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NCCN Task Force Report: mTOR Inhibition in Solid Tumors

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- Identify strategies for the rational combination of mTOR inhibitors
- Summarize the toxicity profiles of these mTOR inhibitors
- Identify subsets of patients who are good candidates for this new class of agent
- Recognize the known toxicities of these agents and strategies to ameliorate them

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NCCN Task Force Report: mTOR Inhibition in Solid Tumors

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NCCN Clinical Practice Guidelines, mTOR, mTOR inhibition, targeted therapy, cancer, malignancy, rapamycin, temsirolimus, everolimus, deforolimus

Abstract

The mammalian target of rapamycin (mTOR) protein complex functions as an integration center for various intracellular signaling pathways involving cell cycle progression, proliferation, and angiogenesis. These pathways are frequently dysregulated in cancer, and therefore mTOR inhibition is a potentially important antitumor target. Commercially available mTOR inhibitors include rapamycin (i.e., sirolimus) and temsirolimus. Other agents under investigation include everolimus and deforolimus. mTOR inhibition has been studied in various solid tumors, including breast, gynecologic, gastrointestinal, prostate, lung, and head and neck cancers. Studies have focused on mTOR inhibition as a monotherapy or in combination with other drugs based on the principle that inhibiting as many targets as possible reduces the emergence of drug resistance. Temsirolimus is currently the only mTOR inhibitor that is specifically labeled for treatment of solid tumors. However, preclinical studies and early-phase trials are rapidly evolving. Additionally, research is further defining the complicated mTOR pathways and how they may be disordered in specific malignancies. To address these issues, NCCN convened a task force to review the underlying physiology of mTOR and related cellular pathways, and to review the current status of research of mTOR inhibition in solid tumors. (*JNCCN* 2008;6[Suppl 5]:S1–S20)

Rapamycin, a natural product identified in the soil of the Easter Island Rapui Nui in the 1970s, has been of medical interest because of its antiproliferative effects, and has been used as a component of immunosuppressive regimens for patients who have undergone solid organ transplantation. In the late 1990s, the mammalian target of rapamycin (mTOR) was identified as a 250 kd serine/threonine protein kinase. Interest in mTOR has since exploded, as further investigation has shown that mTOR is a highly conserved regulatory protein involved in cell cycle progression, proliferation, and angiogenesis.[1] Furthermore, both upstream and downstream mTOR signaling pathways are commonly dysregulated in human cancers, suggesting that the mTOR pathway plays a key role in tumorigenesis and that mTOR inhibition is a potentially important antitumor target.

This report focuses on the biology of the mTOR pathway, its relevance to cancer biology, and current research exploring the role of TOR inhibition in various solid tumors and presents the views of the task force as discussed at the December 7, 2007, meeting.

The mTOR Pathway

Key Points

- The mTOR complex 1(mTORC1) drives cellular growth by controlling various processes that control protein synthesis and angiogenesis.
- Upstream signaling pathways of mTORC1 include the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, which is frequently dysregulated in cancer.
- Inhibition of mTORC1 represents a very attractive antitumor target, either as a monotherapy or in combination with other strategies targeting other pathways.

Major signaling pathways in cancer include the PI3K/Akt pathway; protein kinase C pathway; and mitogen-activated protein kinase/RAS pathway. mTOR has been identified as a key kinase functioning downstream of PI3K/Akt, and thus its inhibition has emerged as a potentially important therapeutic target.

mTOR functions in 2 ways, depending on the associated protein complexes. For example, mTORC1 consists of mTOR, regulatory associated protein of TOR, and various other proteins. mTORC1 is sensitive to both sirolimus and nutrient levels. In contrast, mTOR complex 2 (mTORC2) is associated with sirolimus-insensitive companion of TOR (RICTOR) and, as its name suggests, this complex is relatively insensitive to sirolimus in many model systems. Of the 2, the mTORC1 pathway has been most extensively investigated as an antitumor target, based on both upstream and downstream effects of mTORC1 inhibition by sirolimus and sirolimus analogues. Specifically, mTORC1 drives cellular growth by controlling various processes that control protein synthesis, such as the translation of specific mRNA, the templates for ribosomal proteins and proteins that regulate the G1 to S-phase cell cycle transition (e.g., cyclin D), and angiogenesis. Upstream through the PI3K/Akt pathway, mTORC1 is regulated by various signals, including growth factors, hormones, intracellular energy levels, and hypoxia.[1,2]

The complicated network of mTORC1 signaling pathways is summarized in Figure 1, and the following discussion focuses on the components of the signaling pathway that are believed to be particularly important for tumorigenesis. For the purposes of this discussion, mTOR will refer to mTORC1 unless otherwise specified.

Upstream Signaling Pathways

The complexity of mTOR signaling pathways can be overwhelming, but essentially mTOR functions to integrate growth factor and nutrient signals. Growth factor signaling ultimately activates mTOR, whereas low nutrient availability, such as low glucose or hypoxia, inhibits mTOR. This system ensures that cell proliferation and growth will only occur under favorable conditions. When growth conditions are not favorable, mTOR is not activated and protein synthesis is reduced.

These upstream factors are mediated by the PI3K/Akt pathway.[3] For example, PI3K is initially activated by various growth factors through its interaction with receptor tyrosine kinases, such as the insulin-like growth factor receptor and insulin receptor/insulin receptor substrates (IRS proteins). PI3K can also be indirectly activated by Ras. Activation of PI3K generates phosphatidylinositol 3,4,5 phosphates, which localize the phosphoinositol-dependent kinase 1 and Akt proteins to the plasma membrane, and results in activation of Akt and further signaling downstream. Akt may regulate mTOR directly through an activating phosphorylation or indirectly through an inhibitory phosphorylation of the tuberous sclerosis complex (TSC), consisting of the TSC1 and -2 proteins.

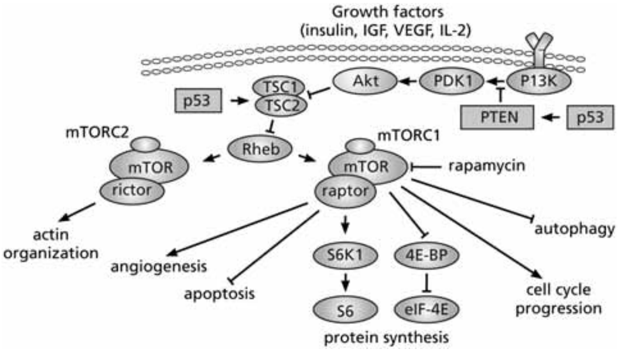

Figure 1 mTOR signaling network.
Abbreviations: IGF, insulin-like growth factor; IL-2, interleukin-2; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RAPTOR, regulatory associated protein of TOR; Rheb, ras homology expressed in brain; RICTOR, sirolimus-insensitive companion of mTOR; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor.
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TSC negatively regulates mTOR through its inhibition of a small protein referred to as Rheb (ras homology expressed in brain), so the inhibitory phosphorylation of TSC by Akt results in conversion of Rheb to an active form that phosphorylates and activates mTOR. It is well-known that mutations in either of the TSC genes can result in tuberous sclerosis, characterized by hamartomatous tumors, perhaps the first discovery linking mTOR to tumorigenesis.

The presence of negative feedback pathways is not surprising in such a complicated network. One negative feedback loop involves S6K proteins, which, when activated by mTOR, phosphorylate IRS-1 and IRS-2. IRS-1 and -2 are activated by insulin and insulin-like growth factor binding to their receptors. The IRS proteins bind and activate the regulatory subunit of PI3K, but phosphorylation by S6K leads to degradation of IRS-1 and -2, and consequently to suppression of PI3K/Akt signaling. Therefore, if mTOR inhibition results in a disruption of the negative feedback loop, it could ultimately be associated with a deleterious effect. For example, in vitro studies have shown that prolonged inhibition of mTOR can lead to enhanced PI3K/Akt activation. However, combined therapies designed to inhibit mTOR and PI3K/Akt pathways may provide a synergistic effect.[4] Other studies have shown that prolonged exposure to sirolimus may lead to tissue-specific Akt inhibition through depletion of mTORC2 (mTOR-RICTOR complex), which normally appears upstream and activates Akt.[5] The relevance of these feedback mechanisms to clinical sensitivity or resistance to mTOR inhibition is unclear.

Aberrant PI3K/Akt activation is commonly observed in tumors because of various causes, including activation of oncogenes, loss of tumor suppressors (e.g., phosphatase and tensin homolog [PTEN]), overexpression of Akt, overexpression or amplification of S6K (a downstream kinase), or mutations of the TSC (Table 1).

