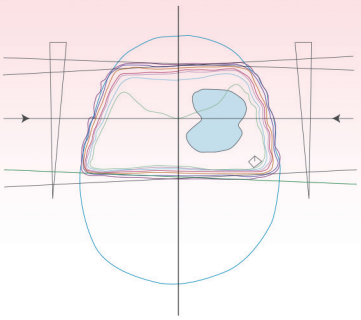
CHAPTER

5 Biologics and Their Interactions with Radiation



Timothy V. Waxweiler and David Raben

Over the past decade, radiation oncology researchers have rapidly developed the capability of delivering "targeted" therapy using photon- or proton-based energies with the evolution of intensity-modulated radiation therapy (IMRT) and the incorporation of image-guided motion tracking. During this same decade, advances in molecular pathology have resulted in powerful predictive biomarkers such as KRAS, ALK, and BRAF that have ushered in a new era in cancer therapy, that of personalized cancer therapeutics. Improved understanding of the molecular mechanisms underlying malignant processes has allowed the development of novel therapeutic agents that target specific cellular processes. Molecularly targeted therapeutic agents, or biologics, are a class of agents that have been developed to specifically interfere with functions central to the pathophysiology of the malignant phenotype.

Although initially targeted in the single-agent setting and then with chemotherapy, many of these biologic pathways are also central to the radiation response; this finding raised the likelihood that some biologic agents may be effective radiosensitizers. A radiosensitizing effect has indeed been demonstrated clinically and in model systems. As biologically targeted drugs continue to gain prominence in the cancer therapy landscape, radiation oncologists should be familiar with therapies that their patients are likely to be receiving in conjunction with radiation. Furthermore, the potential for radiosensitization mandates that radiation oncologists become educated regarding the potential for these agents to exacerbate toxicity or improve efficacy in the standard radiotherapy setting. Finally, the potential to take advantage of the new biologics as radiosensitizers offers an exciting opportunity to improve local control for many tumor types treated with radiotherapy.

Before we can move forward we must ask why many of our preclinical studies, that at first glance appear promising, have failed to translate successfully in phase III clinical trials when combined with chemoradiation.[1,2] Over the past decade or so, we have, in fact, only demonstrated success in one phase III trial using an anti-EGFR antibody with radiation alone.[3] Perhaps the optimal preclinical studies were never performed to adequately evaluate optimal sequencing and whether adding a targeted agent to a chemoradiation backbone in fact could be antagonistic. Perhaps some of these targeted drugs might be best used as maintenance therapies after completion of chemoradiation. Perhaps dual targeting of specific pathways might provide improved local-regional control over traditional chemoradiation combinations and should be tested against standard of care. Do we need to dose biologically based approaches in a maximum tolerated drug (MTD) format similar to what we traditionally do with chemoradiation phase I trials? We have even gone as far as adding dual biologics to a chemoradiation backbone with little or no preclinical data to support its clinical translation, resulting in added toxicity.[4-7] These are critical questions that must be answered if we are to realistically move our field forward in a molecular age.

In a previous version of this chapter, the authors emphasized developments in the laboratory and in the clinic related to epidermal growth factor inhibition, antiangiogenesis, proteasome interference, DNA repair inhibitors, mTOR inhibitors, and insulin growth factor receptor inhibitors; and how these factors cooperate with radiation. In this edition, the clinical experience with familiar molecularly targeted drugs and radiation has been updated and streamlined; however, we intend to place an emphasis on selected newer agents that appear promising in a variety of disease sites. These include PI3K/Akt/mTOR pathway inhibitors, PARP inhibitors, and immunomodulating agents. Many additional agents are under investigation including "dirty" biologics, agents such as the multityrosine kinase inhibitors that target multiple pathways, although a detailed discussion of these agents is beyond the scope of this chapter. To provide some perspective, since the previous edition of this chapter was published in 2011, the number of biologics approved by the Food and Drug Administration (FDA) has more than tripled, from ~14 to more than 50 agents (Table 5-1). This update particularly focuses on emerging biologics believed likely to play an increasing role in clinical radiation oncology in the near future. What does the practicing radiation oncologist need to know as we witness a remarkable evolution in our understanding of molecular oncology and develop new insights in to radio-genomics to predict response and toxicity to radiation therapy? Next-generation sequencing is assisting in defining new targets within a cancer to personalize our therapeutic approaches and this information may be valuable in finding the optimal agents to combine with radiation within a specific disease site.

EPIDERMAL GROWTH FACTOR RECEPTOR FAMILY INHIBITORS

Epidermal Growth Factor Receptor Family Biology

The epidermal growth factor receptor (EGFR) family signaling process has captured significant attention clinically over the past 15 years as a targetable pathway that could be used in conjunction with radiation. Why is it important and how does it work? EGFR signaling regulates mesenchymal-epithelial interactions during growth and development, transmitting extracellular cues to intracellular signaling cascades.[10-12] The family has four known members: EGFR, HER2 (erbB2), HER3 (erbB3), and HER4 (erbB4). These membrane-spanning tyrosine kinase receptors contain an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. These normally quiescent receptors are activated when ligand binds to the extracellular domain of a receptor monomer. Ligand binding induces a structural change that favors dimerization with the same member (homodimer) or a different member (heterodimer) of the family. When dimerized, the tyrosine kinase domains are activated and they phosphorylate key tyrosine residues in the intracellular domain, resulting in activation of several downstream signaling cascades.

The end result of receptor activation, proliferation, differentiation, migration, or survival signaling depends on many factors, including which receptor pairs are formed and for

TABLE 5-1 FDA-Approved Biologic Modifiers[8,9]

Agent	Class	Approved Indication	Key Toxicities
Afatinib[RT] (Gilotrif)	mTKI: HER2, EGFR	Metastatic NSCLC	Skin effects
Aflibercept[RT] (Zaltrap)	VEGFR fusion-protein antibody	Metastatic CRC	Hemorrhage, GI perforation, poor wound healing
Alectinib*	ALK inhibitor	Crizotinib-refractory NSCLC	Fatigue, GI effects
Alemtuzumab[RT] (Campath)	CD52 antibody	B-cell CLL	Cytopenias, infusion reactions
Axitinib[RT] (Inlyta)	mTKI: VEGFR, PDGFR, CKIT	Metastatic RCC	HTN, GI effects, fatigue
Belinostat (Beleodaq)	HDAC inhibitor	Peripheral T-cell lymphoma	Fatigue, GI effects, anemia, fever
Bevacizumab[RT] (Avastin)	Anti-VEGFR antibody	CRC; advanced NSCLC; metastatic RCC; recurrent glioblastoma multiforme; metastatic HER2-negative breast cancer	Hemorrhage
Bortezomib[RT] (Velcade)	Proteasome inhibitor	Multiple myeloma; mantle cell lymphoma	Cytopenias, GI effects, neuropathy
Bosutinib (Bosulif)	Src-Abl TKI	Ph(+) CML	GI effects
Brentuximab vedotin[RT] (Adcetris)	CD30 antibody-drug conjugate	Refractory Hodgkin lymphoma; anaplastic large cell lymphoma	Cytopenias, GI effects
Cabozantinib[RT] (Cometriq)	mTKI: RET, VEGFR, TIE2, MET, TRKB	Metastatic medullary thyroid cancer	Hemorrhage, GI perforation or fistula
Carfilzomib (Kyprolis)	Proteasome inhibitor	Refractory multiple myeloma	Cytopenias, GI effects
Catumaxomab (Removab)	CD3, EpCAM antibody	Malignant ascites	Fever, GI effects
Ceritinib* (Zykadia)	ALK inhibitor	ALK(+) metastatic NSCLC after crizotinib	Fatigue, GI effects, hyperglycemia
Cetuximab[RT] (Erbitux)	Anti-EGFR antibody	Irinotecan-refractory metastatic CRC with KRAS wt; HNSCC	Anaphylaxis, skin rash
CO-1686*	EGFR	EGFR(+) NSCLC with T790M resistance mutation	Hyperglycemia, GI effects, fatigue
Crizotinib[RT] (Xalkori)	ALK inhibitor	ALK(+) locally advanced or metastatic NSCLC	GI effects
Dabrafenib (Tafinlar)	BRAF inhibitor	Advanced melanoma with BRAF mutation	Skin effects
Daratumumab*	CD38 antibody	Refractory multiple myeloma	Cytopenias
Dasatinib[RT] (Sprycel)	mTKI: Src, BCR-ABL, CKIT, PDGFR, TKI	Refractory CML or Ph(+) ALL	Myelosuppression
Denileukin diftitox (Ontak)	CD25-directed diptheria cytotoxin	CD25(+) cutaneous T-cell lymphoma	Infusion reaction, capillary leak syndrome, vision loss
Denosumab (Xgeva)	RANK ligand inhibitor	Bone metastases prevention; giant cell tumor of bone	Musculoskeletal effects
Entinostat*	HDAC inhibitor	Advanced ER(+) breast cancer	Fatigue, neutropenia
Erlotinib[RT] (Tarceva)	EGFR TKI	Chemotherapy-refractory NSCLC; advanced pancreatic cancer with gemcitabine	Skin rash, diarrhea
Everolimus[RT] (Afinitor)	mTOR inhibitor	Advanced RCC; progressive neuroendocrine tumors of pancreatic origin; subependymal giant-cell astrocytoma in tuberous sclerosis; advanced breast cancer with exemestane	GI effects
Gefitinib[RT] (Iressa)	EGFR TKI	Advanced or chemotherapy-refractory NSCLC	Skin rash, diarrhea
Ibrutinib*	Bruton TKI	Mantle cell lymphoma; Waldenstrom macroglobulinemia; CLL	GI effects
Ibritumomab (Zevalin)	CD20 RIT	Relapsed or refractory low-grade or follicular B-cell NHL; follicular NHL with response to first-line chemotherapy	Infusion reactions, cytopenias, mucocutaneous reactions

Continued

TABLE 5-1 FDA-Approved Biologic Modifiers—cont'd

Agent	Class	Approved Indication	Key Toxicities
Imatinib[RT] (Gleevec)	BCR-ABL TKI	Philadelphia chromosome–positive CML or ALL; GIST; dermatofibrosarcoma protuberans; myelodysplastic/myeloproliferative disorders; systemic mastocytosis	Myelosuppression
Ipilimumab[RT] (Yervoy)	CTLA-4 antibody	Advanced melanoma	Autoimmune reactions
Lapatinib[RT] (Tykerb)	mTKI: EGFR, HER2	Refractory HER2 overexpressing advanced breast cancer with capecitabine	Rash, diarrhea
MPDL3280A*	PD-L1	Metastatic bladder cancer	Auto-immune reactions
Nilotinib[RT] (Tasigna)	BCR-ABL TKI	Ph(+) CML	QT prolongation
Obinutuzumab* (Gazyva)	CD20 antibody	CLL	Infusion reactions, cytopenias, PML
Ofatumumab* (Arzerra)	CD20 antibody	Refractory CLL	Cytopenias, GI effects
Palbociclib*	CDK4, CDK6	ER(+), HER2(–) advanced breast cancer	Cytopenias
Panitumumab[RT] (Vectibix)	Anti-EGFR antibody	Irinotecan-refractory metastatic CRC without KRAS mutation	Anaphylaxis, skin rash
Pazopanib[RT] (Votrient)	mTKI: VEGFR, PDGFR, CKIT	Metastatic RCC; advanced soft tissue sarcoma	Hepatotoxicity
Pembrolizumab* (aka lambrolizumab)	PD-1 antibody	Advanced melanoma	Autoimmune reactions
Pertuzumab[RT] (Perjeta)	Anti-HER2 antibody	HER2 overexpressing breast cancer with trastuzumab or docetaxel	Cardiomyopathy, embryo-fetal toxicity
Radium-223 (Xofigo)	Calcium mimetic	Castration-resistant prostate cancer with bone metastases	GI effects
Ramucirumab (Cyramza)	VEGFR	Advanced or metastatic gastric or gastroesophageal adenocarcinoma	GI effects, HTN, headaches
Regorafenib (Stivarga)	mTKI: VEGFR, PDGFR,KIT, RET, TIE2, FGFR, RAF, MAPK	Unresectable or refractory GIST; previously treated CRC	Hepatotoxicity
Rituximab[RT] (Rituxan)	CD20 antibody	Non-Hodgkin lymphoma; CLL	Infusion reactions, tumor lysis, mucocutaneous effects, PML
Romidepsin[RT] (Istodax)	HDAC inhibitor	Cutaneous T-cell lymphoma	GI effects
Temsirolimus[RT] (Torisel)	mTOR inhibitor	Advanced RCC	Rash, asthenia, GI effects
Tositumomab I-131 (Bexxar)	CD20 RIT	Relapsed or refractory follicular NHL	Hypersensitivity, cytopenias
Trametinib[RT] (Mekinist)	MEK inhibitor	Advanced melanoma	Cardiomyopathy, skin effects, GI effects
Trastuzumab[RT] (Herceptin)	Anti-HER2 antibody	HER2-neu overexpressing breast cancer	Cardiomyopathy
Sorafenib[RT] (Nexavar)	mTKI: VEGFR, PDGFR, Raf, c-KIT	Unresectable hepatocellular carcinoma; advanced RCC	Rash, diarrhea, HTN
Sunitinib[RT] (Sutent)	mTKI: VEGFR, PDGFR, c-KIT, Flt3	Advanced RCC; GIST; pancreatic neuroendocrine tumors	Rash, diarrhea, HTN
Vandetanib[RT] (Caprelsa)	mTKI: RET, VEGFR, EGFR	Unresectable or metastatic medullary thyroid cancer	QT prolongation
Volasertib*	Polo-like kinase inhibitor	AML	Cytopenias
Vorinostat[RT] (Zolinza)	HDAC inhibitor	Cutaneous T-cell lymphoma	GI effects
Vemurafenib[RT] (Zelboraf)	BRAF kinase	Unresectable or metastatic melanoma with BRAF mutation	Photosensitivity and other skin effects

[RT]Concurrent trials with radiation are ongoing or have been completed.

