. He was therefore continued on surveillance. He continues to do well without evidence of disease 17 months post chemoradiation and has successfully been spared from undergoing salvage surgery.

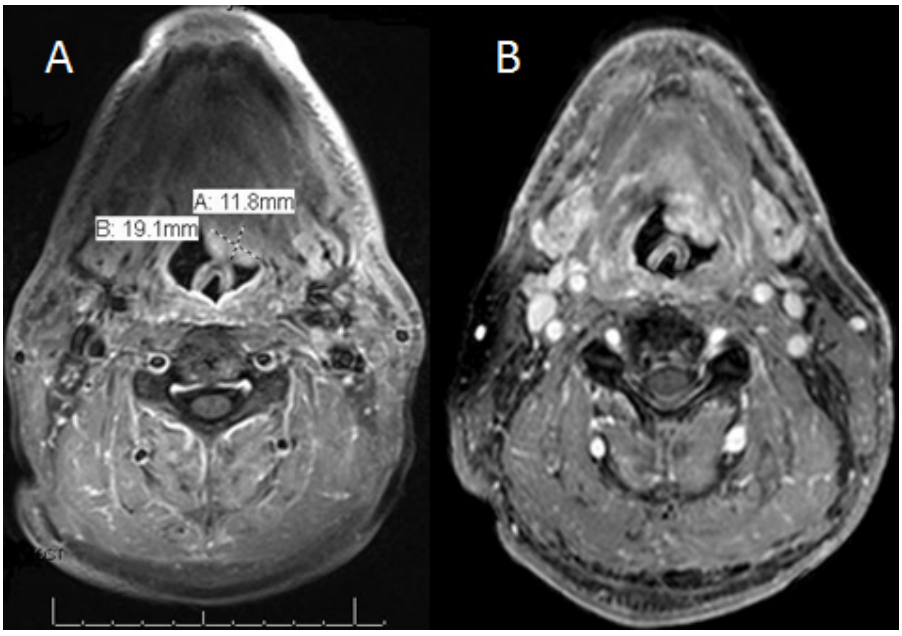
Discussion

How often does residual disease post chemoradiation occur in the first place? Data on residual disease post chemoradiation for HNSCC have been focused on residual disease in the neck. This is due to the fact that planned neck dissection post chemoradiation had been a common practice in many centers across United States and around the world. In a retrospective review from the University of Chicago, 69 patients with stage III or IV HNSCC all underwent neck dissection 5 to 17 weeks post chemoradiation completion. Twenty-four of 69 (35%) had residual disease in the neck lymph nodes. Positive nodes were seen in 10 out of 20 (50%) in N3 disease and 14 out of 39 (36%) in N2. N3 denotes when a lymph node metastasis has exceeded 6 cm in size. N2 denotes when a single ipsilateral lymph node is larger than 3 cm but has not exceeded 6 cm in size, or, in the case of multiple ipsilateral nodes, none exceed 6 cm in size; or, in bilateral/contralateral nodes, none exceed 6 cm in size. On the other, data on residual disease in the primary tumor post chemoradiotherapy are limited, but such disease seems to occur in about 10% to 25% of patients.[1]

The PET-NECK trial is perhaps the most recognized study regarding the use of PET scans and HNSCC. The trial's main focus was the management of neck disease in HNSCC in patients undergoing

chemoradiation. In this trial, a total of 564 patients with HNSCC N2 or N3 disease were randomly assigned to undergo either a planned neck dissection (planned-surgery control group) or a PET scan (surveillance) 12 weeks after completion of chemoradiation. The authors concluded that the PET scan–guided watch-and-wait policy was noninferior to planned neck dissection when comparing overall survival (OS) and disease-specific survival. The researchers found that only 20% in the PET scan arm ultimately required neck dissection, which resulted in fewer complications related to neck dissection, similar quality of life, and more cost-effective management.[2] In this study, the PET scan–guided surveillance approach was shown to be noninferior in terms of survival, and it resulted in fewer neck dissections.

FIGURE 1. Posttreatment Changes on MRIs.



(1A) Magnetic resonance imaging (MRI) findings 12 weeks post chemoradiation showed a bulky enhancement at the base of tongue, 1.2 cm × 1.9 cm × 1.2 cm in size.
(1B) A repeat MRI done at 20 weeks did not show improvement.

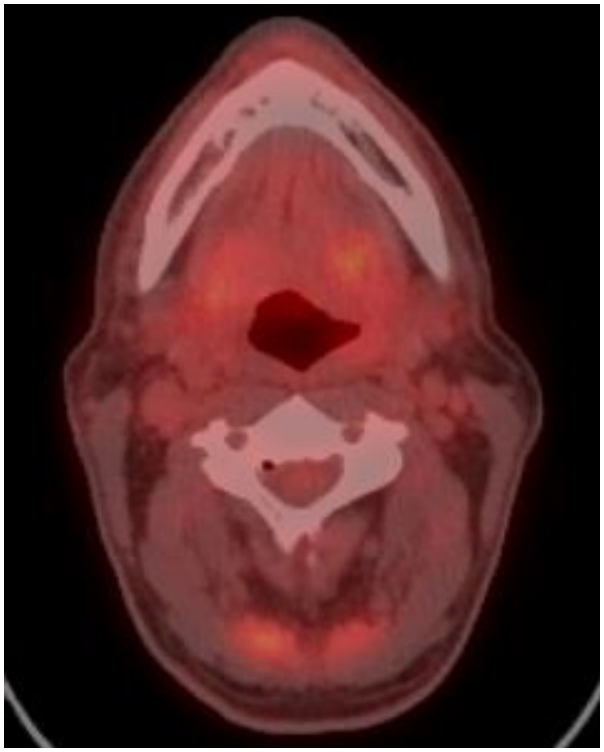
What about the use of PET scans to detect residual disease in the primary tumor? In the PET-NECK trial described above, the surveillance group was the arm that underwent PET scans 12 weeks after completion of chemoradiation; PET detected 28 of 276 patients (10%) with PET-positive disease at the primary site. However, the outcomes of these patients are not available in the manuscript nor in the supplemental material. In a retrospective review by Sjövall et al, a total of 82 patients with HNSCC, with a positive baseline PET-CT scan before start of treatment, were evaluated with a PET-CT scan 6 to 7 weeks after radiation therapy, and again with a clinical examination/endoscopy (with or without biopsy) 1 to 2 weeks later.