For example, PTEN is a tumor suppressor that opposes the action of PI3K, and thus reduces Akt activation. Loss of PTEN function results in activation of Akt, and thus up-regulation of mTOR-dependent pathways that increase cell proliferation and angiogenesis. PTEN loss is seen in many tumors, such as renal cell cancer, hepatocellular cancer, glioma, melanoma, prostate cancer, and endometrial cancer. Inhibition of mTOR can thus interrupt this disordered pathway. Other malignancies associated with activation of the PI3K/Akt pathway include chronic myelogenous leukemia, and breast, pancreatic, and ovarian cancers.[6]

The RAS signaling pathway is also commonly dysregulated in human tumors. This pathway is linked with the mTOR complex through downstream activation of Raf and ERK (extracellular signal-regulated kinase) pathways. The *p53* gene encodes a critical tumor suppressor protein that is activated under conditions that induce cell stress, such as DNA damage and low nutrient levels. This protein is frequently deleted or mutated in human malignancies, and loss of its function is associated with resistance to chemotherapy. Recent evidence indicates that mTOR signaling is inhibited by p53, and loss of p53 function results in mTOR activation.[7]

Downstream Signaling Pathways

mTOR and its protein complexes have several important functions that generally involve coupling growth stimuli to cell cycle progression. One important function of mTOR is the regulation of the protein translational machinery of the cell. Components of this regulation include initiation of mRNA translation, organization of the actin cytoskeleton, ribosome biogenesis, and protein degradation. For example, mTOR activates ribosomal protein S6K and suppresses eukaryotic translation initiation factor 4E binding protein (4EBP-1), ultimately resulting in an increased translation of ribosomal and mRNA. In fact, the phosphorylation status of S6K in the peripheral blood has served as a research tool to assess the activity of the mTOR pathway. The mTOR pathway also regulates proteins that are involved in the progression of the G1 phase and initiation of the S phase of the cell cycle. One of these proteins is cyclin D1, a cell-cycle regulator. Cyclin D1 up-regulation is seen in

Table 1 Abnormalities in the PI3K/AKT/mTOR Pathway Associated with Malignancies

Protein	Dysfunction/Effect	Tumor Type
K-Ras	Mutation resulting in activations	Pancreatic, gastric, colon, lung
Receptor tyrosine kinases	Receptor activation	Many
p110	Gene amplification Gene mutation	Head and neck, ovarian Gastrointestinal, brain
p85	Gene mutation	Colon, ovarian
PTEN	Gene mutation, deletion, or promoter methylation (loss of function)	Endometrial, gliobastoma, thyroid, hepatocellular carcinoma, Cowden disease, prostate
Akt	Gene amplification Protein overexpression	Breast, ovarian, colon Ovarian, breast, renal cell, prostate
TSC1/2	Gene mutation	TSC syndrome
4EBP1 and eIF4E	Gene amplification Protein overexpression	Breast Squamous cell, adenocarcinoma
S6K1	Gene amplification	Breast, ovarian

Abbreviations: 4EBP1, eukaryotic translation initiation factor 4E binding protein; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homolog; TSC, tuberous sclerosis complex.
Adapted by permission from Macmillan Publishers Ltd: Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov 2006;5:671—688, © 2006.

mantle cell lymphoma and breast cancer. Therefore, mTOR inhibition may result in a prolonged G1 phase of the cell cycle, thus producing cytostasis, an effect that may complement cytotoxic agents.

mTOR is also believed to regulate angiogenesis, which is commonly increased in malignancy. The underlying pathways most extensively investigated are those of renal cell cancers that are associated with the loss of von Hippel-Lindau (VHL) tumor suppressor function. The normal VHL protein is considered a master regulator and ultimately destroys the hypoxia-inducible factors (HIF)-1α and HIF-β.[8] In the absence of the VHL protein, tumors overproduce HIF-1α, resulting in an increase in the gene products, including vascular endothelial growth factor (VEGF), platelet-derived growth factors, transforming growth factor, and erythropoietin. HIF also impacts cell proliferation through its regulation of cyclin D levels. mTOR regulates the mRNA translation of HIF; therefore, inhibition of mTOR has antiangiogenic and antiproliferative properties through its regulation of HIF.[9] The antiangiogenic effects may also be mediated through the mTORC2 pathways. mTOR inhibition may have differential effects on endothelial cells compared with tumor cells with respect to Akt activity and pharmacodynamic effects.[10]

As noted in Figure 1, mTOR functions as an integration center for various signaling pathways; thus, inhibition of mTOR can affect multiple upstream and downstream pathways that may be dysregulated in various cancers. Therefore, inhibition of mTOR could reduce cell proliferation, angiogenesis, and metastases, and induce apoptosis. Emerging information on the complex mTOR pathways also suggests that mTOR inhibition could provide synergy with other cancer therapies. For example, antiangiogenic agents, such as bevacizumab, sorafenib, or sunitinib, could complement the downstream antiangiogenic effect of mTOR inhibition. Various tyrosine kinase inhibitors could interrupt the activation of various upstream mTOR signaling pathways, thus complementing direct inhibition of the mTOR complex itself.

Both Raf and ERK are components of RAS pathway, which is frequently dysregulated in tumors. The RAS pathway intersects with the mTOR pathway upstream through inhibition of TSC, and downstream at the level of mRNA transcription. Therefore, dysregulation of the RAS pathway would ultimately contribute to mTOR dysregulation. Sorafenib, a RAF-kinase inhibitor, and CI-1040, a mitogen-activated extracellular kinase (MEK) inhibitor, could thus complement mTOR inhibition.[11]

mTOR Inhibitors

Key Points

- Four mTOR inhibitors are available for clinical trials: rapamycin and 3 derivatives: temsirolimus, everolimus, and deforolimus.
- All 4 drugs are structurally similar; differences are related to route of administration and schedule of administration.
- It is anticipated that mTOR inhibitors will eventually be used as part of combination therapy.

Four mTOR inhibitors are available for clinical trials: the prototype rapamycin (i.e., sirolimus) and 3 derivatives: temsirolimus RAD001 (everolimus), and AP23573 (deforolimus; Figure 2).

Currently, only sirolimus and intravenous temsirolimus are commercially available. Although early studies showed that sirolimus had antiproliferative effects, this drug was not further developed as a cancer therapy because of solubility and stability issues that prevented its use as a parenteral agent, which was then considered an important attribute. However, renewed interest has been shown in sirolimus and is being investigated in several phase I studies for different tumors. Temsirolimus is a sirolimus ester that is rapidly converted to sirolimus and is delivered intravenously. Everolimus is delivered orally, and both oral and intravenous formulations of deforolimus are under development. The schedules and doses of sirolimus analogues used in clinical trials are in Table 2.

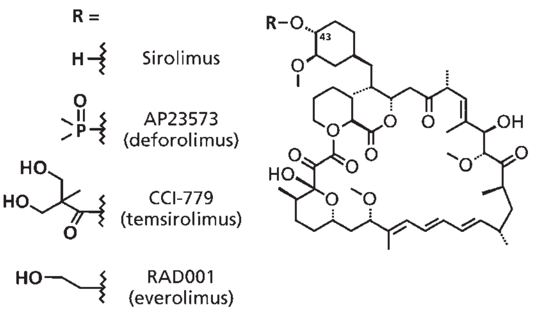

Figure 2 Sirolimus analogs in the clinic.
Adapted from Sehgal SN. Sirolimus: its discovery, biological properties, and mechanism of action. Transplant Proc 2003;35(Suppl 3):7S–14S, © 2003, with permission from Elsevier.

Table 2 Doses and Schedules of Sirolimus and Its Analogues

Drug	Dose and Schedule
Sirolimus[12,13]	Daily oral dosage: 3–6 mg/d Weekly oral dosage: 30–60 mg
Temsirolimus	Daily intravenous dosage x 5: 15 (19.2) mg/d Weekly intravenous dosage: 25–250 mg
Everolimus	Daily oral dosage: 10 mg/d Weekly oral dosage: 50–70 mg
Deforolimus	Daily intravenous dosage x 5: 12.5 mg Weekly intravenous dosage: 50–75 mg Daily oral dosage: 40 mg/d, days 1–5, every week

The pharmacokinetics of the 4 drugs are very similar, and tumor biopsies and studies of peripheral blood cells have shown that the drugs inhibit mTOR at the dosages listed in Table 2, as evidenced by downstream inhibition of the molecular marker S6K. Any differences among them are not related to efficacy, but rather to route of delivery (intravenous vs. oral), schedule, and toxicity. It is important to note that the adverse effects profile of mTOR inhibitors have primarily been assessed in patients treated with monotherapy, but seem to be similar. Dose-limiting toxicities consist of mucositis, asthenia, and thrombocytopenia. Other side effects include reversible cutaneous side effects, such as an acneiform rash. Metabolic abnormalities include hyperglycemia and elevations of cholesterol and triglycerides, which must be monitored during therapy. Infection, weight loss, and anemia are other adverse events.

It is anticipated that mTOR inhibitors will frequently be used in combination with other targeted therapies, based on the basic principle that inhibiting as many targets as possible may reduce the emergence of drug resistance. Therefore, unanticipated adverse effects could emerge because the potential for beneficial additive or synergistic activity will be coupled with a similar potential for deleterious additive or synergistic adverse events. Potential toxicities include significant immunosuppression requiring prophylactic antibiotic therapy or drug interactions based on common metabolic pathways.

Various preclinical studies using cell lines and tumor models have explored the activity of mTOR inhibitors both as a monotherapy and in combination with other agents. Characterization of downstream substrates creates the possibility for sensitive molecular and imaging markers that could be useful in vivo for diagnosis and prognosis and for monitoring the biologic effects of mTOR inhibition (Table 3).[14,15] Biomarkers that could be assessed in the skin, peripheral blood, or tumor itself are key research tools. Peripheral blood biomarkers would be the most convenient because performing multiple biopsies of tumor tissue over time is impractical. However, some early-phase studies of mTOR inhibitors have evaluated patients before and after surgery, with assessment of mTOR inhibition in the biopsy and subsequent post-treatment surgical specimen. Molecular imaging has emerged as an important component of drug development for agents that alter the glycolytic pathways. Additionally, dynamic contrast-enhanced MRI could be used to assess the antiangiogenic effect of mTOR inhibition.