ALL, *Acute lymphocytic leukemia;* ALK, *anaplastic lymphoma kinas;* CLL, *chronic lymphocytic leukemia;* CML, *chronic myelogenous leukemia;* CRC, *colorectal cancer;* EGFR, *epidermal growth factor receptor;* FGFR, *fibroblast growth factor receptor;* GI, *gastrointestinal;* GIST, *gastrointestinal stromal tumor;* HDAC, *histone deacetylase;* HNSCC, *head and neck squamous cell carcinoma;* HTN, *hypertension;* mTKI, *multiple tyrosine kinase inhibitor;* NHL, *non-Hodgkin lymphoma;* NSCLC, *non–small cell lung cancer;* PDGFR, *platelet-derived growth factor receptor;* Ph(+), *Philadelphia chromosome positive mutation;* PML, *progressive multifocal leukoencephalopathy;* RCC, *renal cell carcinoma;* RIT, *radioimmunotherapy;* SEGA, *subependymal giant cell astrocytoma;* TKI, *tyrosine kinase inhibitor;* VEGFR, *vascular endothelial growth factor receptor.*

**Initial approval by FDA Breakthrough Therapies program for expedited drug development under the FDA Safety and Innovation Act of 2012.*

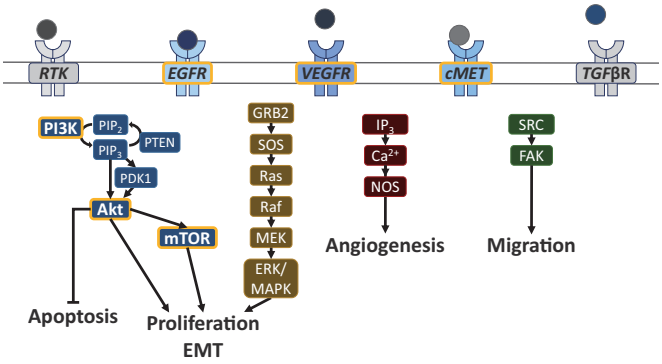


Figure 5-1 General overview of major signaling pathways involved in oncogenesis (many of the molecular targets emphasized in this chapter are outlined in yellow). Cell surface receptors (e.g., EGFR, VEGFR, cMET) normally bind various growth factors and small molecules initiating cell signaling pathways, which lead to maintenance of various cell processes. Mutations involved in up-regulated oncogene or down-regulated tumor suppressor activity lead to inhibition of apoptosis, cell proliferation, epithelial mesenchymal transition (EMT), angiogenesis, migration/invasion, and a variety of additional pro-oncogenic processes.

how long they are activated.[13] This in turn depends on which receptors are predominantly present in the cell and which ligand is involved in activation. There are two ligand families that activate the EGFR family receptors, the EGF-like and heregulin families.[14,15] The EGF-like family includes EGF, TGF-α, amphiregulin, betacellulin, and HB-EGF. The heregulin (neuregulin) family includes many proteins resulting from splice variations of two different genes, all designated as heregulin with different subtypes. The ligands exhibit preference for particular receptors and induce different receptor combinations. HER2 has no known ligand. Instead, HER2 is the favored partner of the other receptors when ligand binds to EGFR, HER3, or HER4.[16] This complex interplay of receptors is important for understanding and interpreting the effect of an inhibitor of a single member of the EGF family.

The effect of receptor activation also depends on which downstream signals are activated involving DNA synthesis and repair, apoptosis evasion, growth factor signaling, and proliferation. EGFR family members signal via a diverse network of signal transduction pathways, including the protein kinase C (PKC), Ras-Raf-ERK, PI3K-Akt, and STAT pathways[17] (Figure 5-1). Furthermore, various receptor pairs recruit different downstream effectors. For instance, HER3 contains multiple PI3K-binding motifs, resulting in strong signaling via PI3K, which plays a role in cell survival, invasion, and proliferation. Interestingly, HER3 alone among the receptors has an inefficient kinase domain, requiring heterodimerization with other family members to become phosphorylated. The need for heterodimerization juxtaposes the PI3K signal emanating from HER3 with the Ras-Raf-ERK or STAT signal emanating from EGFR, HER2, or HER4.

EGFR Family and Tumor Pathogenesis

In 1986, the Nobel Prize was awarded to Stanley Cohen for the discovery of growth factors, resulting from his work in identifying EGF and its receptor.[18] EGFR was first identified as a proto-oncogene because of its homology to the avian erythroblastosis (v-erb) oncogene.[19] Aberrant function of EGFR or HER2 occurs frequently in human tumors; gene amplification results in massive overexpression in a proportion of gliomas and breast cancers.[20-22] Alternatively, dysregulation occurs at more modest levels of expression when the receptor is activated as the result of autocrine stimulation, in which the tumor produces its own ligand to activate the receptor. This type of dysregulation occurs frequently in cancers of the head and neck, gastrointestinal system, and prostate gland.[23-26] Another mechanism of dysregulation is the development of mutations in the kinase domain that render the kinase activity more potent, most clearly demonstrated in lung cancer.[27] Likewise, mutations in the ligand-binding domain can cause the receptor to be constitutively active even in the absence of ligand, as occurs in a significant proportion of gliomas.[28] Dysregulation via mechanisms other than amplification may not always result in overexpression as detected by standard immunohistochemical techniques, raising the issue of how best to identify all tumors in which EGFR dysregulation promotes tumor proliferation and resistance to therapy.

EGFR Family Inhibitors

The frequent dysregulation of the EGFR family in tumors makes the family an attractive target for exploitation. Remarkable progress has been made in the development of EGFR family inhibitors.[29] Many antibodies directed against the extracellular domains of EGFR and HER2 and small-molecule tyrosine kinase inhibitors have now been approved by the FDA for clinical application, and many more are in various stages of development.[8,30]

The first EGFR family–targeted agent to be approved with radiation was cetuximab (Erbitux), an anti-EGFR monoclonal antibody that binds to the extracellular domain of EGFR, interferes with ligand binding and, hence, dimerization and activation.[31] Cetuximab has modest activity as a single agent but gives more encouraging results when it is combined with cytotoxic therapy. Cetuximab is typically given intravenously on a weekly schedule. When combined with radiation therapy, a loading dose is given the week before initiation of radiation treatment. Cetuximab has gained FDA approval for use in treating metastatic colorectal cancer and in locally advanced head and neck cancers. Initial approval for cetuximab in treating metastatic colorectal cancer was based largely on the results of a positive trial including 329 patients randomized to receive either cetuximab and irinotecan or cetuximab alone.[32] Further molecular analysis demonstrated that patients with KRAS mutations in codons 12 or 13 did not respond to cetuximab with survival benefits seen only in patients with wild type KRAS; this is presumably related to alternate activation of signal transduction pathways.[33,34] In head and neck cancers, we have yet to determine biomarkers that predict response to anti-EGFR therapy. This finding underscores the complexity of cancer biogenetics and marks an important turning point toward an era of personalized cancer therapy.

The anti-Her2 antibody trastuzumab and more recently pertuzumab, an antibody that inhibits Her2 heterodimerization, have been shown to improve outcomes for patients with Her2 overexpressing breast cancer.[35-37] These agents are currently recommended for combined use by the National Comprehensive Cancer Network (NCCN) in a variety of stages and settings. Additional investigations are ongoing in other cancers with Her2 overexpressing, including esophageal and salivary duct tumors.

Further discussions regarding the use of EGFR inhibitors alone or with chemotherapy are beyond the scope of this chapter, however, we provide references related to these avenues.[38-44] Our focus is a review of the use of these agents with radiation preclinically and the successes and failures in the clinical arena. One of the most frustrating aspects of this combination is the fact that we still struggle with a lack of predictive biomarkers related to response to EGFR inhibitors. Is it the ligand presence that predicts response? Does the

presence of an EGFR mutation always predict response to EGFR inhibitors in diseases like lung cancer, or do we need to dig deeper?[45] Is it based on gene amplification or high gene copy numbers in EGFR wild type cancers?[46]

Several small-molecule tyrosine kinase inhibitors targeting EGFR have gained FDA approval: gefitinib (Iressa), erlotinib (Tarceva), afatinib (Gilotrif), and lapatinib (Tykerb). These compounds specifically inhibit the tyrosine kinase activity of an EGFR family receptor while relatively sparing the other EGFR family members and related tyrosine kinases. Gefitinib and erlotinib act on EGFR; lapatinib is active against HER2; and afatinib targets both EGFR and HER2. These small-molecule agents have shown modest benefits in patients with advanced malignancies (primarily in patients with EGFR mutations); however, they have yet to demonstrate any significant benefits in phase II/III clinical trials with irradiation. One phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme (GBM) exhibited unacceptable toxicity with multiple treatment related deaths and no evidence of increased efficacy.[47]

The primary toxicity of both gefitinib and erlotinib occurs in the skin, similar to cetuximab, and as diarrhea. However, infrequent cases of serious, life-threatening interstitial lung disease have also been reported for both agents, as well as anaphylactic reactions in approximately 3% of patients treated with cetuximab. Because these reactions can be life threatening, careful monitoring is required with these agents.

EGFR Family and Radiation Response

EGFR family members play an important role in radiation response. Preclinical studies showed that cells made to express v-erb were rendered radioresistant.[48] Similarly, breast cancer cell lines become more radioresistant when made to overexpress HER2, and head and neck cancer cell radioresistance correlates with EGFR expression levels.[49-52]

Clinical studies also suggest that EGFR family dysregulation influences radiation response. A study of 170 gliomas treated with primary radiotherapy demonstrated lower response rates in tumors that overexpressed EGFR; the response rate was 33% in EGFR-negative tumors, 18% in EGFR-intermediate tumors, and 9% in EGFR-positive tumors.[53] In smaller series of patients with head and neck cancers, locoregional recurrence after radiotherapy was associated with EGFR overexpression.[54,55] In breast cancer, a case-control series of patients with in-breast tumor recurrence after breast-conserving surgery and radiotherapy found that the proportion of patients with HER2 overexpression was higher in the recurrence group than in the controls.[56]

Preclinical Studies of EGFR Family Inhibitors as Radiosensitizers

The role of EGFR family members in radiation response was further clarified by studies using newly developed EGFR family inhibitors. In virtually every study, EGFR or HER2 inhibitors demonstrated modest radiosensitization.[57-59] Radiosensitization is more pronounced in vivo than in vitro and with fractionated-dose than with single-dose irradiation. The understanding of the mechanisms underlying enhanced radiosensitization is evolving.[60-62] In every case, the combination of EGFR or HER2 inhibitors and radiation resulted in increased cell cycle arrest, predominately in the G1 phase, but with a substantial decrease in S phase, which translates in vivo to decreased proliferation. Radiosensitization with EGFR family inhibitors also causes decreased angiogenesis. It is not yet clear whether this is an additive result of combined antiangiogenesis effects from both radiation and EGFR inhibitors, or whether the EGFR inhibitors further increase the susceptibility of the vascular elements of the tumor to radiation. The combination of EGFR inhibitors and radiation increases apoptosis in some, but not all, models. Finally, EGFR inhibitors appear to directly interfere with EGFR induced DNA-PK-dependent nonhomologous end joining repair of radiation-induced DNA damage.[63,64] What is lacking in past studies were designs that actually mimicked what we do in the clinic, including comparing chemoradiation to chemoradiation plus an EGFR inhibitor for example in the disease setting to be clinically studied rather than extrapolating from another tumor type. These types of studies, albeit performed in somewhat artificial systems, might have provided valuable information as to the best way to move forward in a clinical trial.

