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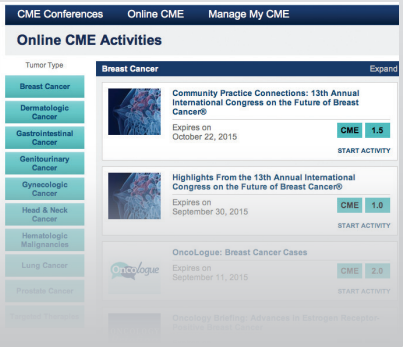

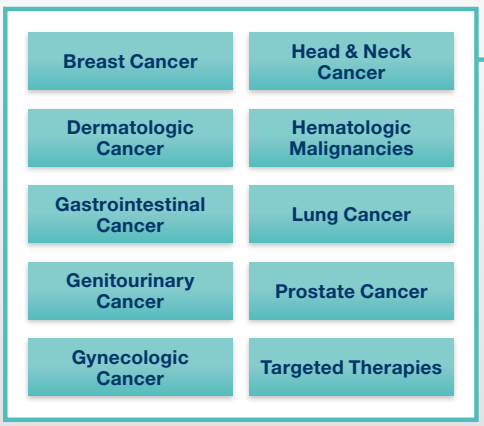

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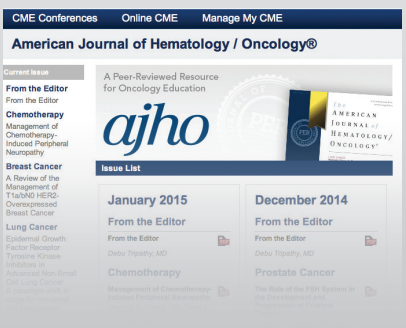

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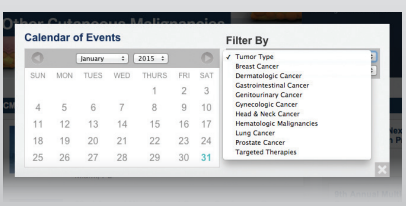

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From the Editor

The March issue of *AJHO* has a biological bent—which of course is an understandable trend as new drugs entering into clinical practice are now more commonly biological agents. Moreover, the movement of effective biological agents from advanced to adjuvant settings often (but not always) has the potential to save more lives. In this vein, Drs Asmar and Halmos provide a perspective on the use of targeted agents for early-stage lung cancer. Earlier trials were not using genomic assays to select patients, so the results of ongoing trials—in particular, multifaceted trials such as the ALCHEMIST suite of trials—could change our approaches significantly.

Adjuvant therapy for colon cancer still consists of cytotoxic agents, despite the effectiveness of angiogenesis and EGFR-targeting drugs in the advanced setting. However, a significant proportion of patients are elderly, and Drs Williams and Sanoff provide us with tools for decision support to factor age and other associated factors that predict toxicities and recurrence risk reduction, as well as alternative regimens to optimize benefit/risk tradeoffs in this area.

Our hematological feature also illustrates the biological revolution as multiple myeloma is now approached with both cytotoxic and biological drugs that are deployed in specific combinations based on patient characteristics and treatment goals. Dr Chari provides a very useful summary of the current state of the art and a preview of a new generation of targeted drugs under investigation.

Our pathway-based biology and targeted therapy review by Dr Ma focuses on PI3 kinase and downstream mTOR inhibitors in breast cancer. Recent results of PI3K inhibition are not producing the same results seen with approved mTOR inhibitor therapy with everolimus, yet we cannot clearly explain this with our current biological understanding of these pathways. The number of genomic-guided therapies is rapidly growing, both in the clinical practice setting after FDA approval and in the clinical research setting, where treatment decisions must be made rapidly.

Is next-generation sequencing that encompasses hundreds of genes the answer? Certainly, as the cost drops and accuracy improves, we are very likely moving to high-throughput, broad-scale analytic platforms that will require oncologists to become fluent with these technologies. Drs Basho, Eterovic, and Meric-Bernstam provide a timely primer on the methods, applications, future potential, and current limitations of next-generation sequencing.

Our CME article reviews updates from the ASCO GI symposium that revolve around one of the more successfully targeted pathways—angiogenesis. Following the initial demonstration of bevacizumab of improving survival in advanced colorectal cancer, two newer anti-angiogenic drugs have been approved beyond second line therapy with several others under investigation and showing various degrees of promise. Chosen studies are highlighted and in this piece and provide a snapshot of the future landscape in this area.

Debu Tripathy, MD
Editor-in-Chief

Adjuvant Chemotherapy in Older Adults With Colon Cancer

Grant R. Williams, MD, and Hanna K. Sanoff, MD, MPH

Abstract

Colorectal cancer (CRC) is predominately a disease of older adults, with the median age of diagnosis 68 years; 59% of all cases are diagnosed in persons age 65 years or older. Although older patients have been underrepresented in clinical trials, pooled analysis suggests similar treatment benefits with fluorouracil and leucovorin (5FU/LV) for older patients compared with younger patients, while the evidence of treatment benefit from oxaliplatin-containing regimens is less compelling. Many helpful tools that provide estimates of prognosis, risk of recurrence, risk of severe toxicity with chemotherapy, and functional age can aid clinical decisions. In this review, we present the evidence for adjuvant chemotherapy in older adults, discuss helpful tools that can aid in adjuvant decision making, and present a suggested treatment approach to adjuvant chemotherapy in older adults with CRC.

Key words: Colon cancer, adjuvant chemotherapy, elderly, geriatric oncology, treatment-related decision making

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States, with an estimated 136,000 new cases and over 50,000 deaths in 2014 alone.[1] As with most cancers, CRC is predominately a disease of older adults. The median age of a CRC diagnosis is 68 years, and 59% of all cases of CRC are diagnosed in persons over the age of 65 years, with 35% older than 75 years.[2] As the US population continues to age, the absolute number of cancer cases and the proportion of cancers occurring in the elderly (≥65 years) will both increase.[3] Thus, providing optimal care for older adults with CRC is a pressing issue.

A hallmark of aging is the gradual loss of physiologic reserve, with a resultant reduced ability to compensate when exposed to stressors such as infection, cancer, and chemotherapy.[4,5] This loss of physiologic reserve is accompanied by a gradual decline in normal organ function, such as reduced cardiac motility, glomerular filtration rate, and hepatic volume.[6-8] At the same time, older adults also have a shift in priorities and social support networks. Older adults are often less willing to initiate or continue treatments with severe adverse effects (AEs), and value their current time feeling well more than they value the potential for increased longevity.[9,10] Thus, even when the likelihood of benefit may be the same, the AEs of adjuvant chemotherapy may be less appealing to older patients. Adjuvant treatment decisions require a careful balance of changes in physiology and priorities, with decisions being thoughtfully individualized.

Since a 1990 National Institutes of Health consensus conference, adjuvant 5-fluoropyrimidine-based chemotherapy for node-positive CRC has become the standard of care in the United States.[11] However, despite these recommendations, increasing age is significantly associated with lower likelihood of receiving any adjuvant chemotherapy.[12] The use of any chemotherapy drops off quickly with advancing age, with only 63% of those aged 75 to 79 years, 43% of those aged 80 to 84 years, and 14% of patients 85 years and older receiving adjuvant therapy.[13] Whether this trend represents thoughtful and appropriate treatment on the part of patients and physicians based on comorbid disease and limited life expectancy or an inappropriate reflection of ageism is unclear.

Evidence for Adjuvant Therapy in Older Adults

In light of the aforementioned age-related changes, it is very reasonable for patients and physicians to question whether adjuvant therapy is equally safe and effective in the elderly. However, because only a small minority of clinical trial participants are over age 65 years, and even fewer over age 70, subset analyses by age within individual trials are underpowered.[14] To overcome this challenge, multiple pooled analyses and meta-analyses have been performed. In 2001, a pooled analysis of 7 trials that randomly assigned participants to fluorouracil (5FU) with leucovorin (LV) versus observation in the adjuvant setting showed a similar beneficial treatment effect in older and younger patients.[15] 5FU/LV appears to be well tolerated in both the adjuvant and metastatic

TABLE 1. Useful Resources for Management of Older Patients With Colorectal Cancer

	Site	Web Address
Geriatric Assessment	International Society of Geriatric Oncology	www.siog.org
	Portal of Geriatrics Online Education (POGOe)	www.pogoe.org
	ASCO University	www.university.asco.org/search/site/geriatrics
	UNC Lineberger Geriatric Oncology Program	http://unclineberger.org/patientcare/programs/geriatric/educational-materials-tools
Estimating Survival	ePrognosis	www.eprognosis.org
Estimating Benefit	Adjuvant! Online	www.adjuvantonline.org
	Mayo Clinic Stage III Colon Cancer Calculator	http://www.mayoclinic.org/medical-professionals/adjuvant-systemic-therapy-tools/colon-cancer
Predicting Toxicity	Cancer and Aging Research Group	www.mycarg.org
	Moffitt Cancer Center CRASH score	http://eforms.moffitt.org/crashScore.aspx

ASCO indicates American Society of Clinical Oncology; UNC, University of North Carolina.

settings with similar grade 3 and 4 toxicity between younger and older patients with the exception of leukopenia without excess infection.[15,16] These data have been confirmed in independent studies for both 5FU/LV and capecitabine.[17-19]

Based on the results of 3 adjuvant trials (MOSAIC,[20] NSABP-C-07,[21] and XELOXA[22]), oxaliplatin-based combinational chemotherapy is considered the standard of care for patients with stage III colon cancer, offering a 4% overall survival (OS) benefit over 5FU/LV at 6 years. However, the additional benefit of oxaliplatin in older patients appears to be attenuated. Subset analyses of MOSAIC and NSABP C-07 trials showed no significant benefit in OS with the addition of oxaliplatin in patients age 70 or older (MOSAIC mortality hazard ratio [HR] = 1.10; 95% confidence interval [CI], 0.73-1.65; for NSABP-C-07, HR = 1.32; 95% CI, 1.03-1.70).[20,21] In contrast, a subgroup analysis of XELOXA, a study that evaluated the use of oral capecitabine in combination with oxaliplatin versus bolus 5FU/LV, the benefits of disease-free survival (DFS) were maintained regardless of age, but no significant OS benefit was shown.[22] In an analysis using the ACCENT[23] database of 2575 patients age ≥70 years using oxaliplatin-based regimens versus 5FU/LV, although there was a trend toward improved time to recurrence in oxaliplatin-treated patients over age 70, there was no DFS or OS significant efficacy benefit. However, there was only a borderline significant interaction by age for OS (P = .05) and no significant interaction for DFS (P = .09), suggesting a lack of significant effect modification by age.[23]

Further evaluation of this in the MOSAIC subgroup saw the same trend toward prolongation of time to relapse; however, much shorter post-recurrence survival in oxaliplatin-treated patients may have mitigated the benefit of any delay in recurrence.[20] The benefit of oxaliplatin seems to be reduced compared with younger patients in terms of DFS and OS, while maintaining time to recurrence. A recent large population-based analysis of patients treated outside the context of a clinical trial, presumably representing a broader range of patient population than clinical trial enrollees, showed a marginal improvement in survival with the addition of adjuvant oxaliplatin in patients age 75 and older.[13] Overall, these findings suggest there is minimal or no survival benefit of additional oxaliplatin to adjuvant 5FU/LV in adults age 70 or older with stage III CRC.[24] In the management of patients with stage II CRC, the benefit of adjuvant chemotherapy is small regardless of age, and oxaliplatin does not improve outcomes over 5FU/LV.[20]

Evaluating Older Adults With Cancer

Given the potential for increased serious AEs and the long-term impacts associated with chemotherapy, the choice of whether to recommend adjuvant therapy to older adults should depend on an individual's risk of recurrence, estimated life expectancy (without recurrence), and estimated risk of toxicity with treatment (see **Table 1** for a list of useful online resources). These factors must all be considered in order to adequately weigh the risk/benefit of adjuvant treatment. Regardless of the objective measures of treatment benefit and toxicity, older patients may arrive at different personal trade-offs in terms of the benefits and risks of treatment, whether fit or frail.[9] When discussing potential treatment options, it is critical to consider a patient's values and preferences to inform the decision-making process.

Due to the heterogeneous aging process, age alone is not an adequate measure of physiologic or functional age and is a poor determinant of cancer outcomes. Traditionally, tools such as the Karnofsky Performance Status (KPS) and Eastern Cooperative Oncology Group (ECOG) Performance Status have been used by oncologists to assess functional status, but these tools are limited to simple numeric scales that although helpful, do not accurately assess the function of older patients with cancer. Many tools have been developed to better evaluate a person's *functional* age.