Translational Research

Key Points

- mTOR inhibitors show promising results in preclinical models of breast, head and neck, and prostate cancers in combination with:
    - Chemotherapy; doxorubicin and cisplatin
    - Sorafenib
    - SAHA (suberoylanilide hydroxamic acid), a deacetylase inhibitor

Preclinical studies in cell lines and tumor models have searched for possible synergies with antineoplastic agents. For example, neither cisplatin nor temsirolimus alone are considered active agents in breast cancer, but results of cell line studies suggest that the combination may be more effective. Similarly, some synergism may exist between doxorubicin, which acts in the S phase of the cell cycle, and temsirolimus, which acts during the G1 cycle. Similar studies in cell lines from head and neck cancers have shown a potential synergy between cisplatin and either temsirolimus and everolimus.

Taxanes are another candidate class for combination therapy with TOR inhibition because these agents act at the G2/M phases of the cell cycle. Breast cell lines studies have shown a synergism between temsirolimus and abraxane.

Another consideration is the complicated signaling pathways that could involve activation of

Table 3 Summary of Potential Biomarkers of mTOR Inhibition

Biologic Pathway	Marker	Peripheral Blood	Skin Biopsy	Imaging and Tumor Biopsies
Activation of PI3K/Akt/mTOR	Akt phosphorylation (Serine 473), PTEN	Not done	Akt activity	Akt activity in biopsy
Anabolism/catabolism	S6, S6K1, 4EBP1, LDH	S6, S6K1, 4EBP1, LDH	S6, S6K1	S6, S6K1 in tumor biopsy; 18FDG-scan
Cell cycle control (cytostatic effects)	G1-S arrest ↓ cyclin D Ki-67 reduction	Not done	Not done	↓ cyclin D
Antiangiogenic effect	↑ VEGF ↑ HIF-1α Tumor blood flow	Not applicable	Not done	Vascular density in biopsy; CT vascularity; MRI; Doppler

Abbreviations: 4EBP1, eukaryotic translation initiation factor 4E binding protein; HIF, hypoxia-inducible factor; LDH, lactate dehydrogenase; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homolog; VEGF, vascular endothelial growth factor.

Adapted from Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. Nat Rev 2006;5:671–688; with permission.

negative feedback loops that activate upstream components of the TOR complex. This possibility suggests inhibition of multiple targets in the signaling pathways, such as tyrosine kinase, angiogenesis, or histone deacetylase inhibitors. Figure 3 illustrates potential targets for therapy.

Cell line studies examining different combination therapies are summarized in Table 4. The combination index reflects the synergy between agents; a combination index of 0.3 to 0.7 represents a synergistic effect, 0.7 to 0.9 is a slight synergy, 0.9 to 1.1 is an additive effect, and a value above 1.1 represents an antagonism. The lack of synergy with SAHA or sorafenib for any tumor is notable.

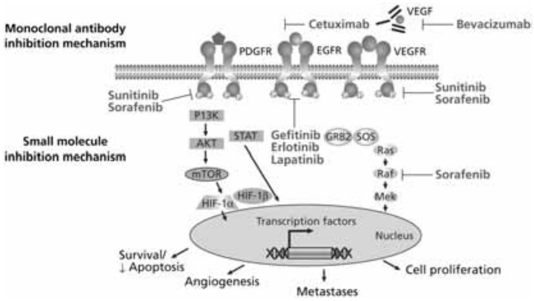

Figure 3 Kinase inhibitors and antibodies combination with mTOR inhibitors in cancer therapy.
Abbreviations: EGFR, epithelial growth factor receptor; HIF, hypoxia-inducible factors; MEK, mitogen-activated extracellular kinase; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; VEGF, vascular endothelial growth factor.
Courtesy of Andrew Zhu, MD, 2008.

mTOR Inhibition

Kidney Cancer
Key Points

- mTOR inhibition has emerged as an important target in the treatment of renal cancer.
- A phase III trial in patients with advanced renal cancer of poor prognosis showed a survival benefit with temsirolimus compared with interferon.
- Preliminary results of a phase III trial have also shown that everolimus is associated with improved progression-free survival compared with best supportive care in patients with metastatic renal disease that has progressed after therapy with either sunitinib or sorafenib.
- Clinical trials exploring tumor characteristics and molecular context are of high priority to identify patients most likely to benefit from mTOR inhibition.

Until recently, systemic treatment options for kidney cancer were limited, consisting of α-interferon or interleukin-2. Over the past several years, options have expanded with the introduction of the tyrosine kinase inhibitors sorafenib and sunitinib, both specifically labeled for use in kidney cancer; bevacizumab was also investigated in kidney cancer. All of these agents function to inhibit VEGF receptor, and sorafenib also inhibits the intracellular kinase activity of Raf, which regulates an upstream pathway of mTOR. In 2007, temsirolimus, an mTOR inhibitor, received FDA approval for use in patients with kidney cancer. Additional mTOR inhibitors in clinical trials

Table 4 Preclinical Studies of mTOR Inhibitors and Other Cancer Therapeutic Agents

Cancer Cell Lines	Drug Combinations	Combination Indices
Breast	Adriamycin and Torisel	0.63
Breast	Cisplatin and Torisel	0.30
Breast	SAHA and Torisel	> 1.0
Breast	Sorafenib and Torisel	> 1.0
Breast	Sorafenib and Torisel	0.9
Breast	SAHA and Torisel	> 1.0
Head and neck	Cisplatin and RAD001*	0.69
Head and neck	Cisplatin and Torisel	0.47
Head and neck	Adriamycin and Torisel	0.89
Head and neck	SAHA and Torisel	0.86
Head and neck	Sorafenib and Torisel	> 1.0
Head and neck	SAHA and Torisel	> 1.0
Pancreas	SAHA and Torisel	> 1.0
Pancreas	SAHA and RAD001*	> 1.0
Pancreas	Sorafenib and Torisel	0.85
Pancreas	SAHA and Torisel	> 1.0
Pancreas	SAHA and RAD001*	> 1.0
Pancreas	Sorafenib and Torisel	> 1.0
Prostate	Sorafenib and Torisel	0.82
Prostate	SAHA and Torisel	0.76

*Everolimus
0.1–0.3: Strong synergism
0.3–0.7: Synergism
0.7–0.9: Slight synergism
0.9–1.1: Additive
1.1–1.2: Slight antagonism
1.2–1.5: Antagonism
> 1.5L Strong antagonism
Abbreviation: SAHA, suberoylanilide hydroxamic acid.

include everolimus (RAD001) and deforolimus (AP23573).

Based on the positive results of a phase I study in patients with kidney cancer, temsirolimus moved to a phase II study in which 111 patients with cytokine-refractory metastatic renal cancer were randomized to receive 1 of 3 doses of temsirolimus, 25, 75, or 250 mg/m^2 intravenously once weekly.[16] Median time to progression was 5.8 months, which compared favorably with the typical 2.5- to 3.0-month time to progression seen with other investigational agents after cytokine therapy. A total of 51% of patients benefited clinically, defined as partial response, minor response, or stable disease lasting at least 24 weeks. A total of 46% of patients had 3 or more poor risk factors,[17] defined as time from diagnosis to first therapy of less than 1 year, performance status of less than 80%, low hemoglobin, or high calcium or lactate dehydrogenase level.

Of particular interest was that patients with poor-risk factors had a median survival of 8.2 months, considerably better than the 4.9 month median survival reported in a previous study of first-line interferon therapy in patients classified as poor risk by a prognostic factor model developed by investigators at Memorial Sloan-Kettering Cancer Center.[17] This observation suggested that temsirolimus as first-line treatment may have a greater benefit than interferon in this group.

Results of another phase I trial of the combination of temsirolimus and interferon-α were reported at the same time as this phase II study.[18] This trial showed the combination was feasible, and these drugs were included as an arm in the subsequent pivotal phase III trial. Additionally, this phase I trial also included a tissue microarray analysis that suggested that Akt activation was more frequently observed in patients with poor prognostic factors, providing a biologic basis for the observed clinical benefit in the poor risk groups.

Given the similar outcomes with the 3 doses of temsirolimus, the lowest dose of 25 mg/m^2 intravenously once weekly was chosen for the subsequent phase III trial.[19] Moreover, no significant differences were seen in the safety profiles for the 3 different doses, although patients receiving the highest doses of 250 mg/m^2 reported the most dose reductions, primarily because of grade 2 fatigue or rash. However, this trial showed that dose escalation is possible, which may be relevant for other diseases.

