Exploiting novel molecular targets in gastrointestinal cancers

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Abstract

Novel molecular targets are being discovered as we learn more about the aberrant processes underlying various cancers. Efforts to translate this knowledge are starting to impact on the care of patients with gastrointestinal cancers. The epidermal growth factor receptor (EGFR) pathway and angiogenesis have been targeted successfully in colorectal cancer with cetuximab, panitumumab and bevacizumab. Similarly, EGFR-targeting with erlotinib yielded significant survival benefit in pancreatic cancer when combined with gemcitabine. The multi-targeting approach with sorafenib has made it the first agent to achieve significant survival benefit in hepatocellular carcinoma. Efforts to exploit the dysregulated Akt/mTOR pathway in GI cancer therapy are ongoing. These molecular targets can be disrupted by various approaches, including the use of monoclonal antibody to intercept extracellular ligands and disrupt receptor-ligand binding, and small molecule inhibitors that interrupt the activation of intracellular kinases.

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Key words: Colorectal; Pancreatic; Liver cancers; Targeted therapy; Epidermal growth factor receptor; mTOR; Angiogenesis

Ma WW, Hidalgo M. Exploiting novel molecular targets in gastrointestinal cancers. World J Gastroenterol 2007; 13(44): 5845-5856

http://www.wjgnet.com/1007-9327/13/5845.asp

INTRODUCTION

Cellular proliferation, differentiation and death are regulated by a number of extracellular factors, such as hormones, cytokines and growth hormones. Interactions between extracellular stimuli and the nucleus is mediated by a complex and interconnecting network of signaling pathways. This process is often abnormal in cancer cells and our understanding of these molecular events led to the identification of novel targets for therapy development. Various approaches are been used to target these dysfunctional elements, including ligand neutralization, disruption of receptor binding, and inhibition of receptor kinases and intracellular signal messengers.

A plethora of compounds are now under development that targets these aberrant processes. Almost all of these biological agents have limited single agent activity but are synergistic when combined with conventional cytotoxic agents. Therefore, they are usually tested in combination with standard therapy in specific cancer types. In colorectal cancers, fluorouracil-based regimens form the backbone of therapy in both adjuvant and metastatic settings. Likewise, gemcitabine based therapy remains the cornerstone for untreated advanced pancreatic cancer and sorafenib is likely to become the standard therapy for hepatocellular carcinoma.

Successful targeting of angiogenesis and the epidermal growth factor pathway has made colorectal cancer a prototypical model for the development of signaling pathway-specific agents in gastrointestinal (GI) cancers. Akt/mTOR pathway is another candidate target in anti-cancer therapies. This paper will review the approaches currently used to exploit these novel targets in the development of GI cancer therapy. The review will focus specifically on colorectal, pancreatic and primary liver cancers (hepatocellular carcinoma, or HCC).

EPIDERMAL GROWTH FACTOR RECEPTOR PATHWAY

Epidermal growth factor receptor (EGFR) is a member of the HER-family kinases, which includes EGFR, HER2, Erbb3 and Erbb4. Upon ligand binding, EGFR homodimerizes with another EGFR or other members of the HER-family (heterodimerization), and lead to the activation of proliferative and survival signaling pathways, such as the Ras/Raf/MEK (mitogen-activated protein kinase, or MAPK) and Akt/mTOR cascades.

Abnormal expression or regulation of epidermal growth factors (EGF) and the receptors are implicated in the pathogenesis of many malignancies. EGFR is overexpressed or up-regulated in colorectal cancers and pancreatic cancers, and is associated with early progression
and poor survival[17-22]. Similarly, EGFR is overexpressed in HCC and is associated with aggressive features with increased cellular proliferation and reduced apoptosis. In vitro inhibition of EGFR in HCC cell lines results in cell cycle arrest and apoptosis[23-25]. These led to the clinical development of anti-EGFR agents as single agent, or in combination therapy in view of their in vitro and in vivo synergistic activity with cytotoxic agents[26].

Cetuximab

Cetuximab is a chimeric murine/human IgG1 monoclonal antibody that blocks ligand-dependant EGFR receptor activation. The antibody has a higher affinity for the receptor than the ligands, such as EGF and transforming growth factor (TGF-α)[27-29]. The drug is cytostatic when administered alone but highly synergistic with irinotecan in refractory colorectal cancer xenografts, leading to clinical development in irinotecan-refractory colorectal cancer patients[30,31]. In the pivotal multi-center randomized phase III trial, 329 patients with metastatic colorectal cancer who progressed on irinotecan-based therapy were randomized to receive cetuximab alone or a combination of cetuximab and irinotecan[9]. The patients in the combination arm achieved a superior response rate of 22.9% and median time to progression of 4.1 mo compared to 10.8% and 1.5 mo in the monotherapy arm respectively. The median survival was not statistically different between the two groups.

Compared to best supportive care, metastatic colorectal cancer patients who failed multiple previous regimens achieved better overall survival, time to progression and quality of life with cetuximab monotherapy in the recent study by National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) and Australasian Gastro-Intestinal Trials Group (AGITG)[32]. In the first line setting, Cetuximab improved response rate and time to progression when administered in combination with irinotecan-based regimen (FOLFIRI) in the CRYSTAL trial[33].

The efficacy of cetuximab with oxaliplatin-based regimen (such as FOLFOX) in second- and first-line settings is being evaluated in randomized trials (the EXPLORE and OPUS trials, respectively)[34-36]. However, the addition of cetuximab to oxaliplatin based fluoropyrimidine regimens (FOLFOX or CapOx) seemed to increase the frequency of grade 3/4 adverse events, specifically gastrointestinal toxicities, rash and lethargy[37]. The role of cetuximab in adjuvant, or postoperative, setting is being studied in 2 ongoing randomized trials (PETACC-8, Intergroup 0147) in combination with oxaliplatin-containing regimens[38,39].

Cetuximab is approved by FDA in U.S. for use in patients with EGFR-expressing colorectal cancer who failed previous irinotecan-based therapy. This was due to the fact that the trials mentioned enrolled only patients with EGFR-expressing tumors, based on preclinical data suggesting the predictive value of EGFR expression for cetuximab efficacy. However, patients with EGFR-negative colorectal cancer were later found to benefit from cetuximab therapy as well, suggesting that EGFR expression level does not correlate with cetuximab response[40,41]. This is an important lesson for the development of biological agents: patient selection based on expression, or non-expression, of specific molecular markers can be faulty. Such hypothesis should be validated vigorously in well-designed clinical trials.

The side effects of cetuximab are fairly tolerable with appropriate management. Hypersensitive infusion reaction was reported in about 3% of the patients. About 75% of patients receiving cetuximab developed a mild aceneiform-like rash. The development of cetuximab-related rash seemed to correlate with response but this needs to be studied further[42].

Cetuximab was evaluated in combination with gemcitabine in advanced pancreatic cancer. Despite encouraging phase II results, the recent randomized phase III trial (SWOG S0205) failed to confirm the superiority of cetuximab plus gemcitabine combination over gemcitabine monotherapy in this patient population[43].