Clinical Studies of EGFR Family Inhibitors as Radiosensitizers

Promising preclinical studies with EGFR family inhibitors have translated into improved patient outcomes in randomized controlled trials when added to radiation alone primarily in patients with locally advanced head and neck squamous cell carcinoma (LA-HNSCC). The most mature of these is a phase III trial that compared the efficacy of standard radiotherapy with standard radiotherapy plus the anti-EGFR antibody cetuximab. In this study, 424 patients with LA-HNSCC of the oropharynx, hypopharynx, or larynx were stratified by T stage, nodal status, and performance status and then were randomly assigned to receive radiotherapy alone or radiotherapy plus weekly concurrent cetuximab. Radiotherapy was delivered using one of three fractionation regimens (stratified): a once-daily regimen (2 Gy × 35 fractions over 7 weeks), a twice-daily regimen (1.2 Gy × 60 to 64 fractions over 5 to 5.5 weeks), or a concomitant-boost regimen (1.8 Gy × 30 fractions with a second daily fraction of 1.5 Gy for the last 12 treatment days over 6 weeks). Concurrent chemotherapy was not allowed. Two-year local control was 56% in the radiotherapy-plus-cetuximab arm versus 48% in the radiotherapy-alone arm, with a median duration of local control of 36 months versus 19 months, respectively ($p = 0.02$).[65] Overall survival was also significantly enhanced with combined therapy. A recent update reported 45.6% 5-year overall survival in the radiotherapy-plus-cetuximab arm versus 36.4% in the radiotherapy-alone arm, with median survival of 49 months versus 29 months, respectively ($p = 0.018$).[3]

Although concern for unwanted normal tissue effects when combining targeted drugs against EGFR and radiation therapy alone is justifiable, the additional morbidity of this approach appears minimal based on the experiences in the clinic to date. In the study by Bonner et al,[65] the improvement in outcome was associated with an increase in acute skin, but not mucosal, toxicity. Furthermore, there were no differences between the groups on standardized quality-of-life assessment scores. Interestingly, a grade 2 or greater acneiform rash correlated with better survival rates (HR 0.49, $p = 0.002$). This correlation, seen in other studies in multiple disease sites, has been hypothesized to be reflective of an anticancer immune response.[66,67] In a separate trial, concurrent administration of adjuvant radiotherapy with trastuzumab in patients with early-stage breast cancer did not increase the incidence of acute radiation toxicity.[68]

Results have been mixed in trials of EGFR inhibitors added to combination chemotherapy and radiation. RTOG 0234, a phase II randomized trial comparing radiation plus cetuximab and either weekly cisplatin or weekly docetaxel in patients with LA-HNSCC, suggest that both regimens are feasible with outcomes superior to results from RTOG 9501 that used high-dose cisplatin on days 1, 22, and 43.[69] Grade 3 to 4 myelosuppression was observed in 28% (cisplatin) and 14% (docetaxel) of patients. Dermatitis was seen in 39% of patients in each

group. The rates of grade 3 or higher mucositis were 37% and 33% in the cisplatin and docetaxel arms, respectively; these rates seem low but are encouraging compared with historical controls. The 2-year distant metastasis rate was 13% in the group that received docetaxel and cetuximab versus 26% with cisplatin and cetuximab. One of the conclusions from this trial is that perturbing growth factor signaling may allow us, under the right circumstances, to reduce administration of high doses of standard chemotherapy and reduce patient morbidity. The addition of cetuximab to the current standard of cisplatin and radiotherapy for LA-HNSCC was evaluated in the phase III RTOG 0522 with abstract only results thus far showing no improvement in progression-free survival (PFS) or overall survival (OS), equivalent overall grade 3 to 5 toxicities, and increased grade 3 or higher mucositis and skin reactions in patients receiving chemoradiation with cetuximab versus chemoradiation alone.[1] In the phase II trial ACOSOG Z4051, 70 patients with locally advanced esophageal adenocarcinoma received preoperative therapy with the EGFR monoclonal antibody panitumumab added to docetaxel, cisplatin, and radiation.[7] Despite 54% of patients showing at least a near pathologic complete response, nearly half of all patients had grade 4 or higher toxicities making this regimen unsuitable for further study. To our knowledge, this type of combination was not studied preclinically to assess its efficacy and safety before going forward into a clinical trial.

An alternative strategy is to evaluate the use of induction chemotherapy before combination EGFR inhibition and radiation. The TREMPLIN study, a phase II randomized trial, directly compared the addition of cisplatin versus cetuximab to radiation for 116 patients with LA-HNSCC of the larynx/hypopharynx with a greater than 50% response following three cycles of traditional induction chemotherapy.[70] Larynx preservation at 3 months, larynx function at 18 months, and overall survival at 3 years demonstrated equivalent outcomes. Local failures were slightly higher in the cetuximab arm (8 vs. 5), but only the patients in the cetuximab arm eventually underwent salvage surgery (7 vs. 0). Grade 3 or higher rates of mucositis were similar between arms, and skin toxicity was roughly doubled in the cetuximab arm; however, acute renal toxicity was substantial in the cisplatin plus radiotherapy arm. This suggests that perhaps by using induction chemotherapy we may be able to study combinations of biologically targeted agents without the added toxicity of conventional chemotherapy agents, assuming the preclinical studies support this approach. An additional trial, the Gruppo di Studio Tumori della Testa e del Collo (GSTCC) trial, employed a 2×2 factorial design for 421 patients with LA-HNSCC randomized with and without induction chemotherapy and randomized to concurrent radiation with either cetuximab or cisplatin.[71] Abstract results showed similar response rates, PFS, and OS between patients receiving cetuximab versus cisplatin. Toxicity rates were also similar between the two groups and patients receiving cetuximab actually required more treatment interruptions with a median radiation therapy (RT) duration of 8 weeks versus 7 weeks in the cisplatin arms. Additional retrospective analyses examining cetuximab versus cisplatin have suggested that cisplatin provides better local control but similar overall survival outcomes.[72,73]

Why the failures to date? As mentioned at the beginning of this chapter, perhaps the optimal preclinical studies were never performed. Often, biologic agents like EGFR inhibitors are combined with radiation in the laboratory and the assumption is made that this will be just as effective, if not more so, when combined clinically with chemoradiation. Preclinical studies must seek to optimize clinically relevant standards to truly understand the optimal sequencing and combinations for integrating EGFR inhibitors with, and perhaps without, radiation, chemotherapy, and additional biologics. Currently, multiple RTOG trials continue to address these issues in head and neck cancers in the setting of intermediate or high-risk postoperative settings (i.e., 0920, 1216) and oropharynx cancers that are positive for human papillomavirus (HPV) (i.e., 1016). Additional RTOG trials examining EGFR targeting concurrently with radiation include RTOG 0839 using panitumumab in locally advanced NSCLC, RTOG 0974 with trastuzumab for Her2 positive breast ductal carcinoma in situ, and RTOG 1010 employing trastuzumab for locally advanced esophageal adenocarcinoma. These are a handful of examples of the ongoing efforts needed to cull out which patients benefit from targeting a particular pathway such as epidermal growth factor signaling and which patients, based on specific mutations, may need interference with Akt, mTOR, or DNA repair pathways in addition to or in lieu of traditional chemotherapy approaches.

ANGIOGENESIS INHIBITORS

Angiogenesis and Tumor Pathogenesis

All tumors require development (or expansion) of blood vessels to promote further tumor growth and nutritional support beyond a 2-mm diameter.[74] Molecules such as vascular endothelial growth factor (VEGF) mediate stimulation of angiogenic signaling and neovascularization. Elevated levels of specific isoforms of VEGF and other indirect markers predict for a worse prognosis in many types of cancer, including those of the gastrointestinal tract, such as pancreatic and esophageal cancers.[75,76] VEGF expression is affected by both the genetic aberrancies of the particular cancer as well as the microenvironmental changes, including hypoxia.

Once VEGF binding activates VEGF-receptor signaling, a cascade of transcriptional signals to promote blood vessel formation is set in motion (see Figure 5-1). Tumors develop a nutritional support system by borrowing existing blood vessels, growing new vessels from surrounding endothelium, and entrapping circulating endothelial stem cells. In contrast to mature vessels, developing tumors display vessels that are immature and chaotic, with a resultant lack of cohesion within the vessel matrix. As a result of increased permeability, blood perfusion through the tumor can be heterogeneous. This can lend itself to areas of hypoxia, which in turn results in activation of pro-angiogenic molecules such as HIF1 and nuclear factor kappa B (NFκB). Transcriptional activation occurs with further production of VEGF and additional pro-angiogenic proteins such as cyclooxygenase (COX)–2, Tie-2, osteopontin, histone deacetylase, and hepatocyte growth factor. An autocrine and paracrine cascade is created to further tumor growth and invasion.

Angiogenesis Inhibitors

Three main strategies under preclinical or clinical investigation exemplify the important concepts underlying angiogenic inhibition: (1) small-molecule tyrosine kinase inhibitors (TKIs) that target vascular endothelial growth factor receptor (VEGFR) signaling, (2) agents that directly target the tumor vasculature, or vascular targeting agents (VTAs), and (3) agents that inhibit VEGF.

Multiple VEGFR-TKIs have now gained FDA approval including sorafenib (Nexavar), sunitinib (Sutent), axitinib (Inlyta), pazopanib (Votrient), regorafenib (Stivarga), vandetanib (Caprelsa), and cabozantinib (Cometriq). Many of these biologics target multiple TKIs in addition to VEGFR signaling. They are indicated for treatment of a variety of cancers including unresectable hepatocellular carcinoma, advanced or metastatic renal cell carcinoma, metastatic colorectal cancer,

gastrointestinal stromal tumors, advanced soft-tissue sarcomas, and metastatic medullary thyroid cancer based on positive results of phase III randomized studies. Vandetanib and cabozantinib are active against VEGFR, EGFR, and more specifically RET kinase, a mutation present in the majority of patients with sporadic or inherited medullary thyroid cancer. These drugs have now been FDA approved for advanced or metastatic medullary thyroid carcinoma following phase III trial results with each significantly prolonging PFS.[76-80]

Agents that target the microtubule formation of intratumoral vasculature, thus destabilizing vessels and causing rapid necrosis within the central part of the tumor, might be effective compounds to combine with radiation. The rationale for pursuing agents that attack intratumoral vessels is based on the premise that endothelial cells in tumors display a different growth pattern than those in normal tissues; it is a chaotic, rapidly expanding pattern. VTAs were developed to take advantage of this differential to selectively occlude or destroy tumor blood vessels. Little progress has been achieved in clinical trials with VTAs, in part because of issues related to cardiac toxicity; however, this remains a potentially promising approach.

An alternative approach, inhibiting VEGF using an anti-VEGF monoclonal antibody is akin to scooping up the keys rather than blocking the lock. Antibodies against VEGF have shown anticancer activity in a variety of preclinical models.[81] Bevacizamab (Avastin) is a recombinant humanized version of the murine antihuman VEGF monoclonal antibody rhuMAb VEGF. Bevacizumab is FDA approved for use in patients with metastatic colorectal cancer when used in combination with 5-fluorouracil (5-FU). Two separate clinical trials demonstrated superior response rates, PFS, and OS in patients treated with combined 5-FU–based therapy with bevacizumab compared with others treated with 5-FU–based therapy alone in either the first- or second-line setting.[82,83] The addition of bevacizumab to carboplatin and paclitaxel also improved overall survival in chemotherapy-naïve patients with metastatic or recurrent, nonsquamous non–small cell lung cancer (NSCLC) and has been approved for use in patients with recurrent GBM, based on trials demonstrating good rates of radiographic response and stable to decreased corticosteroid requirement.[84,85] Improvements in PFS have also been demonstrated in patients with renal cell carcinoma and breast cancer receiving combinations of traditional systemic therapy and bevacizumab.[86] These promising clinical results in the advanced setting have prompted further investigations in the locally advanced setting with radiation.

Preclinical Studies of Angiogenesis Inhibitors as Radiosensitizers

At first glance, one might hesitate to consider blocking angiogenesis from a radiation oncology perspective. If sequencing is not optimal, blocking angiogenesis might to lead to increased hypoxia and reduced tumor control. In fact, the last 10 years have demonstrated the opposite effect: enhanced radiation sensitivity with anti-VEGF agents in the laboratory. Early work by Teicher et al[87-90] demonstrated enhanced radiation cytotoxic effects in a variety of antiangiogenic models. Antiangiogenic agents may enhance the effect of radiation by stabilizing tumor vasculature, thereby enhancing tumor oxygenation.[91] By inhibiting proangiogenic signaling, regulation of antiapoptotic proteins such as amplified NFκB may be improved. Many of these molecules, including VEGF, are activated by radiation, so reversing this process seems logical.

In the clinical trial setting, we have typically combined antiangiogenic agents with chemoradiation; however, we struggle with determining the optimal sequencing. To this end, there have been preclinical studies looking at this issue. ZD6474, a dual VEGFR/EGFR inhibitor, was evaluated with radiation in a xenograft model bearing EGFR-TKI–insensitive NSCLC Calu-6 tumors.[92] Two combined treatment schedules were examined: (1) a concurrent schedule using ZD6474 (50 mg/kg) dosing given 2 hours before the first dose of radiation, and (2) a sequential schedule using ZD6474 dosing given 30 minutes after the last dose of radiotherapy. The sequential approach was superior in terms of the time for treated tumors to quadruple in volume (RTV4) from their pretreatment size ($p < 0.0001$). Importantly, the reduced RTV4 (30 ± 1 day) in the concurrent schedule was also significantly better than either ZD6474 or radiation alone ($p < 0.02$). Nevertheless, clinical trials tend to simply combine these agents concurrently with chemoradiation, ignoring preclinical results.