The phase III trial included 626 previously untreated patients with advanced renal cancer randomized to 1 of 3 arms; a control group receiving escalating doses of interferon-α alone, a treatment arm receiving 25 mg/m^2 of temsirolimus intravenously once weekly, and another arm receiving a lower dose of temsirolimus (15 mg/m^2) intravenously once weekly combined with interferon-α, 6 mcg subcutaneously 3 times per week.[19] Eligibility criteria included advanced or metastatic renal cell cancer and a Karnofsky performance score of 60 or greater. At least 3 of 6 of the following poor prognostic factors were also required:

1) Serum lactate dehydrogenase level of more than 1.5 times the upper limit of the normal range
2) Hemoglobin level below the lower limit of the normal range

3) A corrected serum calcium level of more than 10 mg/dL (2.5 mmol/L)
4) Less than 1 year from initial diagnosis of renal cell carcinoma to randomization
5) A Karnofsky performance score of 60 or 70
6) Metastases in multiple organs

The requirement that all patients have multiple factors predictive of shorter survival was a unique feature of this trial. The inclusion of patients with tumors other than clear cell (e.g., chromophobe, papillary histology) and those who had not undergone nephrectomy (one third of patients enrolled) were additional unique features. The primary end point of the trial was overall survival, with 2 comparisons: temsirolimus compared with interferon, and temsirolimus plus interferon compared with interferon.

The overall survival was significantly greater in the temsirolimus group (median value, 10.9 months) compared with the interferon monotherapy group (median value, 7.3 months). The overall survival of patients receiving the combination of temsirolimus and interferon was not greater than that of patients receiving interferon alone. Possible reasons why the combination therapy group was not associated with significantly longer survival include the lower dose of temsirolimus in the combination arm (15 mg vs. 25 mg weekly for the temsirolimus group) or greater toxicity in this arm.

A planned exploratory subgroup analyses compared the survival advantage of temsirolimus with that of interferon in patients with clear cell and non–clear cell histology; the latter histology constituted 20% to 25% of renal cell carcinomas. The 75 patients with non–clear cell tumors is the largest such population in any randomized trial. In this group, the median overall and progression-free survivals of those receiving temsirolimus (11.6 and 7.0 months, respectively) were significantly better than those receiving interferon (4.3 and 1.8 months, respectively).[20]

Tables 5 and 6 summarize selected adverse effects. Among the grade 3 and 4 toxicities, asthenia was the most common. Temsirolimus was also associated with various laboratory anomalies, including anemia, hyperglycemia, hyperlipidemia, and hypercholesterolemia. The metabolic abnormalities are consistent with mTOR's role in the regulation of metabolism.

Based on the results of this study, temsirolimus received FDA approval with a labeled indication similar to sorafenib and sunitinib (i.e., for the treatment of advanced renal cell cancer). The NCCN Clinical Practice Guidelines in Oncology now recognize temsirolimus as an option for first-line therapy of patients with advanced renal cell cancer with at least 3 of 6 poor prognostic features.

The data on second-line therapy are more limited, but results of the phase II study show that temsirolimus has activity in this setting. The most common scenario for second-line therapy is after failed initial treatment with sunitinib. Currently, sorafenib is typically the next choice of therapy, but it is likely to be minimally effective because sunitinib and sorafenib are tyrosine kinase inhibitors that target VEGF receptors. An ongoing phase II and III study of temsirolimus compared with sorafenib as second-line therapy is designed to address this issue. The targeted enrollment is 476 patients who will be randomized to receive 25 mg weekly intravenous doses of temsirolimus or 400 mg of oral sorafenib twice daily. Patients are stratified according to prognostic factors, nephrectomy status, and time to sunitinib failure. The primary objective of the study is progression-free survival; it is designed to detect a 33% increase in progressive-free survival (i.e., 4 to 5.3 months).

Combination therapy was the next step in the evolution of temsirolimus as a treatment of renal cell cancer. A phase I trial of temsirolimus combined with sunitinib closed after the first 3 patients experienced significant toxicity, including grade 3 groin and axillary acneiform rash and severe thrombocytopenia. The toxicity may be related to unexpected unfavorable pharmacokinetic interactions. The combination could be potentially tolerable at much lower doses of either drug or using sunitinib and temsirolimus in alternate cycles.

An ongoing phase III trial is comparing bevacizumab combined with either temsirolimus or interferon as first-line therapy for advanced renal cell cancer. The trial, with a planned enrollment of 800 patients, is designed to detect a 30% difference in the primary outcome of progressive-free survival. Unfortunately, interferon as a routine component of first-line therapy has been supplanted by sunitinib or sorafenib, and therefore the "control arm" of bevacizumab and interferon is not considered ideal.

Although the ideal outcome would be overall survival, this is difficult to study in trials of renal cell cancer because patients for whom initial assigned therapy fails will presumably transition to alternative therapies. Therefore, the possibility exists that the improved progression-free survival seen with temsirolimus, or

Table 5 Percent of Patients Experiencing Selected Adverse Events in a Phase III Study of Temsirolimus and IFN-α

Phase III Study of Temsirolimus and IFN-α: Selected Adverse Events (% of Patients)

	IFN (n = 200)		Temsirolimus (n = 208)		Temsirolimus + IFN (n = 208)	
Adverse Event	**All Grades**	**Grade 3 or 4**	**All Grades**	**Grade 3 or 4**	**All Grades**	**Grade 3 or 4**
Asthenia	64	26	51	11	62	28
Nausea	41	4	37	2	40	3
Rash	6	0	47	4	21	1
Dyspnea	24	6	28	9	26	10
Diarrhea	20	2	27	1	27	5
Peripheral edema	8	0	27	2	16	0
Vomiting	28	2	19	2	30	2
Stomatitis	4	0	20	1	21	5

Abbreviation: IFN, interferon.
Adapted from: Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007;356:2278. Copyright © 2007 Massachusetts Medical Society. All rights reserved.

any therapy for that matter, will not translate into improved overall survival.

Everolimus (RAD001) is an oral mTOR inhibitor that has been studied in renal cell cancer. A phase II study was conducted in 41 patients with metastatic renal cell cancer who received 10 mg/d in a 28-day cycle.[21] The population was evenly divided among those with and without poor risk factors, or with clear cell or non–clear cell histology. The overall response rate was 32%, with a median progression-free survival of 11.4 months. The toxicities in this trial were similar to those for temsirolimus, although an increased incidence of pnemonitis occurred. This study established the activity of everolimus in renal cell cancer.

Everolimus was then studied in a phase III placebo-controlled, randomized trial of 362 patients with metastatic renal cell cancer who had progressive disease after undergoing sunitinib or sorafenib therapy.[22] Patients were randomized to receive either everolimus or best supportive care. On disease progression, patients in the control arm were able to crossover to the everolimus arm. The median progression-free survival in the RAD001 group was 4.0 months compared with 1.9 months in the placebo group. Of patients in the RAD001

Table 6 Percent of Patients Experiencing Selected Hematologic and Laboratory Abnormalities in a Phase III Study of Temsirolimus and IFN-α

Phase III Study of Temsirolimus and IFN-α: Selected Hematologic and Laboratory Abnormalities (% of Patients)

	IFN (n = 200)		Temsirolimus (n = 208)		Temsirolimus + IFN (n = 208)	
Adverse Event	**All Grades**	**Grade 3 or 4**	**All Grades**	**Grade 3 or 4**	**All Grades**	**Grade 3 or 4**
Anemia	42	22	45	20	61	38
Hyperlipidemia	14	1	27	3	38	8
Hyperglycemia	11	2	26	11	17	6
Hypercholesteremia	4	0	24	1	26	2
Thrombocytopenia	8	0	14	1	38	9
Neutropenia	12	7	7	3	27	15

Abbreviation: IFN, interferon.
Adapted from: Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007;356:2278. Copyright © 2007 Massachusetts Medical Society. All rights reserved.

group, 10% experienced adverse events leading to drug discontinuation versus 4% in the placebo group. These results indicate that RAD001 produces a statistically and clinically significant improvement in progression-free survival with a favorable safety profile. Based on these results, an independent data monitoring committee halted this trial to allow the remaining patients on placebo to receive RAD001. Study follow-up is ongoing to assess the secondary end point of overall survival.

Different combination drug regimens have also been considered for renal cell cancer. Renal cell cancers are frequently characterized by increased activity of VEGF, which can be interrupted either through mTOR inhibition from its down-regulation of HIF, or through direct inhibition by bevacizumab or the receptor kinase inhibitors sunitinib or sorafenib. Therefore, a rationale exists for combining mTOR inhibitors with any of these antiangiogenic drugs. However, an important consideration is that bevacizumab and receptor kinase inhibitors are currently administered near their maximum tolerated dosages, and therefore any combination therapy would likely require dose reductions because of the overlapping toxicities. The key question, then, is which drug should have a dose reduction to permit tolerable toxicities of combined therapy.

A few ongoing early-phase studies are examining the combinations of temsirolimus with bevacizumab or sorafenib. The recently opened BeST study (bevacizumab, sorafenib, temsirolimus) is a 4-arm phase II study that will randomize 360 patients with advanced renal cell cancer to receive bevacizumab as a single agent, bevacizumab plus sorafenib, bevacizumab plus temsirolimus, or sorafenib plus temsirolimus. The results of this study will be used to select the best combination therapy for further study in a phase III trial.

Sequential therapy is another treatment strategy that will be studied in the upcoming STAR trial (sunitinib trial in advanced renal cancer). This phase II study will randomize patients to receive single-agent bevacizumab, temsirolimus, or sunitinib with crossover permitted at tumor progression, with further crossover to the remaining agent at second tumor progression.

Correlative studies of tumor tissue samples have been included in randomized studies of temsirolimus. Tissue correlates of mTOR inhibition can help guide further studies and refine patient selection criteria. For example, patients whose tumors express a high level of phosphorylated S6 or Akt seem more likely to respond to temsirolimus.