The Comprehensive Geriatric Assessment (CGA) involves a careful review of a patient's health status, including functional status, cognition, psychological state, nutritional status, social support, polypharmacy, and comorbid illnesses (see **Table 2** for more details and suggested measures).[25] The feasibility of an abbreviated, primarily self-administered CGA has been shown in the oncology setting, in both community and academic outpatient clinics.[26,27] CGA is able to detect impairments not typically identified in routine history or physical examinations, and can be predictive of survival, chemotherapy toxicity, postoperative morbidity, and mortality in older patients with cancer.[28] The CGA is a useful tool in the assessment of a patient's overall health status and potential frailty, a state of decreased physiologic reserve.[4,29] Although there is no clear consensus or definition of frailty, a number of criteria can be used to identify vulnerable older adults, such as the presence of geriatric syndromes (such as falls or dementia) or dependence in activities of daily living.[5] Given the high risk of adverse clinical outcomes for frail older adults,[30] further research is under way to better identify this population. Current evidence supports evaluating elements of the CGA as a part of a routine evaluation of older adults with cancer.[28]

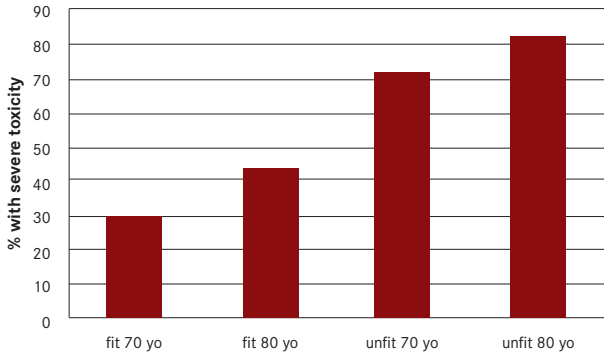
Estimating life expectancy (without recurrence) is a critical step in assessing the potential benefit of adjuvant treatment. Several tools are available to assist in life expectancy estimation for use within a variety of different populations.[31] The vast majority of CRC recurrences occur within the first 3 years, and nearly all patients with recurrences will die within 5 years. However, in patients with significant competing comorbidities that limit overall survival, 6 months of adjuvant therapy for even a 15% improvement in survival may not be warranted.[32] Also, the use of Web-based tools for assessing the benefit from adjuvant therapy can help quantify outcomes with and without adjuvant therapy. Tools such as *Adjuvant! Online* and the calculator derived from the ACCENT database can be useful aids in the discussion of the risk of tumor recurrence and potential benefits from adjuvant chemotherapy, but they need to be interpreted in the context of physiologic age.[33,34]

Although adjuvant chemotherapy is generally well tolerated in older adults, due to diversity in overall health and physical reserve inherent in older populations, the range of potential toxicities can vary greatly. In a seminal publication by Hurria et al,[35] a chemotherapy toxicity prediction model was developed for use in older adults that incorporates features commonly known to increase risk of toxicity (age, creatinine clearance, baseline hemoglobin), as well as CGA measures such as hearing status, falls, and dependence on assistance with instrumental activities of daily living (IADL). For example, using this predictive tool, a fit 70-year-old male with no physical impairments would have an estimated 30% risk of grade 3-4 toxicity undergoing single-agent chemotherapy for CRC, whereas a similar 70-year-old male with fair hearing, a recent fall, and difficulty taking his own medica-

TABLE 2. Domains Typically Evaluated in a Comprehensive Geriatric Assessment and Suggested Measures to Use

Domain	Potential Measures
Function Status	Instrumental Activities of Daily Living (IADL)
	Activities of Daily Living (ADL)
	Timed Up and Go (TUG) or gait speed
	Vision and hearing assessment
	Falls assessment
Comorbidity	Number of comorbidities
	Number of medications
Cognition	Mini-Cog, Mini-Mental State Exam (MMSE), or Montreal Cognitive Assessment (MoCA)
Nutrition	Unintentional weight loss in past 6 months, Mini Nutritional Assessment (MNA)
Body Composition	Body mass index (BMI), Lean body mass assessment
Psychological	Geriatric Depression Scale (GDS), Patient Health Questionnaire-9 (PHQ-9)
Social Support	Medical Outcomes Survey (MOS) social support survey

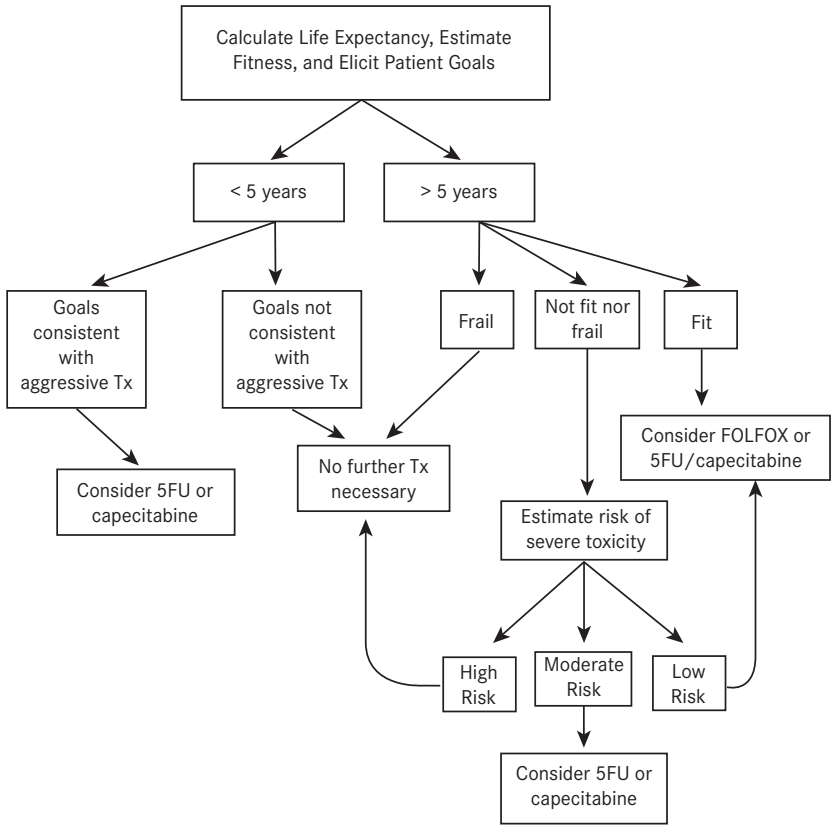
FIGURE 1. Comparison of Predicted Chemotherapy Toxicity for 4 Hypothetical Older Adults



Comparison of the risk of severe toxicity (≥grade 3) related to chemotherapy of 4 hypothetical patients with colorectal cancer with varying age and fitness levels using chemotherapy toxicity calculator developed by Hurria et al.[35] Fit patients have no physical impairments and no laboratory abnormalities, and unfit patients have fair hearing, a recent fall, and difficulty taking their own medications (IADL dependency), with no lab abnormalities.

YO indicates years old.

FIGURE 2. Overview of the Approach to Adjuvant Treatment Decisions in Older Adults With Colon Cancer



Suggested adjuvant treatment algorithm for use in older adults with colorectal cancer based on life expectancy, overall health status, risk of toxicity, and patient goals.
5FU indicates 5-fluorouracil; FOLFOX, 5-fluorouracil + oxaliplatin; Tx, treatment.

tions (IADL dependency) would be estimated to have a 72% risk of toxicity (**Figure 1**). Although the mortality risk from adjuvant chemotherapy itself is quite low, chemotherapy is associated with AEs that may potentiate or be potentiated by other comorbidities, thus resulting in morbidity or worsened quality of life. In particular, oxaliplatin is associated with peripheral neuropathy that can be made potentially worse by pre-existing diabetes or lumbar stenosis, resulting in increased risk of falls and/or disability.[36]

Adjuvant Treatment Approach in Older Adults With CRC

There is general agreement that fit, older adults who are active and without comorbidity and who have a life expectancy of at least 5 years should be offered adjuvant chemotherapy. Frail older adults with significant functional impairments and limited life expectancies are not suitable candidates for chemotherapy. In older adults who are neither fit nor frail, the decision-making process is the most complex and requires a delicate balance of the risks and benefits informed by patient preferences. (**Figure 2** provides an overview of the approach to adjuvant treatment decisions in older patients with CRC.)

In general, we recommend adjuvant chemotherapy in all fit older patients with stage III and many with stage II high-risk CRC. For fit patients with stage III disease with life expectancies substantially greater than 5 years—typically patients in their late 60s and early 70s—we suggest considering an oxaliplatin-based chemotherapy regimen, with the caveat that the absolute survival benefit is quite small. Given concern for relative 5FU/LV resistance in tumors deficient in mismatch repair enzymes, we use FOLFOX or omit chemotherapy altogether given good prognosis. Oxaliplatin should be avoided in patients with pre-existing neuropathy, and it should be immediately stopped if significant toxicity emerges, given the uncertainty of the OS benefit of oxaliplatin-based regimens in older adults. Due to the lack of compelling benefit of oxaliplatin, we generally recommend using 5FU/LV or single-agent capecitabine. For 5FU, we recommend using the infusional modified de Gramont regimen. If an oxaliplatin-based regimen is chosen, we prefer the modified FOLFOX6 regimen, and frequently begin at 20% dose reduction and escalate subsequently if there are any concerns about tolerance. For patients with issues with count recovery, which is more frequent with older patients, we omit 5FU bolus.[37,38] In patients who are unable to tolerate an ambulatory infusion pump, capecitabine plus oxaliplatin (used in the XELOX trial) is also reasonable, but we typically use 825 mg/m^2 to 850 mg/m^2 twice daily rather than 1000 mg/m^2.[22]

For patients who are deemed less fit or who have comorbid conditions that are likely to limit 5-year survival, as well as those with high-risk stage II disease that are mismatch repair enzyme proficient, we recommend single-agent fluoropyrimidine. Our preference in elderly patients is for infusional 5FU rather than capecitabine, both as a single agent and in combination, in light of capecitabine-based regimens being associated with greater toxicity in older adults than infusional 5FU, at least in the metastatic setting where this has been tested.[39] Eliminating the bolus

of 5FU from the infusion regimen may significantly mitigate hematologic toxicity.[40] Although 6 months of therapy is the optimal duration of adjuvant chemotherapy, there is evidence that 3 months of adjuvant chemotherapy may be adequate,[41] so we feel comfortable discontinuing adjuvant therapy between 4 to 6 months if toxicity impairs quality of life.

Conclusions

In general, we recommend adjuvant chemotherapy in all fit older patients with stage III and stage II high-risk CRC, and recommend carefully considering the risks and benefits of adjuvant therapy in nonfit and nonfrail older adults. We also believe that it is critical to elicit the values of our older patients to better understand their preferences with regard to how they balance the potential for life prolongation and risk of AEs. Older patients receive similar benefits from 5FU-based chemotherapy as younger patients, but the incremental benefit from oxaliplatin is reduced. Treating older adults with CRC requires a carefully crafted individualized plan that balances an individual's risk of recurrence, estimated life expectancy and risk of toxicity, and personal preferences.

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Novel Targets in Multiple Myeloma

Ajai Chari, MD

Abstract

Despite the availability of 5 classes of drugs for the treatment of multiple myeloma (MM), the disease remains incurable in most cases, with patients eventually relapsing on each of these agents. The benchmark for drug approval is likely to be an overall response rate (ORR) of approximately 25% to 30% and progression-free survival (PFS) of approximately 4 months. This article reviews the targets, mechanisms of action (MOA), safety, and efficacy of the novel agents in MM that are currently thought to have the best chance of meeting these benchmarks, including the recently approved panobinostat. After a brief update on putative MOA of immunomodulatory (IMiDs) drugs, the following monoclonal antibodies (mAbs) are discussed: the CD38 antibodies daratumumab and SAR650982, the CS1 antibody elotuzumab, and indatuximab ravtansine—a CD138 antibody conjugated to maytansinoid. Non-mAbs discussed include inhibitors of histone deacetylases, kinesin spindle protein, AKT3, PIM kinase, and nuclear transport (XPO1).