Cetuximab monotherapy proved to be tolerable in patients with advanced HCC though activity was lacking in phase II trials[37,44]. Gruenwald et al enrolled 32 unresectable HCC patients and 27 were evaluable. Seventy-two percent (23 of 32) had Child-Pugh Stage A cirrhosis, 25% Stage B and 3% Stage C. Previously treated patients were eligible for this trial and 44% achieved stable disease for at least 8 wk and median time to progression was 22.5 wk. The agent is been evaluated in combination with cytotoxic chemotherapy in HCC[45].

Panitumumab

Panitumumab is a fully humanized anti-EGFR monoclonal antibody that is being evaluated in metastatic colorectal cancer. The agent has the advantage of avoiding the hypersensitive reaction typical of chimeric murine proteins, such as cetuximab. In a multi-institutional phase III trial, patients with refractory metastatic colorectal cancer were randomized to receive panitumumab plus best supportive care or best supportive care alone[46]. Eight percent (8%) of patients receiving panitumumab achieved partial response. About 90% developed the characteristic acneiform rash comparable to cetuximab monotherapy. As expected and importantly, hypersensitivity infusion reaction for the humanized monoclonal antibody was lower than that reported for cetuximab. Combination regimens containing panitumumab are been evaluated clinically.

Erlotinib

Erlotinib is an oral quinazoline that reversibly inhibits EGFR receptor tyrosine kinase. The small molecule induces in vitro cell cycle arrest and apoptosis, and has in vivo anti-tumor effects[47,48]. Major side effects are rash and diarrhea. Characteristic of this class of drug, Erlotinib was approved in 2004 by FDA in U.S. for use as single agent in previously treated non-small cell lung cancer (NSCLC) following the demonstration of survival benefit in a randomized phase III trial (NCIC-CTG BR.21)[49]. EGFR mutations seems to correlate with the efficacy of anti-EGFR therapy in NSCLC though effort to uncover additional molecular predictors continues[50].

Among GI cancers, erlotinib is furthest along clinical development in pancreatic cancer. Gemcitabine has been
the standard first-line therapy for advanced pancreatic cancer in improving symptoms and survival, but not curative. In the NCIC-CTG sponsored multi-institutional trial, 569 patients with untreated advanced pancreatic adenocarcinoma were randomized to receive gemcitabine plus erlotinib or gemcitabine plus placebo. Intention-to-treat analysis showed longer survival in patients receiving erlotinib plus gemcitabine (6.24 mo vs 5.91 mo; HR 0.82, P = 0.038) compared to gemcitabine only. One year survival was also higher in the erlotinib-containing arm (23% vs 17%, P = 0.023). Unlike colorectal cancer, tumor EGFR expression was not a pre-requisite in this trial. There was more frequent mild grade rash, diarrhea and hematological toxicity in the combination arm but the frequency of moderate and severe toxicities were comparable in both arms. However, routine use of erlotinib and gemcitabine combination cannot be recommended in patients with advanced pancreatic cancer in view of the high cost of erlotinib.

Erlotinib use in colorectal cancer remains investigational. The drug showed encouraging result when used in combination with capcitabine and oxaliplatin in previously treated disease in phase II trial. The result needs to be validated in a larger randomized trial. The drug had unacceptably high rate of toxicity when combined with dose-reduced FOLFIRI in patients with metastatic colorectal cancer.

Erlotinib is being tested in untreated advanced HCC patients in an ongoing open-labeled phase II trial. Tumor EGFR expression is not an exclusion criteria in this trial. Interim analysis of 25 patients suggested a longer median survival among erlotinib-responding patients of 44 wk compared to 25 wk in erlotinib-non-responders. All responders developed rashes. The trial aims to accrue a total of 40 patients.

**Lapatinib**

Lapatinib is an interesting oral inhibitor of two tyrosine kinases: ErbB1 (EGFR) and ErbB2 (HER-2/neu). The agent has significant efficacy in advanced breast cancer when combined with capecitabine. Both EGFR and HER-2/neu are co-expressed in colorectal cancer cells and simultaneous targeting of these receptors in preclinical studies enhanced apoptosis. Lapatinib is currently being tested in previously treated colorectal cancer patients.

EGFR pathway proves to be a valid target in GI cancers, especially in colorectal cancer with cetuximab and panitumumab. The small but statistically significant survival improvement by erlotinib in pancreatic cancer has been more a demonstration of “proof-in-principle” and the optimal approach to using anti-EGFR agents in pancreatic cancer still needs to be defined. Lapatinib development will hopefully shed light on whether dual-targeting of the ErbB receptor family is a successful approach in colorectal cancer (Table 1).

**ANGIOGENESIS**

Angiogenesis is vital to cellular growth, reproduction and development. The process is often pathological in cancers, driven by an imbalance of pro- and anti-angiogenic factors in tumors. The resulting tumor-induced vasculature is often leaky and dysfunctional, leading to increase interstitial pressure that impedes the delivery of both oxygen and chemotherapeutic agents.

VEGF-A (commonly known as VEGF) is among the first angiogenic factor discovered and shares sequence homology to the platelet-derived growth factor (PDGF) superfamily. VEGF-A interacts with two transmembrane receptor tyrosine kinases: VEGFR-1 (Flt-1) and VEGFR-2 (KDE, Flk-1). VEGFR-2 is the primary mediator of VEGF-A and is often overexpressed in tumor vasculatures. Activation of VEGFR-2 promotes endothelial cell proliferation, survival and migration. As such, VEGFR-2 has been a major anti-angiogenic target.

VEGF over-expression and increased microvessel growth:
density correlated with disease recurrence, metastases and survival in colorectal cancers\textsuperscript{[48-84]}. Similarly, increased VEGF expression in pancreatic adenocarcinoma was also associated with poor prognosis though some studies suggest that PDGF and bFGF, instead of VEGF-A, are more important in the modulation of angiogenesis in pancreatic cancer\textsuperscript{[85-88]}. HCC is highly vascular and patients with the liver neoplasm have higher serum VEGF levels than those with benign liver tumors\textsuperscript{[89-91]}. In addition, increased VEGF expression following surgical resection or prior to transarterial chemoembolization correlated with poor prognosis\textsuperscript{[92-95]}.

As such, angiogenesis has been a focus of GI cancer therapy and can be accomplished by monoclonal antibody and small molecule tyrosine kinase inhibitor. These antiangiogenic agents are believed to exert their anti-tumor effects by either affecting the tumor directly, inhibiting neovascularization, or enhancing chemotherapy delivery by normalizing the tumor vasculature\textsuperscript{[96,97]}.

**Bevacizumab**

Bevacizumab is a humanized monoclonal VEGF-binding antibody with anti-angiogenic properties that is the furthest along clinical development in its class. The drug was approved by FDA in U.S. for use with intravenous fluorouracil-containing regimens in patients with metastatic colorectal cancer\textsuperscript{[98]}.

The hint for bevacizumab efficacy in colorectal cancer in first-line setting was observed in a phase II trial. 104 patients with metastatic colorectal cancer were randomized to receive fluorouracil and leucovorin (5FU/LV) (control arm), 5FU/LV plus “low dose” bevacizumab (5 mg/kg) and 5FU/LV plus “high dose” bevacizumab (10 mg/kg)\textsuperscript{[99]}. Patients in both bevacizumab-containing arms achieved higher response rate (control: 17%; “low dose” bevacizumab: 40%; “high dose”: 24%), longer time to progression and median survival (13.8 mo; 21.5 mo; 16.1 mo, respectively). Interestingly, outcome was better in the “low dose” bevacizumab arm than the “high dose” arm and was attributed partly to a higher proportion of poor risk patients in the “high dose” arm. Bevacizumab-related toxicities in this trial included thrombosis, hypertension, proteinuria and epistaxis. Bevacizumab at 5 mg/kg was thus chosen as the recommended dose for further development.