Results showed that post radiation, 77% of the patients had no visible FDG uptake, meaning they did not have gross residual disease that would have been detected on a PET scan. When equivocal PET scans are regarded as positive, the sensitivity, specificity, negative and positive predictive values, and accuracy were 100%, 78%, 100%, 6%, and 78%, respectively.[3] Positive predictive value is the probability that subjects with a positive test truly have the disease. Negative predictive value is the probability that subjects with a negative test truly do not have the disease.

In a meta-analysis of 27 studies, most of which were retrospective studies involving 11 to 80 patients per trial, Isles et al described the role of PET scans in the follow-up of HNSCC following radiation or chemoradiation. The pooled mean positive and negative predictive values for the detection of residual disease at the primary site were 75% (95% confidence interval [CI], 68%-82%) and 95% (95% CI, 92%-97%), respectively.[4]

Residual disease may be difficult to distinguish from posttreatment

FIGURE 2. Posttreatment Changes on PET Scan.



A positron emission tomography scan at 25 weeks did not show any fluorodeoxyglucose uptake.

changes when CT or MRI are used. In a prospective study from The University of Texas MD Anderson Cancer Center, which included 98 patients with stage III or IV disease, the positive and negative predictive values of PET-CT versus CT were compared 8 weeks post chemoradiation. In high-risk patients, PET-CT was superior to CT in detecting residual primary disease with a positive predictive value (PPV) of 100% versus 71.4%; for residual lymph node disease (as opposed to residual primary disease), the PPVs were 75% versus 37.5% in the PET-CT and CT arms, respectively. High risk was defined as human papillomavirus (HPV)-negative tumors; nonoropharyngeal primaries such as laryngeal, oral cavity, and hypopharyngeal cancer; or history of significant tobacco use.[5]

HPV status may be the most important factor when considering imaging modalities and their timing. HPV status is highly prognostic of survival. The PET-NECK trial showed 2-year OS of approximately 90% and 55% for patients whose tumors were p16 positive and negative, respectively, regardless of whether they were in the PET scan surveillance or upfront neck dissection arm.[2] Studies have also shown that HPV-positive tumors may take more time to involute.[6] The research thus indicates that in HPV-positive patients, a more relaxed approach to intervention is appropriate, whereas in HPV-negative patients, aggressive interventions may be required.

Last but not least, cost considerations must also be taken into account. A prospective single-institution study from Australia indicated that the cost per patient was A$ (Australian dollars) 16,502 for planned neck dissection, A$8014 for CT, and A$2573 for PET surveillance. A policy in which PET was used only for incomplete response on CT was the least-cost strategy (A$2111).[7] The cost analysis reported by the PET-NECK trial group noted that the per-person cost saving was £1492 (approximately $2,190 in US dollars), with an additional 0.08 quality-adjusted life-year per person.[2]

The presented case illustrates a typical example of a patient with HPV-positive, locally advanced HNSCC who underwent chemoradiation. Suspicious findings on the MRI 12 weeks post treatment were worrisome. The issue became even more challenging when the suspicious findings on imaging persisted 20 weeks post therapy despite a negative biopsy. As this patient demonstrated good compliance with follow-up appointments, and as his tumor was HPV-positive (which is known to take more time to involute), we had opted for further surveillance utilizing PET scans. The PET scan performed 25 weeks post therapy did not reveal any FDG uptake, and the patient has been successfully followed on surveillance since then, without being subjected to salvage surgery.

Conclusion

PET scans may have a role in identifying those who could be safely followed post definitive therapy even with residual findings on CTs or MRIs. Currently available compelling evidence on PET scans in HNSCC comes from studies focusing on neck disease. Further studies could focus on the utilization of PET scans in the management of primary HNSCC tumor sites post definitive chemoradiation.

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Optimizing Sequencing Beyond Disease Progression After Second-Line Therapy in Metastatic Colorectal Cancer

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Abstract

Colorectal cancer (CRC) remains a significant cause of morbidity and mortality worldwide with high disease incidence. Additionally, despite large-scale screening efforts targeting US adults, significant numbers of patients present with advanced, metastatic disease. Over the past 20 years, the therapeutic armamentarium for metastatic disease has increased significantly; 10 new drug approvals include targeted biologics and tyrosine kinase inhibitors. With this increase in options, median overall survival (OS) for patients with metastatic CRC has increased to more than 30 months. With more drug options, and even more combination options now available, optimal sequencing of these options to maximize their proportional OS benefit for patients is of utmost importance; it remains a topic of continued investigation. Here, we review the current evidence on optimizing sequencing, particularly as it relates to regorafenib and trifluridine-tipiracil.

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Introduction

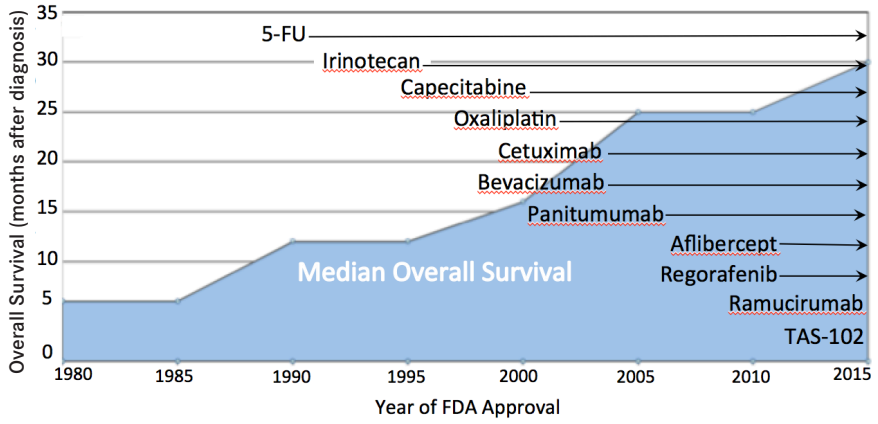
Colorectal cancer (CRC) remains a significant cause of morbidity and mortality worldwide with high disease incidence and, despite large-scale screening efforts recommended for all US adults, significant numbers of patients presenting with advanced, metastatic disease. In 2016, it represented 8% of both new US cases of cancer and cancer-related deaths.[1] Metastatic disease is considered incurable, with the exception of patients presenting with oligometastatic lesions confined to the liver or lung who may be amenable to resection, or metastasectomy.[2,3] When treatment with curative intent is not possible, patients are typically given a combination of cytotoxic chemotherapy often in conjunction with a targeted therapy. In spite of advances in systemic therapy, the 5-year survival rate is still a mere 13%.[1,4]