Similar to studies showing enhanced radiation effects with VEGFR signaling interference, studies combining anti-VEGF antibodies with radiation confirmed that although exposure of human tumor xenografts to radiation promoted induction of VEGF expression, inhibiting VEGF with anti-VEGF antibodies supplanted this effect and resulted in increased endothelial cell killing and synergized antitumor effects in murine tumor model systems.[93]

Clinical Studies of Angiogenesis Inhibitors as Radiosensitizers

Unfortunately limited success has been realized in the clinic. In locally advanced rectal adenocarcinoma, initial phase I studies by Willett et al[94] in rectal cancer brought to light clinical evidence of the vascular normalization hypothesis in which regulation of tumor vasculature may impair entry of metastatic cells into circulation while improving drug delivery and tumor oxygenation for radiosensitization. Multiple subsequent trials have examined the efficacy of either anti-VEGF or anti-EGFR therapies added to standard preoperative chemoradiation regimens for rectal cancer with modest pathologic complete response (pCR) rates ranging from ~10% to 20%.[5,95,96] One recent phase I/II trial examined the combination of bevacizumab and erlotinib with 5-FU and radiation in this setting with an encouraging pCR rate of 33% (9/27) although ~47% of patients experienced at least one grade 3 to 4 toxicity.[5]

In patients with GBM, two recently published major randomized phase III trials examined the addition of bevacizumab versus placebo to standard temozolomide and radiation in newly diagnosed patients.[97,98] Both RTOG 0825 ($n = 637$) and AVAglio ($n = 921$) showed a significant PFS improvement of ~4 months with bevacizumab, but similar overall survival outcomes between arms with a median survival of ~16 to 17 months. MGMT status did not influence response rates between arms in both trials. In the RTOG trial, patients receiving bevacizumab showed higher rates of decline in neurocognitive function and quality of life; whereas in the AVAglio trial, quality-of-life outcomes were improved in patients receiving bevacizumab. Adverse events were modestly increased in the bevacizumab arms of both trials. In the context of these results, the role of bevacizumab in upfront GBM treatment remains unclear.

Results have been more cautionary in combining bevacizumab with EGFR targeting and radiation in lung cancer. Based in part on promising results with bevacizumab and erlotinib in patients with metastatic NCSCL, several phase I/II trials in locally advanced lung cancer have been completed. Bevacizumab and chemoradiotherapy were associated with a concerning incidence of tracheoesophageal fistula and aerodigestive hemorrhage in 3 of 29 patients with small cell lung cancer and NSCLC.[99] A more recent phase I/II trial treated 45

patients with stage III NSCLC with induction, concurrent, and consolidative regimens of bevacizumab and erlotinib with radiation and chemotherapy.[4] Both PFS and OS results were not significantly different from current published results with standard chemoradiation, and on the downside, a significantly higher than expected 29% of patients on this trial had grade 3 to 4 esophagitis. Of note, the radiation in this trial treated elective mediastinal lymph nodes and used three-dimensional conformal radiotherapy to a total dose of 74 Gy (a dose recently shown to have inferior OS when compared to the standard 60 Gy by RTOG 0617); these combined factors likely contributed at least partially to the increased esophagitis rate.[100] The SWOG trial S0533 also attempted to integrate bevacizumab with chemoradiation in a three-step design with administration with docetaxel in the consolidation phase after cisplatin-etoposide and radiation.[101] Because of poor accrual and Cancer Therapy Evaluation Program (CTEP) warnings regarding toxicity, the trial was stopped early. Two of seven patients in the high risk cohort experienced grade 5 hemoptysis.

Bevacizumab as a radiosensitizer has also been studied in cervical cancer with a recently completed RTOG 0417 trial giving the agent every 2 weeks for three cycles during concurrent cisplatin and radiotherapy in 49 patients with stage IB-III disease.[102] With primary endpoints focused on adverse events, this trial noted minimal protocol-defined toxicity with 13 patients (26.5%) having grade 3 toxicities; 5 patients (10.2%) experiencing grade 4 toxicity; and no grade 5 toxicities. The majority of all toxicities were hematologic. In terms of efficacy, 3-year rates showed an 81% OS, 69% disease free-survival, and 23% loco-regional failure.[103] These outcomes are on par with published major trials using standard chemoradiation in cervical cancer.[104]

Thus, in 2014, we are still searching for successful clinical combinations of antiangiogenic agents with radiation in a multitude of disease sites with no positive phase III trials to demonstrate that this strategy is working. Jackson et al[105] further discuss the current use of bevacizumab in combined modality settings with an emphasis on cervical cancer in their recent review, providing the reader with a comprehensive discussion of current and future avenues.

PI3K/Akt/mTOR PATHWAY, INHIBITORS, AND RADIOSENSITIZATION

One of the most important pathways related to cancer cell survival, the PI3K/Akt/mTOR kinase pathway (see Figure 5-1), is a central regulator of cell metabolism, proliferation, and survival by preventing apoptosis. Furthermore, PI3K/Akt/mTOR is up-regulated in many tumors and can also be up-regulated with radiation.[106] Selective and pan-PI3K inhibitors, PI3K and mTOR dual inhibitors, Akt inhibitors, and mTOR inhibitors are all being developed. Given the variety of targets and specificities of these agents, current research has focused on their efficacy, resistance, and toxicity profiles in a wide range of tumors, especially those with pathway alterations (e.g., loss of PTEN, KRAS mutations, and TSC1/2 alterations).[107]

The agents furthest along in development to date are mTOR inhibitors, primarily rapamycin analogs. These include temsirolimus and everolimus, which block signal transduction downstream of mTOR and facilitate apoptosis, suggesting effectiveness as radiosensitizing agents. Currently these two drugs are approved for clinical use in advanced renal cell carcinoma, and everolimus is also approved for pancreatic neuroendocrine tumors, subependymal giant-cell astrocytomas, and advanced breast cancer with exemestane.[108-110] Their clinical role as radiosensitizers is less established but is an active area of research.

To date, preclinical cancer cell models have demonstrated radiosensitizing effects of mTOR inhibitors.[111,112] Temsirolimus is as effective as cisplatin in radiosensitizing HNSCC lines, and triple-combination therapy did not provide additional cooperative effects over temsirolimus with radiation.[112] The in vivo effects were more dramatic partly because of an antiangiogenic byproduct of mTOR inhibition.

The radiosensitizing role of mTOR inhibitors continues to be investigated through clinical studies with mixed results. In a phase I trial of temsirolimus combined with palliative thoracic radiation in patients with NSCLC, dose-limiting toxicities included sudden death, pneumonitis, and pulmonary hemorrhage; however a safe maximum tolerable dose level was achieved.[113] Three separate phase I trials have examined the combination of radiation, temozolomide, and an mTOR inhibitor (i.e., temsirolimus or everolimus) in patients with GBMs, and infectious complications and stomatitis were the primary toxicities.[114-116] These trials have provided the basis for the phase I/II RTOG 0913 trial (i.e., everolimus with radiation and temozolomide up front and in combination with adjuvant temozolomide in GBMs) and the phase II EORTC 26082 (i.e., temsirolimus versus temozolomide in both the concurrent radiation and adjuvant settings for patients lacking methylation of the MGMT promoter with gliomas). In LA-HNSCC, the mTOR pathway has been shown to mediate expression of eukaryotic protein synthesis initiation factor 4E (eIF4E), with elevated eIF4E expression in histologically cancer-free margins associated with increased risk for recurrence. Based on this evidence, Fury et al[117] performed a phase I trial adding everolimus to standard of care concurrent cisplatin and radiation therapy in 13 patients with LA-HNSCC. Three patients experienced dose-limiting toxicities (i.e., two with mucositis, one with failure to thrive), and lymphopenia was the most common grade ≥3 adverse event seen in 12 patients (92%). Overall, a tolerable everolimus dose of 5 mg daily was found, and at 19.4 months follow-up, only 2 patients (15%) had experienced recurrent disease. Translation of these results into phase II and III trials and beyond is anticipated.

The remaining classes of PI3K/Akt/mTOR pathway inhibitors are still in their infancy with many additional phase I/II trials under way.[118] Several investigations are specifically examining their potential as radiosensitizers in both curative and palliative settings including LA-HNSCC, NSCLC, and malignant gliomas. Emerging data in HPV-positive head and neck cancers suggest an increased prevalence of PI3 kinase (PI3K) pathway mutation and copy number alterations.[119] In HPV-positive tumors harboring PI3K mutations mTOR appears activated rather than AKT downstream and thus may be a reasonable target to combine with radiation for this subgroup of patients.[120] A phase I trial of BKM120 or buparlisib, an oral PI3K inhibitor with cisplatin-radiation, is in the process of opening in patients who are HPV positive with locally advanced head and neck cancers.[8] Next-generation sequencing is a powerful tool that continues to deliver new information on the genomic landscape of many cancers including head and neck and should lead to more personalized cancer care.[121]

DNA REPAIR INHIBITORS: FOCUS ON PARP

PARP and DNA Repair

Up-regulated DNA repair within cancers contributes to radioresistance and is a concept across all histologies. DNA damage from radiation or chemotherapy results in a variety of mechanisms that attempt to fix both single- and double-stranded breaks quickly, so cancer cells can continue to replicate and grow. To counteract this repair, one promising strategy incorporates the use of poly(ADP-ribose) polymerase (PARP)

inhibitors. The PARP family consists of 17 proteins with PARP-1, 2, 3, and 4 and Tankerase 1 and 2 playing key roles in posttranslational modification of proteins involved in multiple pathways.[122,123] PARP-1 is the most heavily studied of these enzymes and is activated by base damage, single-stranded DNA breaks, and double-stranded DNA breaks caused by insults, including chemotherapy and ionizing radiation.[124] Activation leads to poly(ADP-ribosyl)ation of PARP-1, nicotinamide adenine dinucleotide (NAD+) consumption, a localized negative charge, and direct enzyme interactions with subsequent interaction of multiple pathways involving DNA repair (especially XRCC1), chromatin restructuring, and cell-cycle check points (Figure 5-2).[125]

Initial interest in PARP inhibition in oncology stemmed from the concept of synthetic lethality in which cancer cells with preexisting deficiencies in homologous-recombination pathways (e.g., BRCA mutations) exhibit selective cytotoxicity to single agent PARP inhibitors. Although many complex interactions occur with PARP inhibition, these cancer cells' susceptibility is in part attributed to their reliance on PARP-dependent DNA repair pathways such as base excision repair.[126] Remarkable activity has been observed in patients with BRCA1/2 mutations in a phase I study using olaparib (AZD2281), an orally bioavailable PARP inhibitor.[127] Importantly, minimal toxicity was observed in doses up to 600 mg twice a day. Objective antitumor activity was reported only in mutation carriers (22/60 patients entered, all of whom had refractory ovarian, breast, or prostate cancer). Two subsequent international phase II trials in patients with metastatic BRCA1/2 mutated breast ($n = 57$) and ovarian cancers ($n = 54$), respectively, treated two cohorts with the oral PARP inhibitor olaparib at doses of 400 mg twice daily (i.e., the maximum tolerated dose) and 100 mg twice daily.[128,129] These trials saw objective response rates of ~30% to 40% in the high-dose groups and ~10% to 20% in the low-dose groups. Both trials reported mostly low grade toxicities with grade 3 to 4 toxicities limited to fatigue, nausea, vomiting, and anemia.

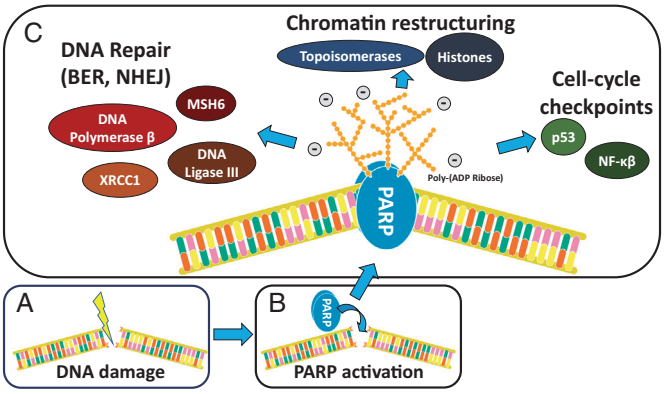


Figure 5-2 Overview of the role of poly(ADP-ribose) polymerase (PARP) activation in response to DNA damage from chemotherapy or irradiation. Activation of PARP triggers various DNA repair pathways, cell cycle regulation, and regulation of gene expression facilitated by chromatin restructuring.