Key words: Multiple myeloma, prednisone, dexamethasone, thalidomide, lenalidomide, pomalidomide, bortezomib, carfilzomib, panobinostat, melphalan, cyclophosphamide, doxorubicin, DCEP/D-PACE, BCNU, bendamustine

Practical Application

- A review of currently approved drug classes for the treatment of multiple myeloma (MM)
- A review of novel agents under development for the treatment of MM, including their:
  - mechanisms of action
  - safety and toxicity profiles
  - efficacy

Although there currently are 5 classes of drugs available for the treatment of multiple myeloma (MM; **Table 1**), the disease remains incurable in most cases, with patients eventually relapsing on each of these agents. The benchmark for drug approval based on 2 recent agents approved by the FDA (carfilzomib and pomalidomide) in the relapsed/refractory space is likely to be an overall response rate (ORR) of approximately 25% to 30% and progression-free survival (PFS) of approximately 4 months.[1,2] The goal of this review is to discuss the targets, mechanisms of action (MOA), safety, and efficacy of the novel agents in MM that are currently thought to have the best chance of meeting these benchmarks.

Targets of IMiDs

Of note, there also have been developments in our understanding of the targets of immunomodulatory drugs (IMiDs). Until recently, it has been unclear how these oral medications achieve antimyeloma benefits without significant toxicity at the site of drug absorption in the gut or other off-target organ toxicities. Recently, cereblon, a member of the E3 ubiquitin ligase family, was identified as the likely mediator of teratogenicity of IMiDs. Cereblon also implicates IMiDs in the dysregulation of the ubiquitination process that targets proteins for proteasomal degradation.[3] Malignant plasma cells, by virtue of being antibody/protein factories, have been demonstrated to be extremely vulnerable to such dysregulation by the proteasome inhibitor (PI) drug class. Moreover, additional work suggests that the immunologic changes associated with IMiDs may be due to Aiolos and Ikaros, which are not only substrates of cereblon, but also transcription factors involved in interleukin 2–mediated T cell activation.[4] A better understanding of the MOA of these agents in MM will be essential to overcoming drug resistance and to developing the next generation of IMiDs and PIs.

Fortunately there are also several promising new drug targets in MM (**Table 2**). To date, there has been no rituximab—that is, a monoclonal antibody (mAb) with single-agent activity in MM. CD38 is a transmembrane glycoprotein and ectoenzyme with high receptor density on MM cells.[5] The CD38 antibodies daratumumab and SAR650982 are both humanized IgG1 mAbs with multiple MOA, including antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis, complement-dependent cytotoxicity, direct apoptosis induction, and inhibition of CD38 enzymatic activity.[6] Other than manageable infusional

TABLE 1. Currently Available Anti-Myeloma Therapies

Steroids	IMiDs	Proteasome Inhibitors	HDAC Inhibitors	Conventional Chemotherapy
Prednisone	Thalidomide	Bortezomib	Panobinostat	Melphalan
Dexamethasone	Lenalidomide	Carfilzomib		Cyclophosphamide
	Pomalidomide			Doxorubicin
				DCEP/D-PACE
				BCNU
				Bendamustine

BCNU indicates bis-chloroethylnitrosourea (carmustine); DCEP, dexamethasone + continuous IV infusion of cyclophosphamide + etoposide + cisplatin; D-PACE, dexamethasone + continuous IV infusion of cisplatin + doxorubicin + cyclophosphamide + etoposide; HDAC, histone deacetylase; IMiDs, immunomodulatory drugs.

toxicities expected with mAbs, both agents are well tolerated. In small phase 2 studies, both agents have shown single-agent activity with an overall response rate (ORR) in the range of 30% to 35% and 60% to 70% when combined with lenalidomide (**Table 3**).[7-10] Daratumumab received breakthrough designation in May 2013 for patients with MM who have received more than 3 lines of therapy or are IMiD- and PI-refractory. Once these agents are approved, it is expected that they quickly will be moved throughout the disease spectrum, and also, based on preliminary data from combination studies demonstrating non-overlapping toxicity and improved efficacy, eventually be approved for administration with many of the agents in **Table 1**.

Another mAb that has already entered phase 3 studies in MM is elotuzumab, which is a humanized IgG1 mAb that targets cell surface 1 (CS1), a member of the SLAM family, which is uniformly and highly expressed in more than 95% of patients with primary MM, but not on stem cells or other normal tissues. The MOA appears to be ADCC-mediated by natural killer (NK) cells.[11] Although elotuzumab has no significant single-agent activity, it has an excellent safety profile when given weekly for two 28-day cycles, and thereafter on days 1 and 15 in combination with a lenalidomide and dexamethasone backbone. Grades 3 and 4 toxicities are primarily hematologic and range from 10% to 15%, likely due to lenalidomide, except perhaps for lymphopenia. However, there was no significant increase in infections. In a phase 2 study of 36 lenalidomide-naïve patients who received elotuzumab 10 mg/kg in combination with lenalidomide and dexamethasone, the ORR was 92% and PFS was 33 months,[12] relative to historic controls of lenalidomide-dexamethasone with an ORR of approximately 60% and PFS of 11 months.[13,14] Elotuzumab received breakthrough designation by the FDA in May 2014 for more than 1 line of therapy given with lenalidomide-dexamethasone.

TABLE 2. Novel Targets in Multiple Myeloma

Target	Agent
CS1	elotuzumab
CD38	daratumumab SAR650982
CD138	indatuximab ravtansine (BT-062 + maytansinoid)
HDAC6	ricolinostat
KSP1	filanesib
AKT3	afuresertib
XPO1	selinexor
PIM kinase	LGH447

The last mAb with an encouraging signal in MM is indatuximab ravtansine (formerly known as BT062), an anti-CD138 chimerized monoclonal IgG4 linked to the microtubule inhibitor maytansinoid (DM4). CD138 is primarily expressed on MM cells and also on epithelial cells, albeit to a lesser extent. Once the mAb binds to CD38, internalization and lyosomal processing of the linker releases the cytotoxic DM4 metabolites that result in apoptosis due to inhibition of tubulin polymerization.[15] A phase 1 dose-escalation study showed dose-limiting toxicities of elevated gamma-glutamyltransferase, mucositis, and hand-foot syndrome. Serious adverse events also included gastrointestinal bleeding and corneal damage. Although monotherapy produced stable disease or better in 50% of patients, the ORR was only 4%, and therefore it is unlikely to move forward as a single agent.[16] When combined with lenalidomide-dexamethasone, 100% of patients had stable disease or better, including an ORR of 75% in a small number of lenalidomide-refractory patients.[17]

Although MM cells are initially susceptible to PI therapy, the aggresome pathway is also responsible for the destruction of misfolded proteins. Dual pathway inhibition has demonstrated preclinical synergy.[18] Two oral pan-histone deacetylase inhibitors (HDACs) have completed large phase 3 studies. The VANTAGE study[19] comparing bortezomib and dexamethasone with either vorinostat or placebo showed only a disappointing 0.8-month improvement in PFS over the control arm. More recently, the PANORAMA 1 study[20] with bortezomib (intravenous) and dexamethasone with either panobinostat or placebo showed a 3.9-month improvement in PFS and an improvement in complete response (CR) rates from 15.7% to 27.6%. However, the rates of grade 3 or 4 diarrhea increased from 8% to 25%. Most

TABLE 3. Summary of Efficacy of Monoclonal Antibodies(at recommended phase 2/phase 3 dosages)

Treatment	N	Eligibility (% Refractory)	Response Rate	PFS (mos)	DOR (mos)	OS (mos)
Elotuzumab Len Dex[12]	36	0%, Len sensitivity required	92%	33	17.8	NR
Daratumumab[7]	20	50%/75%/38% (% Refr: Bor/Len/Bor+Len)	35% (n = 20)	NR	NR	NR
SAR650984[8]	13	69% Car+Pom exposed	30.8%	NR	5+	NR
Daratumumab Len Dex[9]	30	6.7% Len Refr	87%	NR	NR	NR
SAR650984 Len Dex[10]	24	80% Len/20% Pom ~60% Bor/~50% Car	63% (48% in Len Refr, n = 25)	5.8	NR	NR
BT062 Len Dex[17]	21	100% Bor/87% Len	73% (75% in Len Refr, n = 8)	NR	NR	NR

Daratumumab granted FDA breakthrough designation May 2013 for >3 lines of therapy or PI- and IMiD-refractory disease.
Elotuzumab granted FDA breakthrough designation May 2014 for >1 line of therapy given with Len-Dex.

Bor indicates bortezomib; Car, carfilzomib; Dex, dexamethasone; DOR, duration of response; IMiD, immunomodulatory drug; Mos, months; Len, lenalidomide; NR, not reported; PFS, progression-free survival; OS, overall survival; PI, proteasome inhibitor; Refr, refractory.

likely, the diarrhea is attributable to the combination of panobinostat with intravenous bortezomib, as these rates of diarrhea have not been seen when panobinostat is combined with subcutaneous bortezomib, carfilzomib, or lenalidomide.[21-23] Moreover, PANORAMA 2[24] demonstrated that the addition of panobinostat to bortezomib in bortezomib-refractory patients resulted in a response rate (RR) of 34.5% and PFS of 5.4 months. Panobinostat was approved by the FDA on February 23, 2015, in combination with bortezomib and dexamethasone in patients who have received at least 2 prior regimens (including bortezomib and an IMiD). Ricolinostat, an oral specific inhibitor of HDAC6, a key component of the aggresome pathway, is also under investigation (**Table 4**).

There are additional non-mAb novel agents with single-agent activity in development (**Table 5**). Filanesib is a highly selective allosteric inhibitor of kinesin spindle protein (KSP), which is a microtubule motor protein. KSP inhibition prevents formation of a bipolar spindle, leading to apoptosis particularly in Mcl-1–dependent MM cells. As a single agent, the main toxicity is neutropenia, which is manageable with prophylactic granulocyte-colony stimulating factor (GCSF). As a single agent, the ORR is 16% and the median PFS is 3.7 months (n=32), which is comparable to the 15% and 3.4 months with the addition of dexamethasone to filanesib (n=55), albeit this was in a much more heavily pretreated, IMiD- and PI-refractory population. Of particular interest though, is the potential biomarker alpha 1-acid glycoprotein (AAG), an acute-phase protein used to monitor inflammatory conditions. AAG binds to filanesib such that high AAG concentrations result in increased IC50 for filanesib in vitro. Lower AAG levels seem to correlate with clinical outcomes, as such patients had an ORR without dexamethasone of 24% and a median PFS of 5.1 months.[25] Al-

TABLE 4. Summary of Combination Therapy With Histone Deacetylase Inhibitors

Regimen	Phase (N)	Outcomes
Panobinostat or placebo + Bor Dex[20]	3 (768)	ORR: 60.7% vs 54.6% (P = .09) PFS: 12.0 vs 8.1 mos (P < .0001) OS: 33.6 vs 30.4 mos (P = .26)
Panobinostat + Bor Dex[24]	2 (55 Bor refr)	ORR 34.5%; CBR 52.7%
Panobinostat + Len Dex[23]	2 (19)	ORR: 31%; CBR: 52 % N = 14; Len Refr : ORR: 21% CBR: 42%
Panobinostat + Carfilzomib[22]	1/2 (26)	ORR: 46%; CBR: 52 % Bor Refr: ORR: 44%; n = 26
Vorinostat or placebo + Bor[19] (no Dex)	3 (637)	ORR: 54% vs 41% (P < .0001) PFS: 7.6 vs 6.8 mos (P < .01); OS: no difference
Ricolinostat ± Bor Dex[32]	1 (20)	ORR: 25%; CBR: 60%
Ricolinostat + Len Dex[33]	1 (22)	ORR: 64%; CBR: 100%

Bor indicates bortezomib; CBR, clinical benefit rate; Dex, dexamethasone; DOR, duration of response; IMiD, immunomodulatory drug; Len, lenalidomide; Mos, months; ORR, overall response rate; OS, overall survival; PI, proteasome inhibitor; PFS, progression-free survival; Refr, refractory.

TABLE 5. Summary of Non-Monoclonal Antibody Novel Agents With Single-Agent Activity

Treatment	N	Eligibility	ORR	PFS (mos)	DOR (mos)	OS
Ixazomib Dex	33	88% Len Exp; Bor Sens	34%	12.4	NR	96% @ 6 mos
Filanesib in low AAG[25]	32 21	75% IMiD Refr 53% PI Refr	16% 24%	3.7 5.3	8.6 8.6	19 mos 23.3 mos
Filanesib Dex in low AAG[25]	55 36	100% IMiD Refr 98% PI Refr	15% 19%	3.4 5.1	5.1 5.1	10.5 mos 10.8 mos
Afuresertib[27]	34	97% IMiD Exp; 88% PI Exp	8.8%	NR	NR	NR
Selinexor Dex[32]	8	Refr to all classes	50%	NR	NR	NR
LGH 447[29]	59	NR	10.5%	NR	5.8	NR

Filanesib granted orphan drug approval by FDA May 2014.