Until 2000, systemic therapy options for metastatic CRC were extraordinarily limited, consisting simply of 5-fluorouracil. In 1996, the drug irinotecan was approved for patients with recurrent/refractory disease, and then in 2000 for first-line therapy. Since then, the armamentarium has increased significantly, with 9 new drug approvals for the disease. These include monoclonal antibodies that target the vascular endothelial growth factor (VEGF) (bevacizumab), the VEGF receptor 2 (ramucirumab), and the epidermal growth factor receptor (EGFR; cetuximab and panitumumab); a fusion protein that targets VEGFs A and B and placental growth factor (aflibercept); an orally active inhibitor of angiogenic, stromal, and oncogenic kinases (regorafenib); and an oral cytotoxic agent that consists of a nucleoside analog and a thymidylate synthetase inhibitor (trifluridine-tipiracil) (**Figure 1**). With this increase in options, median overall survival (OS) for patients with metastatic CRC has increased as well, from 12 months in 1990 to more than 30 months in 2015.

Despite the significant increase in median OS, however, the proportional increase for each individual regimen's median OS has ranged from just 1.4 to 4.2 months (**Figure 2**).[5-14] With more drug options, and even more combination options now available, optimal sequencing of these options to maximize their proportional OS benefit for patients is of utmost importance; it remains a topic of continued investigation. Options for the refractory setting, after standard therapy has ceased—regorafenib and trifluridine-tipiracil—have emerged in the last 2 to 3 years with overall, small proportional increases in OS. Studies of both agents have included exploration of the effects of prior therapies in different ways, with results demonstrating little in the way of significant benefits in any particular situation (**Table**).

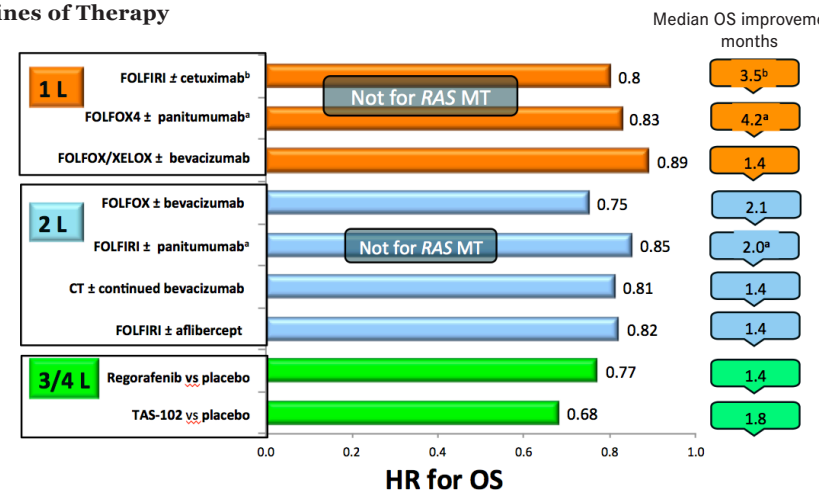
Regorafenib is an oral multikinase inhibitor that targets tumor-cell proliferation via targeting of the KIT, PDGFR-β, and RET kinases; tumor microenvironment signaling via targeting of PDGFR-β and FGFR1; and neoangiogenesis via targeting of VEGF receptors 1-3 and TIE2.[15-17] In the global, multicenter phase III CORRECT study, 760 patients with metastatic CRC were randomized after failure of standard therapy with regorafenib or placebo.[12] Regorafenib demonstrated a significant increase in the primary endpoint, median OS, compared with placebo, at 6.4 versus 5 months (HR, 0.77; $P = .0052$).

FIGURE 1. Advances in Drugs for the Treatment of Metastatic CRC



5-FU indicates fluorouracil; CRC, colorectal cancer; TAS-102, trifluridine + tipiracil.

FIGURE 2. Proportional Impact on Magnitude of OS Benefit Achieved Across Lines of Therapy



CT, chemotherapy, FOLFIRI, 5-fluorouracil and irinotecan; FOLFOX, 5-fluorouracil and oxaliplatin; HR, hazard ratio; L, line of therapy; OS, overall survival; PFS, progression-free survival; TAS-102, trifluridine and tipiracil; *RAS* MT, any *RAS* (*KRAS*, *NRAS*, *BRAF*) mutated; XELOX, capecitabine and oxaliplatin.

[a]*KRAS* WT subset; *P* value = not significant.

[b]*KRAS* WT subset; *P* value = significant

Median progression-free survival (PFS) was also improved at 1.9 versus 1.7 months, for regorafenib versus placebo, respectively. The HR was significantly increased at 0.49 (*P* <.0001). Disease control rate was significantly increased for regorafenib at 41%, compared with placebo at 14.9%.

In subgroup analyses, regorafenib demonstrated superiority in all subgroups, including patients who were diagnosed <18 versus ≥18 months prior to study entry (HR, 0.816 vs 0.760) and patients receiving ≤3 versus >3 prior treatment lines for metastatic disease (HR, 0.788 vs 0.747). In the CONCUR study, a smaller trial conducted in Asia alone, regorafenib was compared with placebo in patients with metastatic CRC post standard therapies. It demonstrated an OS benefit (HR, 0.55).[18] Notably, patients in this study who had less exposure to prior biologic therapeutics had a differential response to regorafenib. In a subgroup analysis, patients who had not or had received prior targeted therapy (anti-VEGF or anti-EGFR) were noted to fare differently on regorafenib versus placebo, with median OS times of 9.7 versus 7.4 months (HR, 0.31 vs 0.78), respectively (**Table**).

Trifluridine is an old cytotoxic agent, first synthesized in 1964, that now has new life as part of a novel drug, trifluridine-tipiracil (TAS-102), for the management of metastatic CRC. Trifluridine is the active agent, acting as a nucleoside analog. After intracellular triphosphorylation, it is incorporated into DNA, causing DNA strand-breaks and inhibition of tumor cell growth. The tipiracil component inhibits thymidine phosphorylase in the liver, which would normally immediately metabolize trifluridine; it thus enables adequate and sustained serum levels of trifluridine.