Bevacizumab was subsequently tested in metastatic colorectal cancer patients in combination with 5FU, leucovorin, leucovorin and irinotecan (IFL) in the pivotal phase III trial. 813 patients with untreated metastatic colorectal cancer were randomized to receive IFL plus placebo (control arm), IFL plus bevacizumab 5 mg/kg or 5FU/LV plus bevacizumab 5 mg/kg\textsuperscript{[100]}. IFL superseded 5FU/LV as the standard first-line regimen in U.S. by the time this trial was planned and was chosen as the control arm. The 5FU/LV plus bevacizumab arm was added as a backup since the safety of IFL plus bevacizumab was unknown. The 5FU/LV/bevacizumab arm was discontinued later during the planned interim analysis when IFL plus bevacizumab proved to be safe. The superior survival of 20.3 mo in the IFL plus bevacizumab over the IFL plus placebo arm of 15.6 mo supported the use of bevacizumab in the first-line treatment of metastatic colorectal cancer. Consistent with the earlier phase II trial, reversible hypertension and proteinuria were more frequent with bevacizumab use. Other rare but serious side effects include gastrointestinal perforation, thrombosis and wound dehiscence.

Bevacizumab was also tested in metastatic colorectal cancer combined with oxaliplatin-based regimen in second-line setting. In the randomized phase III trial (E3200), patients with previously treated colorectal cancer were randomized to 3 arms: FOLFOX4 plus bevacizumab, FOLFOX4 and bevacizumab only. The dose of bevacizumab chosen was 10 mg/kg\textsuperscript{[101]}. The patients were not exposed to bevacizumab previously. Preliminary result showed superior survival and progression free survival in the FOLFOX4 plus bevacizumab arm. In a separate analysis, 56% of patients receiving FOLFOX4 plus bevacizumab had bevacizumab dose reduction but the survival was not significantly different from those without dose reduction\textsuperscript{[102]}. Preliminary results indicate that bevacizumab is equally effective with oxaliplatin-based regimen and should be considered in second-line setting for metastatic colorectal cancer patients without previous bevacizumab exposure.

Despite the progress with bevacizumab in metastatic colorectal cancer therapy, many clinical questions remained unanswered, such as the role of continuing bevacizumab from first- into second-line setting and the synergism of bevacizumab with oral fluoropyrimidines. The combination of bevacizumab, erlotinib plus FOLFOX was examined in a phase II trial but 40% of patients developed unacceptable toxicity and the treatment was stopped\textsuperscript{[103]}. Bevacizumab has been tested with FOLFIRI in an ongoing phase II trial involving patients with metastatic colorectal cancer\textsuperscript{[104]}.

The combination of bevacizumab and gemcitabine was been evaluated in pancreatic cancer. The multi-center phase II trial demonstrated a modest partial response rate of 21% in untreated advanced pancreatic cancer patients treated with the combination\textsuperscript{[105]}. Unfortunately, the combination failed to achieve survival improvement compared to gemcitabine only therapy in the subsequent phase III randomized trial (CALGB 80303)\textsuperscript{[104]}. The combination of bevacizumab with gemcitabine plus oxaliplatin (GemOx) is being evaluated in an ongoing North Central Cancer Treatment Group phase II trial\textsuperscript{[106]}.

**VEGF-Traps**

VEGF-Traps (Regeneron) is a novel chimeric decoy receptor with higher affinity for VEGF-A than monoclonal antibodies\textsuperscript{[107]}. The molecule consists of the extracellular domains of VEGFR-1 and -2 fused to the constant region (Fc) of IgG1\textsuperscript{[108]}. Preclinical studies demonstrated potent anti-tumor and anti-angiogenic activities in various cancer models, prompting further clinical testing of the agent\textsuperscript{[109]}. Phase I study of the agent in patients with advanced solid tumors showed that the agent is well-tolerated and the toxicities, including fatigue, pain, constipation and arthralgia, can be managed safely\textsuperscript{[110]}. VEGF-Traps is being tested with fluorouracil-based regimens in phase I trials\textsuperscript{[111,112]}.
**Table 2 Agents targeting angiogenesis in GI cancers**

| Agents                   | Tumor types                  | Regimen                                      | Study Design | References   |
|--------------------------|------------------------------|----------------------------------------------|--------------|--------------|
| Monoclonal antibodies    |                              |                                              |              |              |
| Bevacizumab              | Colorectal cancer            | Bevacizumab/IFL                              | Phase III    | [10]         |
|                          |                              | Bevacizumab/FOLFIRI                          | Phase III    | [99]         |
|                          |                              | Bevacizumab/FOLFIRI                          | Phase II     | [102]        |
| VEGF decoy               |                              | I-LV5FU2/ VEGF-Trap                          | Phase I      | [111]        |
|                          |                              | Bevacizumab/ioxalplatin/Bevacizumab/FOLFIRI  | Phase I      | [112]        |
| Tyrosine kinase inhibitors|                               |                                              |              |              |
| Sorafenib                | Hepatocellular carcinoma     | Sorafenib                                    | Phase III    | [8]          |
|                          | Pancreatic cancer            | Gencitabine/sorafenib                        | Phase I      | [116]        |
|                          | Colorectal cancer            | Oxalplatin/sorafenib                         | Phase I      | [115]        |
|                          | Hepatocellular carcinoma     | Sunitinib                                     | Phase I      | [122]        |
|                          |                              |                                             | Phase I      | [121,123]    |

**References**

Sorafenib (BAY43-9006) is an oral bi-aryl urea initially developed as a potent inhibitor of Raf protein\(^{[113]}\). The agent is also a multi-target kinase inhibitor and has significant activity against VEGFR-1, VEGFR-2, VEGFR-3 and PDGFR. As such, sorafenib is also been evaluated for its anti-angiogenic properties. The drug significantly inhibits neovascularization in colon, breast and non-small cell lung cancer xenografts in preclinical studies, marked by decreased tumor microvesSEL density.

Phase I trial involving patients with refractory solid tumors showed that sorafenib is fairly well tolerated. The main toxicities were diarrhea, skin rash and fatigue\(^{[114]}\). Downstream ERK protein was significantly inhibited at sorafenib \(\geq 200\) mg bid dose, indicating Raf inhibition. Partial response was observed in one (of 6) patients with HCC (400 mg bid dose) and stable disease for more than 6 mo in 6 (of 26) of colorectal cancer patients\(^{[115,116]}\).

Sorafenib became the first agent to achieve significant survival benefit in advanced HCC in a multi-center randomized trial (SHAPR trial)\(^{[8]}\). 602 patients with previously untreated advanced disease with Child-Pugh Stage A cirrhosis and good performance status (ECOG PS 0-2) were randomized to receive sorafenib or placebo. Compared to the placebo arm, patients receiving sorafenib had a longer median survival (10.7 mo \( vs \) 7.9 mo; HR 0.69, \( P < 0.01 \)) and time to progression (HR 0.58, \( P < 0.01 \)). Serious side effects were similar in both groups though diarrhea and hand-foot syndrome were more frequent in those receiving sorafenib. Criticisms of the study include the generalisability of the result since majority of the patients enrolled were European and had minimal liver dysfunction. The benefit in Child’s B and C patients remains unclear. Moreover, the therapy is quite costly and is a significant financial burden for most HCC patients who live in poorer developing countries. Sorafenib continues to be evaluated in HCC in combination therapy.