AAG indicates alpha 1-acid glycoprotein; Bor, bortezomib; CBR, clinical benefit rate; Dex, dexamethasone; DOR, duration of response; Exp, exposed; IMiD, immunomodulatory drug; Len, lenalidomide; Mos, months; ORR, overall response rate; OS, overall survival; PI, proteasome inhibitor; PFS, progression-free survival; Refr, refractory; Sens, sensitive.

though linking drug approval to a novel biomarker is not an easy path forward, to date, there are no biomarkers predictive of response in MM, and KSP inhibition is a novel MOA in MM. Filanesib was granted orphan drug approval by the FDA in May 2014.

The phosphatidylinositide 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) signaling pathway is activated in MM, and preclinically, inhibition of this pathway leads to apoptosis.[26] The oral AKT3 inhibitor afuresertib showed a single-agent RR of 8.8%.[27] PIM kinases also promote tumor cell proliferation and survival, and are overexpressed in hematologic malignancies.[28] The pan-PIM kinase inhibitor LGH 447 recently demonstrated a monotherapy RR of 10.5%.[29] While the single agent responses for afuresertib and LGH 447 are modest, better patient selection or combination strategies may be more efficacious.

Finally, the nuclear protein exportin 1 (XP01) is a promising target in oncology. Preclinically, inhibition of XPO1 induced MM cytotoxicity and impaired osteoclastogenesis.[30] Although the data are very preliminary, the nuclear transport inhibitor known as selinexor (KPT-330) has already demonstrated activity. In 8 patients with MM who were refractory to all drug classes and were treated with selinexor and dexamethasone, the response rate was 50%.[31]

Although PIs and IMiDs have yielded significant improvement in MM outcomes, the currently available classes of drugs can take us only so far, as evidenced by the modest ORR and PFS of recently approved agents. Further improvements will require a better understanding of myelomagenesis, a better understanding of the mechanisms of resistance to current agents, biologically guided/risk-adapted therapy, and finally, the development of agents with novel targets. Fortunately, as reviewed here, there are many such promising agents.

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Clinical Applications and Limitations of Next-Generation Sequencing

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Abstract

Growing interest in personalized cancer therapy has led to numerous advances in the field of cancer genomics. Next-generation sequencing (NGS) is one such development that has allowed for lower cost, higher-throughput genome sequencing. However, the vast number and types of genomic aberrations found in cancer means that interpretation of the data generated by NGS requires substantial analytical complexity. Here, we discuss the clinical applications of NGS and the obstacles that must be overcome prior to widespread use in clinical decision making.

Key words: Next-generation sequencing, review, genomics, cancer

Introduction

Personalized cancer therapy requires the use of molecular diagnostics to tailor treatments to individuals. At this time, only a few molecular biomarker-based therapies, such as erlotinib in *EGFR*-mutated lung cancer and vemurafenib in *BRAF*-mutated melanoma, have been widely accepted.[1,2] Next-generation sequencing (NGS) has the potential to revolutionize oncology through the classification of tumors and identification of biomarkers that can predict response to individualized therapy.

Until recently, the Sanger sequencing method was the most widely used sequencing method, and resulted in the only complete human genome sequence.[3] This technology relies on incorporation of chain-terminating dideoxynucleotides during DNA replication.[4] Fluorescently labeled terminators, capillary electrophoresis separation, and laser signal detection have improved the throughput of Sanger sequencing.[5] However, it remains labor-intensive, time-consuming, and expensive when done in large scale.[6] Therefore, the demand for faster, more accurate, and more cost-effective genomic information has led to the development of NGS methods.

NGS methods are high-throughput technologies with capabilities of sequencing large numbers of different DNA (massively parallel) sequences at once. NGS technologies monitor the sequential addition of nucleotides to immobilized DNA templates generated from target tissue.[7] Unfortunately, the increased throughput of NGS reactions comes at the cost of shorter sequences, as most sequencing platforms (Illumina, Roche, SoLiD) offer shorter read lengths (30–400 bp) than the conventional Sanger-based method.[8] These shorter sequences are then assembled into longer sequences such as complete genomes.

Common approaches to DNA sequencing include whole-genome sequencing, whole-exome sequencing, targeted exome sequencing, and "hotspot" sequencing. Whole-genome sequencing sequences the complete genome of a sample (ie, chromosomal DNA and mitochondrial DNA, which includes intronic and exonic regions). Whole-exome sequencing is a technique that sequences all of the protein-coding genes (ie, all exons in the genome). Targeted exome sequencing uses target-enrichment methods to capture genes of interest. This approach is becoming increasingly popular in oncology for assessing the full sequence of cancer-related gene panels. Targeted exome sequencing also facilitates sequencing at a greater depth, and thus the identification of subclonal mutations. Alternately, rather than sequencing the full sequence of selected genes, only selected regions of selected genes can be sequenced, focusing on cancer gene "hotspots"—regions with recurrent mutations. Although hotspot mutation testing facilitates large-scale sequencing of many samples, it does limit the knowledge that is acquired through sequencing because it limits the evaluation to small regions in selected genes. Consequently, it increases the possibility of omitting relevant mutations for which evaluation is not being conducted, thus limiting the clinical knowledge that is gained through NGS. Despite its drawbacks, it is becoming a widely accepted form of NGS.

In addition to nucleotide change detection (mutations and small insertions and deletions), NGS allows for DNA-copy number predictions. Further, NGS technology also can be applied to RNA in order to evaluate the transcriptome of a tumor. RNA-sequencing (RNA-seq) allows for the assessment of gene expression and transcriptional splice variant analysis in addition

Practical Application

- Provide a brief introduction to the methods of next-generation sequencing (NGS)
- Identify clinical applications of NGS including the identification of various molecular aberrations in different tumor types, the resultant design of molecular biomarker-driven clinical trials, and the potential to identify molecular aberrations that lead to disease progression and resistance
- Identify limitations of NGS, including the need for extensive analytic capabilities, the difficulties in identification of driver mutations, and the confounding factor of tumor heterogeneity
- Identify potential future applications of NGS

to detection of mutations. A typical NGS work flow from sample collection to the capture and sequencing of genes of interest and data analysis is illustrated in **Figure 1**.

Identification of Cancer Genomics

In recent years, NGS has been used to characterize genomic alterations such as mutations, insertions/deletions, and copy number changes, and the frequency with which they occur in various tumor types. Efforts such as the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) aim to catalog such genomic alterations across many tumor types.[9,10] However, the wealth of information that is generated through this process unveils potentially the largest hurdle of genomic medicine: How do we analyze the abundance of information that is generated to make informed decisions regarding therapy? Analysis of cancer genomes reveals that most tumors contain multiple alterations.[11-14] As a result, it is very important to distinguish the "driver" mutations that contribute to tumor development from the "passengers" that do not.[15]

Comparison of sequenced genomes to reference genomes allows for the identification of genome alterations that may be relevant in disease development and progression.[16] However, such comparison depends on the establishment of extensive and accurate reference genomes, which is a cumbersome task. Further, the complexity of genomic aberrations in cancer makes it difficult to rely on standard reference genomes.[8] Therefore, simplified methods of identifying driver mutations are required. Several theories exist for the potential identification of driver mutations. One such hypothesis is that mutations that occur with higher frequency are more likely to contribute to tumor development and growth.[17] Genome-wide association studies (GWAS) aim to compare the incidence of commonly known single nucleotide polymorphisms (SNPs) in genomes from patients with and without a specific disease. SNPs that occur at a higher frequency in the diseased population are identified as potentially causative. If a specific mutation is not found in high frequency, but the same molecular pathway contains frequent genomic alterations, those alterations may also be relevant.

Another theory is that alterations present in both germline and tumor tissue of the same patient are likely to be integral to tumor development. For example, some mutations in cancer-predisposition genes such as *BRCA1/2* clearly do contribute to the development and maintenance of cancer. This, however, requires that germline tissue be collected in each patient. Yet another theory is that sequencing DNA and RNA from the same sample will identify mutations that subsequently alter expression, and are thus significant. However, all of these methods only begin to narrow the spectrum of genomic alterations that may be clinically relevant. Chromosome-scale changes and epigenomic changes cannot be evaluated in this manner. Many studies are now focusing on the development of bioinformatic tools to aid in the identification of driver mutations.[18]

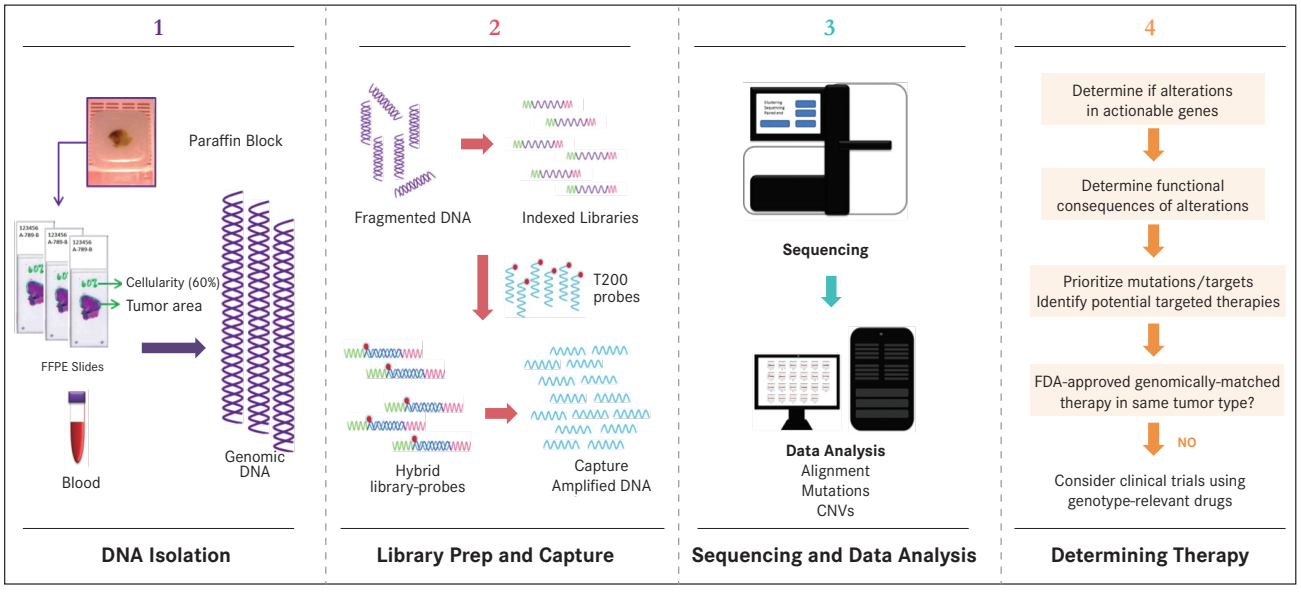
Clinical Decision Support

Once driver mutations have been identified in a tumor, the next step is to assess whether those mutations are "actionable." Actionable alterations affect the function of a cancer-related gene and can be targeted with approved or investigational therapies. Assessing functionality is a difficult task and requires predictive knowledge of genome alterations. Often, early-phase studies are used to assess the role various mutations based on rates of response to targeted therapies. However, enrollment in such studies requires that physicians be aware of genome alterations and potential trials for each patient.

A survey of 160 physicians at a tertiary-care National Cancer Institute (NCI)-designated comprehensive cancer center revealed that a considerable percentage of physicians have low confidence in their genomic knowledge.[19] As a result, many institutions have instituted tumor boards to increase awareness of and access to appropriately targeted therapies.[20] Similarly, the American Society of Clinical Oncology has monthly presentations that explore current treatment strategies and novel therapeutics in various tumor types to increase knowledge of newer targeted therapies. Other trials such as NCI Molecular Analysis for Therapy Choice Program (NCI-MATCH) has streamlined the decision making by designing algorithms and creating rules to designate alterations as actionable, and to prioritize targets if more than one target is identified. In this signal-seeking trial, 3000 patients will undergo tumor NGS to match genomic alterations to smaller histology-agnostic phase 2 trials of Food and Drug Administration (FDA)-approved agents (in other diseases) and investigational therapeutics (**Figure 2**).