The global, multicenter phase III study RECOURSE enrolled 800 patients whose disease had progressed on at least 2 prior regimens. The study compared results of treatment with TAS-102 with placebo. Median OS was significantly prolonged for patients on TAS-102 compared with placebo, at 7.1 months versus 5.3 months, respectively (HR, 0.68, *P* <.0001). Median PFS was also improved at 2 versus 1.7 months, respectively (HR, 0.48, *P* <.0001).[13] The results of a subgroup analyses from the RECOURSE study showed similar OS benefit regardless of whether patients had or had not previously been treated with regorafenib (HR, 0.69 vs 0.69, respectively).[13] However, the researchers found that the subgroups were unbalanced, because more than 4 times as many patients had not received prior regorafenib than those who had. Patients who had received more prior therapy seemed to have benefited more from

TAS-102 (≤4 prior regimens: HR, 0.59; 3 prior regimens: HR, 0.74; 2 prior regimens, HR, 1.05).

The TERRA study of 316 Asian patients also demonstrated a benefit for TAS-102 versus placebo with a median OS of 7.8 months and 7.1 months, respectively (HR, 0.79; *P* = .035).[19] Median PFS was similarly prolonged at 2 versus 1.8 months, respectively (HR, 0.43; *P* <.001).[19] Notably, only about 20% of patients in this study had received prior anti-VEGF therapy and 18% prior anti-EGFR therapy. Thus, it is inferred that TAS-102 is not differentially effective with earlier use in the treatment sequence.

With knowledge gained from these large, prospective studies across a wide spectrum of ethnicities, how might we inform our decision-making process when it comes to sequencing these newer agents? We know that patients derive benefit from access to all agents with activity in metastatic CRC, including regorafenib and TAS-102. In the CORRECT study, patients derived similar benefit regardless of the number of prior lines of therapy they had received and regardless of the time from diagnosis of metastatic disease. However, slightly more benefit may have been seen in those having received prior anti-EGFR therapies.

In the CONCUR study, we learned that regorafenib appears to provide more benefit in patients who have not received any prior targeted therapies. Though this may be the case, now in the age of molecular subtypes of metastatic CRC (ie, the all-*RAS* wild-type versus *RAS* mutated subtypes), targeted therapies do have their place given their demonstrated benefit in earlier lines of therapy. We learned in the RECOURSE and TERRA studies that TAS-102 does not appear to confer significant benefit in less pretreated patients, with patients receiving ≤4 prior regimens faring better than those receiving 2 prior regimens, and to some degree also those having received 3 prior regimens. We also have noted that TAS-102 activity does not seem to be affected by prior exposure to regorafenib.

Without clear, significant data on particular situations in which 1 drug (regorafenib or TAS-102) produces better outcomes than another, there is no definitive instruction on how to sequence these newer agents. The data above may suggest that regorafenib should be considered first in sequence, although this observation is limited by a noticeable absence of direct comparative studies. In deciding when to employ each of the particular therapies, it seems most appropriate to consider clinical patient-specific information, such as performance status, liver function, bone marrow function, and reserve, and

TABLE. Regorafenib and TAS-102 Studies

	Regorafenib				TAS-102			
Study name	CORRECT		CONCUR		RECOURSE		TERRA	
Prior biologics	100% BEV 100% EGFR mAbs		60%		100% BEV 100% EGFR mAbs		20% BEV 18% EGFR mAbs	
	Rego	BSC	Rego	BSC	TAS-102	BSC	TAS-102	BSC
N (patients)	505	255	136	68	534	266	271	135
mOS (months)	6.4	5.0	8.8	6.3	7.1	5.3	7.8	7.1
	HR 0.77 *P* = .0052		HR 0.55 *P* <.0006		HR 0.68 *P* <.0001		HR = 0.79 *P* = .035	
mPFS (months)	1.9	1.7	3.2	1.7	2.0	1.7	2.0	1.8
	HR 0.49 *P* <.0001		HR 0.31 *P* <.0001		HR 0.68 *P* <.0001		HR 0.43 *P* <.0001	
RR (%)	1.0	0.4	4.4	0	1.6	0.4	1.1	0
Outcomes & prior therapies	Rego: prior anti-EGFR	Rego: no prior anti-EGFR	Rego: no prior targeted tx	Rego: any prior targeted tx	TAS: prior Rego	TAS: no prior Rego	n/a	n/a
	HR 0.71	HR 0.825	HR 0.31	HR 0.78	HR 0.69	HR 0.69		
Outcomes & prior therapies	≤3	>3	n/a	n/a	3	≥4	n/a	n/a
	HR 0.788	HR 0.747			HR 0.74	HR 0.59		
Main AEs	HFSR, fatigue				Neutropenia, GI toxicities			

AEs indicates adverse events; BEV, bevacizumab; BSC, best supportive care; EGFR, epidermal growth factor receptor; GI, gastrointestinal; HFSR, hand-foot skin reaction; HR, hazard ratio; mAbs, monoclonal antibodies; mOS, median overall survival; mPFS, median progression-free survival; rego, regorafenib; RR, response rate; TAS-102, trifluridine and tipiracil; tx, therapy.

adverse events from prior therapies/drug classes, etc.

It is unlikely that situational, comparative randomized studies of regorafenib and TAS-102 will be pursued, especially now, since enthusiastic progress is being made in identifying the qualities of a growing number of CRC subtypes. For example, in *HER2*-amplified CRC, some success has been demonstrated in studies such as HERACLES and MyPathway.[20,21] Additionally, immunotherapy strategies for both the mismatch repair-deficient and -proficient subpopulations continue to develop, with therapies in various stages of development being investigated in both treatment-naïve and pretreated patient populations, in addition to the adjuvant setting. Pembrolizumab now has breakthrough status with the FDA, based on data from the microsatellite-instability–high CRC patient population.[22] These strategies include immunotherapy combinations, for example, with agents targeting the indoleamine 2,3-dioxygenase pathway, hypomethylating agents, histone deacetylase inhibitors, and vaccine-based therapies such as GVAX. Also, of course, strategies for managing *RAS*- and *RAF*-mutated disease continue to evolve. During this time of continuing advances, even more questions regarding appropriate sequencing of therapies for our patients with CRC will arise and need to be considered carefully.

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Optimizing Sequencing in Patients with NSCLC and Actionable Mutations

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Overview
This activity is designed to inform physicians about optimizing sequencing in patients with non–small cell lung cancer (NSCLC) and actionable mutations.

Target Audience
This activity is directed towards medical oncologists, primary care physicians, nurses, and nurse practitioners who treat and/or manage patients with NSCLC. Surgical oncologists, radiation oncologists, pathologists, internists, fellows, physician assistants, and other healthcare providers are also invited to participate.