Sunitinib (SU11248) is an orally available inhibitor of VEGFR-2, PDGFR, c-kit and FLT-3. Preclinical studies showed anti-tumor activity in various malignancies, including leukemia, breast and lung cancer models\(^{[117-119]}\). In a phase I study, the recommended dose for sunitinib was determined to be \(50\) mg/d on a “4-wk-on/2-wk-off” schedule\(^{[120]}\). The toxicities include hypertension, thrombocytopenia, neutropenia, diarrhea, hair and skin changes. Sunitinib is being tested in HCC and in combination with irinotecan and cetuximab in previously treated metastatic colorectal cancer\(^{[121-123]}\).

Of the anti-angiogenic agents discussed, bevacizumab proved to be an exceptionally efficacious agent in colorectal cancer when combined with conventional cytotoxic agents. However, this monoclonal antibody failed to achieve the clinical benefit expected in pancreatic cancer in combination therapy. More excitingly, sorafenib becomes the first chemotherapeutic agent to achieve significant clinical benefit in HCC (Table 2).

**AKT/mTOR PATHWAY**

The mammalian target of rapamycin (mTOR) is a cytosolic serine/threonine kinase that plays a central role in cell proliferation and survival\(^{[125]}\). The kinase is downstream to the phosphatidylinositol 3’-kinase (PI3K)/Akt signaling pathway. Activated mTOR interacts with downstream effectors, such as 4E-BP1 and p70s6K, to modulate various growth and survival-related cellular functions. The pathway is sensitive to extracellular growth factors (EGF, VEGF and IGF) and nutrients (amino-acids, glucose and oxygen).

In a series of 101 resected primary hepaticoma (with 73 HCC), 15% had overexpression of phospho-mTOR and 5% had increased total mTOR protein expression\(^{[126]}\). In pancreatic cancers, more than 90% of the tumors contain an activating upstream ras mutation and about half of the surgically resected pancreatic cancer specimens had mTOR activation\(^{[127-131]}\).

Loss of the suppressive PTEN gene expression, PI3K gene mutations and amplification of Akt result in constitutive activation of the upstream PI3K/Akt pathway.
observed in some tumors. Such activation increases the tumors’ susceptibility to mTOR inhibitors and provided the rationale in developing rapamycin (mTOR inhibitor) analogs in various cancer types. In addition, inhibition of mTOR reversed gemcitabine resistance in gemcitabine-resistant pancreatic cancer cell lines in preclinical xenograft models. These preclinical data support the clinical testing of mTOR inhibitors in HCC and pancreatic cancer.

**Rapamycin**

Rapamycin (sirolimus) is an oral macrolide derived from *Streptomyces hygroscopicus* that is widely used as immunosuppressant in organ transplantation. Rapamycin and its analogs also inhibit cellular proliferation in a wide range of human tumors. The drug complexes with FKBP12, a member of the immunophilin family of FK506-binding proteins, intracellularly which in turn inhibits the mTOR kinase activity, leading to G1 phase cell cycle arrest and apoptosis. However, the drugs poor aqueous solubility, chemical stability and lack of investor interest impeded its clinical development as an anti-neoplastic agent. Currently, rapamycin is being tested in a pharmacodynamic-guided dose-finding study involving patients with advanced solid tumor and also in a phase II trial involving patients with advanced pancreatic cancer.

**Temsirolimus**

Temsirolimus (CCI-779) is a water-soluble synthetic rapamycin ester with significant anti-proliferative properties that can be administered via both oral and intravenous routes. The drug demonstrated comparable in vitro anti-tumor effect to rapamycin against a wide range of human cancer cell lines, including prostate, breast, small-cell lung carcinoma, melanoma, glioblastoma and T-cell leukemia. The agent inhibits tumor growth, or is cytostatic, in a variety of cancer xenograft models but did not achieve tumor shrinkage.

Two dosing schedules of temsirolimus were tested in separate phase I trials: weekly intravenous dose versus the 30 minute intravenous infusion administered daily for 5 d on a bi-weekly schedule. Toxicities observed include skin changes, mucosomatitis, asthenia, myelosuppression (thrombocytopenia, neutropenia), dyslipidemia and elevated liver enzymes. Dose escalation for the weekly regimen was stopped at 220 mg/m², which was the highest planned dose. Toxicities were fairly manageable and reversible at this dose. Interestingly, tumor shrinkages (partial and minor responses) were observed clinically, contrary to the cytostatic phenomenon seen in preclinical studies. Two patients achieved partial response: one with renal cell carcinoma and another with breast cancer. This led to further testing of temsirolimus in various cancer types. Temsirolimus was recently approved by FDA in U.S. for the treatment of poor risk renal cell carcinoma patients based on the positive result from a randomized phase III trial.

**Everolimus**

Everolimus (RAD001) is an oral rapamycin analog that inhibits tumor growth and angiogenesis in a dose-dependent manner and has anti-proliferative activity against a wide range of human cancers. The optimal biologically active dose of everolimus was studied in two phase I trials. Everolimus 20 mg weekly was determined to be biologically active and toxicities associated with weekly everolimus administration were well tolerated and included anorexia, fatigue, rash, mucositis, headache, hyperlipidemia and gastrointestinal disturbance. The dose-limiting toxicities of daily everolimus were stomatitis, neutropenia and hyperglycemia. Pre-treatment and during-treatment tumor biopsies were done to evaluate pharmacodynamic effects of everolimus and a 10 mg daily dose was recommended as the optimal dose. Partial response was seen in one colorectal cancer patient and everolimus is in phase II development as single agent in refractory colorectal cancer. The agent is being developed in other cancer types as well, such as gastrointestinal stromal tumor, neuroendocrine tumors, renal cell carcinoma, non-small cell lung cancer and melanoma.

The Akt/mTOR pathway seems to be an important survival and pro-growth pathway in GI cancers. Temsirolimus is the first of its class to achieve significant anti-tumor efficacy and clinical development of the class of mTOR inhibitors in pancreatic cancer and HCC continues.

**CONCLUSION**

Angiogenesis and EGFR pathways were hypothesized as targets for anticancer therapy more than three decades ago. Efforts to translate this knowledge to bedside are just starting to benefit patients with GI cancers. Successful development of cetuximab and bevacizumab in colorectal cancer ushered in the era of biologically targeted agents in the fight against GI cancers. More milestones were later achieved when the survival of previously difficult-to-treat GI cancers were improved by these novel biological agents, as in the case of erlotinib in pancreatic cancer and sorafenib in HCC. More molecular targets will become apparent as our knowledge of the complex neoplastic processes increases, and will provide exciting translational opportunities in the development of GI cancer therapy.