If a response signal is seen in early-phase trials, the clinical relevance and therapeutic implications of actionable mutations can be assessed through thoughtful biomarker-driven research. Hypothesis-driven preclinical studies and clinical trials to assess targeted therapies in various tumor types can be designed. Such trials allow for the recruitment of selected patients into clinical trials to enhance the assessment of those targeted therapies. Ultimately, the goal is to implement randomized clinical trials to assess molecularly targeted therapy in a biomarker-se-

FIGURE 1. Overview of a Potential Next-Generation Sequencing Work Flow



(1) Slides are cut from tumor samples embedded in paraffin blocks. For each tumor sample, hematoxylin and eosin stains are performed and cellularity is assessed. Matching peripheral blood is also collected for each patient. Genomic DNA is isolated from both the formalin-fixed, paraffin-embedded tissue and blood. Alternately, frozen fresh tissue samples as well as other normal DNA sources such as saliva, buccal swab, or normal tissue can be used. (2) DNA is fragmented and libraries are made by ligating indexed adaptors (Indexed Libraries) that allow for sample pooling. Hybridization with probes is performed; the captured DNA is washed and amplified and proceeded to DNA sequencing. (3) Captured DNA is sequenced; after sequencing, samples are demultiplexed, or separated, and the raw data is submitted to data analysis for mutations and copy number variations identification. (4) The genomic alterations are reviewed and alterations in actionable genes are identified. Functional impact of alterations in actionable genes is assessed and therapeutic implications of known and predicted functional alterations are determined.

Modified from Chen et al. Clin Chem.2015.[33]

lected or biomarker-stratified fashion. The Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trials (ALCHEMIST) is an example of such a trial in which patients with early-stage adenocarcinoma of the lung are screened for *EGFR* and *ALK* mutations, and subsequently randomized into trials of relevant targeted therapy if mutations are found. With NGS technology, a high throughput of patients can undergo testing to assess their eligibility for clinical trials within a clinically reasonable timeframe.

Genomic Evolution and Intertumor and Intratumor Heterogeneity

Further complicating the implementation of genomic medicine is the fact that driver mutations can evolve during the course of cancer. As tumors are treated or as they grow, a variety of acquired genomic alterations may emerge. For example, melanoma treated with BRAF or MEK inhibitors has been shown to acquire *BRAF* amplifications and downstream alterations that lead to reactivation of the MAP kinase pathway.[21-23] Similarly, increased signaling via the phosphatidylinositol 3-kinase/Akt pathway may contribute to trastuzumab resistance in HER2-positive breast cancer.[24] Thus, the dynamic nature of cancer requires that genomic information be applicable in real time in order for clinical use. As a result, archived tissue from biopsies may not be relevant for therapy selection at the time of progression.

In addition to genomic evolution, tumors may also develop intertumor and intratumor heterogeneity. Intertumor heterogeneity refers to differences in alterations of tumors at different sites, while intratumor heterogeneity refers to differences in alterations within a tumor. Both intertumor and intratumor heterogeneity can further complicate the determination of relevant mutations because it means that tissue for NGS has to be obtained from relevant sites as well as at a relevant time point in the treatment course. This can result in repeated biopsies. Additionally, metastatic sites such as bone and brain can be difficult to test. However, comparison of primary tumors with matched

metastases has shown relatively high concordance in their mutational profiles, suggesting that additional biopsies may not always be necessary.[25,26]

Although genomic evaluation makes it difficult to identify relevant aberrations, recognizing genomic evolution is a powerful tool to better understand the progression of cancer. Genomic analysis of cancer at different stages, from precancerous lesions to localized tumors to metastatic disease, can identify genetic events that drive tumor growth. For example, genomic studies that analyze genomic alterations in breast ductal carcinoma in situ (DCIS) can help to design a predictive model for lesions that are likely to progress to carcinoma versus those that are not.[27] Similarly, NGS-based analysis of drug-resistant cells can help identify mechanisms of resistance. For instance, sequencing tumors from patients with estrogen receptor (ER)–positive breast cancer that recurred or progressed after treatment with antiestrogen therapy revealed mutations in the *ESR1* gene; these mutations were constitutively active.[28] Interestingly, *ESR1* gene mutations were not seen in TCGA analysis, which included primary tumors only.[11] Together, these studies suggest that activating mutations in the *ESR1* gene are an acquired mechanism of resistance to antiestrogen therapy. Similarly, RNA sequencing of tamoxifen-sensitive and -resistant breast cancer cells revealed gene expression changes implicating a series of resistance mechanisms that could be grouped in ER functions, cell cycle regulation, transcription/translation, and mitochondrial dysfunction.[29]

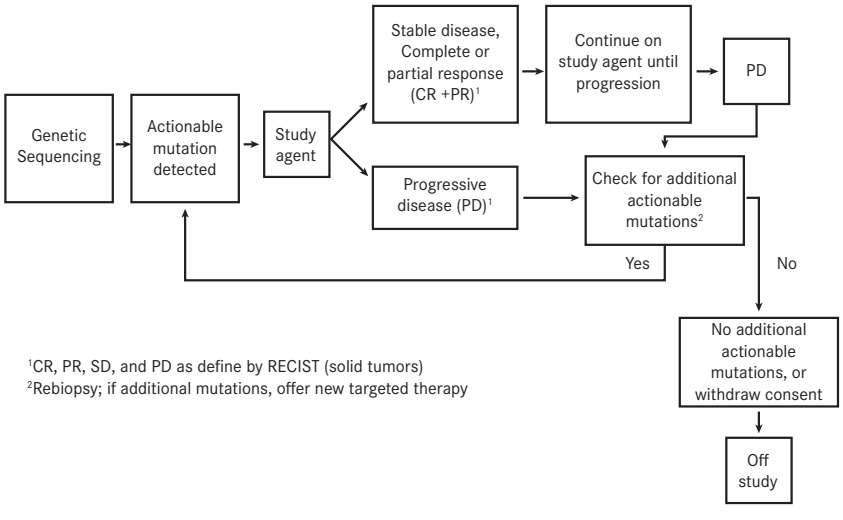
Future Applications and Directions

Several additional applications of NGS are under development. One potential future application of NGS is the evaluation of circulating tumor cells or free-plasma DNA to detect early relapse or residual cancer.[20] Once tumor-specific genome alterations have been identified by NGS, PCR assays could be used to detect circulating tumor cells or free-plasma DNA harboring the same alterations. Disease status, drug responsiveness, and relapse could be serially assessed. The monitoring strategy would, however, require that the mutation being tested be present in all tumor cells and remain present throughout the course of disease. As discussed previously, due to genomic evolution and tumor heterogeneity, such mutations are difficult to identify. Optimally, mutations used for monitoring would be truncal mutations—mutations in the "trunk-branch" model of heterogeneity, and thus representing ubiquitous driver mutations present in every tumor subclone and region.[31] However, serial monitoring could also identify new alterations that occur under the selection pressure of treatment, which could give insights into mechanisms of acquired resistance.

Another potential application of NGS is to improve the diagnosis of cancer. Poor tissue sampling and processing can often make a histological diagnosis difficult. Additionally, mixed tumor phenotypes can sometimes make it difficult to determine the origin of the tumor. However, NGS-based analysis of tissue can be performed on small amounts of viable tissue and is accurate when sufficient information regarding causative mutations is known. An evaluation of 143 benign and malignant thyroid nodules revealed that genotyping of fine-needle aspiration (FNA) samples of the nodules using a broad NGS panel provided high sensitivity and specificity in the diagnosis of these samples.[32] Such diagnoses would require clinical validation prior to widespread use. Furthermore, as the genomics of different tumors become apparent, NGS can be used to identify different molecular subtypes, which is already becoming commonplace with sarcoma fusion proteins.

Finally, NGS can identify molecular aberrations that render tumors exquisitely sensitive to certain therapies, resulting

FIGURE 2. Overview of NCI-MATCH Trial Design



A schematic of the NCI-MATCH trial design. Patient tumors undergo genetic sequencing and are assessed for actionable mutations. If found, patients are enrolled in smaller phase 2 trials of approved or investigational therapeutics until progression of disease. At the time of disease progression, patient tumors are again assessed for other actionable mutations. If found, patients are again enrolled in another phase 2 trial. If no further actionable mutations are found, patients are taken off study.

Figure adapted from Abrams et al. ASCO *Educational Book*. 2014.[34]

in exceptional responses. Such extraordinary outcomes can improve our understanding of molecular features that can predict response to certain drugs. For this purpose, the NCI has undertaken the Exceptional Responders Initiative, through which tumors of exceptional responders will undergo DNA and RNA sequencing to define genetic alterations that might have resulted in such responses.

Conclusion

Next-generation sequencing has opened a broad new area of research with the potential to revolutionize personalized cancer medicine. However, further development of this field requires real-time knowledge of genome alterations that can be used in clinical decision making. This requires a robust data infrastructure, continuous improvement in sequencing technology, development of analytical tools, and ongoing biomarker-driven preclinical and clinical trials. Ultimately, however, NGS data have the potential to guide clinicians in tailoring treatment to dynamic genomic changes in individual tumors.

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The PI3K Pathway as a Therapeutic Target in Breast Cancer

Cynthia X. Ma, MD, PhD

Abstract

The phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway is crucial to many aspects of cell growth and survival. The recognition of its importance in tumorigenesis and cancer progression has led to the development of a number of agents that target various components of this pathway as cancer therapeutics. Promising results with these agents have been observed in the treatment of advanced estrogen receptor–positive (ER+) breast cancer. However, the therapeutic efficacy of single-agent PI3K pathway inhibitors is likely limited by feedback regulations among its pathway components and crosstalk with other signaling pathways. There are ongoing efforts to investigate predictors of response and mechanisms of treatment resistance.

Key words: phosphatidylinositol-3-kinase, PI3K, breast cancer, targeted therapy

Function of PI3K Pathway and Alterations in Breast Cancer

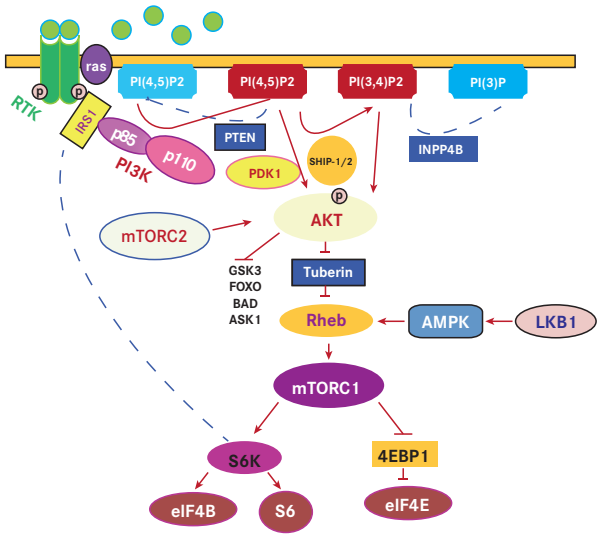
Class I phosphatidylinositol-3-kinases (PI3Ks) are heterodimers of a 110 KD catalytic subunit (p110α, p110β, p110γ or p110δ) and a regulatory subunit, which receive activation signals from receptor tyrosine kinases (RTKs), RAS, and G protein–coupled receptors (**Figure 1**).[1] Activated PI3K catalyzes the conversion of phosphatidylinositol bisphosphate, PI(4,5)P2, to phosphatidylinositol triphosphate, PI(3,4,5)P3, which recruits phosphoinositide-dependent kinase-1 (PDK1) and protein kinase B, also known as AKT, leading to a cascade of signaling events that regulate cell survival, proliferation, metabolism, motility, and genomic stability.[1] This pathway is also important in regulating tumor-associated immune response and angiogenesis.[2]

Genetic or epigenetic alterations in PI3K pathway components, including activating mutations in *PIK3CA*, the gene encoding the p110α catalytic subunit of PI3K, and *AKT1*, and loss-of-function mutations or epigenetic silencing of phosphatase and tensin homolog (*PTEN*), the negative regulator of the pathway, are commonly observed in cancer, leading to activation of PI3K pathway signaling. In estrogen receptor–positive (ER+) breast cancer, mutations in *PIK3CA* represent the most common genetic events, occurring at a frequency of 30% to 50%. Less commonly observed are mutations in *PTEN* (2% to 4%), *AKT1* (2% to 3%), and phosphatidylinositol-3-kinase regulatory subunit alpha (*PIK3R1*: 1% to 2%).[3,4] Similar findings were observed in *HER2*-positive breast cancer.[4] In contrast, triple-negative breast cancer (TNBC) is associated with a lower incidence of *PIK3CA* mutations (<10%), but much higher frequency of loss of PTEN (about 30% to 50%).[4] The frequent occurrence of PI3K pathway activation makes it an attractive therapeutic target in breast cancer.