Breast Cancer

Key Points

- Among all upstream signaling pathways in the mTOR complex, the PI3K pathway is believed to be the most relevant in breast cancer.
- However, available data have not shown a significant effect of mTOR inhibition in patients with advanced or metastatic breast cancer.

Among all the upstream signaling pathways in the mTOR complex, the PI3K pathway is believed to be the most relevant in breast cancer. An estimated 10% to 50% of breast cancers are associated with mutations affecting this pathway. Additionally, the traditional growth factors and hormone receptors are also believed to funnel through the PI3K pathway. Dysregulation of the PTEN pathway is involved in a smaller proportion of breast cancers, including those associated with Cowden's syndrome.

Several studies have focused on either temsirolimus or everolimus in patients with breast cancer. For example, in a phase II study of 109 patients with heavily pretreated locally advanced metastatic breast cancer who were randomized to receive a weekly dose of either 75 or 250 mg of intravenous temsirolimus, Chan et al.[23] reported no significant differences in response between the groups. An objective response rate of 9.2% was seen, with a median time to progression of 12 weeks. This response rate is similar to that seen with other therapies in patients with refractory disease.

Preclinical studies of cell lines suggested that the antiestrogen therapy letrozole could be synergistic with mTOR inhibition. In 2004, results of a phase II study were presented in which 55 patients with heavily pretreated locally advanced or metastatic breast cancer were randomized to receive letrozole with daily (10 mg) or intermittent temsirolimus (30 mg) for 5 days every 2 weeks.[24] Both dose schedules showed favorable results for tolerability, which supported a subsequent large phase III international study to further evaluate the efficacy.

In this study, 1112 patients with advanced or metastatic breast cancer were randomized in a double-blind fashion to receive either letrozole alone or letrozole combined with intermittent temsirolimus. The therapy represented first-line treatment for many women. Patients were treated until evidence was seen of disease progression or the therapy was no longer tolerated.

Preliminary results were presented in 2006.[25] The treatment groups had similar objective tumor responses and clinical benefit rates. An independent data monitoring committee advised that treatment goals were unlikely to be achieved with continuation of the trial, and the study was stopped. Although the efficacy was disappointing, this large trial provided valuable information on the toxicities of oral temsirolimus. In terms of major toxicities, the oral temsirolimus arm was associated with a slightly increased rate of neutropenia, diarrhea, fatigue, and stomatitis. However, few of these toxicities resulted in dose reductions, and oral temsirolimus was considered feasible for most women.

Everolimus with and without letrozole has also been investigated in breast cancer. Baselga et al.[26] reported on a phase II study randomizing 270 patients undergoing neoadjuvant therapy to receive either letrozole alone or letrozole and 10 mg of daily oral everolimus. The overall response rate in the everolimus arm was 68.1% compared with 59.1% in the letrozole alone arm, a difference that approached statistical significance ($P = .062$). However, the rate of grade 3/4 adverse events was significantly higher in women undergoing combined therapy (22.6%) compared with those treated with letrozole alone (3.8%). The most frequent adverse events included hyperglycemia, stomatitis, interstitial lung disease, infections, and fatigue. Needle biopsies of tumor specimens were mandatory before treatment and after 15 days of therapy for biomarker analysis to be performed. These analyses showed significant changes between the baseline and 15-day biopsies for mTOR biomarkers such as S6, Akt, and cyclin 1, confirming mTOR inhibition.[27]

Another area of research interest is the combination of inhibitors of human epithelial growth factor receptor 2 (HER2)/neu and mTOR because of the interaction of HER2/neu and the PI3K pathway, which ultimately funnels into the mTOR pathway. One phase II study is examining the safety, tolerability, and response rate of the combination of trastuzumab and everolimus in patients who have HER2/neu-positive metastatic breast cancer for whom previous trastuzumab therapy failed.

In summary, available data have not shown a significant effect of mTOR inhibition in patients with advanced or metastatic breast cancer, most of whom have had estrogen-positive postmenopausal breast cancer. These disappointing results have led researchers to question whether intravenous temsirolimus might be better tolerated than oral mTOR inhibition, or whether other breast cancers with different combinations of prognostic factors might be more sensitive to mTOR inhibition.

Head and Neck Cancer

Key Points

- mTOR inhibitors may have clinical activity in head and neck cancers.
- Preclinical data support the combination of mTOR inhibitors with inhibitors of epithelial growth factor receptor (EGFR) and VEGF in a clinical setting.
- mTOR inhibitors may play a role in the adjuvant setting for patients with squamous cell head and neck cancer with high-risk features, such as positive surgical margins.

Cetuximab is currently the only biologic agent that has been specifically FDA-approved for the treatment of head and neck cancer. This approval was based partly on the results of a study comparing radiation therapy with and without cetuximab.[28] However, cisplatin combined with radiation therapy is standard therapy for patients with locoregional head and neck cancer. Therefore, the combination of cisplatin or carboplatin with or without cetuximab is being studied in a phase III randomized study (EXTREME).[29]

Preliminary results of the EXTREME study showed an improved overall survival in patients undergoing combination therapy. Other studies have examined the role of gefitinib and erlotinib in patients with recurrent or metastatic head and neck cancer, with promising results.[30,31] These biologic agents have response rates ranging from 4% to 10%, with median survivals ranging from 6 to 8 months.

mTOR inhibition also has a potential role in head and neck cancer, given that approximately 30% to 50% of squamous cell cancers are associated with alterations in the upstream PI3K, Akt, or PTEN pathways. Several preclinical studies have examined various combination therapies. For example, Jimeno et al.[32] studied 2 squamous cell cancer cell lines, 1 that was resistant to EGFR inhibitors and the other that had intermediate susceptibility to EGFR inhibitors. The cell lines were xenografted in vivo and then treated with either temsirolimus or erlotinib monotherapy, or combination therapy.

Superior growth arrest was associated with temsirolimus therapy in both cell lines. Additionally, tumor cell regression was observed in the cell line with intermediate susceptibility treated with combined therapy. This treatment effect was also reflected in immunohistochemical assessment of the pharmacodynamic effects, which showed abrogation of the mTOR pathways in the susceptible cell lines. The authors concluded that mTOR inhibition shows antitumor activity in EGFR-resistant squamous cell cancer cell lines. Additionally, marked antitumor effects were associated with dual pathway inhibition.

Nathan et al.[33] used a model of minimal residual disease to study temsirolimus as a possible adjuvant therapy. In vitro studies showed the growth inhibitory effects of temsirolimus, and in vivo studies showed a dose-dependent decrease in phosphorylation in S6, indicating mTOR inhibition. Finally, in a minimal residual animal model, temsirolimus was associated with improved survival curves in the treated versus control mice. The authors concluded that the results of this model suggest that temsirolimus is promising as a single agent for long-term treatment of patients with positive surgical margins that are positive for activated downstream mTOR pathways.

Patients scheduled to undergo salvage surgery for recurrent disease represent a convenient group in whom strategies suggested by preclinical studies can be tested. For example, these patients could be treated preoperatively with mTOR inhibition and undergo baseline studies, with follow-up studies performed on surgical specimens. This strategy would allow response data to be collected and correlated with tissue markers, which could help define the most optimal candidates.

Research interest has also been shown in mTOR inhibition as a treatment for thyroid cancer, based partly on reports that the PI3K signaling cascade is a common feature of follicular thyroid neoplasms in animal models.[34]

Gastrointestinal Cancers
Key Points

- Everolimus is in phase III studies as a treatment of neuroendocrine tumors.
- Anecdotal reports and preclinical studies provide the rationale for studying mTOR inhibitors in hepatocellular cancer.
- Minimal data are available on mTOR inhibition in other types of gastrointestinal cancer.

Research interest in mTOR inhibition for treating gastrointestinal malignancies has primarily focused on neuroendocrine cancer. The underlying biologic rationale is the intersection between neuroendocrine tumors and tuberous sclerosis. Tuberous sclerosis is a familial syndrome related to germline mutations in the *TSC* genes, which are involved in the upstream regulation of the mTOR pathway. The condition occurs in 1 in 6000 live births and is characterized by benign tissue overgrowths, angiomyolipomas, and, less commonly, renal cell cancer.

Tuberous sclerosis belongs to the same family as neurofibromatosis, which is also associated with midgut neuroendocrine tumors, such as carcinoid tumors of Vater's ampulla, duodenum, and mediastinum. Familial neurofibromatosis is related to germline mutations in the neurofibromatosis 1 gene (NF-1), whose products also funnel through the TSC complex. For example, loss of function of NF-1 is associated with mTOR activation. These observations formed the basis for early preclinical studies of mTOR inhibition in neuroendocrine tumors.

Subsequently, Duran et al.[35] reported on the results of a phase II study of everolimus and octreotide in 37 patients with advanced islet cell cancer or carcinoid tumor who received intravenous weekly doses of 25 mg of temsirolimus. Correlative tissue studies showed effective inhibition of mTOR, based on the phosphorylation status of S6. Two patients experienced a partial response, whereas 20 patients showed stable disease, which the authors considered evidence of only minimal activity.