**REFERENCES**

1. Adjei AA, Hidalgo M. Intracellular signal transduction pathway proteins as targets for cancer therapy. *J Clin Oncol* 2005; 23: 5386-5403
2. Meropol NJ. Turning point for colorectal cancer clinical trials. *J Clin Oncol* 2006; 24: 3322-3324
3. Andre T, Colin P, Louvet C, Gamelin E, Bouche O, Achilles E, Colbert N, Boaziz C, Piedbois P, Tubiana-Mathieu N, Boutan-Larozé A, Flesch M, Buyse M, de Gramont A. Semimonthly versus monthly regimen of fluorouracil and leucovorin administered for 24 or 36 weeks as adjuvant therapy in stage II and III colon cancer: results of a randomized trial. *J Clin Oncol* 2003; 21: 2809-2903
4. Chau I, Norman AR, Cunningham D, Tait D, Ross PJ, Iveson T, Hill M, Hickish T, Loots F, Jodrell D, Webb A, Oates JR. A randomised comparison between 6 months of bolus fluorouracil/leucovorin and 12 weeks of protracted venous infusin fluorouracil as adjuvant treatment in colorectal cancer. *Ann Oncol* 2005; 16: 549-557
5. Poplin EA, Benedetti JK, Estes NC, Haller DG, Mayer RJ,
Goldberg RM, Weiss GR, Rivkin SE, Macdonald JS. Phase III Southwest Oncology Group 9415/Intergroup 0153 randomized trial of fluorouracil, leucovorin, and levamisole versus fluorouracil continuous infusion and levamisole for adjuvant treatment of stage III and high-risk stage II colon cancer. J Clin Oncol 2005; 23: 1819-1825

6 Burris HA, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 1997; 15; 2403-2413

7 Thomas MB, Abbruzzese JL. Opportunities for targeted therapies in hepatocellular carcinoma. J Clin Oncol 2005; 23: 8093-8108

8 Llovet J, Ricci S, Mazzaferro V, Hilgard P, Raoul J, Zeuzem S, Poulin-Costello M, Moscovici. Voliotis D, Bruix J. For the SHARP Investigators Study Group. Sorafenib improves survival in advanced Hepatocellular Carcinoma (HCC): Results of a Phase III Randomized placebo-controlled trial (SHARP trial). J Clin Oncol 2007; 25 Suppl: 185. LBA1

9 Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser A, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004; 351: 337-345

10Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Gifford S, Holmgren E, Ferrara N, Fyle G, Rogers B, Ross R, Kabbavavna F, Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350: 2323-2342

11Scaltriti M, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res 2006; 12: 5268-5272

12Rubio-Viqueira B, Hidalgo M. Targeting mTOR for cancer treatment. Curr Opin Investig Drugs 2006; 7: 501-512

13Reai FX, Rettig WJ, Chesa PG, Melamed MR, Old LJ, Mendelsohn J. Expression of epidermal growth factor receptor in human cultured cells and tissues: relationship to cell lineage and stage of differentiation. Cancer Res 1986; 46: 4726-4731

14Carpenter G, Cohen S. Epidermal growth factor. J Biol Chem 1990; 265: 7709-7712

15Basega J, Arteaga CL. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. J Clin Oncol 2005; 23: 2445-2459

16Aaronson SA. Growth factors and cancer. Science 1991; 254: 1146-1153

17Messa C, Russo F, Caruso MO, Di Leo A. EGF, TGF-alpha, and EGFR in human colorectal adenocarcinoma. Acta Oncol 1998; 37: 285-289

18Porebska I, Harlozińska A, Bojarowski T. Expression of the tyrosine kinase activity growth factor receptors (EGFR, ERB B2, ERB B3) in colorectal adenocarcinomas and adenomas. Tumour Biol 2000; 21: 105-115

19Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995; 19: 183-232

20 Mayer A, Takimoto M, Fritz E, Schellander G, Kofler K, Ludwig H. The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor, and mdr gene expression in colorectal cancer. Cancer 1993; 71: 2454-2460

21Lockhart AC, Berlin JD. The epidermal growth factor receptor as a target for colorectal cancer therapy. Semin Oncol 2005; 32: 52-60

22Yamanaka Y, Friess H, Kobrin MS, Buchler M, Beger HG, Korc M. Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. Anticancer Res 1993; 13: 565-569

23Foster JM, Black J, LeVeau C, Khoury T, Javel M, Kuvshinoff B, Gibb JF. EGF pathway activation in hepatocellular carcinoma is a prognostic predictor of survival and a potential target for biologic therapy. 2006 ASCO Gastrointestinal Cancers Symposium Abstract 212; 2006

24Huether A, Höpner M, Sutter AP, Schuppan D, Scherübl H. Erlotinib induces cell cycle arrest and apoptosis in hepatic cell carcinoma lines and enhances chemosensitivity towards cytostatics. J Hepatol 2005; 43: 661-669

25 Höpner M, Sutter AP, Huether A, Schuppan D, Zeitz M, Scherübl H. Targeting the epidermal growth factor receptor by gefitinib for treatment of hepatocellular carcinoma. J Hepatol 2004; 41: 1008-1016

26Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res 2001; 7: 2967-2970

27Gruenwald V, Willens L, Gebel MG, Greten TF, Kubicka S, Ganser A, Manns MP, Malek NP. A phase II open-label study of cetuximab in unresectable hepatocellular carcinoma: Final results. J Clin Oncol 2007; 25: 4598

28Xiong HQ, Rosenberg A, LoBuglio A, Schmidt W, Wolff RA, Deutsch J, Needle M, Abbruzzese JL. Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with radiotherapy and chemotherapy for patients with locally advanced pancreatic cancer-PARC: study protocol E1605. J Natl Cancer Inst 2005; 97: 2265

29Saltz LB, Meropol NJ, Loehrer PJ, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 2004; 22: 1201-1208

30Saltz L, Rubin MS, Hochster HS. Cetuximab (IMC-C225) Plus Irinotecan (CPT-11) is Active in CPT-11-Refractory Colorectal Cancer (CRC) that Expresses Epidermal Growth Factor Receptor (EGFR). Proc Am Soc Clin Oncol 2001; 20; 7 (Abstract)

31Jonker DJ, Karapetis CS, Moore M, Zalcberg JR, Tu D, Berry S, Koski S, Krahm M, Simes J, Tebbutt N, Van Hazel G, O’Callaghan CJ. Randomized phase III trial of cetuximab monotherapy plus best supportive care [BSC] versus BSC alone in patients with pretreated metastatic epidermal growth factor receptor-positive colorectal cancer. A trial of the National Cancer Institute of Canada Clinical Trials Group and the Australasian Gastro-Intestinal Trials Group. Proc Am Assoc Cancer Res 2007; 48

32Van Custem E, Nowacki MP, Lang I, Cascini S, Shchepotin I, Maurel J, Rouquier P, Cunningham D, Nippen J, Kohne CH. Randomized phase III study of irinotecan and 5-FU/FA with or without cetuximab in the first-line treatment of patients with metastatic colorectal cancer (mCRC): The CRYSTAL trial. J Clin Oncol 2007; 25: 4000

33Badarith S, Mitchell EO, Hennis CD, Graham VL, Hansen CA, Henderson TT, Langer C. Cetuximab plus FOLFOX for colorectal cancer (EXPLORE): Preliminary safety analysis of a randomized phase III trial. J Clin Oncol 2004; 22: 3531