Beyond synthetic lethality, PARP inhibitors show promise as chemosensitizers and radiosensitizers by directly preventing cancer cells from repairing induced DNA damage. In the preclinical setting, many groups have shown the ability of PARP inhibitors to sensitize a variety of histologies, both p53 wild type and null, to radiation in both in vitro and in vivo settings.[130-136] These results are rapidly being translated into the clinic with open phase I and II trial using a variety of PARP inhibitors with radiation and additional systemic agents in sites including central nervous system (CNS), head and neck, breast, lung, esophagus, pancreas, and rectum (Table 5-2).[8] Additional trials have focused on PARP inhibitors with DNA damaging chemotherapies, particularly temozolomide. Specifically, the current RTOG 0929 trial is exploring the use of the oral PARP inhibitor ABT-888 with temozolomide for patients with recurrent GBMs.[137] In development through the NRG (National Surgical Adjuvant Breast and Bowel Project (NSABP), the Radiation Therapy Oncology Group (RTOG), and the Gynecologic Oncology Group (GOG)) cooperative group mechanism is a trial that will evaluate this compound with radiation in patients with newly diagnosed GBM. Of note is the fact that PI3K inhibitors can down-regulate BRCA1/2, suggesting a possible dual targeting approach with these agents and PARP inhibitors to prevent homologous recombination and DNA damage repair.[138]

CHK1 and WEE1

In addition to PARP inhibitors, recent investigations into targeting DNA repair related cell-cycle proteins CHK1 and WEE1 have begun to generate excitement for their role as radiosensitizers. In response to DNA damage, these proteins help mediate both the S and G_2 phase checkpoints.[139] Both CHK1 and WEE1 are up-regulated in P53 mutated cancers, which already bypass the G1 checkpoint.[140] Theoretically, inhibition of either CHK1 or WEE1 would render P53 mutant cancer cells susceptible to DNA damaging treatments such as radiation resulting from critical checkpoint failures, while allowing normal tissues, with intact G1 checkpoints, to remain relatively unaffected. Although lacking preclinical data, several phase I

TABLE 5-2 Select PARP Inhibitors Currently Undergoing Clinical Trials[8,9]

Agent	Manufacturer	Sites
BMN-673	Biomarin	BRCA(+) breast, solid tumors, hematologic malignancies
CEP-9722	Cephalon	NSCLC
E7449	Eisai Incorporated	Advanced solid tumors or B-cell malignancies
Iniparib	Sanofi	Breast, NSCLC
Niraparib	Tesaro	Ovarian, BRCA(+) breast
Olaparib	AstraZeneca	BRCA(+) ovarian, gastric, NSCLC, glioblastoma, esophagus, HNSCC, CRC
Rucaparib	Clovis Oncology	Ovarian, fallopian tube, peritoneal,
Veliparib	Abbott Labs	Pancreatic, ovarian, cervical, breast, NSCLC, DPG, SCLC, liver, prostate, melanoma, metastatic solid tumors, leukemia, myeloma,

CRC, *Colorectal carcinoma;* DPG, *diffuse pontine glioma;* HNSCC, *head and neck squamous cell carcinoma;* NSCLC, *non–small cell lung cancer;* SCLC, *small-cell lung carcinoma.*

trials are evaluating WEE1 inhibitors with concurrent radiation and chemotherapy in patients with recurrent GBMs, cervical cancer, and unresectable pancreatic adenocarcinomas.[8]

IMMUNE TARGETED BIOLOGICS

Cancer and the Immune System

In the past decade, increasing research emphasis has been placed on understanding the role the immune system plays in preventing and controlling cancers and the system's response to radiation. Despite maintaining a multitude of distinctly nonself-antigens among clonal cell populations, many cancers still develop the capability to circumvent the immune system's surveillance of nonself-antigens. Recent research has elucidated that these cancers reach a state of immune tolerance through halting the immune system at immune checkpoints and preventing T-cell activation through alteration of both stimulatory and inhibitory signaling.[141] Two clinically significant regulators of the immune checkpoint are cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), which inhibits initial T-cell activation, and programmed cell death protein 1 (PD-1), which suppresses subsequent T-cell activity in peripheral tissues and tumors.[142-144] Evolving quickly in many different disease sites, we may be entering a new frontier of durable responses never quite seen before in deadly diseases. Can the efficacy of immunologic strategy be amplified with radiation?

CTLA-4 Inhibitors

Ipilimumab, a CTLA-4 antibody, is the first FDA-approved targeted therapy to specifically promote the body's antitumor immune response, rather than directly targeting cancer cells. This human monoclonal antibody's approval for use in unresectable or metastatic melanoma was based on results from two recent phase III trials.[145,146] One trial in 676 patients with metastatic melanoma saw a 3.6-month improvement in median OS from 6.4 months with placebo to 10.0 months with ipilimumab, regardless of whether patients received an additional vaccine that was also part of the study.[145] The 2-year OS nearly doubled for patients on ipilimumab versus placebo (23.5% vs. 13.7%). Regarding toxicity, 14 treatment-related deaths occurred and 10% to 15% of patients had grade 3 to 4 immune-related adverse events. A subsequent trial tested the addition of ipilimumab versus placebo to dacarbazine in 502 patients with metastatic melanoma and again saw a significant improvement in OS.[146] Patients receiving dacarbazine with ipilimumab versus placebo had median OS of 11.2 months versus 9.1 months and 3-year OS of 20.8% versus 12.2%. Although no drug-related deaths occurred, grade 3 to 4 adverse events occurred in 56% of patients in the ipilimumab arm versus 27.5% of those in the placebo arm. As previously seen, this difference was primarily as a result of increased immune-related events including skin, gastrointestinal, and hepatic toxicity. Another CTLA-4 inhibitor, tremelimumab, also underwent multiple phase I-III trials but was not approved because of dose levels inducing unacceptable toxicities and lack of a proven survival advantage.[147]

Ipilimumab, Radiation, and the Abscopal Effect

First proposed in 1953, the abscopal effect has since been elucidated as an immune-mediated systemic cancer response induced by localized radiotherapy.[148,149] Since ipilimumab's FDA approval in 2011, at least four cases have been published linking its use with radiation to the abscopal effect.[150-152] In each case, the patients received ipilimumab either before, concurrent with, or subsequent to stereotactic body radiotherapy or radiosurgery. The high doses of radiation given to each patient have been postulated as necessary to induce sufficient tumor necrosis to allow for an immune response.

A group from Memorial Sloan-Kettering initially reported on a case of a woman with metastatic melanoma who received ipilimumab for four doses followed roughly 1 year later by palliative radiotherapy to a pleural-based spinal para mass to 2850 cGy in three fractions. Following one additional ipilimumab dose 2 months after radiation, the patient was noted to have radiographic regression of both the irradiated lesion and multiple additional distant metastases that were previously progressing.[150] This same group subsequently reported on a patient with metastatic melanoma receiving radiation to an internal mammary lymph node with regression of distant, nonirradiated, left axillary lymph node metastases. In a separate report, a patient with metastatic melanoma received stereotactic radiosurgery to a brain metastasis with concurrent ipilimumab and had a complete clinical response of all metastatic lesions.[151] Notably, his titers of melanoma autoantibodies to melanoma antigen A3 increased from 1:300 to 1:700 following radiation and ipilimumab, supporting a systemic immune response. A Stanford group noted a complete systemic response in a patient with metastatic melanoma receiving stereotactic radiotherapy to two of eight hepatic lesions sandwiched in the middle of four cycles of ipilimumab.[152] Despite the promise of these cases and the growing use of radiation and ipilimumab in patients with melanoma, the abscopal effect remains a rare phenomenon. Ongoing research is investigating the optimal radiation and ipilimumab dosing and timing and the possibility of a biomarker indicating which patients will be most suitable for these treatments. Currently, the National Cancer Institute reports six clinical phase I or II trials hoping to further elucidate the underlying immune mechanisms of the abscopal effect and possibly finding a consistently reproducible method of inducing this phenomenon.[118]

PD-1 and PDL-1 Inhibitors

In a similar but separate immune suppressing vein, the primary effect of PDL-1 binding of PD-1 is suppression of the cytotoxic T-cell response in peripheral tissues and tumors.[153] This mechanism was initially elucidated in the setting of chronic viral infections and inflammatory states, but in the past decade, basic and translational research has shown that PD-1 pathway activation provides an alternative approach to CTLA4 through which cancers can achieve a durable state of immune tolerance.[144,154,155] Notably, cancers have been shown to activate this pathway via two different mechanisms. In one method, a tumor develops constitutive overexpression of PDL-1 on its membrane providing immune suppression regardless of tumor microenvironment. Separately, tumor cells can be induced to express PDL-1 by cytokines (e.g., interferon-γ [IFN-γ]) in a local inflammatory state.[156]

Several molecular therapeutics targeting PD-1 or its ligand PDL-1 are currently under investigation in multiple disease sites with published results appearing promising to date. Three separate phase I studies of more than 600 total patients with advanced cancers of eight separate disease sites receiving an anti-PD-1 or anti-PDL-1 antibody showed objective responses in ~10% to 50% of patients with grade 3 to 4 toxicities in the range of 10% primarily involving immune-related complications.[157-159] In each study, a subset of patients was noted to have durable response off therapy. A recent report notes two patients who achieved eventual durable complete responses lasting more than 3 years off therapy and one patient with 16 months of a stable off-therapy partial response, eventual relapse, and subsequent partial response for at least

16 months following reinduction of anti-PD-1 therapy.[160] In a subset immunohistochemical analysis of tumors for one of these trials, only those tumors expressing PDL-1 by immunohistochemical analysis achieved a response.[159] Based on results of one of these trials focusing on melanoma patients, pembrolizumab (previously lambrolizumab) has gained expedited FDA approval through the FDA Breakthrough Therapies program.[9] In addition, many more trials are ongoing, and further research with these agents is needed to determine optimal dosing and timing; whether patients can be selected for these therapies based on molecular studies; whether these agents can be combined with complementary immunomodulatory biologics such as ipilimumab; and whether these agents have a role in conjunction with radiation including the interesting possibility of an alternative mechanism for generating an abscopal effect. A more in-depth review of immune-targeted biologics by Pardoll et al is provided for reference.[141]

FUTURE DIRECTIONS

Several other classes of agents have not yet received FDA approval but have significant potential as radiosensitizers. The central role of signal transduction, DNA repair, and cell cycle control in radiation response leads to obvious interest in investigating agents that selectively perturb these processes in tumor cells as radiosensitizers.[161,162] Additionally, newer agents targeting cancer stem cells (CSCs) may help impair tumor resistance and relapse following radiation, surgery, and systemic therapies. Here we briefly discuss the bright future of several of these classes.

c-MET

c-MET, another receptor tyrosine kinase, binds hepatocyte growth factor (HGF) and has been associated with a variety of oncogenic pathways including angiogenesis, cell proliferation, DNA damage repair, epithelial-mesenchymal transition (EMT), and the related metastatic capabilities of cell motility and invasion.[163,164] Recent in vitro and in vivo studies have shown that c-MET is up-regulated in many cancer histologies and also up-regulated further in irradiated cells possibly also playing a role in radioresistance.[165,166] At present a variety of these biologics are in varying stages of development as far as phase III trials; however, minimal clinical applications have attempted to use these in conjunction with radiation. Further preclinical investigations examining the radiosensitizing effects of these agents will be necessary before reaching the clinical stage as radiosensitizers.

TGF-β

An intriguing area of anticancer research that is associated with chronic inflammation and potentially tied into the immune-modulating story is the transforming growth factor β (TGF-β) pathway.[167] The TGF-β pathway is found in abundance in solid tumors and is associated with malignant progression through a variety of interactions on the tumor cell and in the surrounding microenvironment. What is its specific mechanism of action? TGF-β1 signaling occurs primarily through a heteromeric complex of type II and type I TGF-β receptors that activates the Smad pathway by TβRI-mediated phosphorylation of Smad2 and Smad3. Subsequent nuclear relocation of receptor-associated phosphorylated Smads bound to Smad4, results in activated TGF-β-driven transcriptional responses.[168] The TGF-β receptor complex can also signal via non-Smad pathways to affect cell survival and EMT.