Learning Objectives
After participating in this CME/CE activity, learners should be better prepared to:
- Explain the key unmet needs in the treatment of advanced NSCLC for patients who have actionable mutations
- Describe the advantages and disadvantages of different types of molecular testing to identify patients with actionable mutations
- Discuss the most common genomic alterations that have been identified in NSCLC

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Introduction

Background

Lung cancer is the leading cause of cancer-related mortality in the United States.[1] In 2017 there will be an estimated 222,500 new cases of lung cancer (non–small cell lung cancer [NSCLC] and small cell lung cancer combined) and 155,870 related deaths.[1]

The initial treatment of NSCLC usually relies on surgical resection followed by systemic cytotoxic chemotherapy and/or radiation therapy. Advances in understanding NSCLC pathophysiology and immunology have led to the development of numerous targeted therapeutic approaches, improving patient outcomes.[2] Several targeted therapies are approved by the US FDA for use in various settings in NSCLC.

Epidermal growth factor receptor (EGFR)–targeted therapies affect activated tyrosine kinase receptors. FDA-approved drugs with an NSCLC indication include erlotinib, afatanib, gefitinib, necitumumab, and osimertinib. Erlotinib, afatanib, and gefitinib are small molecule tyrosine kinase inhibitors (TKIs) approved for treatment of *EGFR*-mutant lung tumors in the first-line setting. Osimertinib is indicated for those patients who have progressed on EGFR TKI therapy when the tumor has acquired resistance due to the T790M mutation. Necitumumab, an anti-EGFR monoclonal antibody, is used in combination with gemcitabine and cisplatin for first-line treatment of patients with metastatic squamous cell NSCLC. Anaplastic lymphoma kinase (ALK), a tyrosine kinase, is activated by translocation in approximately 5% of patients with NSCLC.[2] There are 3 FDA-approved ALK-targeted TKIs: crizotinib, ceritinib, and alectinib. Crizotinib is a first-generation ALK inhibitor. Ceritinib is indicated for patients with *ALK*-positive metastatic NSCLC who have progressed on or are intolerant to crizotinib. Alectinib is a second-generation inhibitor used in the crizotinib-resistant population. Targeted therapy is a promising approach for patients with lung cancer.

Molecular Testing

The testing of patients with molecular technology has become increasingly more important to the treatment of patients with NSCLC, in part due to the recognized effectiveness of targeted therapies. The importance of molecular analysis continues to be highlighted by the large National Cancer Institute Molecular Analysis for Therapy Choice (NCI-MATCH) trial, which plans on screening up to 6000 patients with various tumor types to examine for gene abnormalities for which a targeted therapy exists. This trial opened enrollment in August 2015, and will have 30 treatment arms. In an interim analysis of 645 patients screened for the NCI-MATCH trial, 48 patients with NSCLC (7.4%) were screened. As part of this analysis, 33 patients with various cancer types have been assigned therapy, of which 5 (15.2%) patients with NSCLC were assigned.[3] The primary endpoint for the NCI-MATCH trial is the objective response rate (ORR). This study highlights the importance of incorporating molecular testing into the determination of treatment approach.

Molecular testing should be ordered at the time of diagnosis for patients with advanced-stage NSCLC[4]; testing in patients with stage I-III NSCLC is controversial. When surgery or surgery followed by adjuvant chemotherapy is the initial plan for treatment, molecular testing for targeted therapy is not clearly indicated.[5] Testing early-stage NSCLC may identify targeted therapy, which could be useful for patients who experience recurrent NSCLC. It has been recommended to prioritize *EGFR, ALK,* and *ROS1* testing over other molecular predictive tests, due to the relative frequency and availability of effective therapies.[4] Molecular testing for *EGFR*, *ALK*, and *ROS1* is recommended to select patients for targeted therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.[4] Testing can also be performed to evaluate for genomic alterations such as *KRAS, BRAF, MET, RET,* neurotrophic tyrosine receptor kinase (*NTRK*), and human epidermal growth factor receptor 2 (*HER2*).

The primary tumor or metastatic lesions are suitable for molecular testing. However, in 2016, the FDA approved the first liquid biopsy test. Liquid biopsy makes it possible to determine a patient's suitability for EGFR-targeted therapy by analyzing circulating-free tumor DNA in peripheral blood samples. A liquid biopsy is minimally invasive, easily repeatable, and can be used for single-gene molecular testing. Both methods of testing have been shown to be effective, with a high rate of similarity.[6] Liquid biopsy has a high concordance of 88% to 90% with results from standard tests in the V600 mutation of *BRAF*.[7]

The cost of universal molecular testing of NSCLC is substantial, and it has been suggested that universal *EGFR* and *ALK* testing is not needed at the time of initial diagnosis.[8] At one facility, the estimated additional cost of *EGFR* and *ALK* testing for all newly diagnosed patients with NSCLC was $75,200 per year. The suggestion by these authors is to focus testing only on patients with locally advanced and advanced-stage disease.[8] In a retrospective analysis, it has been demonstrated that blood-based testing is significantly less costly than tissue-based biopsy methods, with a potential savings of $3000 to $7400 per patient with liquid biopsy compared with tissue-based biopsy.[9]

Actionable Mutations in NSCLC

Tumor molecular subtyping is paramount for advanced-stage NSCLC therapy guidance. Different types of genomic alterations have been identified involving multiple kinase genes, such as *EGFR, KRAS, ALK, ROS1, BRAF, MET, RET, NTRK,* and *HER2*.[10] These genomic alterations represent specific molecular subtypes of pulmonary adenocarcinomas, each with its own distinct biology, epidemiology, prognosis, and therapeutic susceptibility.

EGFR

The most common *EGFR*-activating mutations are the exon 19 deletion and exon 21 point L858R mutation, accounting for 85% to 90% of *EGFR* clinical mutations.[11] The effectiveness of EGFR TKIs in patients with *EGFR* variations has been demonstrated by several FDA-approved therapies (ie, erlotinib, afatanib, gefitinib, and osimertinib). The Iressa Pan-Asia Study (IPASS) trial examined gefitinib compared with platinum-based chemotherapy in patients with advanced pulmonary

adenocarcinoma. Progression-free survival (PFS) was significantly longer with gefitinib for patients whose tumors had both high *EGFR* gene copy number and *EGFR* mutation (hazard ratio [HR], 0.48; 95% confidence interval [CI], 0.34 to 0.67).[12] The IPASS trial supports recommendations that patients with advanced NSCLC who might be candidates for first-line EGFR TKIs erlotinib, afatinib, or gefitinib be tested for *EGFR* mutation status, and treated if positive.