Targeting the PI3K Pathway in ER+ Breast Cancer

The importance of *PIK3CA* mutation in the etiology of ER+ breast cancer is supported by several lines of evidence. A majority of the mutations, including the 3 hotspot mutations E542K, E545K, and H1047R, are missense "activating" mutations that cluster in the evolutionarily conserved accessory domain and the kinase domain.[5] The oncogenic property of the common *PIK3CA* mutations was demonstrated by their ability to induce cellular transformation and xenograft tumor formation when overexpressed in mammary epithelial cells.[6,7] In preclinical studies, cancer cells carrying *PIK3CA* mutation depend on the alpha catalytic subunit of PI3K for cell growth.[8] Although the presence of *PIK3CA* mutation in ER+ breast cancer has not been associated with de novo resistance to endocrine therapy,[9] upregulation of PI3K pathway signaling has been observed in tumor cells grown under long-term estrogen deprivation in experimental models.[10] In clinical samples, higher PI3K activity, based on the levels of phosphorylated forms of AKT, mammalian target of rapamycin (mTOR), glycogen synthase kinase 3 (GSK3), and the ribosomal protein S6 kinase (S6K), and loss of PTEN was associated with a lower ER level and with luminal B status.[11] Importantly, a synthetic lethal interaction, or synergistic apoptotic induction, was observed between estrogen deprivation and inhibition of PI3K, either molecularly by knockdown of *PIK3CA* or pharmacologically with inhibitors of PI3K or AKT, in ER+ breast cancer cell

FIGURE 1. PI3K Pathway Signaling



Class IA PI3K is composed of a p85 regulatory subunit and a p110 catalytic subunit. The inhibitory effect of p85 on the catalytic subunit is released following activation of the receptor tyrosine kinase by ligand binding, or by RAS. PI3K converts phosphatidylinositol bisphosphate, PI(4,5)P2, to phosphatidylinositol triphosphate, PI(3,4,5)P3, which serves as the docking sites for the membrane localization of the PH domain-containing molecules including phosphoinositide-dependent kinase-1 (PDK1) and AKT. PDK1 phosphorylates AKT at threonine 308. Mammalian target of rapamycin complex 2 (mTORC2) phosphorylates AKT at serine 473, leading to full activation of AKT, which phosphorylates and inhibits tuberous sclerosis complex, causing accumulation of Rheb GTP and activation of mammalian target of rapamycin complex 1 (mTORC1). S6 kinase (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) are important to protein synthesis. AKT also phosphorylates glycogen synthase 3β (GSK3β), Bcl-2-associated agonist of cell death (BAD), the forkhead transcription factors (FOXO), and other molecules. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) dephosphorylates PI(3,4,5)P3 to the inactive diphosphate form, while inositol polyphosphate 4-phosphatase type II (INPP4B) removes the phosphate group at position 4 of the inositol ring from the inositol 3,4-bisphosphate, thus negatively regulating the PI3K pathway. In addition, a negative feedback loop exists between mTORC1 and AKT through S6K1 induced IRS1 phosphorylation.

lines.[8,12] These studies provided the rationale for combining endocrine therapy and inhibitors of PI3K pathways in ER+ breast cancer.

Initial success from combing endocrine therapy with inhibitors against the PI3K pathway was demonstrated in clinical trials of rapamycin analogues for the treatment of advanced ER+ breast cancer resistant to an aromatase inhibitor (AI). These agents inhibit the activity of mTORC1 by interacting with FKBP12. In the TAMRAD trial, a randomized phase 2 trial of tamoxifen with or without everolimus in 111 postmenopausal women with AI-resistant, ER+, advanced breast cancer, the combination arm was associated with a significantly improved progression-free survival (PFS; 4.5 vs 8.6 months; hazard ratio [HR] = 0.54; P =.002) and overall survival (OS).[13] Similarly, BOLERO-2, a phase 3 trial of exemestane in combination with either everolimus or placebo in postmenopausal women with advanced ER+, *HER2*-negative (HER2-) breast cancer resistant to letrozole or anastrozole, demonstrated a significant improvement in PFS (3.2 months in the placebo/exemestane arm vs 7.8 months in the everolimus/exemestane arm; HR = 0.45; P <.0001),[14,15] leading to FDA approval of this combination in AI-resistant, advanced ER+ disease. However, the OS was no different in both arms (26.6 months in the placebo/exemestane arm vs 31 months in the everolimus/exemestane arm; HR = 0.89; P =.14).[15] The OS data were disappointing; however, this may not be too surprising, as rapalogs lack the ability to inhibit mTORC2, leading to feedback upregulation of AKT activity and treatment failure.[16] Nonetheless, the improvement in PFS is meaningful, and everolimus remains a treatment option for the AI-resistant population.

Everolimus was also combined with fulvestrant in a single-arm, phase 2 trial in AI-resistant, metastatic, ER+ breast cancer.[17] Among the 31 evaluable patients, the objective response rate was 13% and the clinical benefit rate was 49%, with median time to progression (TTP) of 7.4 months, suggesting clinical efficacy. However, a randomized trial is required to define the activity of this combination in AI-resistant, ER+ breast cancer.

A number of agents that target the PI3K pathway (**Table 1**), including mTOR kinase inhibitors, direct PI3K or AKT inhibitors, and dual inhibitors of PI3K and mTOR, are in clinical development and have the potential to more effectively inhibit the PI3K pathway than rapamycin analogues.[18] The mTOR kinase inhibitors and AKT inhibitors are in early phases of clinical trial development, while PI3K inhibitors have advanced to phase 3 trials and have shown promise in early-phase trials for ER+ disease.[19]

PI3K inhibitors are classified into pan-PI3K inhibitors and isoform-specific inhibitors, depending on their specificities to the 4 isoforms of the p110 catalytic domain, p110α, p110β, p110γ, and p110δ. Isoform-specific inhibitors may have the advantage of more effective isoform inhibition at tolerable doses than pan-PI3K inhibitors. However, patient selection may be particularly important for these agents, as cancers may rely on different p110 isoforms for cell growth. For example, p110α is critical for *PIK3CA*-mutant breast cancers, while p110β appears to be particularly important for those with loss of PTEN. Therefore, p110α-specific inhibitors may not be effective in tumors deficient of PTEN.

Early trials of PI3K inhibitors have shown promising activity in

ER+ breast cancer. In a phase 1b study of buparlisib, a pan-PI3K inhibitor, plus letrozole in patients with metastatic ER+ breast cancer refractory to endocrine therapy, 16 of 51 patients (31%) enrolled in the study derived clinical benefit (lack of disease progression, ≥6 months).[19] Buparlisib has also been combined with fulvestrant, with activity observed in a phase 1 trial that enrolled patients with metastatic ER+ breast cancer.[20] The combination of buparlisib and fulvestrant is being evaluated in phase 3 trials, including BELLE-2 (NCT01610284) for AI-resistant, metastatic, ER+ breast cancer, and BELLE-3(NCT01633060) for AI-resistant, metastatic, ER+ breast cancer that has progressed on or after a mTOR inhibitor.

Pictilisib is the first pan-PI3K inhibitor for which results of randomized trials in ER+ breast cancer have been reported.[21,22] OPPORTUNE is a preoperative window study that randomized 75 postmenopausal patients with newly diagnosed, operable, ER+, *HER2*-negative breast cancer at a 2:1 ratio to receive 2-week preoperative treatment with anastrozole plus pictilisib (n = 49, 44 evaluable) or anastrozole alone (n = 26, all evaluable), with the primary endpoint of inhibition of tumor-cell proliferation, as measured by change in Ki67 expression at surgery following 2-week treatment.[21] A higher degree of Ki67 suppression was observed with the combination therapy (83.8%) compared with anastrozole alone (66%; *P* =.004), indicating a superior efficacy of the combination arm.[21] The FERGI phase 2 study of pictilisib plus fulvestrant versus fulvestrant plus placebo in patients with AI-resistant, ER+, advanced or metastatic breast cancer was reported at the 2014 San Antonio Breast Cancer Symposium (SABCS). The median PFS was 5.1 months (fulvestrant + placebo arm) vs 6.6 months (fulvestrant + pictilisib arm; *P* =.0959), which did not differ statistically. However, in the progesterone receptor–positive (PR+) subgroup, the addition of pictilisib to fulvestrant resulted in an improvement of PFS from 3.7 months to 7.4 months (HR = 0.440; 95% CI, 0.281-0.689).[22] In this trial, dose reductions of pictilisib due to skin and gastrointestinal (GI) toxicities were frequent, and few patients experienced the on-target side effect of PI3K inhibition with hyperglycemia, arguing that perhaps the dose of pictilisib might not have been optimal. We await the release of the BELLE2 data, which is anticipated early 2015, and data from other trials of isoform-selective PI3K inhibitors to define the role of various PI3K inhibitors in the treatment of ER+ breast cancer.

TABLE 1. PI3K Pathway Inhibitors in Clinical Trials

Rapalogs
RAD001 (everolimus) CCI-779 (temsirolimus) AP23573 (deforolimus)
mTOR kinase inhibitors
MLN0128 AZD2014 OSI-027 CC-223
Pan-PI3K inhibitors
GDC-0941 (pictilisib) BKM120 (buparlisib) XL147 PX-866 BAY 80-6946 CH5132799
p110α-specific PI3K inhibitors
BYL719 (alpelisib) MLN1117 GDC-0032 (taselisib, p110b sparing, also targets p110g and d)
p110β-specific PI3K inhibitors
AZD8186 SAR260301 GSK2636771
Dual PI3K/mTOR inhibitors
BEZ235 BGT226 XL765 GDC-0980
AKT inhibitors
Perifosine MK2206 XL418 GDC-0068 (ipatasertib) GSK2141795 GSK2110183 AZD5363

Targeting the PI3K Pathway in HER2-Positive Breast Cancer

It is well established that *HER2*-amplified tumors show significant dependence on the PI3K pathway, and the antitumor effect of HER2-targeted agents is at least in part mediated by inhibition of PI3K pathway activity.[23] Preclinical data demonstrated that the presence of *PIK3CA* mutations in *HER2*-positive breast cancer uncouples HER2 and PI3K signaling, rendering tumor cells resistant to HER2-targeted agents such as trastuzumab, while dual targeting of the HER2 and PI3K pathways was effective in overcoming trastuzumab resistance.[23]

However, addition of the mTOR inhibitor everolimus to trastuzumab-containing chemotherapy regimens for the treatment of metastatic *HER2*+ breast cancer has led to disappointing results in clinical trials. In the setting of the treatment of trastuzumab- and taxane-resistant metastatic *HER2*+ breast cancer, addition of everolimus to the combination of trastuzumab and vinorelbine led to a statistically significant but small improvement in median TTP (7.0 months with everolimus vs 5.8 months without everolimus; *P* <.01) in the randomized phase 3 BOLERO-3 tri-

al, reported at the 2014 American Society of Clinical Oncology Annual Meeting.[24] In the first-line metastatic setting, addition of everolimus to the combination of trastuzumab and paclitaxel showed no improvement in PFS (14.95 months with everolimus vs 14.49 months without everolimus) in the phase 3 BOLERO-1 trial, reported at the 2014 SABCS.[25] However, in both trials, the ER-negative (ER-) population derived more benefit, arguing the potential role of everolimus in the ER- population.

Clinical trials of other PI3K pathway inhibitors are under way for the treatment of *HER2+* breast cancer.[26] In a phase 1b trial of buparlisib plus trastuzumab in patients with *HER2+* advanced or metastatic breast cancer that progressed on trastuzumab-based therapy, the combination was well tolerated; at the recommended phase 2 dosage, there were 2 (17%) partial responses and 7 (58%) patients had stable disease (≥6 weeks), suggesting clinical activity.[27] A phase 2 study of this combination is ongoing to define the role of PI3K inhibitors in the treatment of *HER2+* breast cancer.

Targeting the PI3K Pathway in TNBC

The potential role of the PI3K pathway in the pathogenesis of TNBC is supported by the frequent detection of PTEN loss and the evidence of activated PI3K pathway signaling in this subtype of breast cancer in an analysis of The Cancer Genome Atlas (TCGA) samples.[4] In preclinical studies, inhibitors against mTOR and AKT induced growth arrest of patient-derived xenograft (PDX) models of TNBC.[28] In addition, in the phase 1 study of single-agent BKM120, a pan-PI3K inhibitor, a partial response was observed in a patient with TNBC.[29] Further preclinical and clinical studies that define the role of PI3K in TNBC are under way. However, frequent mutations or copy number changes in genes important for cell cycle arrest or apoptosis, such as *RB*, *MYC*, and *TP53*, likely limit the antitumor activity of PI3K pathway inhibitors in this tumor type. Rational combination therapies likely are needed. Of particular interest is the discovery that the PI3K pathway plays an important role in maintaining the genomic stability, and that inhibitors of the PI3K pathway may increase DNA damage and sensitize TNBC to inhibitors of poly ADP ribose polymerase (PARP).[30,31] The combination of inhibitors against PI3K and PARP is being tested in clinical trials for the treatment of advanced TNBC.