The paradox lies in the fact that TGF-β actually demonstrates tumor suppressive effects that cancer cells attempt to get around; yet later, TGF-β promotes cancer cell proliferation and invasion once the suppressor activity is blocked (Figure 5-3). Evidence exists that radiation can activate the TGF-β signaling pathway with cross-talk activation of COX-2.[169] This phenomenon has been observed in normal tissue injury in patients treated with radiation.[170] For a wonderful overview of TGF-β signaling and its association with cancer progression as well as opportunities to exploit this pathway for improved anticancer effects we point you to an extensive review by Dancea et al.[171]

Targeting Cancer Stem Cells

In the CSC model, a select group of CSCs drive the renewal, differentiation (including epithelial-to-mesenchymal transition), invasion, and often treatment resistance of an overall heterogeneous population of a cancer.[172] Notch, WNT, and the hedgehog (HH) pathways play central roles in the livelihood

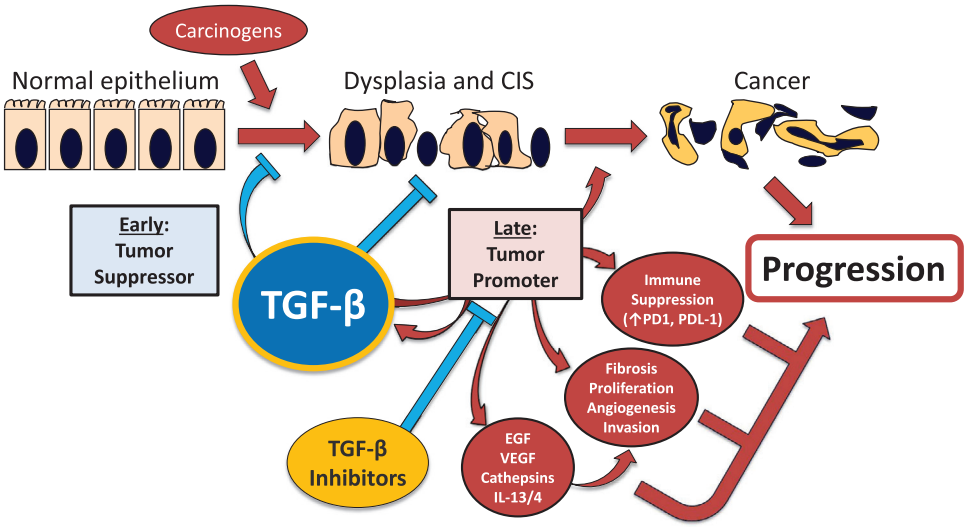


Figure 5-3 The TGF-β pathway exhibits tumor suppressive effects through cell cycle regulation and apoptosis early on in carcinogenesis. Eventually, cancer cells bypass the inhibitory signaling, and TGF-β becomes tumor promoting via a variety of hallmark oncogenic pathways, while concurrently up-regulating TGF-β production.

of these CSCs, offering a handful of promising biologic targets. Multiple phase I and II clinical trials testing inhibitors of these pathways are under way in a variety of solid tumors with early results showing general tolerability but mixed efficacy.[173-175] Furthermore, the potential to impair repopulation makes CSC pathway inhibitors attractive agents as radiosensitizers, especially in the setting of cancers known to exhibit radiation-induced accelerated repopulation such as HNSCC. An early in vivo study presented at ASCO 2014 found that the combination of a HH antibody with radiation in mice with primary cervical cancer xenografts led to better tumor growth delay, reduced lymph node metastasis, increased survival, and had no overt toxicity when compared to radiation alone.[176]

The heterogeneous population of cancers and CSCs may be responsible for the mixed efficacy of biologics previously discussed in this chapter. Nevertheless, this challenge offers an opportunity founded in the hypothesis that cancers may be more vulnerable to combined modality therapies including combinations of complementary biologics, not necessarily limited to CSC pathway inhibitors.[172] In the clinical setting, increased expression GLI-1, a zinc finger protein induced in HH pathway activation, was recently shown to be associated with increased metastasis, progression, and overall survival independent of stage and EGFR expression in a retrospective analysis of HNSCC tumors from patients treated on RTOG 9003.[177] Subsequently, Keysar et al[178] showed that in vitro and in vivo HH pathway inhibition drove HNSCC cells into an EGFR-dependent state thereby preventing cetuximab resistance and significantly impairing tumor growth when combined with cetuximab. A better understanding of such cross-talk signaling pathways among CSCs and the collective cancer cell population will be critical in determining the most clinically effective and tolerable combinations.

Designing Clinical Trials

If the past few years are any indication, we can expect continued exponential growth of targeted therapeutic agents in oncology. With endless permutations of dosing, timing, and combinations of these agents and modern radiation and chemotherapies, increasing emphasis must be placed on careful design and implementation of clinical trials. The NCI and RTOG recently released collaborative strategic guidelines for early stage development of radiosensitizers.[179] The guidelines highlight multiple hurdles in the development of radiosensitizers including difficulties in translating preclinical studies, poor endpoints such as tumor response rates which are expected to occur with radiation alone, and attributing toxicity to an agent versus radiation in a single-arm phase I trial. Furthermore, they provide recommendations for optimizing radiosensitizer trials including rigorous evaluation of preclinical and single-agent clinical data; avoidance of redundant pharmacokinetic studies; efficient methods of dose-escalation; and accurate assessment of expected toxicities. Given the extensive costs of these modern targeted therapies, we must continue to investigate biomarkers for optimal selection of patients whose tumors are most likely to respond to a specific intervention. This sentiment has been echoed by ASCO's recent Choosing Wisely recommendations and recently pursued by large-scale screening efforts.[180,181] Large-scale phase III trials in unselected patients are an inefficient and extremely costly means for scientific progress in our field. A notable alternative to the current trial design paradigm is the concept of the basket study design. In this setting, trials would test targeted therapies in patients with specific mutations regardless of cancer site or histology or within a specific disease site such as lung or rectal cancer with therapy directed based on the mutation status of each patient. Advances in next-generation sequencing will play a central role in making these types of trials feasible.

In addition, and very exciting, is the recent discoveries using cancer personalized profiling by deep sequencing (CAPP-Seq), an efficient and relatively inexpensive ultrasensitive method for quantifying circulating tumor DNA (ctDNA). As an example, Diehn et al[182] at Stanford applied CAPP-Seq for NSCLC with a design covering multiple classes of somatic alterations that identified mutations in >95% of tumors. Subsequently, they detected ctDNA in 100% of patients with stage II-IV NSCLC and in 50% of patients with stage I, with a remarkable 96% specificity for mutant allele fractions down to ~0.02%. Importantly, the measured levels of ctDNA were highly correlated with tumor volume and were accurate in distinguishing between residual disease after radiation and treatment-related imaging changes by computed tomography (CT) or positron emission tomography (PET). ctDNA levels also assisted in determining earlier response to therapy compared to radiographic measurements that can often be difficult to assess. This type of emerging technology offers a glimpse into future possibilities when combining novel agents with radiation and will help us determine if patients are developing resistance to a particular drug. Additionally, integrating radiation into this new framework would need to account for site-specific targeting and toxicity factors (Figure 5-4). With less than 15% of current phase I trials involving radiation, much

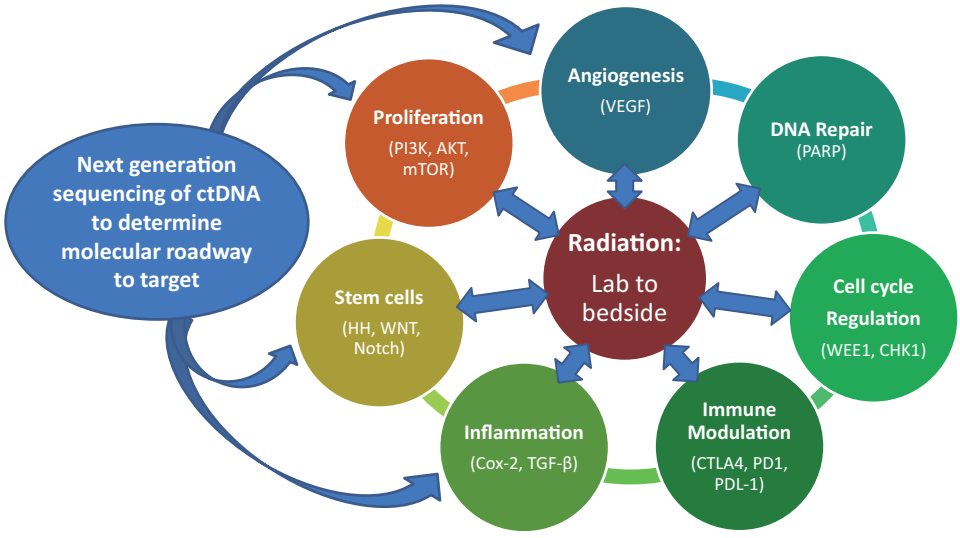


Figure 5-4 Preclinical studies leading to clinical translation using molecular discoveries such as next-generation sequencing to rationally tailor and sequence various biologics during and after radiation therapy for locally advanced disease—getting away from traditional chemoradiation approaches.

work lies ahead for our field toward better understanding the role of targeted therapeutics as radiosensitizers, presenting exciting opportunities to improve treatment results.[118]

CRITICAL REFERENCES

A full list of cited references is published online at www.expertconsult.com.

3. Bonner JA, Harari PM, Giralt J, et al: Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol 11(1):21–28, 2010.
8. National Institutes of Health: ClinicalTrials.gov Database. [Website], 2014. Available from: <www.clinicaltrials.gov>. Accessed May 20, 2014.
10. Earp HS, 3rd, Calvo BF, Sartor CI: The EGF receptor family—multiple roles in proliferation, differentiation, and neoplasia with an emphasis on HER4. Trans Am Clin Climatol Assoc 11315–11333, discussion 333–334, 2003.
11. Yarden Y: The EGFR family and its ligands in human cancer. Signaling mechanisms and therapeutic opportunities. Eur J Cancer 37(Suppl 4):S3–S8, 2001.
12. Ullrich A, Schlessinger J: Signal transduction by receptors with tyrosine kinase activity. Cell 61(2):203–212, 1990.
17. Schlessinger J: Cell signaling by receptor tyrosine kinases. Cell 103(2):211–225, 2000.
23. Grandis JR, Tweardy DJ: Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res 53(15):3579–3584, 1993.
40. Inoue A, Kobayashi K, Maemondo M, et al: Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naive non–small cell lung cancer with sensitive EGFR gene mutations (NEJ002). Ann Oncol 24(1):54–59, 2012.
41. Shepherd FA, Pereira JR, Ciuleanu T, et al: Erlotinib in previously treated non–small-cell lung cancer. N Engl J Med 353(2):123–132, 2005.
43. Gatzemeier U, Pluzanska A, Szczesna A, et al: Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non–small-cell lung cancer: The Tarceva Lung Cancer Investigation Trial. J Clin Oncol 25(12):1545–1552, 2007.
55. Ang KK, Berkey BA, Tu X, et al: Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res 62(24):7350–7356, 2002.
58. Harari PM, Huang SM: Radiation response modification following molecular inhibition of epidermal growth factor receptor signaling. Semin Radiat Oncol 11(4):281–289, 2001.
65. Bonner JA, Harari PM, Giralt J, et al: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 354(6):567–578, 2006.
68. Halyard MY, et al: Radiotherapy and adjuvant trastuzumab in operable breast cancer: Tolerability and adverse event data from the NCCTG Phase III Trial N9831. J Clin Oncol 27(16):2638–2644, 2009.
72. Riaz N, Sherman E, Koutcher L, et al: Concurrent chemoradiotherapy with cisplatin versus cetuximab for squamous cell carcinoma of the head and neck. Am J Clin Oncol Jan 7, 2014. [epub ahead of print].
84. Sandler A, Gray R, Perry MC, et al: Paclitaxel-carboplatin alone or with bevacizumab for non–small-cell lung cancer. N Engl J Med 355(24):2542–2550, 2006.
94. Willett CG, Boucher Y, di Tomaso E, et al: Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 10(2):145–147, 2004.
97. Chinot OL, Wick W, Mason W, et al: Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med 370(8):709–722, 2014.
98. Gilbert MR, Dignam JJ, Armstrong TS, et al: A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med 370(8):699–708, 2014.
105. Jackson MW, Rusthoven CG, Fisher CM, et al: Clinical potential of bevacizumab in the treatment of metastatic and locally advanced cervical cancer: Current evidence. Onco Targets Ther 7751-759:2014.
107. Dienstmann R, Rodon J, Serra V, et al: Picking the point of inhibition: A comparative review of PI3K/AKT/mTOR pathway inhibitors. Mol Cancer Ther 13(5):1021–1031, 2014.
118. National Cancer Institute: Clinical trial database, 2014. Available from: <http://www.cancer.gov/ClinicalTrials>. March 3, 2014.
121. Rizzo G, Black M, Mymryk J, et al: Defining the genomic landscape of head and neck cancers through next-generation sequencing. Oral Dis April 12, 2014. doi: 10.1111/odi.12246. [epub ahead of print].
126. Helleday T: The underlying mechanism for the PARP and BRCA synthetic lethality: Clearing up the misunderstandings. Mol Oncol 5(4):387–393, 2011.
139. Morgan MA, Parsels LA, Maybaum J, et al: Improving the efficacy of chemoradiation with targeted agents. Cancer Discov 4(3):280–291, 2014.
141. Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12(4):252–264, 2012.
150. Postow MA, Callahan MK, Barker CA, et al: Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 366(10):925–931, 2012.
151. Stamell EF, Wolchok JD, Gnjatic S, et al: The abscopal effect associated with a systemic anti-melanoma immune response. Int J Radiat Oncol Biol Phys 85(2):293–295, 2013.
158. Hamid O, Robert C, Daud A, et al: Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 369(2):134–144, 2013.
160. Lipson EJ, Sharfman WH, Drake CG, et al: Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clin Cancer Res 19(2):462–468, 2013.
162. Maity A, Kao GD, Muschel RJ, et al: Potential molecular targets for manipulating the radiation response. Int J Radiat Oncol Biol Phys 37(3):639–653, 1997.
163. Bhardwaj V, Cascone T, Cortez MA, et al: Modulation of c-Met signaling and cellular sensitivity to radiation: Potential implications for therapy. Cancer 119(10):1768–1775, 2013.
166. Qian LW, Mizumoto K, Inadome N, et al: Radiation stimulates HGF receptor/c-Met expression that leads to amplifying cellular response to HGF stimulation via upregulated receptor tyrosine phosphorylation and MAP kinase activity in pancreatic cancer cells. Int J Cancer 104(5):542–549, 2003.
171. Dancea H, Shareef M, Ahmed MM: Role of radiation-induced TGF-beta signaling in cancer therapy. Mol Cell Pharmacol 1(1):44–56, 2009.
172. Takebe N, Harris PJ, Warren RQ, et al: Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol 8(2):97–106, 2011.
179. Lawrence YR, Vikram B, Dignam JJ, et al: NCI-RTOG translational program strategic guidelines for the early-stage development of radiosensitizers. J Natl Cancer Inst 105(1):11–24, 2012.
181. ASCO: ASCO's 2013 choosing wisely: Top five list in oncology, 2014. Available from: <http://www.asco.org/practice-research/ascos-2013-top-five-list-oncology>. May 20, 2014.
182. Newman AM, Bratman SV, To J, et al: An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 20(5):548–554, 2014.