Although temsirolimus has not been vigorously pursued as treatment of neuroendocrine tumors, phase II data are available for everolimus in patients with advanced neuroendocrine cancer.[36] A total of 60 patients with either islet cell or carcinoid tumors received depot octreotide and oral everolimus at either 5 or 10 mg/d. The partial response rate was 17%, with stable disease reported in 75% of patients. Of particular interest is the relative independence from dextrose infusions in patients with islet cell tumors producing insulin. Everolimus is being studied in a phase III placebo-controlled trial of patients with advanced neuroendocrine tumors.

A few hints from preclinical data suggest that mTOR inhibition may be active in hepatocellular cancer. For example, anecdotal reports exist of patients who underwent liver transplantation whose recurrent hepatocellular cancer regressed when treated with sirolimus as an immunosuppressant. Preclinical xenograft modeling studies of hepatocellular cell lines have shown mTOR pathway activation, with a marked decrease in tumor burden associated with mTOR inhibition. Additionally, a phase III randomized, placebo-controlled trial enrolling 600 patients showed that sorafenib, which may also indirectly inhibit mTOR, improved survival in advanced hepatocellular cancer.[37]

Gynecologic Malignancies
Key Points

- Endometrial cancer is frequently associated with loss of PTEN activity, and it therefore may be a good candidate for mTOR inhibitor therapy.
- PI3K amplification and PTEN mutations are common in ovarian cancer.
- Any potential role of mTOR inhibition in ovarian cancer must be considered in relation to the standard combination of paclitaxel and carboplatin and to bevacizumab, given the overlapping effects on angiogenesis of both mTOR inhibition and bevacizumab.

Endometrial Cancer: A strong rationale exists for mTOR inhibition as a treatment strategy in endometrial cancer because of the frequency of PTEN mutations in these tumors.[38] Loss of PTEN function is associated with a disruption of the P13K/Akt upstream signaling pathway. Furthermore, preclinical data have shown that mTOR inhibition results in inhibition of endometrial cell lines and tumor development in murine models of endometrial cancer. Oza et al.[39] published results of a phase II trial in 23 patients with metastatic or recurrent endometrial cancer who were treated with 25 mg/wk of temsirolimus. Of the 19 patients evaluable for response analysis, 5 (26%) experienced a partial response and 12 (63%) showed stable disease. Unexpectedly, responses and stable disease seemed independent of PTEN status.

Deforolimus has also been studied in endometrial cancer. In a phase II trial, Columbo et al.[40] treated women with advanced or recurrent pretreated endometrial cancer who received 12.5 mg/d of intravenous deforolimus for 5 days every other week. Of the 27 evaluable patients, 9 (33%) experienced clinical benefit, defined as either a complete response, partial response, or prolonged stable disease. Toxicity included fatigue and anemia in 33%, and mouth sores, nausea, and vomiting in 30%. Sixteen grade III/IV adverse events were reported. The authors concluded that deforolimus showed encouraging single agent activity in this population.

Given the lack of correlation between PTEN function and response, future studies should focus on molecular predictors of response. In addition, combination therapies require further exploration, particularly because chemotherapy with paclitaxel and carboplatin is being used more frequently in patients with advanced endometrial cancer.

Ovarian Cancer: Several lines of evidence indicate a possible role of mTOR inhibition in the treatment of ovarian cancer. PI3K is amplified in an estimated 30% of tumors with serous histology. PTEN mutations are common in ovarian cancer, which may result in tumor insensitivity to cisplatin. The Akt pathway is frequently up-regulated in ovarian cancer, leading to up-regulation of mTOR.

Results from preclinical studies with everolimus are promising. In cell lines, everolimus markedly inhibited cell proliferation with high Akt activation, and also enhanced cisplatin-induced apoptosis in cells with high mTOR activity. In xenograft models, everolimus inhibits tumor growth, angiogenesis, and intraperitoneal dissemination, and was associated with improved survival. When combined with cisplatin, improved therapeutic efficacy is seen.[41] In a transgenic mouse model at risk for the development of serous adenocarcinoma, treatment with everolimus delayed tumor development, suggesting a possible chemoprevention strategy. The mice treated with everolimus that developed tumors had a reduction in ascites.[42]

One research interest is a strategy involving combination therapy of mTOR inhibition with bevacizumab therapy, particularly given that several recent phase II trials have shown that bevacizumab has single-agent activity in patients with platinum-resistant ovarian cancer. For example, Burger et al.[43] treated 62 patients with persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer with single-agent bevacizumab. The overall response rate was 21%, median response duration was 10 months, and progression-free survival at 6 months was 42%. This progression-free survival is superior to that associated with cytotoxic drugs in similar patient populations.

Two other phase II studies have concluded that bevacizumab is an active agent in ovarian cancer.[44,45] Studies of bevacizumab monotherapy in other tumors have generally reported limited activity, so the response rates in ovarian cancer are a little surprising. One possible explanation is that bevacizumab may be particularly effective in treating malignant ascites, which is a feature of ovarian cancer.

The combination of carboplatin/paclitaxel with or without bevacizumab as a first-line treatment or maintenance therapy of stage III or IV ovarian cancer is now being studied in a randomized phase III trial. Given the strongly positive results of the phase II trials, bevacizumab is considered likely to because a standard component of ovarian cancer therapy. Therefore, any potential role of mTOR inhibition in ovarian cancer must be considered in relation to not only the standard combination of paclitaxel and carboplatin but also bevacizumab, given the overlapping effects on angiogenesis of mTOR inhibition and bevacizumab.

Lung Cancer
Key Points

- mTOR inhibition as monotherapy in lung cancer has minimal activity.
- Research interest has been shown in combination mTOR inhibition and chemotherapy.
- mTOR inhibition may improve EGFR tyrosine kinase efficacy in lung cancer in all populations.
- mTOR inhibition may augment tumor responses with irreversible, dual EGFR inhibitors or other novel target agents (i.e., EGF1R inhibitors, PI3K inhibitors, Akt inhibitors, MEK inhibitors).

Non–Small Cell Lung Cancer: Although PTEN mutations are rare in lung cancer, the PI3K/Akt pathway is frequently activated. Preclinical studies have shown that overexpression of phosphorylated Akt is associated with poor-prognosis lung cancer.[46] Constitutive Akt pathway activation results in decreased tumor sensitivity to chemotherapy, radiation, and EGFR inhibition.[47,48] Furthermore, mTOR inhibition can sensitize lung cancer cells to chemotherapy and radiation.[49,50]

EGFR inhibition with erlotinib has shown improved survival and has emerged as a standard treatment for non–small cell lung cancer (NSCLC).[51] Tumors with EGFR or *kRAS* gene mutations may have increased mTOR pathway activation in NSCLC.[52] Additional interest has been shown in combining EGFR inhibition therapy with mTOR inhibition based partly on the hypothesis that EGFR and mTOR inhibition can work synergistically to down-regulate Akt activity.[53] For example, mTOR inhibition as a monotherapy triggers rapid and sustained initiation of PI3K/Akt pathways through feedback mechanisms. PI3K/Akt pathways may be inhibited by combined mTOR and EGFR inhibition, suggesting a role for combination therapy. Additionally, combining mTOR inhibition with novel dual EGFR inhibitors may overcome acquired erlotinib resistance.[49]

Several lines of preclinical studies support the role of mTOR inhibition in early-stage lung cancer. For example, CXC chemokine receptor 4 (CXCR4) is a cell surface receptor that has been shown to mediate metastasis in many solid tumors. In patients with advanced lung cancer, high levels of circulating CXCR4+/pancytokeratin cells are associated with decreased overall survival. In a study of lung cancer tumor cells, both hypoxia and EGFR activation induced CXCR4 expression, thus potentially increasing their metastatic potential. CXCR4 expression leads to invasion and metastasis. This process is mediated by the PI3K/PTEN/Akt pathway, and is thus modulated by mTOR.[54] Therefore, a combination of low oxygen tension, which stimulates the HIF pathway, and EGFR activation may ultimately promote metastasis. These processes can be interrupted by mTOR or EGFR inhibition, and may be most effectively blocked by the combination.

This research has prompted a clinical study to evaluate CXCR4 as a potential marker of micrometastatic disease in patients with resectable stage I through IIIA NSCLC. Patients will receive either erlotinib or temsirolimus for 2 weeks before surgery, with imaging and biomarker analysis performed before and after surgery to determine the levels of CXCR4 expression and whether CXCR4 can be blocked by either erlotinib or temsirolimus.

Other clinical studies have examined mTOR inhibition in patients with advanced NSCLC. One phase II study enrolled 85 patients whose disease had progressed on platinum-based therapy with or without EGFR inhibitors.[55] Patients received 10 mg/d of everolimus. Among patients who had and had not previously received an EGFR inhibitor, partial remissions were experienced in 3% and 5%, respectively. The median progression-free survivals were 11.3 and 9.7 weeks, respectively. The most common grade 3/4 adverse events were fatigue, mucositis, and dyspnea.

The authors concluded that everolimus monotherapy was minimally active in pretreated advanced NSCLC and that combination therapy is a more promising approach. A phase I study of everolimus and gefitinib in advanced NSCLC evaluated the safety and maximum tolerated dose of the combination. Everolimus at 5 or 10 mg/d in combination with gefitinib at 250 mg/d was administered to 10 patients. In the cohort receiving the 10 mg dosage, 2 patients experienced dose-limiting toxicity (grade 5 hypotension and grade 3 stomatitis), 8 were evaluable for response and 2 experienced partial responses. Based on this study, the recommended phase II dose was determined to be 5 mg of everolimus combined with 250 mg of gefitinib daily.