Adverse Events of PI3K Pathway Inhibitors

In general, PI3K pathway inhibitors including mTOR, AKT, and PI3K inhibitors have been shown to be well tolerated. Common adverse events (AEs) associated with everolimus include stomatitis, rash, fatigue, GI side effects, pneumonitis, and hyperglycemia. However, most of these AEs are grade 1 and 2. Among these AEs, stomatitis is the most common cause for dose interruption/dosage adjustment. In the BOLERO 2 trial, stomatitis occurred in 59% of patients (8% grade 3).[32] Everolimus-induced stomatitis often presents as aphthous-like ulcers that develop acutely within the first 2 weeks of treatment and quickly resolves with dose interruption. Prophylactic measures to promote good oral hygiene are recommended, including the use of a soft toothbrush that is changed on a regular basis; daily flossing; frequent rinsing with bland rinses such as sterile water, normal saline, or sodium bicarbonate. Avoidance of acidic, spicy, and hard or crunchy foods and alcoholic mouthwash are highly recommended. In addition, close follow-up of patients after initiation of treatment with dose interruption and dosage reduction after resolution is important.[32] In contrast to stomatitis associated with chemotherapy or radiation therapy, everolimus-induced stomatitis has an inflammatory component. The use of topical high-potency corticosteroids (eg, dexamethasone 0.1 mg/mL; clobetasol gel 0.05 %), topical nonsteroidal anti-inflammatories (eg, amlexanox 5 % oral paste), and topical anesthetics ("miracle" or "magic" mouthwashes typically containing lidocaine viscous, diphenhydramine, and an antacid such as aluminum hydroxide or magnesium hydroxide) are recommended.[32,33]

In clinical trials of PI3K inhibitors, common AEs included hyperglycemia, GI toxicity, fatigue, transaminitis, and skin rash.[19,22] Mood disorder was also seen in trials of buparlisib. Hyperglycemia is an on-target side effect of PI3K inhibition, as a result of more sustained inhibition of p110a, and is often manageable with metformin.[19,21,29] It remains to be determined whether isoform-specific inhibitors are more advantageous in widening the therapeutic window of PI3K inhibitors.

Predictors of Response

The identification of genetic predictors of response to PI3K pathway inhibitors has been complicated by the presence of a multitude of genetic or epigenetic alterations of the pathway components and the lack of relevant tumor specimens.[34] Single-gene alterations such as *PIK3CA* mutation have not been predictive of treatment response in studies of everolimus and pan-PI3K inhibitors.[19] In an effort to identify the target population of everolimus, archival tumor specimens from patients enrolled in the BOLERO-2 trial were subjected to targeted next-generation sequencing (NGS) of 182 cancer-related genes. No correlation with PFS was observed with each of the 9 genes with a mutation rate >10% (eg, *PIK3CA*, *FGFR1*, and *CCND1*), or when less frequently mutated genes (eg, *PTEN*, *AKT1*) were included in their respective pathways.[34] However, most of the archival tumor specimens analyzed were obtained from the primary rather than the metastatic disease. Hypothetically, RNA or protein signatures that measure the signaling output of the PI3K pathway could be more informative. However, there is no established clinical assay for this approach at this time. Limited protein markers of PI3K pathway activity were evaluated in the translational study of TAMRAD, in which p4EBP1, LKB1, or PI3K protein expression were found to be predictive of everolimus benefit.[35] Larger stud-

ies are needed to confirm the results. At this time, there are no established biomarkers available for patient selection.

Similarly, in clinical trials of pan-PI3K inhibitors, *PIK3CA* mutation or PTEN status has not been associated with the antitumor response in clinical trials.[19,21,22] Interestingly, in the neoadjuvant pictilisib trial, luminal B cancers derived more benefit from the addition of pictilisib in Ki67 suppression.[21] An exploratory subgroup analysis in the FERGI trial demonstrated that addition of pictilisib to fulvestrant significantly improved PFS in the subgroup of patients with PR+ tumors.[22] However, these results need to be validated in other trials. Perhaps isoform-specific inhibitors are more likely to function in genetically defined populations based on their mechanisms of action.

Combination Treatment Strategies

The antitumor activity of single-agent PI3K pathway inhibitors is likely limited due to the presence of feedback loops within the PI3K pathway and crosstalk between PI3K pathway and signaling pathways. Successful tumor control likely demands combination therapy approaches. Based on preclinical data, potential candidate partners include inhibitors against RTKs, MEK, MYC, PARP, or the STAT3 pathway, and strategies to enhance autophagy and apoptosis (**Table 2**).[18] Inhibition of the PI3K pathway leads to FOXO nuclear accumulation that upregulates the expression of RTK.[36,37] Examples of ongoing clinical trials that target both the RTK and PI3K pathways include HER2-targeted agents in combination with PI3K inhibitors in *HER2+* breast cancer.[38] However, combining RTK inhibitors with PI3K pathway inhibitors may be challenging, as several RTKs could be upregulated. An alternative approach is to combine PI3K pathway inhibitors with inhibitors of the RAS/RAF/MEK/ERK cascade, as both mediate RTK signaling and promote cell survival and proliferation. The extensive crosstalk between these 2 pathways has been demonstrated in preclinical studies. PI3K pathway inhibition activates ERK,[39] while MEK inhibition increases AKT activity.[40] Clinical trials are ongoing to test the tolerability of this strategy.[41] Data indicated increased toxicity with dual blockade of the PI3K and MEK pathways, but treatment could be particularly effective in tumors with genetic alterations in both pathways.[42] Inhibitors of bromodomain and extraterminal domain (BET), which regulate the transcription level of MYC, may be reasonable approaches in tumors with MYC overexpression, as it has been shown to confer resistance to PI3K pathway inhibitors.[43] BCL2 family proteins produce an antiapoptotic effect by maintaining mitochondrial integrity. Preclinical evidence supports the use of BCL2 antagonists, to prime for mitochondrial death, in combination with PI3K pathway inhibitors.[44]

Conclusion

The PI3K pathway is an important therapeutic target in breast cancer. In ER+ breast cancer, initial success has been observed with mTOR inhibitors. In addition, clinical trials of mTOR kinase inhibitors and direct inhibitors of PI3K and AKT, which potentially inhibit the pathway more effectively, are ongoing. However, as single agents, the antitumor activity of PI3K pathway inhibitors is likely limited as a result of feedback regulation and crosstalk with RTK and other signaling pathways. Strategies that combine PI3K pathway inhibitors with inhibitors against RTKs, or inhibitors against MEK, MYC, PARP, or STAT3 pathways, or agents that activate autophagy and apoptosis machineries, are being explored. In addition, there is continued effort to identify resistance mechanisms and predictors of therapeutic response.

TABLE 2. Potential Targets for Combination Therapy With PI3K Pathway Inhibitors

Target for Combination Therapy	Examples
Receptor tyrosine kinases	HER2, EGFR, IGF-1R, FGFR
Estrogen receptor	Hormonal therapy
MEK	MEK inhibitor
MYC	BET inhibitor
PARP	PARP inhibitor
Autophagy	Inducers of autophagy
BCL2	BCL2 inhibitor
STAT3	JAK2 inhibitor

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To Treat or Not to Treat: When Is Adjuvant EGFR TKI Therapy Appropriate?

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Abstract

The use of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) therapy in the adjuvant setting for nonmetastatic *EGFR*-mutated lung cancer remains controversial, as conclusive data to support this practice are lacking. Recent prospective trials suggest an improvement in disease-free survival with these agents; however, an overall survival advantage is yet to be demonstrated. Expedited adjuvant trials are sorely needed to determine whether EGFR TKIs are beneficial for patients with earlier stages of disease.

Key words: Lung cancer, EGFR, adjuvant

Introduction

Roughly 10% to 15% of lung adenocarcinomas diagnosed in the United States harbor activating mutations in the epidermal growth factor receptor (*EGFR*) gene.[1] The remarkable efficacy of small-molecule EGFR tyrosine kinase inhibitors (TKIs) in this unique subset of patients has revolutionized the therapeutic approach to lung cancer over the past 10 years, and has created a treatment paradigm for other molecularly defined subsets of cancer. Multiple randomized phase 3 studies have demonstrated that EGFR TKIs are superior to chemotherapy in patients with stage IV *EGFR*-mutated disease, with excellent response rates (58%-75%) and, on average, a doubling of progression-free survival (PFS).[2-5] Thus, agents such as erlotinib, gefitinib, or afatinib are now standard first-line therapy for advanced non-small cell lung carcinoma (NSCLC) with sensitizing *EGFR* mutations. More recently, the second-generation EGFR TKI afatinib also has shown a significant overall survival (OS) benefit compared with first-line chemotherapy, specifically in patients with *EGFR* exon 19 deletion–positive tumors.[6]

The unprecedented success of these agents in the metastatic setting logically leads to the clinical question: Are EGFR TKIs beneficial as adjuvant therapy for patients with earlier stages of disease? The importance of this question cannot be overstated. Although early-stage lung cancers are treated surgically with curative intent, recurrence rates after complete anatomic resection remain unacceptably high, ranging from 30% to 70%.[7] Tumor recurrence is in fact the primary obstacle to long-term survival. Only 36% to 73% of patients with stage IA-IIB lung cancer are alive at 5 years; for those with stage III disease, the 2-year survival is less than 50% despite definitive therapy.[8] We have learned that adjuvant/neoadjuvant cisplatin-based doublet chemotherapy can marginally improve survival by eradicating occult micrometastases. The Lung Adjuvant Cisplatin Evaluation (LACE) trial,[7] a pooled analysis of 5 large trials (4584 patients), demonstrated a 5-year OS benefit of 5.4% with chemotherapy. This is a fairly modest gain considering the toxicity associated with cisplatin-based chemotherapy, and leaves us in dire need of novel adjuvant approaches to improve cure rates.

Success in Other Tumor Types

The use of molecularly targeted therapies in an adjuvant setting is not unprecedented, and there are lessons to be learned from successes in other tumor types. One example is imatinib mesylate therapy for resected gastrointestinal stromal tumors (GIST) that express constitutively activated mutant isoforms of KIT protein. In a landmark phase 3 trial of 770 patients, adjuvant imatinib demonstrated dramatically improved disease-free survival (DFS) compared with placebo for resected GIST (hazard ratio [HR] = 0.35; P <.0001).[9] More recently, a randomized study showed improved OS in patients with GIST who received 3 (vs 1) years of adjuvant imatinib after resection, with survival curves remaining apart well past the point of TKI discontinuation.[10] A second important example is adjuvant trastuzumab, which significantly improves OS after resection of *HER2*-positive breast cancer, with benefits that exceeded expectations based on its value in the metastatic setting.[11] These experiences provide compelling reasons to investigate the role of targeted agents in the adjuvant management of NSCLC.

Early Clinical Trials of Adjuvant EGFR Inhibition

The earliest exploration of adjuvant EGFR inhibition involved 2 large randomized trials initiated over 10 years ago. Although both were negative studies, it is important to recognize that nei-

ther trial was enriched for patients with *EGFR* mutations. The first was SWOG S0023,[12] a phase 3 study designed to enroll 672 patients with unresectable, locally advanced (stage III) NSCLC receiving definitive chemoradiation. Patients whose disease did not progress after treatment were subsequently randomized to gefitinib 250 mg per day or placebo. However, an unplanned interim analysis (of 243 patients) in 2005 demonstrated an unexpectedly inferior survival for those receiving gefitinib (median of 23 vs 35 months; $P = .013$). The study was thus closed prematurely, and routine use of maintenance EGFR TKIs in stage III disease is not currently recommended outside of a clinical trial.