34Jennis A, Polikoff J, Mitchell EO, Badarith S, Graham C, Chen T, Gustafson T, Langer C. Eributix (Cetuximab) Plus FOLFOX for Colorectal Cancer (EXPLORE). Proc Am Soc Clin Oncol 2005; 8; 131

35Bokemeyer C, Bondarenko I, Makhson A, Hartmann C, Aripcico J, Zampino M, Donea S, Ludwig H, Zubeck A, Koralewski P. Cetuximab plus 5-FU/FA/oxaliplatin (FOLFOX-4) versus FOLFOX-4 in the first-line treatment of metastatic colorectal cancer (mCRC): OPUS, a randomized phase II study. J Clin Oncol 2007; 25: 4035

36Maughar T. Cetuximab (C), oxaliplatin (Ox) and fluoropyrimidine (Fp): Toxicity during the first 12 weeks of treatment for the first 804 patients entered into the MRC COIN (CR10) trial. J Clin Oncol 2007; 25: 4077

37Comparison of Combination Chemotherapy Regimens With or Without Cetuximab in Treating Patients Who Have
Undergone Surgery For Stage III Colon Cancer (Intergroup 0147). Available from: URL: http://www.clinicaltrials.gov/ct/show/NCT00879274

39 Taieb J. Combination Chemotherapy With or Without Cetuximab in Treating Patients With Stage III Colon Cancer That Was Completely Removed By Surgery (PETACC-8). Available from: URL: http://www.clinicaltrials.gov/ct/show/NCT00265811

40 Chau I, Cunningham D. Adjuvant therapy in colon cancer: when, what and how? Ann Oncol 2006; 17: 1347-1359

41 Lenz H, Mayer RJ, Gold PJ. Activity of cetuximab in patients with colorectal cancer refractory to both irinotecan and oxaliplatin. J Clin Oncol 2004; 22: 3510

42 Chung KY, Shia J, Kemeny NE, Shah M, Schwartz GK, Tsao A, Hamilton A, Pan D, Schrag D, Schwartz L, Klimstra DS, Fridman D, Kelsen DP, Saltz LB. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005; 23: 1803-1810

43 Pérez-Soler R, Saltz L. Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining? J Clin Oncol 2005; 23: 5235-5246

44 Philip PA, Benedetti JK, Fenoglio-Preiser CM, Zalupski MM, Lenz H, O'Reilly E, Wong R, Atkins J, Abbruzzese JL, Blanke C. Phase III study of gemcitabine [G] plus cetuximab [C] versus gemcitabine in patients [pts] with locally advanced or metastatic pancreatic adenocarcinoma [PC]. SWOG 50205 study. J Clin Oncol 2007; 25: LBA4598

45 Zhu A, Blaskowsky L, Enzinger PC, Bhargava P, Ryan DP, Meyerhardt JA, Horgan K, Hale K, Sheehan S, Stuart KE. Phase II study of cetuximab in patients with unresectable or metastatic hepatocellular carcinoma. J Clin Oncol 2006; 24: 14096

46 Louafi S, Hebar M, Rosmorduc O, Tesmoingt C, Asnacios A, Romano O, Fartoux L, Artur P, Poynard T, Taieb J. Gemcitabine, oxaliplatin (GEMOX) and cetuximab for treatment of hepatocellular carcinoma (HCC). Results of the Phase II study ERGO. J Clin Oncol 2007; 25: 4594

47 Gibson TB, Ranganathan A, Grothey A. Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer. Clin Colorectal Cancer 2006; 6: 29-31

48 Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, Barbacci EG, Iwata KK, Arnold L, Boman B, Fisher D, Zalupski MM, Lenz H, O'Reilly E, Wong R, Atkins J, Abbruzzese JL, Blanke C. Phase III study of gemcitabine [G] plus cetuximab [C] versus gemcitabine in patients [pts] with locally advanced or metastatic pancreatic adenocarcinoma [PC]. SWOG 50205 study. J Clin Oncol 2007; 25: LBA4598

49 Pollock VA, Savage DM, Baker DA, Tsaparakos KE, Sloan DE, Moyer JD, Barbacci EG, Pustilnik LR, Smolarek TA, Kindler HL, Blaszkowsky LS, Enzinger PC, Benevoli L, Napolitano M, Adkins N, Goguen P, Martin S, Zalupski MM, Lenz H, O'Reilly E, Wong R, Atkins J, Abbruzzese JL, Blanke C. Phase III study of gemcitabine [G] plus cetuximab [C] versus gemcitabine in patients [pts] with locally advanced or metastatic pancreatic adenocarcinoma [PC]. SWOG 50205 study. J Clin Oncol 2007; 25: LBA4598

50 Gibson TB, Ranganathan A, Grothey A. Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer. Clin Colorectal Cancer 2006; 6: 29-31

51 Pao W, Miller VA. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions. J Clin Oncol 2005; 23: 2556-2568

52 Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawka P, Walde D, Wolf LA, Campos D, Lim R, Ding K, Clark G, Voskoglu-Nomikos T, Plasinsky M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007; 25: 1960-1966

53 Grubb SS, Grusenmeyer NJ, Petrelli NJ, Gralla RJ. Is it cost-effective to add erlotinib to gemcitabine in advanced pancreatic cancer? J Clin Oncol 2006; 24: 6048

54 Meyerhardt JA, Zhu AX, Enzinger PC, Ryan DP, Clark JW, Kulkhe MH, Earle CC, Vinciorel M, Michellini A, Sheehan S, Fuchs C, Phase II trial of Cetuximab, oxaliplatin, and erlotinib in previously treated patients with metastatic colorectal cancer. J Clin Oncol 2006; 24: 1892-1897

55 Messersmith WA, Laheru DA, Senzer NN, Donehower RC, Grouleff P, Rogers T, Kelley SK, Ramies DA, Lum BL, Hidalgo M. Phase I trial of irinotecan, infusional 5-fluorouracil, and leucovorin, with erlotinib (FOLFIRI) with erlotinib (OSI-774): early termination due to increased toxicities. Clin Cancer Res 2004; 10: 6522-6527

56 Thomas MB, Dutta A, Brown T, Charuvascavej C, Rashid A, Hoff PM, Dancey J, Abbruzzese JL. A Phase II Open-label Study of OSI-774 (NSC 718781) in Unresectable Hepatocellular Carcinoma. J Clin Oncol 2005; 23: 4038

57 Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Grusfeld A, Crown J, Chan A, Kaufman B, Skarlos D, Campone M, Davidson N, Berger M, Oliva C, Rubin SD, Stein S, Cameron D. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006; 355: 2733-2743

58 Zhou Y, Li S, Hu YP, Wang J, Hauser J, Conway AN, Vinci MA, Humphrey L, Zborowska E, Willss K, Brattain MG. Blockade of EGFR and ErbB2 by the novel dual EGFR and ErbB2 tyrosine kinase inhibitor GW572016 sensitizes human colon carcinoma GEO cells to apoptosis. Cancer Res 2006; 66: 4041-4048

59 Fields LA, Rinaldi D, Henderson CA, JG, Chu L, Brill KJ, Leopold LH, Berger MS. An Open-Label Multicenter Phase II Study of Oral Lapatinib (GW572016) as Single Agent, Second-Line Therapy in Patients with Metastatic Colorectal Cancer. J Clin Oncol 2005; 23: 3583