A head-to-head comparison of a first-generation EGFR TKI and a second-generation EGFR TKI has been evaluated. The LUX-Lung 7 trial compared gefitinib with afatinib and found that afatinib significantly increased response rate (RR; 70% vs 56%, *P* = .0083), median PFS (11 vs 10.9 months; HR, 0.73; 95% CI, 0.57-0.95; *P* = .017), and median time to treatment failure (13.7 months vs 11.5 months; HR, 0.73; 95% CI, 0.58-0.92; *P* = .0073) over gefinitib.[13] Although the results of this trial might suggest that second-generation EGFR TKIs are more favorable compared with first-generation EGFR TKIs, there was no difference in overall survival (OS) between the 2 EGFR TKIs. Further studies are required to determine clinical outcomes of first-generation EGFR TKIs versus second-generation EGFR TKIs.

Treatment with EGFR TKIs improves outcomes for patients whose tumors harbor these *EGFR* mutations, but their efficacy is limited by the development of acquired resistance. The acquisition of a secondary mutation in exon 20 (T790M) is the most common *EGFR*-dependent acquired resistance mechanism. The T790M mutation is observed in up to 50% to 60% of resistant patients.[14] A third-generation EGFR TKI, osimertinib, is an *EGFR*-mutant–selective inhibitor with activity against the T790M mutant kinase and sensitizing *EGFR* mutations. Other novel third-generation TKIs are in early phases of development, including HM61713, ASP8273, EGF816, AZD3759, and HMPL-813.[15]

ALK

Chromosomal *ALK* rearrangements are found in approximately 3% to 7% of NSCLCs.[15] Crizotinib, a first-generation ALK inhibitor, targets ALK, ROS1, and MET tyrosine kinases, and is indicated for locally advanced or metastatic NSCLC that is *ALK*-positive as detected by an FDA-approved test. More recently, it has been indicated for metastatic NSCLC that is *ROS1*-rearrangement positive.[16]

The acquisition of a secondary *ALK* mutation is common with patients who develop ALK TKI resistance.[14] There have been many identified secondary *ALK* mutations, including but not limited to L1152R, L1196M, C1156Y, and F1174L.[14,17] Two drugs are FDA approved as second-generation ALK TKIs to overcome crizotinib resistance: ceritinib and alectinib. Ceritinib has demonstrated a significant improvement over chemotherapy in patients previously treated with crizotinib, with a reported RR of 39.1% compared with 6.9% with chemotherapy (ASCEND-5 trial). PFS was 5.4 months compared with 1.6 months with chemotherapy (HR, 0.49; *P* <.001). The most frequent grade 3/4 adverse eventts (AEs) with ceritinib were nausea (7.8%), vomiting (7.8%), and diarrhea (4.3%); with chemotherapy, they were neutropenia (15.5%), fatigue (4.4%), and nausea (1.8%).[18] However, treatment-related AEs were more frequent in the ceritinib arm than in the chemotherapy arm.

There are investigational drugs for those patients who acquire resistance to second-generation ALK inhibitors. It was recently shown that the use of lorlatinib may overcome resistance to ALK inhibitors, which remains a significant challenge for patients with *ALK*-positive NSCLC. In a dose-escalation phase I study of patients with *ALK*-positive or *ROS1*-positive NSCLC who were treatment-naïve or had disease progression after at least 1 prior TKI, the ORR and PFS with lorlatinib were 46% and 11.4 months, respectively, in patients treated with 1 prior TKI.[19] The most common treatment-related AEs were hypercholesterolemia (69%) and peripheral edema (37%).[19] Most patients had received 2 or more prior ALK TKIs. In these patients, the RR was 42% and PFS was 9.2 months.[15] The phase II ALTA trial (NCT02094573) of brigatinib in patients with crizotinib-refractory *ALK*-positive NSCLC reported interim analysis indicating 46% and 54% ORRs, respectively, in 2 groups: The first continuously took 90 mg of brigatinib per day in a 28-day cycle; the second took 90 mg of brigatinib per day for 7 days followed by 180 mg of brigatinib per day for the 28-day cycle. Reported AEs included increased elevated creatinine phosphokinase, hypertension, rash, pneumonia, and increased lipase.[20] Ensartinib has demonstrated clinical activity in the same crizotinib-refractory *ALK*-positive NSCLC patient population, with the most common AEs being rash (47%), nausea (28%), vomiting (25%), and fatigue (23%).[21] Lastly, an arm in the open-label, multicenter, global phase 2 basket study (STARTRK-2) is for patients with *ALK*- or *ROS1*-rearranged NSCLC previously treated with crizotinib. The STARTRK-2 trial is examining entrectinib in this patient population (NCT02568267).

The next generation of investigational ALK inhibitors in patients resistant to ALK TKIs are not ALK-selective inhibitors, and instead target other kinases such as ROS1 and MET.

ROS1

ROS1 gene rearrangements are found in 1% to 2% of NSCLCs.[15] ROS1 rearrangements in lung cancer share common carcinogenic properties to *ALK* rearrangements in terms of clinical characteristics, therapeutic susceptibilities, and acquired resistance mechanisms. Clinical development of next-generation dual ALK and ROS1 inhibitors (lorlatinib, ceritinib, brigatinib, and entrectinib) and other ROS1 inhibitors (cabozantinib and foretinib) is currently ongoing.[22] As previously mentioned, lorlatinib is being examined in *ROS1*-positive NSCLC and has demonstrated the ability to overcome crizotinib resistance. *ROS1*-positive NSCLC patients (n = 12) achieved ORRs of 33% and 66% in the crizotinib-pretreated and crizotinib-naïve subsets, respectively.[23]

KRAS

There are no FDA-approved therapies for *KRAS*-mutated tumors, and this represents an area of required research and development. *KRAS* has been referred to as clinically difficult to inhibit; therefore, strategies have focused on inhibition of downstream therapeutic approaches. The use of mitogen-activated extracellular signal-regulated kinase (MEK) inhibitors has shown some promise in *KRAS*-positive NSCLC. A randomized, open-label phase II study in patients with advanced NSCLC, refractory to more than 1 prior therapy, and *KRAS*-positive examined 2 therapeutic

agents, MK-2206 and AZD6244. These agents demonstrated promise against *KRAS*-positive cancers. The disease control rate in *KRAS*-positive patients was 25% in those who took MK-2206, and 62% in those who took MK-2206 and AZD6244. The most common grade 3/4 AE seen in the combined MK-2206 plus AZD6244 arm was maculopapular rash.[24]