The National Cancer Institute of Canada (NCIC) phase 3 BR.19 trial[13] was the first randomized, double-blind, placebo-controlled investigation of a targeted agent (gefitinib) delivered in the adjuvant setting for completely resected NSCLC. Patients with stage IB-IIIA NSCLC were randomized, following surgical resection and optional adjuvant chemotherapy, to either 2 years of adjuvant gefitinib or placebo. Unfortunately, this study was also terminated early due to safety concerns based on the negative phase 3, placebo-controlled ISEL trial[14] that demonstrated no survival benefit with gefitinib as second- or third-line treatment for metastatic disease, as well as the aforementioned S0023 interim report.[12] Thus, BR.19 accrued only 503 of the planned 1160 patients, and the median duration of study therapy was less than 5 months. Only 76 patients had *EGFR*-mutated disease (36 in the gefitinib arm, 40 in the placebo arm). An exploratory analysis of this subgroup showed no difference in DFS (HR = 1.22; $P = .15$) or OS (HR = 1.24; $P = .14$), and a trend towards harm with gefitinib. In summary, BR.19 was underpowered, terminated early, and nonenriched for the relevant population, and had suboptimal duration of therapy. It is thus impossible to draw statistically robust conclusions from these data regarding the impact of adjuvant EGFR inhibition in early-stage NSCLC.

Promising Recent Trial Data

Although these initial studies were disappointing, more recently reported data offer promising insights. In 2011, investigators at Memorial Sloan Kettering Cancer Center (MSKCC) retrospectively reviewed a prospectively maintained surgical database of 167 patients with resected stage I-III NSCLC harboring *EGFR* exon 19 or 21 mutations.[15] They compared 2 cohorts—one with 56 patients who received either adjuvant or neoadjuvant EGFR TKI therapy, and the other including 111 patients who did not receive TKI therapy. In the multivariate analysis, which controlled for stage and adjuvant platinum chemotherapy, the 2-year DFS was 89% for the TKI-treated cohort compared with 72% for the control group (HR = 0.53; $P = .06$). Importantly, however, there was no statistically significant difference in 2-year OS. The retrospective nature of this study introduces the possibility of significant bias, as treatment was primarily based on the preferences of patients and their oncologists. This highlights the crucial need for prospective trials.

The first prospective data to suggest that adjuvant targeted therapy may indeed alter the disease course for early-stage NSCLC were presented at the 2014 American Society of Clinical Oncology (ASCO) Annual Meeting, from the SELECT and RADIANT trials. The SELECT trial[16] was a multicenter, single-arm, phase 2 study of adjuvant erlotinib in resected, early-stage, *EGFR* mutation–positive NSCLC. Patients with stage IA-IIIA disease who completed routine adjuvant chemotherapy and/or radiotherapy subsequently received erlotinib 150 mg daily for 2 years, followed by computed tomography (CT) surveillance. The investigators reported a 2-year DFS of 89%, an improvement over the historical control of 76% that was used to power the study. While this is an encouraging result, a considerable drop-off in DFS was seen by 3 years among these highly selected patients, and with the absence of a comparator arm, conclusions cannot be reached regarding true benefit. Of note, the majority of recurrences (25 of 29) were seen after erlotinib discontinuation, raising important questions about the optimal duration of TKI therapy.

Also reported at ASCO 2014 were the results of RADIANT,[17] a phase 3 study investigating adjuvant erlotinib in patients with resected NSCLC with overexpression of EGFR protein by immunohistochemistry (IHC) or *EGFR* gene amplification by fluorescence in situ hybridization (FISH). These 2 selection biomarkers are no longer considered to be of significant value, and only 16.4% of enrolled patients had tumors with activating *EGFR* mutations. After complete resection (stage IB-IIIA) and optional adjuvant chemotherapy, patients were randomized (2:1) to receive either erlotinib 150 mg daily or placebo for 2 years. Posthoc subset analysis of the *EGFR*-mutated population (161 patients; 102 erlotinib, 59 placebo) favored erlotinib, with a median DFS of 46.4 months compared with 28.5 months with placebo (HR = 0.61; $P = .0391$, though not statistically significant due to hierarchical testing).[18] The OS data remain immature, with only 22% of necessary events having occurred, and the median was thus not reached. RADIANT highlights some of the pitfalls of a posthoc analysis using a biomarker for which the study was not stratified, raising concerns about statistical power and validity as well as potential confounders. For example, among the patients with *EGFR* mutations, 30.5% in the placebo arm had stage IIIA disease compared with only 17.6% in the erlotinib arm. Because most stage III patients will recur, this major imbalance likely biases the results in favor of adjuvant therapy.

Additional prospective data come from a recently published, small, randomized phase 2 Chinese study investigating patients with resected stage IIIA (N2) NSCLC harboring *EGFR* mutations (exon 19 deletions or *L858R* point mutations).[19] Sixty patients were enrolled and received adjuvant carboplatin/pemetrexed chemotherapy, then were randomized to either gefitinib 250 mg daily for 6 months or observation. The primary endpoint was DFS, and the study was powered to show a 20% improvement

after 2 years. The results were quite impressive, with a median DFS of 40 months in the gefitinib arm compared with only 27 months with placebo (HR = 0.37; *P* = .014). The 2-year OS was 92% versus 77%, favoring gefitinib (HR = 0.37; *P* = .076), although the survival data remain premature, and this small study was not powered to show OS benefit.

No Improvement in OS

Despite these strides forward, none of the adjuvant studies described in **Table 1** indicate an improvement in OS—the key measure that must be demonstrated for any adjuvant therapy. This leaves us asking a crucial question: Do EGFR TKIs truly eliminate micrometastases (thereby curing the patient), or do they merely suppress minimal residual disease for a period of time? If the latter is true, one could perhaps argue in favor of reserving the targeted agent until the time of relapse, when it could then be offered as rescue therapy.

In fact, a long-standing concern about adjuvant EGFR inhibition is the notion that exposure to erlotinib or gefitinib may alter the tumor's biology, rendering it resistant at the time of recurrence (via a secondary mutation such as *T790M*, or through other mechanisms). This concern was discussed in a small yet thought-provoking 2011 retrospective MSKCC study in which 22 patients with disease recurrence after adjuvant EGFR TKI

TABLE. Summary of Adjuvant Studies of EGFR TKI Therapy in Lung Cancer

Study	No. of Patients	Stage	Biomarker	Adjuvant Regimen	Prior Definitive Treatment	OS	DFS or PFS
SWOG S0023[12]	243 (of a planned 672)	IIIA – IIIB	Unselected	Gefitinib vs placebo for 5 years	Concurrent chemoradiation	Gefitinib arm inferior (P = .013)	NS, trend toward harm in gefitinib arm for PFS (HR = .08; P = .17)
NCIC BR.19[13]	503 (of a planned 1160)	IB – IIIA	Unselected	Gefitinib vs placebo for 2 years	Resection +/- adjuvant chemotherapy	NS, trend toward harm in gefitinib arm (EGFR+ subset)	NS, trend toward harm in gefitinib arm (EGFR + subset)
MSKCC retrospective[15]	167	I – III	EGFR exon 19 or 21 mutation	Erlotinib/gefitinib (adjuvant or neoadjuvant) vs no TKI	Resection +/- adjuvant chemotherapy	NS	TKI superior (HR = 0.53; P = .06)
SELECT[16]	100	IA – IIIA	Sensitizing EGFR mutation	Erlotinib for 2 years (single-arm)	Resection + standard chemo- and/or radiotherapy	Data not mature	Erlotinib superior for 2-year DFS (89% vs 76% for historical control)
RADIANT[17,18]	973 total (161 EGFR+)	IB – IIIA	EGFR+ by IHC or FISH	Erlotinib vs placebo for 2 years	Resection +/- adjuvant chemotherapy	NS	Erlotinib arm superior in posthoc EGFR+ subset (HR = 0.61; P = .0391) (NS due to hierarchical testing)
Chinese randomized phase 2 study[19]	60	IIIA (N2)	EGFR exon 19 del or exon 21 L858R point mutation	Carboplatin/ pemetrexed +/- gefitinib for 6 months	Resection	Gefitinib arm favored, though underpowered and data not mature (HR = 0.37; P = .076)	Gefitinib arm superior (HR = 0.37; P = .014)

DFS indicates disease-free survival; EGFR+, epidermal growth factor receptor mutation–positive; FISH, fluorescence in situ hybridization; HR, hazard ratio; IHC, immunohistochemistry; NCIC, National Cancer Institute of Canada; NS, not significant; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

therapy were identified.[20] Eleven of these patients were retreated, and 8 of the 11 responded for a median duration of 10 months. Furthermore, repeat biopsies revealed the *T790M* resistance mutation only in patients on active therapy—those who had already completed adjuvant TKI therapy did not have tumor genotypes with secondary resistance mutations. This suggests that retreatment with erlotinib/gefitinib at the time of recurrence is feasible, and implies that longer durations of adjuvant therapy may be necessary.

Questions Remain

Many other unanswered questions remain. First, exactly which "early-stage" patients should qualify for adjuvant EGFR inhibition? Much like with adjuvant chemotherapy, the benefit derived from a TKI will be relative to the absolute risk of disease recurrence, and is thus expected to be less for those with lower stages of disease. With this basic principle in mind, an oncologist may be more inclined to offer adjuvant TKIs to patients with high-risk stage III disease than to those with relatively low-risk stage I disease. Caution must be exercised in this regard, however. As previously discussed, the SWOG S0023 experience showed that gefitinib was actually harmful after definitive chemoradiation for locally advanced disease.[12] Ultimately, we lack the data necessary to construct sound treatment guidelines based on relative risk.

Second, what is the optimal duration of adjuvant TKI therapy? Most recurrences in the SELECT and RADIANT trials occurred after discontinuation of erlotinib.[16,17] Although this may suggest a need for more than 2 years of treatment, the aforementioned Chinese adjuvant study demonstrated positive results with only 6 months of adjuvant gefitinib.[19] An ongoing clinical trial using afatinib (NCT01746251) is evaluating 3 versus 24 months of adjuvant therapy for resected stages I-III NSCLC in an effort to determine whether prolonged treatment courses are superior to shorter ones.

Third, adverse events associated with chronic EGFR TKI therapy must also be considered. Although these agents are generally well tolerated, the side effects are certainly not negligible; rash and diarrhea (each occur in 50% of cases) can have significant impact on quality of life for some, and well-informed decisions must be made before exposing patients to these potential risks.

Finally, the cost of therapy remains a key issue. A 2-year course of erlotinib costs approximately $150,000, and there are roughly 10,000 *EGFR*-mutated lung cancer resections per year in the United States alone. If all these patients were to receive adjuvant erlotinib, it would amount to a staggering $1.5 billion healthcare cost. A conclusive demonstration of clinical benefit is necessary before committing to such an expenditure in the Affordable Care Act era.

Phase 3 Trials Needed

The data from RADIANT and SELECT suggest that adjuvant TKI therapy may offer a consistent and significant reduction in the risk of early recurrence for patients with *EGFR*-mutated disease, potentially improving upon adjuvant chemotherapy. However, as encouraging as this may seem, the data are far from conclusive, and phase 3 prospective trials remain necessary. Several such studies are under way, the most pivotal being the ALCHEMIST study, a suite of integrated precision medicine trials that aim to provide definitive answers. Powered for OS, ALCHEMIST will compare 2 years of adjuvant TKI versus placebo therapy for resected, early-stage lung adenocarcinoma, using erlotinib for *EGFR*-mutated or crizotinib for *ALK*-translocated disease. A trial of this importance should have already been completed by now. As it now stands, mature data will not be available for another 10 years—an unacceptably long time to wait.

In the meantime, our focus is quickly shifting to the more specific third-generation EGFR TKIs—agents in development such as AZD9291 and CO-1686—that have shown higher efficacy, more favorable side-effect profiles, and activity in advanced T790M mutation-bearing disease. These inhibitors will almost certainly be available for mainstream use well ahead of the final data analysis from ALCHEMIST. To this end, a randomized study comparing AZD9291 with placebo in the adjuvant setting is planned. We *strongly* support early initiation of such critical adjuvant trials with newer agents in an effort to answer this question in a far timelier manner. Only then can we take the next steps forward to continue improving cure rates for our patients with this deadly disease—namely, to determine whether EGFR inhibition can replace chemotherapy altogether in the adjuvant space, and to investigate targeted and immunotherapy combination approaches.

Conclusion

While adjuvant EGFR TKI therapy may ultimately prove to be beneficial, current data supporting its use remain limited and an OS advantage has not yet been demonstrated. Nonetheless, molecular testing for *EGFR* (and *ALK*) gene mutations should be seriously considered in patients with resected lung adenocarcinomas so that appropriate patients can be offered enrollment in ongoing adjuvant trials whenever possible. Outside of clinical studies, we must have informed and balanced discussions with our patients with *EGFR*-mutated NSCLC regarding adjuvant TKI therapy, carefully weighing the pros and cons in light of the currently limited available data.

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1. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals: writing and editing for biomedical publication. [Updated April 2010.] http://www.icmje.org/urm_full.pdf. Accessed October 5, 2012.

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