60 Graeven U, Kremmer B, Stöudt K, Krolling B, Rojo F, Weber D, Tilliner J, Ulrich C, Schniegge W. Phase I study of the humanised anti-EGFR monoclonal antibody matuzumab (EMD 72000) combined with gemcitabine in advanced pancreatic cancer. Br J Cancer 2006; 94: 1293-1299

61 Vanhoef J, Oemnes J, Stitt P, Knoer R, Verns H, Hulcrea A, Basgela J. Phase I study of the humanized antiepidermal growth factor receptor monoclonal antibody emd72000 in patients with advanced solid tumors that express the epidermal growth factor receptor. J Clin Oncol 2005; 23: 5705-5714

62 Iannitti D, Dippel P, Akerman P, Barnett JM, Maia-Acuna A, Wirf D, Miner E, Morten D, Haas B, Baselga J. Phase I study of the humanized antiepidermal growth factor receptor monoclonal antibody emd72000 in patients with advanced solid tumors that express the epidermal growth factor receptor. J Clin Oncol 2005; 23: 5705-5714

63 Vanhoef J, Oemnes J, Stitt P, Knoer R, Verns H, Hulcrea A, Basgela J. Phase I study of the humanized antiepidermal growth factor receptor monoclonal antibody emd72000 in patients with advanced solid tumors that express the epidermal growth factor receptor. J Clin Oncol 2005; 23: 5705-5714

64 Kuo T, Cho CD, Halsey J, Wakeslo HA, Advani RH, Ford JM, Fisher GA, Sikic BI. Phase II study of gefitinib, fluorouracil, leucovorin, and oxaliplatin therapy in previously treated patients with metastatic colorectal cancer. J Clin Oncol 2005; 23: 5613-5619

65 Kinder HL, Friberg G, Skoog L, Wade-Olive K, Vokes EE. Phase I/II trial of gefitinib and oxaliplatin in patients with advanced colorectal cancer. Am J Clin Oncol 2005; 28: 340-344

66 Mackenzie MJ, Hirte HW, Glennwood G, Jean M, Goel R, Major PP, Miller WH, Panasci L, Lorimer IA, Batist G, Matthews S, Douglas L, Seymour L. A phase II study of capecitabine, oxaliplatin, and bevacizumab (ZD1839) in patients with advanced colorectal cancer. J Clin Oncol 2005; 23: 3510-3516

67 O'Dwyer PJ, Gantionio BJ, Levy DE, Kauh JS, Fitzgerald DB, Benson AB. Gefitinib in advanced unresectable hepatocellular
canceroma: Results from the Eastern Cooperative Oncology Group’s Study E1203. J Clin Oncol 2006; 24: 4143

68 Czito BG, Willett CG, Bendell JC, Morse MA, Tyler DS, Fernando NH, Mantyh CR, Bobe GC, Honeycutt W, Yu D, Clary BM, Urrutia TN, Ludwig KA, Hurwitz HI. Increased toxicity with gefitinib, cetiniblina, and radiation therapy in pancreatic and rectal cancer: phase I trial results. J Clin Oncol 2006; 24: 656-662

69 Folkman J. Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenisis. N Engl J Med 1995; 333: 1757-1763

70 Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003; 9: 669-676

71 Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nat Med 2001; 7: 987-988

72 Karkkainen MJ, Petrova TV. Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. Oncogene 2000; 19: 5598-5605

73 Cross MJ, Dixielius P, Matsumoto T, Claesson-Welsh L. VEGF-receptor signal transduction. Trends Biochem Sci 2003; 28: 488-494

74 Frank RE, Saclarides TJ, Leurgans S, Speziale NJ, Drab EA, Stuart KE, Zhu A, Fuchs C, Bhargava P, Eng C, Adinin RA, Wolff RA, Lin E, Kopetz S, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Vessel counts and expression of vascular endothelial growth factor and its messenger RNA expression in resectable hepatocellular carcinoma: a prospective study. World J Gastroenterol 2004; 10: 643-648

75 Hiroshishi K, Yamamoto T, Uemishi T, Ogawa M, Sakabe K, Takemura S, Shuto T, Tanaka S, Kubo S, Kinosita H. CD44 and VEGF expression in extrahepatic metastasis of human hepatocellular carcinoma. Hepatogastroenterology 2004; 51: 1121-1123

76 Poon RT, Lau C, Yu WC, Fan ST, Wong J. High serum levels of vascular endothelial growth factor predict poor response to transarterial chemoembolization in hepatocellular carcinoma: a prospective study. Oncol Rep 2004; 11: 1077-1084

77 Jeng KS, Sheen IS, Wang YC, Chu CM, Shih SC, Wang PC, Chang WH, Wang HY. Prognostic significance of preoperative circulating vascular endothelial growth factor messenger RNA expression in resectable hepatocellular carcinoma: a prospective study. J Gastroenterol 1994; 29: 1077-1084

78 Wey JS, Fan F, Gray MJ, Bauer TW, McCarthy MF, Somcio R, Liu W, Evans DB, Wu Y, Hicklin DJ, Ellis LM. Vascular endothelial growth factor receptor-1 promotes migration and invasion of human breast carcinoma of prognostic value after resection? J Clin Oncol 1994; 12: 1437-1438

79 Fujimoto M, Ishiyashiki T, Sugase T, Tarui S. Rapid radioimmunoassay for guanosine 3’,5’-cyclic monophosphate using tritiated ligand. J Biochem 1975; 78: 131-137

80 Kabbinava F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Grifnghn S, Berglends E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 2003; 21: 60-65

81 Gianontio BJ, Catalano PJ, Meropol NJ, O’Dwyer PJ, Mitchell EP, Alberts SR, Schwartz MA, Benson AB. High-dose bevacizumab improves survival when combined with FOLFOX4 in previously treated advanced colorectal cancer: Results from the Eastern Cooperative Oncology Group (ECOG) study E3200. J Clin Oncol 2005; 23: 2

82 Gianontio BJ, Catalano PJ, O’Dwyer PJ, Meropol NJ, Benson AB. Impact of bevacizumab dose reduction on clinical outcomes for patients treated on the Eastern Cooperative Oncology Group’s Study E3200. J Clin Oncol 2006; 24: 3538

83 Meyerhardt JA, Stuart KE, Zhu A, Fuchs C, Bhargava P, Earle C, Blaszkowsky L, Lawrence C, Batu S, Ryan DP. Phase II study of FOLFOX, bevacizumab and erlotinib as initial therapy for patients with metastatic colorectal cancer (MCRC). J Clin Oncol 2006; 24: 3545

84 Hoff PM, Eng C, Adimla RA, Wolff RA, Lin E, Kopetz S, Rodnery A, Chang D, Abbruzzese JL. Preliminary results from a phase II study of FOLFIRI plus bevacizumab as first-line treatment for metastatic colorectal cancer (mCRC). 2006
Rapamycin inhibits the rapamycin ester CCI-779 and tumor growth inhibition. Hollingshead M, Arbuck SG, Travis R, Sausville EA, Dukin L. 2006; J Clin Oncol 23: 91-98.

Rapamycin sensitivity in childhood rhabdomyosarcoma cells through inhibition of proliferation, induces differentiation, and inhibits cdc2 kinase activity in a myogenic cell line. Jayaraman T, Marks AR. 1991; J Biol Chem 266: 17348-17354.