REFERENCES

1. Ang KK, Zhang Q, Rosenthal D, et al: A randomized phase III trial (RTOG 0522) of concurrent accelerated radiation plus cisplatin with or without cetuximab for stage III-IV head and neck squamous cell carcinomas (HNC). ASCO Annual Meeting 2011(5500), 2011.
2. Gregoire V, Hamoir M, Chen C, et al: Gefitinib plus cisplatin and radiotherapy in previously untreated head and neck squamous cell carcinoma: A phase II, randomized, double-blind, placebo-controlled study. Radiother Oncol 100(1):62–69, 2011.
3. Bonner JA, Harari PM, Giralt J, et al: Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol 11(1):21–28, 2010.
4. Socinski MA, Stinchombe TE, Moore DT, et al: Incorporating bevacizumab and erlotinib in the combined-modality treatment of stage III non–small-cell lung cancer: Results of a phase I/II trial. J Clin Oncol 30(32):3953–3959, 2012.
5. Blaszkowsky LS, Ryan DP, Szymonifka J, et al: Phase I/II study of neoadjuvant bevacizumab, erlotinib and 5-fluorouracil with concurrent external beam radiation therapy in locally advanced rectal cancer. Ann Oncol 25(1):121–126, 2014.
6. Clarke JL, Molinaro AM, Phillips JJ, et al: A single-institution phase II trial of radiation, temozolomide, erlotinib, and bevacizumab for initial treatment of glioblastoma. Neuro-Oncol 16:984–990, 2014.
7. Lockhart AC, Reed CE, Decker PA, et al: Phase II study of neoadjuvant therapy with docetaxel, cisplatin, panitumumab, and radiation therapy followed by surgery in patients with locally advanced adenocarcinoma of the distal esophagus (ACOSOG Z4051). Ann Oncol 25(5):1039–1044, 2014.
8. National Institutes of Health: ClinicalTrials.gov Database. [Website], 2014. Available from: <www.clinicaltrials.gov>. Accessed May 20, 2014.
9. Food and Drug Administration: Hematology/Oncology (Cancer) Approvals, 2014. Available from: <http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm>. cited 2014; United States Food and Drug Administration Website.
10. Earp HS, 3rd, Calvo BF, Sartor CI: The EGF receptor family—multiple roles in proliferation, differentiation, and neoplasia with an emphasis on HER4. Trans Am Clin Climatol Assoc 11315–11333, discussion 333–334, 2003.
11. Yarden Y: The EGFR family and its ligands in human cancer. Signaling mechanisms and therapeutic opportunities. Eur J Cancer 37(Suppl 4):S3–S8, 2001.
12. Ullrich A, Schlessinger J: Signal transduction by receptors with tyrosine kinase activity. Cell 61(2):203–212, 1990.
13. Klapper LN, Kirschbaum MH, Sela M, et al: Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. Adv Cancer Res 77:25–79, 2000.
14. Olayioye MA, Neve RM, Lane HA, et al: The ErbB signaling network: Receptor heterodimerization in development and cancer. EMBO J 19(13):3159–3167, 2000.
15. Riese DJ, 2nd, Stern DF: Specificity within the EGF family/ErbB receptor family signaling network. Bioessays 20(1):41–48, 1998.
16. Klapper LN, Glathe S, Vaisman N, et al: The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. Proc Natl Acad Sci U S A 96(9):4995–5000, 1999.
17. Schlessinger J: Cell signaling by receptor tyrosine kinases. Cell 103(2):211–225, 2000.
18. Cohen S, Carpenter G, King L, Jr: Epidermal growth factor-receptor-protein kinase interactions. Co-purification of receptor and epidermal growth factor-enhanced phosphorylation activity. J Biol Chem 255(10):4834–4842, 1980.
19. Downward J, Yarden Y, Mayes E, et al: Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature 307(5951):521–527, 1984.
20. Ekstrand AJ, Sugawa N, James CD, et al: Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proc Natl Acad Sci U S A 89(10):4309–4313, 1992.
21. Slamon DJ, Clark GM, Wong SG, et al: Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235(4785):177–182, 1987.
22. Ohgaki H, Dessen P, Jourde B, et al: Genetic pathways to glioblastoma: A population-based study. Cancer Res 64(19):6892–6899, 2004.
23. Grandis JR, Tweardy DJ: Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res 53(15):3579–3584, 1993.
24. Yamanaka Y, Friess H, Kobrin MS, et al: Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. Anticancer Res 13(3):565–569, 1993.
25. Spano JP, Lagorce C, Atlan D, et al: Impact of EGFR expression on colorectal cancer patient prognosis and survival. Ann Oncol 16(1):102–108, 2005.
26. Scher HI, Sarkis A, Reuter V, et al: Changing pattern of expression of the epidermal growth factor receptor and transforming growth factor alpha in the progression of prostatic neoplasms. Clin Cancer Res 1(5):545–550, 1995.
27. Lynch TJ, Bell DW, Sordella R, et al: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. N Engl J Med 350(21):2129–2139, 2004.
28. Wong AJ, Ruppert JM, Bigner SH, et al: Structural alterations of the epidermal growth factor receptor gene in human gliomas. Proc Natl Acad Sci U S A 89(7):2965–2969, 1992.
29. Arteaga C: Targeting HER1/EGFR: A molecular approach to cancer therapy. Semin Oncol 30(3 Suppl 7):3–14, 2003.
30. Fry DW: Mechanism of action of erbB tyrosine kinase inhibitors. Exp Cell Res 284(1):131–139, 2003.
31. Mendelsohn J: Epidermal growth factor receptor inhibition by a monoclonal antibody as anticancer therapy. Clin Cancer Res 3(12 Pt 2):2703–2707, 1997.
32. Cunningham D, Humblet Y, Siena S, et al: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351(4):337–345, 2004.
33. Lièvre A, Bachet JB, Boige V, et al: KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 26(3):374–379, 2008.
34. Vermorken JB, Trigo J, Hitt R, et al: Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. J Clin Oncol 25(16):2171–2177, 2007.
35. Baselga J, Cortés J, Kim SB, et al: Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 366(2):109–119, 2012.
36. Gianni L, Pienkowski T, Im YH, et al: Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): A randomised multicentre, open-label, phase 2 trial. Lancet Oncol 13(1):25–32, 2012.
37. Buzdar AU, Ibrahim NK, Francis D, et al: Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: Results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 23(16):3676–3685, 2005.
38. Pirker R, Pereira JR, Szczesna A, et al: Cetuximab plus chemotherapy in patients with advanced non–small-cell lung cancer (FLEX): An open-label randomised phase III trial. Lancet 373(9674):1525–1531, 2009.
39. Cohen MH, Williams GA, Sridhara R, et al: United States Food and Drug Administration Drug Approval summary: Gefitinib (ZD1839; Iressa) tablets. Clin Cancer Res 10(4):1212–1218, 2004.
40. Inoue A, Kobayashi K, Maemondo M, et al: Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naive non–small cell lung cancer with sensitive EGFR gene mutations (NEJ002). Ann Oncol 24(1):54–59, 2012.
41. Shepherd FA, Pereira JR, Ciuleanu T, et al: Erlotinib in previously treated non–small-cell lung cancer. N Engl J Med 353(2):123–132, 2005.
42. Pérez-Soler R, Chachoua A, Hammond LA, et al: Determinants of tumor response and survival with erlotinib in patients with non–small-cell lung cancer. J Clin Oncol 22(16):3238–3247, 2004.
43. Gatzemeier U, Pluzanska A, Szczesna A, et al: Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non–small-cell lung cancer: The Tarceva Lung Cancer Investigation Trial. J Clin Oncol 25(12):1545–1552, 2007.
44. Mitsudomi T, Morita S, Yatabe Y, et al: Gefitinib versus cisplatin plus docetaxel in patients with non–small-cell lung cancer harboring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. Lancet Oncol 11(2):121–128, 2010.
45. Tsao AS, Papadimitrakopoulou V: The importance of molecular profiling in predicting response to epidermal growth factor receptor family inhibitors in non–small-cell lung cancer: Focus on clinical trial results. Clin Lung Cancer 14(4):311–321, 2013.
46. Wang F, Fu S, Shao Q, et al: High EGFR copy number predicts benefits from tyrosine kinase inhibitor treatment for non–small cell lung cancer patients with wild-type EGFR. J Transl Med 11(1):90, 2013.
47. Peereboom DM, Shepard DR, Ahluwalia MS, et al: Phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme. J Neurooncol 98(1):93–99, 2009.
48. FitzGerald TJ, Hennault S, Sakakeeny M, et al: Expression of transfected recombinant oncogenes increases radiation resistance of clonal hematopoietic and fibroblast cell lines selectively at clinical low dose rate. Radiat Res 122(1):44–52, 1990.
49. Pietras RJ, Poen JC, Gallardo D, et al: Monoclonal antibody to HER-2/neureceptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells overexpressing this oncogene. Cancer Res 59(6):1347–1355, 1999.
50. Miyaguchi M, Takeuchi T, Morimoto K, et al: Correlation of epidermal growth factor receptor and radiosensitivity in human maxillary carcinoma cell lines. Acta Otolaryngol 118(3):428–431, 1998.
51. Sheridan MT, West CM: Ability to undergo apoptosis does not correlate with the intrinsic radiosensitivity (SF2) of human cervix tumor cell lines. Int J Radiat Oncol Biol Phys 50(2):503–509, 2001.
52. Akimoto T, Hunter NR, Buchmiller L, et al: Inverse relationship between epidermal growth factor receptor expression and radiocurability of murine carcinomas. Clin Cancer Res 5(10):2884–2890, 1999.