The preliminary results of a phase II study of the combination of everolimus and gefitinib in 25 patients with advanced NSCLC were presented in 2007.[56] All enrolled patients were former smokers, a group less likely to respond to gefitinib. The partial response rate was 17%, which was considered promising. Currently, a wide variety of ongoing phase I and II trials are examining mTOR inhibition as a first-line therapy, neoadjuvant therapy, or treatment for advanced disease, either alone or in combination with chemotherapy (i.e., carboplatin, paclitaxel, pemetrexed, docetaxel) or EGFR inhibitors (i.e., erlotinib, gefitinib, sunitinib, sorafenib).

Small Cell Lung Cancer: An interest has been shown in evaluating mTOR inhibition in small cell lung cancer (SCLC), partly because SCLC typically exhibits neuroendocrine features. Temsirolimus was investigated as a potential consolidation therapy in 87 patients with SCLC that was either stable or responding to initial induction chemotherapy.[57] Patients were randomized to receive either 25 or 250 mg of intravenous temsirolimus every week until disease progression or unacceptable toxicity. The primary end point was progression-free survival.

Overall median survival and 1-year progression-free survival rate were 2.2 months and 4.7%, respectively, and no significant difference was seen in progression-free survival between the groups. Median survival was 8 months for all patients. A total of 42% of patients experienced grade 3 toxicities, the most common of which were thrombocytopenia, hypophosphatemia, and fatigue. The incidence of toxicity was higher in the group receiving 250 mg. An additional 12% of patients had grade 4 toxicities, the most common of which was neutropenia. These results did not meet the primary end point, and further investigation of temsirolimus as maintenance therapy for SCLC will not be pursued. Ongoing studies of mTOR inhibition in SCLC include evaluations as monotherapy in relapsed disease and in combination with chemotherapy as first-line therapy.

Prostate Cancer

Key Points

- Sirolimus and its derivatives have growth inhibitory and antiangiogenic activity in prostate cancer models.
- mTOR inhibition can reverse Akt-dependent prostate intraepithelial neoplasia phenotype, glycolysis, survival, and hypoxic response.
- PTEN loss and/or Akt activation may confer sensitivity to mTOR inhibition.

Preclinical Studies: Despite decades of research, treatment for castration-resistant metastatic prostate cancer is limited; only docetaxel has been shown to be associated with a survival benefit in metastatic prostate cancer.[58] Therefore, keen interest has been shown in understanding the underlying biology of castration-resistant growth and developing new therapeutics for recurrent and/or metastatic disease. PTEN loss occurs in 10% to 15% of localized and 30% to 60% of metastatic prostate cancers. This loss correlates with a high Gleason score, advanced stage of disease, and androgen independence.[59–62] Both PTEN loss and resulting Akt activation seem to be involved in the emergence of castration-resistant growth, suggesting a possible role for mTOR inhibition. In a cancer cell model, Trotman et al.[63] found that the extent of PTEN inactivation dictates progression of prostate cancer in a dose-dependent fashion. The dose of PTEN affects key downstream targets, including the FOXO transcription factor, which may then regulate the androgen receptor. PTEN may also regulate DNA stability, thus impacting other chromosomal abnormalities associated with aggressive cancer.[64]

Akt activation has been studied as a prognostic factor for prostate cancer. Ayala et al.[65] studied tissue microarrays consisting of prostate tissue from 640 radical prostatectomy specimens. Tissue samples were selected representing nonneoplastic prostate, hyperplastic, and cancerous tissue stained with antibody to phosphorylated Akt.[65] Increased cytoplasmic Akt correlated with prostate-specific antigen (PSA) relapse after surgery in a multivariate analysis.

A subsequent paper examined PTEN expression and Akt activation in tissue from men undergoing radical prostatectomy who were followed up for 5 years for biochemical recurrence.[66] Combined analysis of PTEN and Akt was predictive of biochemical recurrence. Specifically, 90% of patients whose primary tumor had high Akt expression and were negative for PTEN developed recurrent disease, whereas 88% of those whose tumors had low Akt expression and were positive for PTEN did not. The authors concluded that loss of PTEN expression, together with increased Akt activation and Gleason score, is of significant prognostic value for determining the risk for biochemical progression at prostatectomy. Collectively, these results suggest that PTEN loss and/or Akt activation may be an important molecular event in progression of prostate cancer from localized to metastatic disease.

In vitro studies have suggested that PTEN loss is associated with sensitivity to mTOR inhibition.[67] Preclinical studies of mTOR inhibition in prostate cancer cell lines have shown less-pronounced effects than in tumor xenograft models. For example, in one preclinical study, additive effects of mTOR inhibition with docetaxel were only seen in xenograft models, particularly models with PTEN loss. This pattern of results suggests that mTOR inhibition may actually be mediated more through an antiangiogenic effect on the tumor architecture, which is not assessed in cell lines.[68]

In another study using a mouse model, Majumder et al.[69] showed that treatment with everolimus (RAD001) resulted in decreased activation of the downstream mTOR marker S6 and also reversed the noninvasive prostatic intraepithelial neoplasia phenotype. However, the effects on reducing proliferative rates and apoptosis induction were reduced if the tumor overexpressed BCL-2. The authors then undertook a gene set enrichment analysis to determine which pathway was preferentially inhibited by everolimus. The most significant pathway was that of HIF, which then regulated VEGF and metabolic pathways related to glycolysis. The link between mTOR inhibition and glycolysis shows how glucose uptake, as imaged with FDG-PET scans, may provide a mechanism to assess or predict for clinical benefit with mTOR inhibition in vivo.

Clinical Studies: Various clinical studies have tried to define the optimal dose for different mTOR inhibitors. In phase I studies of temsirolimus, the maximum tolerated dose ranged from 25 to 250 mg/wk intravenously, indicating a wide therapeutic margin. Other studies have attempted to identify optimal candidates for mTOR inhibition by exploring biomarkers, such as PTEN loss, Akt activation, or phosphorylation of the S6 ribosomal protein.[70]

Thomas et al.[71] presented results of a study in which patients were given temsirolimus before undergoing a radical prostatectomy at 3 different oral doses for 4 weeks. The doses ranged from 1 to 15 mg/d. The temsirolimus was well tolerated; only 5 grade 3 and no grade 4 toxicities occurred. Four patients discontinued therapy because of toxicity. The 15-mg dosage arm was expanded to an additional 21 patients based on tissue correlates of S6 activity suggesting that this dose produced the optimal pharmacodynamics. A 75% reduction in S6 phosphorylation occurred in patients with PTEN loss, whereas those who were PTEN–positive experienced only a 21% reduction.

Other preprostatecomy studies of RAD001 and sirolimus are ongoing to determine the optimal dose based on pharmacodynamic analysis. The biologically optimal dose may also vary for different tumor types or molecular profiles. In addition, although preclinical studies have reported various lineage-specific effects of mTOR inhibition in signaling pathways, such as Akt up- or down-regulation, the clinical significance of these effects remain to be determined.[4,5]

Designing phase I and II studies of mTOR inhibition in prostate cancer has many challenges. For example, access to tissue in metastatic prostate cancer is limited, which has previously limited the ability to conduct direct correlative studies. In terms of clinical outcomes, few biomarkers other than PSA can be used to assess intermediate outcomes. Furthermore, traditional response measures of cancer (i.e., tumor size, bone scan changes) have limited sensitivity in prostate cancer.

The optimal end points for phase II studies, such as PSA decline, progression-free survival, or overall survival, are unclear. In an ongoing phase II single arm study in prostate cancer, open-label everolimus (RAD001 at 10 mg/d) is given to patients with metastatic castration-resistant prostate cancer. Tumor biopsies are performed before and during treatment with everolimus, with treatment continuing until disease progression. End points include biochemical (PSA and lactate dehydrogenase levels), radiographic (objective response and time to progression), pathologic (50% increase in apoptosis, 50% decrease in proliferation), genomic (expression profiles of Akt), and genetic (evaluation of PTEN status) findings. One objective of this study is to identify molecular predictors of clinical and pathologic response to mTOR inhibition.

As of January 2008, 19 patients have been evaluated and preliminary data suggest that everolimus is well tolerated at a dosage of 10 mg/d. CT-guided bone biopsies have been feasible, with a 60% to 70% evaluable tissue yield for pathologic correlative studies. Although PSA levels and radiographic studies have shown stable disease in some patients, no evidence has shown objective or PSA responses. The mean time to clinical (non-PSA) progression is 85 days, which compares favorably with other second-line agents in this setting.[72] A second phase II study of everolimus and docetaxel with prednisone is ongoing through the Department of Defense Prostate Cancer Clinical Trials Consortium.