Khan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Ramapun US Study Group. Lancet 2000; 356: 194-202.

Swanson SJ, Hale DA, Mannon RB, Kleiner DE, Cendales LC, Chamberlain CE, Polly SM, Harlan DM, Kirk AD. Kidney transplantation with rabbit antithymocyte globulin induction: a North Central Cancer Treatment Group Study. J Clin Oncol 2005; 23: 9055-9060.

Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest of the constitutively active FRAP-p70s6K pathway in human pancreatic cancer cells. Cancer Res 1999; 59: 3583-3587.

Podsypanina K, Lee RT, Politis C, Hennesey I, Crane A, Puc J, Neshat M, Wang H, Yang L, Gibbons J, Frost P, Dreisbach V, Benis J, Gacigang Z, Fisher P, Sawyers C, Hedrick-Ellenson L, Parsons R. An inhibitor of mTOR degrades neoplasia and normalizes p70/S6 kinase activity in Pten+ mice. Proc Natl Acad Sci USA 2001; 98: 10320-10325.

Akselhard H, Yang MD, Wilson PA. Rapamycin induces spontaneous and fibroblast growth factor beta-stimulated proliferation of endothelial cells and fibroblasts. Transplant Proc 1991; 23: 2833-2836.

Francavilla A, Starzl TE, Carr B, Azzarone A, Carrieri G, Zeng QH, Porter KA. The effects of FK 506, cyclopamine, and rapamycin on liver growth in vivo and in vivo. Transplant Proc 1991; 23: 2817-2820.

Jayaraman T, Marks AR. Rapamycin-FKBP12 blocks proliferation, induces differentiation, and inhibits cdc2 kinase activity in a myogenic cell line. J Biol Chem 1993; 268: 25385-25388.

Dilling MB, Dias P, Shapiro DN, Germain GS, Johnson RK, Hidalgo M, Stadler WM, Logan TF, Dutcher JP. A phase I trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. J Clin Oncol 2005; 23: 2267-2275.

Chiaretti S, Chicchianese A, Alamprese V, Doring M, Peltekian K, Domingues J, Mahalati M, Morris RE. Immunosuppressive drugs after lung transplantation with rabbit antithymocyte globulin induction: a multicentre study. The Rapamune US Study Group. Lancet 2000; 356: 198-203.

Koster PL, Johnson RK, Livi GP. Rapamycin sensitivity in medulloblastoma models as single agent and in combination chemotherapy. Cancer Res 2001; 61: 1527-1532.

Gibbons JJ, Discafani C, Peterson R, Hernandez R, Skotnicki R, Bierer BE. The effect of CCI-779, a novel macroside anti-tumor agent, on the growth of human tumor cells in vitro and in nude mouse xenograft in vivo. Proc Am Assoc Cancer Res 1999; 30: 301.

Raymond E, Alexandre J, Favier S, Vera K, Materman E, Boni J, Leister C, North-Bradley J, Hansaukke A, Armand JP. Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer. J Clin Oncol 2004; 22: 2363-2374.

Hidalgo M, Buckner JC, Erlitchman C, Pollock MS, Boni JP, Dukart G, Marshall B, Speicher L, Moore L, Rowinsky EK. A phase I and pharmacokinetic study of temsirolimus (CCI-779) administered intravenously daily for 5 days every 2 weeks to patients with advanced cancer. Clin Cancer Res 2006; 12: 5755-5763.

Atkins MB, Hidalgo M, Stadler WM, Logan TF, Dutcher JP, Hudes GR, Park Y, Liou SH, Marshall B, Boni JP, Dukart G, Sherman ML. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol 2004; 22: 909-918.

Chan S, Scheulen ME, Johnston S, Mross K, Cardoso F, Dittrich C, Eiermann W, Hess D, Morant R, Semiglazov V, Borner M, Salzberg M, Ostapenko V, Illiger HJ, Behringer D, Bardy-Bouxin N, Boni J, Kong S, Cincotta M, Moore L. Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. J Clin Oncol 2005; 23: 5314-5322.

Galalis E, Buckner JC, Maurer MJ, Kreisberg JL, Ballman K, Boni J, Peralba JM, Jenkins RB, Dakhil SR, Morton RF, Jaeckle KA, Scheithauer BW, Dancey J, Hidalgo M, Walsh DJ. Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. J Clin Oncol 2005; 23: 5294-5304.

Margolin K, Longmate J, Baratta T, Synold T, Christensen S, Weber J, Gajewski T, Quirt I, Doroshov JH. CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. Cancer 2005; 104: 1045-1048.

Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Bossmans J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IG, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ. Temsirolimus, interferon alfa, or both for advanced renal cell carcinoma. N Engl J Med 2007; 356: 2271-2281.

Boulay A, Zumstein-Mecker S, Stephane C, Beuvink I, Zilbermann F, Haller R, Tobler S, Heusser C, O'Reilly T, Stolz B, Marti A, Thomas G, Lane HA. Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. Cancer Res 2004; 64: 252-261.

Lane H, Tanakam C, Kovarik J, O'Reilly EM, Zumstein-Mecker S, McMahon LM, Cohen P, O'Donnell A, Judson I, Raymond E. Preclinical and clinical pharmacodynamic (PK/PD) modeling to help define an optimal biological dose for the oral mTOR inhibitor, RAD001, in oncology. Proc Am
164 **Phase II Trial of RAD001 in Refractory Colorectal Cancer.** Available from: URL: http://www.clinicaltrial.gov/ct/show/NCT00337545

165 Rao RD, Windschitl HE, Allred JB, Lowe VJ, Maples WJ, Gornet MK, Suman VJ, Creagan ET, Pitot HC, Markovic SN. Phase II trial of the mTOR inhibitor everolimus (RAD-001) in metastatic melanoma. *J Clin Oncol* 2006; 24: 8043

166 Milton DT, Kris MG, Azzoli CG, Gomez JE, Heelan R, Krug LM, Pao W, Pizzo B, Rizvi NA, Miller VA. Phase I/II Trial of Gefitinib and RAD001 (Everolimus) in Patients (pts) with Advanced Non-Small Cell Lung Cancer (NSCLC). *J Clin Oncol* 2005; 23: 7104

167 Yao JC, Phan AT, Chang DZ, Jacobs C, Mares JE, Rashid A, Meric-Bernstam F. Phase II study of RAD001 (everolimus) and depot octreotide (Sandostatin LAR) in patients with advanced low grade neuroendocrine carcinoma (LGNET). *J Clin Oncol* 2006; 24: 4042

168 Amato RJ, Miselliati A, Khan M, Chiang S. A phase II trial of RAD001 in patients (Pts) with metastatic renal cell carcinoma (MRCC). *J Clin Oncol* 2006; 24: 4530

169 Van Oosterom A, Dumez H, Desai J, Stoobants S, Van den Abbeele AD, Clement P, Shand N, Kvarik J, Tsyrlova A, Demetri GD. Combination signal transduction inhibition: A phase I/II trial of the oral mTOR-inhibitor everolimus (E, RAD001) and imatinib mesylate (IM) in patients (pts) with gastrointestinal stromal tumor (GIST) refractory to IM. *J Clin Oncol* 2004; 22: 3002

S- Editor Liu Y  E- Editor Yin DH