MET, BRAF, RET, HER2 and NTRK

There are several additional evolving targets in NSCLC including *MET, BRAF, RET, HER2,* and *NTRK*. *MET* oncogene dysregulation is found in approximately 10% of the NSCLC cases in which patients have acquired resistance.[15] Many *MET*-mutated cancers resulting in exon 14 skipping have been described; exon 14 skipping results in enhanced MET signaling.[25] Patients whose NSCLC harbors the exon 14 alteration can achieve clinical benefit from MET inhibitors. Several drug agents have been shown to have activity in patients with high *MET* expression or *MET* mutations, including crizotinib, cabozantinib, and capmatinib.[25-27] RET-targeting TKIs are being used clinically, including vandetanib, cabozantinib, lenvatinib, sunitinib, sorafenib, and alectinib.[15] The response rates to some of these drug agents have been reported to be 16% to 53% in previously treated patients with *RET* rearrangements in NSCLC.[28-30] *HER2* mutations have been identified as oncogenic drivers in lung cancers and are found in 1% to 2% of lung adenocarcinomas.[15] *HER2* mutations in NSCLC demonstrated some responsiveness to trastuzumab and chemotherapy in European cohorts, as well as to such monotherapies as afatinib, dacomitinib, and neratinib.[31,32] BRAF inhibition has shown antitumor activity in patients with *BRAF* V600E-mutant NSCLC. Recently, antitumor activity and safety of dabrafenib plus trametinib in patients with *BRAF* V600E-mutant NSCLC has been demonstrated (NCT01336634). An overall response was observed in 63.2% of patients, with the most common grade 3/4 AEs being neutropenia (9%), hyponatremia (7%), and anemia (5%).[33] Lastly, the frequency of *NTRK* mutations in lung adenocarcinomas is approximately 3.3%.[34] Entrectinib and larotrectinib are pan-tropomyosin receptor kinase inhibitors that are currently under investigation in phase I/II trials.[35,36]

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Moderator: What are some of the unmet needs in the treatment of advanced NSCLC for patients who have actionable mutations?

Dr Reckamp: There are 3 unmet needs that I would describe. First is resistance to targeted therapy. The actionable mutations or gene alterations that occur once a targeted therapy is given can invariably lead to resistance. This leads to progression, and most patients with advanced NSCLC still die of the disease. Compared with other cancer types, for lung cancer, our understanding of mechanisms of resistance for the most common markers of EGFR and ALK is growing. Our researchers are looking at resistance mechanisms and how to overcome and potentially prevent them. However, there remains a need for further research and advances can affect patient outcomes in a major way.

The second unmet need is understanding rare mutations or gene alterations, and how best to study targeted drugs in these patients. Targeted drug treatment options are needed for those patients who will benefit from these approaches. Examples of uncommon gene alterations would be *RET* and *MET*. Although drugs against the targets can be beneficial, drug development is challenging, from requiring large comparative clinical trials to obtaining FDA approval. We need to be forward-thinking about novel trial designs to evaluate these rare mutations. There are many basket trials out there that look at specific mutations in a tumor-agnostic manner, which help us to move the field forward. Trials like the NCI-MATCH trial look at mutations in rarer indications, with the purpose of examining response rates and meaningful efficacy endpoints to understand the benefit of these drugs for patients.

Third, evaluating investigational drugs in the neoadjuvant and adjuvant setting is important. These drugs could potentially lead to a cure when a patient has minimal residual or micrometastatic disease. Currently, we do not have data showing that these drugs prolong survival in the neoadjuvant and adjuvant setting; the primary use of such drugs are in patients with metastatic disease. There are ongoing trials looking at targeted agents, especially for ALK and EGFR, as adjuvant therapy, and those answers hopefully will come in the next decade.

Moderator: What do you feel could be done to further improve facilitation of molecular testing in community settings where patients with advanced NSCLC are managed?

Dr Reckamp: Numerous issues surround this important topic. I may miss some of the challenges a community practitioner encounters, as I do not practice in a community setting. It is likely that the single largest challenge is insurance coverage. Molecular testing is still an expensive endeavor. Molecular testing can arguably change a treatment approach, and potentially quality of life and overall survival for those patients who have alterations. These patients could be given targeted therapy. However, upfront testing when there is low probability of having a gene alteration remains a challenge that is faced, especially in community settings.

The lung cancer community is good about testing for *EGFR, ALK,* and *ROS1*, and now PD-L1, which is not a genetic alteration but also a marker that helps determine therapy. Most practices test for these mutations or markers. With the ability to test blood and tissue, we can do single-gene testing by PCR [polymerase chain reaction] and FISH [fluorescence in situ hybridization]. Next-generation sequencing provides more information on a smaller amount of material. These have differential costs based on insurance and location. The biggest challenge is understanding what the cost to the patient might be; this is often not known. This is further complicated by the fact that the best

test or platform to perform has not been compared or validated. For example, a person who is of Asian descent and a nonsmoker has a high pretest probability to have an *EGFR* mutation. In this scenario, doing single-gene testing may be best option for this patient and the most cost effective. However, if the *EGFR* test is negative for this patient case, then you would have to use more tissue in order to get answers about other genetic alterations. In our practice, we favor moving forward with next-generation sequencing (NGS). This provides the most information with the least amount of tissue, and eliminates the need to go back and do additional tests. In the community, NGS testing may not be standard and challenges remain, most of which are financial.

Blood testing provides multiple choices for patients and providers. These tests have different sensitivities, specificities, and prices. It is an enormous challenge to remain aware of all the various tests and determining which one will be best for your patient. None have been compared directly.

Moderator: What mutational subtypes inform therapeutic sequencing among patients with *EGFR*-mutated NSCLC? What if T790M is found prior to starting frontline therapy?