Strong interest has been shown in combination therapy with mTOR inhibition. Proposed strategies or ongoing studies include combinations with:

- Histone deacetylase inhibitors, in which complementary action is based on HIF modulation, PTEN restoration, and antiangiogenic effects
- EGFR, insulin-like growth factor receptor, HER, and PI3K inhibitors to inhibit parallel or serial signal transduction pathways implicated in the progression to castration-resistant cancer
- Current or novel antiandrogen strategies to modulate androgen receptor dependence of PTEN loss and progression to castration-resistant cancer
- Antiangiogenic agents
- Docetaxel or radiation therapy as chemo- or radiation sensitization

Future Directions of mTOR Inhibitors

Although various phase II and III trials of TOR inhibitors are ongoing in different cancers at different stages, study of mTOR inhibition is still in its infancy. Preclinical research continues to focus the following key aspects of the mTOR pathway to identify the optimal design of subsequent clinical trials:

- Identification of cancers or cancer subtypes that may depend on the mTOR pathway.
- Clarification of the mechanism of action of mTOR inhibition in different tumors. For example, in kidney cancer, mTOR inhibition might act primarily through its angiogenic effects but may have a more antiproliferative effect in tumors with PTEN loss, such as endometrial cancer. Grouping different types of cancer together based on common biologic/molecular parameters may facilitate extrapolating data across histologic tumor types.
- Investigation of combination therapy with cytotoxics, targeted therapies, biologic agents, and radiation therapy, based on hypothesis generating preclinical studies.
- Identification of predictive patient- and tumor-related biomarkers that could be used as a basis of individualized therapy.
- Identification of biomarkers and molecular imaging that can be used to assess mTOR inhibition.

Despite the exciting clinical potential of mTOR inhibition, individual tumor growth may be associated with varying activation of different components of the complex mTOR pathways, and some mTOR substrates may be insensitive to mTOR inhibition. Additionally, depending on the molecular context, mTOR inhibition can result in either cytostasis or apoptosis. Furthermore, the role and activation of mTORC2 pathways that are insensitive to sirolimus are still poorly understood. Therefore, until the tools are available to identify the exact pathways that are disordered in individual tumors, the clinical effect of mTOR inhibition on any individual tumor and the optimal dose may be difficult to predict.

The emergence of multiple new targeted therapies has made clinical trials using overall survival as an end point difficult to design. For example, patients whose disease progressed after one trial of a tyrosine kinase inhibitor may then move to second-line therapy with another, and this type of crossover at disease progression makes overall survival associated with a single regimen very difficult to assess. Therefore, progression-free survival has emerged as a common primary outcome in trials involving mTOR inhibitors and tyrosine kinase inhibitors. Given the cytostatic and antiangiogenic mechanisms of mTOR inhibitors, measures of progression delay may be more reflective of the activity of these agents in a given tumor than traditional response criteria. The use of these end points (progression-free survival, time-to-progression) may facilitate a comparison of combination strategies with and without an mTOR inhibitor. The integration of potential predictive biomarker and molecular imaging studies will also be essential to provide enrichment of those tumor types that may be more sensitive to targeting of mTOR.

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Post-test Please circle the correct answer on the enclosed answer sheet.

1. Which of the below statements regarding why mTOR inhibition is a potentially important antitumor target is/are TRUE?
   a. mTOR functions as an integration center for various signaling pathways.
   b mTOR regulates angiogenesis.
   c. mTOR and its protein complexes generally involve the coupling of growth stimuli to cell cycle progression.
   d. All of the above are true.

2. Which of the following statements regarding the differences between mTORC1 and mTORC2 is/are TRUE?
   a. mTORC2 is sensitive to both sirolimus and nutrient levels; mTORC1 is not.
   b. mTORC1 has been more extensively investigated as an antitumor target than mTORC2.
   c. mTORC2 is a relatively straightforward signaling pathway, while mTORC1 is involved in multiple overlapping pathways.
   d. All of the above are true.

3. Which statement does NOT accurately characterize the PI3/Akt pathway?
   a. The PI3/Akt pathway is an upstream signaling pathway of mTOR.
   b. The PI3K/Akt pathway can be activated by various growth factors through its interaction with receptor tyrosine kinases.
   c. PI3 cannot also be activated by Ras.

4. Which of the below is NOT a mechanism resulting in aberrant PI3K/Akt activation?
   a. activation of oncogenes
   b. under-expression of S6K, a downstream kinase
   c. loss of tumor suppressors
   d. mutations of the tuberous sclerosis complex

5. Which of the following statements about the mTOR inhibitors under investigation for clinical use is/are TRUE?
   a. All of the drugs (siroliums, temsirolimus, everolimus, and deforolimus) are available in both oral and intravenous formulations.
   b. None of these agents are commercially available yet.
   c. Sirolimus has been extensively studied as an anticancer agent.
   d. The pharmacokinetic profiles of the drugs are similar, and differences are primarily related to route of delivery, schedule, and toxicity.
   e. None of the above is true.

6. Which of the following statements regarding biomarkers for mTOR activity is/are TRUE?
   a. Measurement of downstream substrates of mTOR could be used to assess mTOR activity.
   b. Biomarkers could be potentially assessed in either the skin, peripheral blood, or in the tumor itself.
   c. Peripheral blood biomarkers would avoid the necessity of multiple tumor biopsies to assess mTOR activity.
   d. Molecular imaging is another potential technique for assessing biomarker activity.
   e. All of the above are true.

7. Which of the following statements regarding temsirolimus as a treatment of renal cell cancer is/are TRUE?
   a. The pivotal phase III trial of temsirolimus focused on patients with early-stage renal cancer.
   b. The overall survival for patients receiving temsirolimus alone was superior to that of patients receiving interferon.
   c. In the subset of patients with non–clear cell histology, overall survival was lower in the group receiving temsirolimus.
   d. All of the above are true.

8. Which of the following regarding everolimus as a treatment of renal cell cancer is/are TRUE?
   a. Positive preliminary results are available from a randomized study comparing everolimus and best supportive care in patients with metastatic renal cell cancer that progressed after either sunitinib or sorafenib treatment.
   b. Overall survival was markedly improved in patients receiving everolimus.
   c. Everolimus was poorly tolerated.
   d. All of the above are true.

9. Which statement regarding mTOR inhibition and breast cancer is TRUE?
   a. Most patients with breast cancer have aberrant PTEN pathways.
   b. Temsirolimus has shown very promising results in patients with refractory metastatic breast cancer.
   c. Currently available data have not shown a significant effect of mTOR inhibition in patients with advanced or metastatic breast cancer.
   d. Everolimus combined with letrozole is very well tolerated.

10. Which of the following statements regarding mTOR inhibition and head and neck cancer or gastrointestinal cancer is/are TRUE?
    a. Approximately 30% to 50% of squamous cell cancers of the head and neck are associated with abnormalities in the upstream PI3K/Akt/PTEN pathways, suggesting a role for mTOR inhibition.
    b. Research interest in mTOR inhibition as a treatment of gastrointestinal cancer has focused on neuroendocrine tumors.

c. Encouraging phase II data of everolimus in patients with advanced neuroendocrine cancer has led to a phase III placebo controlled trial.
d. All of the above are true.

11. Which of the following statements regarding mTOR inhibition in gynecologic cancers is TRUE?
    a. A strong rationale exists for mTOR inhibition in endometrial cancers because of the frequency of PTEN mutations in these tumors.
    b. Most ovarian cancers are also associated with PTEN mutations.
    c. Any potential role of mTOR inhibition in ovarian cancer must be considered in relation both to the standard combination of paclitaxel and carboplatin, but also in relation to bevacizumab, given the overlapping effects on angiogenesis of both mTOR inhibition and bevacizumab.
    d. Preclinical results of monotherapy with mTOR inhibition have shown no activity.

12. Which of the following statements regarding mTOR inhibition and lung cancer is TRUE?
    a. Temsirolimus has been widely studied in patients with non–small cell lung cancer.
    b. In phase II trials, everolimus monotherapy has shown impressive results in patients with advanced non–small cell lung cancer.
    c. Preclinical data support combining epithelial growth factor receptor inhibition therapy with mTOR inhibition in patients with non-small cell lung cancer, because both of these agents may work synergistically to downregulate Akt activity.
    d. Temsirolimus has been shown to improve progression-free survival as a consolidative therapy for patients with small cell lung cancer.

Post-Test Answer Sheet

Please circle one answer per question. A score of at least 70% on the post-test is required.

1.	a	b	c	d		7.	a	b	c	d
2.	a	b	c	d		8.	a	b	c	d
3.	a	b	c			9.	a	b	c	d
4.	a	b	c	d		10.	a	b	c	d
5.	a	b	c	d	e	11.	a	b	c	d
6.	a	b	c	d	e	12.	a	b	c	d

Please evaluate the achievement of the learning objectives using a scale of 1 to 5.
(1 = Not met; 3 = Partially met; 5 = Completely met)

Outline the mechanism of action of mTOR inhibitors in solid tumors.
1 2 3 4 5

Identify strategies for the rational combination of mTOR inhibitors.
1 2 3 4 5

Summarize the toxicity profiles of these mTOR inhibitors.
1 2 3 4 5

Identify subsets of patients who are good candidates for this new class of agent.
1 2 3 4 5

Recognize the known toxicities of these agents and strategies to ameliorate them.
1 2 3 4 5

Please indicate the extent to which you agree or disagree with the following statements:
(1 = Strongly disagree; 3 = Not sure; 5 = Strongly agree)

The material was presented in a fair and balanced manner.
1 2 3 4 5

The information presented in this monograph was pertinent to my educational needs.
1 2 3 4 5

The information presented was scientifically rigorous and up-to-date.
1 2 3 4 5

The information presented in this monograph has motivated me to modify my practice.
1 2 3 4 5

I would recommend this monograph to my colleagues.
1 2 3 4 5

NCCN Task Force Report:
mTOR Inhibition in Solid Tumors

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