Dr Reckamp: Generally, when performing *EGFR* sequencing, about 90% are going to have the most common exon 19 deletions or exon 21, L858R. There are rarer mutations, some of which have a tendency toward resistance, some with sensitivity to approved EGFR TKIs, and for some mutations there is very little information available. Usually the exon 20 insertions tend to be resistant and some of the exon 18 mutations tend to be sensitive. It all depends on the mutation. We use fewer of the EGFR inhibitors in those patients who have exon 20 mutations. This is different than having a T790 mutation de novo, which is very rare but does occur, and is also an exon 20, but responds to osimertinib. The other exon 20 insertions are different.

Moderator: What are the advantages and disadvantages of a liquid biopsy in order to identify patients with metastatic NSCLC who are eligible for EGFR-targeted therapy?

Dr Reckamp: Liquid biopsy for *EGFR* mutations is FDA approved. An obvious advantage is that liquid biopsy does not require a tissue biopsy, which reduces cost and potential complications. It requires just a blood draw, and that is much easier for a patient and decreases potential risk. This is a clear advantage for patients. The cost of the biopsy is also less.

The main disadvantage is the false negative rate. In order to detect a mutation within the blood, there needs to be circulating cell-free DNA at sufficient levels to be able to detect the mutation. Even if the cancer is present, if there is not enough circulating cell-free DNA, then a liquid biopsy may provide a negative result, but it may be a false negative result. If the result is positive, it is likely to be a true positive and is a very good test for the patient to guide treatment choice.

Moderator: What strategies may soon enter the field of *ALK*-positive NSCLC management and how might that impact sequencing decisions for these patients? How may some of these strategies address the important problem of central nervous system metastases in advanced *ALK*-positive NSCLC?

Dr Reckamp: The strategies entering the field of *ALK*-positive have to do with 2 issues, the first being brain metastases. In general, crizotinib is less effective and less potent in the brain for patients with *ALK*-positive NSCLC. Therefore, patients who have initial brain metastases may respond more favorably with upfront therapy of ALK inhibitors that are more potent in the brain. Both of the approved second-generation inhibitors, ceritinib and alectinib, have excellent penetrance and responses in the brain. This may be reason to use these drugs in the first-line setting. There are data for ceritinib in first-line therapy versus chemotherapy that demonstrate a clinical benefit. However, alectinib has similar benefit in the brain. A provider will have to decide whether to use alectinib or ceritinib as a first-line therapy.

The second issue deals with *ALK*-positive NSCLC management in cases of resistance. Regarding other strategies for sequencing, our lung cancer community is moving toward a better understanding of the mutations that occur upon resistance. As more patients receive these second-generation inhibitors, we will understand more about these mechanisms of resistance, and if there are true patterns of some drugs being able to overcome resistant mutations better than others. There are several ALK inhibitors in development with differential response to various resistance mutations that occur in *ALK*-positive NSCLC. Although there may be a way to understand sequencing of ALK inhibitor therapy, we are not there yet for our patients.

Moderator: *ROS1* has recently been identified as an actionable marker. How can that marker be used to inform sequencing in advanced NSCLC?

Dr Reckamp: There are data indicating that the FDA-approved crizotinib is efficacious for *ROS1* gene alterations in NSCLC. We have new data on ceritinib that were presented in 2016 showing responses in patients with *ROS1* gene rearrangements. The evidence appears to indicate that ceritinib is not effective when patients develop resistance to crizotinib. These are both frontline therapies for patients with *ROS1* gene rearrangements. In my experience, crizotinib is an effective ROS1 inhibitor, and patients have prolonged progression-free survival.

Considering that patients with a *ROS1* gene alteration comprise a small subset of patients with lung cancer, it is going to be hard to develop a full understanding of how best to sequence these drugs. The fact that *ROS1* is a rare gene alteration, and patients do so well on crizotinib, will make it difficult to determine the best sequencing in advanced NSCLC.

Moderator: What role do MEK1/2 inhibitors have in the treatment of NSCLC?

Dr Reckamp: At this point, MEK inhibitors are still investigational, and it has not been determined how these drugs best fit into the treatment paradigm for *KRAS*-mutated NSCLC. There's still interest in drug combinations with MEK inhibitors. The use in *KRAS* patients remains a possibility. There is evidence of prolonged PFS and increased RR in

BRAF-positive patients when MEK inhibitors are used in combination with BRAF inhibitors. Currently, however, these drugs are still investigational, and further studies are required.

Moderator: How do you see the treatment of advanced NSCLC potentially evolving with regard to emerging actionable markers such as *HER2, KRAS, RET,* and *MET?*

Dr Reckamp: As previously mentioned, the less common actionable mutations are definitely an area of unmet need. It is challenging to develop drugs that target an actionable mutation, and enroll enough patients to provide efficacy data that would support an FDA approval. When considering emerging actionable markers, one has to examine them separately. The first question to consider about emerging actionable markers is if they are true actionable mutations or not. In the case of *HER2*, some may be actionable mutations and some may not. Response rates to HER2 TKIs in lung cancer and pan-HER TKIs in lung cancer are less than 20%. Usually when we have a true actionable oncogenic driver and give a targeted therapy, we get response rates at minimum of 50% into the 60% to 70% range. Therefore, *HER2* does not seem to be a straightforward actionable marker at this time. There is some heterogeneity in *HER2* mutations and *HER2* amplification that needs to be understood to help response rates get closer to 50%.

KRAS is another marker that has a lot of heterogeneity. Therefore, no single drug seems to cause large effects in *KRAS*. There are many trials and combination trials looking at various targeted therapies for KRAS, and we're still working to improve outcomes for those patients. Regarding *RET* as a marker, RET inhibitors have been multitargeted TKIs. More specific RET inhibitors may improve responses over the multitargeted RET inhibitors. This seems to be a true oncogenic target, and, again, there may be some heterogeneity in understanding the partner in the translocations that occur with *RET*. We are getting closer to understanding *RET* and moving forward with beneficial treatments for these patients.

MET is another marker that has been more recently studied, and there are multiple ways that we look at *MET*, from amplification, to overexpression, to mutations. Understanding which alteration responds best to the MET inhibitors in the therapeutic armamentarium is important. There are many trials ongoing that are looking at these drugs and various markers for *MET*. This is something that will evolve and provide information that will better help us treat our patients.

And then there are other less common alterations such as *NTRK*. *NTRK* is a marker for which new therapies are being developed. Some of the ALK and ROS1 inhibitors have some activity in these patients as well. Understanding how efficacious these targeted therapies are against these genes, understanding the heterogeneity within the biomarker itself, and understanding whether it is a true oncogenic driver versus a passenger effect, are all important to our field and to improving patient outcomes.

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