53. Barker FG, 2nd, Simmons ML, Change SM, et al: EGFR overexpression and radiation response in glioblastoma multiforme. Int J Radiat Oncol Biol Phys 51(2):410–418, 2001.
54. Gupta AK, McKenna WG, Weber CN, et al: Local recurrence in head and neck cancer: Relationship to radiation resistance and signal transduction. Clin Cancer Res 8(3):885–892, 2002.
55. Ang KK, Berkey BA, Tu X, et al: Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res 62(24):7350–7356, 2002.
56. Haffty BG, Brown F, Carter D, et al: Evaluation of HER-2 neu oncoprotein expression as a prognostic indicator of local recurrence in conservatively treated breast cancer: A case-control study. Int J Radiat Oncol Biol Phys 35(4):751–757, 1996.
57. Sartor CI: Epidermal growth factor family receptors and inhibitors: Radiation response modulators. Semin Radiat Oncol 13(1):22–30, 2003.
58. Harari PM, Huang SM: Radiation response modification following molecular inhibition of epidermal growth factor receptor signaling. Semin Radiat Oncol 11(4):281–289, 2001.
59. Raben D, Helfrich B, Bunn PA, Jr: Targeted therapies for non–small-cell lung cancer: Biology, rationale, and preclinical results from a radiation oncology perspective. Int J Radiat Oncol Biol Phys 59(2 Suppl):27–38, 2004.
60. Sartor CI: Mechanisms of disease: Radiosensitization by epidermal growth factor receptor inhibitors. Nat Clin Pract Oncol 1(2):80–87, 2004.
61. Raben D, Helfrich B, Biardiello F, et al: Understanding the mechanisms of action of EGFR inhibitors in NSCLC: What we know and what we do not know. Lung Cancer 41(Suppl 1):S15–S22, 2003.
62. Schmidt-Ullrich RK, Contessa JN, Lammering G, et al: ERBB receptor tyrosine kinases and cellular radiation responses. Oncogene 22(37):5855–5865, 2003.
63. Huang SM, Harar PM: Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: Inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. Clin Cancer Res 6(6):2166–2174, 2000.
64. Dittmann K, Mayer C, Wanner G, et al: The radioprotector O-phosphotyrosine stimulates DNA-repair via epidermal growth factor receptor—and DNA-dependent kinase phosphorylation. Radiother Oncol 84(3):328–334, 2007.
65. Bonner JA, Harari PM, Giralt J, et al: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 354(6):567–578, 2006.
66. Gatzemeier U, von Pawel J, Vynnychenko I, et al: First-cycle rash and survival in patients with advanced non–small-cell lung cancer receiving cetuximab in combination with first-line chemotherapy: A subgroup analysis of data from the FLEX phase 3 study. Lancet Oncol 12(1):30–37, 2011.
67. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al: Cetuximab for the treatment of colorectal cancer. N Engl J Med 357(20):2040–2048, 2007.
68. Halyard MY, et al: Radiotherapy and adjuvant trastuzumab in operable breast cancer: Tolerability and adverse event data from the NCCTG Phase III Trial N9831. J Clin Oncol 27(16):2638–2644, 2009.
69. Kies MS, Holsinger FC, Lee JJ, et al: Induction chemotherapy and cetuximab for locally advanced squamous cell carcinoma of the head and neck: Results from a phase II prospective trial. J Clin Oncol 28(1):8–14, 2010.
70. Lefebvre JL, Pointreau Y, Rolland F, et al: Induction chemotherapy followed by either chemoradiotherapy or bioradiotherapy for larynx preservation: The TREMPLIN randomized phase II study. J Clin Oncol 31(7):853–859, 2013.
71. Ghi MG, Paccagnella A, Ferrari D, et al: A phase II-III study comparing concomitant chemoradiotherapy (CRT) versus cetuximab/RT (CET/RT) with or without induction docetaxel/cisplatin/5-fluorouracil (TPF) in locally advanced head and neck squamous cell carcinoma (LASCCHN): Efficacy results (NCT01086826). J Clin Oncol (Suppl; abst 6003), 2013.
72. Riaz N, Sherman E, Koutcher L, et al: Concurrent chemoradiotherapy with cisplatin versus cetuximab for squamous cell carcinoma of the head and neck. Am J Clin Oncol Jan 7, 2014. [epub ahead of print].
73. Ye AY, Hay JH, Laskin JJ, et al: Toxicity and outcomes in combined modality treatment of head and neck squamous cell carcinoma: Cisplatin versus cetuximab. J Cancer Res Ther 9(4):607–612, 2013.
74. Wedge SR, Ogilvie DJ, Dukes M, et al: ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Res 62(16):4645–4655, 2002.
75. Millikan KW, Mall JW, Myers JA, et al: Do angiogenesis and growth factor expression predict prognosis of esophageal cancer? Am Surg 66(4):401–405, discussion 405–406, 2000.
76. Niedergethmann M, Hildenbrand R, Wostbrock B, et al: High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas. Pancreas 25(2):122–129, 2002.
77. Wells SA, Jr, Gosnell JE, Gagel RF, et al: Vandetanib for the treatment of patients with locally advanced or metastatic hereditary medullary thyroid cancer. J Clin Oncol 28(5):767–772, 2010.
78. Carlomagno F, Vitagliano D, Guida T, et al: ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. Cancer Res 62(24):7284–7290, 2002.
79. Wells SA, Jr, Robinson BG, Gagel RF, et al: Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: A randomized, double-blind phase III trial. J Clin Oncol 30(2):134141, 2012.
80. Elisei R, Schlumberger MJ, Müller SP, et al: Cabozantinib in progressive medullary thyroid cancer. J Clin Oncol 31(29):3639–3646, 2013.
81. Ferrara N, Davis-Smyth T: The biology of vascular endothelial growth factor. Endocr Rev 18(1):4–25, 1997.
82. Hurwitz H, Fehrenbacher L, Novotny W, et al: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350(23):2335–2342, 2004.
83. Giantonio BJ, Catalano PJ, Meropol NJ, et al: Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: Results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol 25(12):1539–1544, 2007.
84. Sandler A, Gray R, Perry MC, et al: Paclitaxel-carboplatin alone or with bevacizumab for non–small-cell lung cancer. N Engl J Med 355(24):2542–2550, 2006.
85. Friedman HS, Prados MD, Wen PY, et al: Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 27(28):4733–4740, 2009.
86. Miller KD, Chap LI, Holmes FA, et al: Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 23(4):792–799, 2005.
87. Mauceri HJ, Hanna NN, Beckett MA, et al: Combined effects of angiostatin and ionizing radiation in antitumour therapy. Nature 394(6690):287–291, 1998.
88. Kozin SV, Boucher Y, Hicklin DJ, et al: Vascular endothelial growth factor receptor-2-blocking antibody potentiates radiation-induced long-term control of human tumor xenografts. Cancer Res 61(1):39–44, 2001.
89. Ning S, Laird D, Cherrington JM, et al: The antiangiogenic agents SU5416 and SU6668 increase the antitumor effects of fractionated irradiation. Radiat Res 157(1):45–51, 2002.
90. Teicher BA, Emi Y, Kakeji Y, et al: TNP-470/minocycline/cytotoxic therapy: A systems approach to cancer therapy. Eur J Cancer 32A(14):2461–2466, 1996.
91. Lee CG, Heijn M, di Tomaso E, et al: Anti-vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res 60(19):5565–5570, 2000.
92. Williams KJ, Telfer BA, Brave S, et al: ZD6474, a potent inhibitor of vascular endothelial growth factor signaling, combined with radiotherapy: Schedule-dependent enhancement of antitumor activity. Clin Cancer Res 10(24):8587–8593, 2004.
93. Gorski DH, Beckett MA, Jaskowiak NT, et al: Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. Cancer Res 59(14):3374–3378, 1999.
94. Willett CG, Boucher Y, di Tomaso E, et al: Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 10(2):145–147, 2004.
95. Willett CG, Duda DG, di Tomaso E, et al: Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: A multidisciplinary phase II study. J Clin Oncol 27(18):3020–3026, 2009.
96. Rödel C, Liersch T, Hermann RM, et al: Multicenter phase II trial of chemoradiation with oxaliplatin for rectal cancer. J Clin Oncol 25(1):110–117, 2007.
97. Chinot OL, Wick W, Mason W, et al: Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med 370(8):709–722, 2014.
98. Gilbert MR, Dignam JJ, Armstrong TS, et al: A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med 370(8):699–708, 2014.
99. Spigel DR, Hainsworth JD, Yardley DA, et al: Tracheoesophageal fistula formation in patients with lung cancer treated with chemoradiation and bevacizumab. J Clin Oncol 28(1):43–48, 2010.
100. Bradley J, Paulus R, Komaki R, et al: A randomized phase III comparison of standard-dose (60 Gy) versus high-dose (74 Gy) conformal chemoradiotherapy with or without cetuximab for stage III non–small cell lung cancer: Results on radiation dose in RTOG 0617. J Clin Oncol 31(15):7501, 2013.
101. Wozniak AJ, Moon JH, Thomas CR: SWOG S0533: A pilot trial of cisplatin (C)/etoposide (E)/radiotherapy (RT) followed by consolidation docetaxel (D) and bevacizumab (B) (NSC-704865) in three cohorts of patients (pts) with inoperable locally advanced stage III non–small cell lung cancer (NSCLC). J Clin Oncol 30(Suppl; abstr 7018), 2012.
102. Schefter TE, Winter K, Kwon JS, et al: A phase II study of bevacizumab in combination with definitive radiotherapy and cisplatin chemotherapy in untreated patients with locally advanced cervical carcinoma: Preliminary results of RTOG 0417. Int J Radiat Oncol Biol Phys 83(4):1179–1184, 2012.
103. Schefter TE, Winter K, Kwon JS, et al: RTOG 0417: Efficacy of bevacizumab in combination with definitive radiation therapy and cisplatin chemotherapy in untreated patients with locally advanced cervical carcinoma. Int J Radiat Oncol Biol Phys 88(1):101–105, 2014.
104. Chemoradiotherapy for Cervical Cancer Meta-Analysis Collaboration: Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: A systematic review and meta-analysis of individual patient data from 18 randomized trials. J Clin Oncol 26(35):5802–5812, 2008.
105. Jackson MW, Rusthoven CG, Fisher CM, et al: Clinical potential of bevacizumab in the treatment of metastatic and locally advanced cervical cancer: Current evidence. Onco Targets Ther 7751–7759, 2014.

106. Molinolo AA, Hewitt SM, Amornphimoltham P, et al: Dissecting the Akt/mammalian target of rapamycin signaling network: Emerging results from the head and neck cancer tissue array initiative. Clin Cancer Res 13(17):4964–4973, 2007.
107. Dienstmann R, Rodon J, Serra V, et al: Picking the point of inhibition: A comparative review of PI3K/AKT/mTOR pathway inhibitors. Mol Cancer Ther 13(5):1021–1031, 2014.
108. Hudes G, Carducci M, Tomczak P, et al: Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 356(22):2271–2281, 2007.
109. Baselga J, Campone M, Piccart M, et al: Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 366(6):520–529, 2012.
110. Krueger DA, Care MM, Holland K, et al: Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. N Engl J Med 363(19):1801–1811, 2010.
111. Shinohara ET, Cao C, Niemann K, et al: Enhanced radiation damage of tumor vasculature by mTOR inhibitors. Oncogene 24(35):5414–5422, 2005.
112. Ekshyyan O, Rong X, Rong Y, et al: Comparison of radiosensitizing effects of the mammalian target of rapamycin inhibitor CCI-779 to cisplatin in experimental models of head and neck squamous cell carcinoma. Mol Cancer Ther 8(8):2255–2265, 2009.
113. Waqar SN, Robinson C, Bradley J, et al: A phase I study of temsirolimus and thoracic radiation in non–small-cell lung cancer. Clin Lung Cancer 15(2):119–123, 2014.
114. Sarkaria JN, Galanis E, Wu W, et al: Combination of temsirolimus (CCI-779) with chemoradiation in newly diagnosed glioblastoma multiforme (GBM) (NCCTG trial N027D) is associated with increased infectious risks. Clin Cancer Res 16(22):5573–5580, 2010.
115. Mason WP, Macneil M, Kavan P, et al: A phase I study of temozolomide and everolimus (RAD001) in patients with newly diagnosed and progressive glioblastoma either receiving or not receiving enzyme-inducing anticonvulsants: An NCIC CTG study. Invest New Drugs 30(6):2344–2351, 2011.
116. Sarkaria JN, Galanis E, Wu W, et al: North Central Cancer Treatment Group Phase I trial N057K of everolimus (RAD001) and temozolomide in combination with radiation therapy in patients with newly diagnosed glioblastoma multiforme. Int J Radiat Oncol Biol Phys 81(2):468–475, 2011.
117. Fury MG, Lee NY, Sherman E, et al: A phase 1 study of everolimus + weekly cisplatin + intensity modulated radiation therapy in head-and-neck cancer. Int J Radiat Oncol Biol Phys 87(3):479–486, 2013.
118. National Cancer Institute: Clinical trial database, 2014. Available from: <http://www.cancer.gov/ClinicalTrials>. March 3, 2014.
119. Lechner M, Frampton GM, Fenton T, et al: Targeted next-generation sequencing of head and neck squamous cell carcinoma identifies novel genetic alterations in HPV+ and HPV– tumors. Genome Med 5(5):49, 2013.
120. Sewell A, Brown B, Biktasova A, et al: Reverse-phase protein array profiling of oropharyngeal cancer and significance of PIK3CA mutations in HPV-associated head and neck cancer. Clin Cancer Res 20(9):2300–2311, 2014.
121. Rizzo G, Black M, Mymryk J, et al: Defining the genomic landscape of head and neck cancers through next-generation sequencing. Oral Dis April 12, 2014. doi: 10.1111/odi.12246. [epub ahead of print].
122. Rouleau M, Patel A, Hendzel MJ, et al: PARP inhibition: PARP1 and beyond. Nat Rev Cancer 10(4):293–301, 2010.
123. Lindahl T, Wood RD: Quality control by DNA repair. Science 286(5446):1897–1905, 1999.
124. Miwa M, Masutani M: PolyADP-ribosylation and cancer. Cancer Sci 98(10):1528–1535, 2007.
125. Nozaki T, Masutani M, Akagawa T, et al: Suppression of G1 arrest and enhancement of G2 arrest by inhibitors of poly(ADP-ribose) polymerase: Possible involvement of poly(ADP-ribosyl)ation in cell cycle arrest following gamma-irradiation. Jpn J Cancer Res 85(11):1094–1098, 1994.
126. Helleday T: The underlying mechanism for the PARP and BRCA synthetic lethality: Clearing up the misunderstandings. Mol Oncol 5(4):387–393, 2011.
127. Fong PC, Boss DS, Yap TA, et al: Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. New Engl J Med 361(2):123–134, 2009.
128. Audeh MW, Carmichael J, Penson RT, et al: Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: A proof-of-concept trial. Lancet 376(9737):245–251, 2010.
129. Tutt A, Robson M, Garber JE, et al: Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: A proof-of-concept trial. Lancet 376(9737):235–244, 2010.
130. Albert JM, Cao C, Kim KW, et al: Inhibition of poly(ADP-ribose) polymerase enhances cell death and improves tumor growth delay in irradiated lung cancer models. Clin Cancer Res 13(10):3033–3042, 2007.
131. Al-Ejeh F, Shi W, Miranda M, et al: Treatment of triple-negative breast cancer using anti-EGFR-directed radioimmunotherapy combined with radiosensitizing chemotherapy and PARP inhibitor. J Nucl Med 54(6):913–921, 2013.
132. Calabrese CR, Almassy R, Barton S, et al: Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361. J Natl Cancer Inst 96(1):56–67, 2004.
133. Chatterjee P, Choudhary GS, Sharma A, et al: PARP inhibition sensitizes to low dose-rate radiation TMPRSS2-ERG fusion gene-expressing and PTEN-deficient prostate cancer cells. PLoS ONE 8(4):e60408, 2013.
134. Shelton JW, Waxweiler TV, Landry J, et al: In vitro and in vivo enhancement of chemoradiation using the oral PARP inhibitor ABT-888 in colorectal cancer cells. Int J Radiat Oncol Biol Phys 86(3):469–476, 2013.
135. van Vuurden DG, Hulleman E, Meijer OL, et al: PARP inhibition sensitizes childhood high grade glioma, medulloblastoma and ependymoma to radiation. Oncotarget 2(12):984–996, 2011.
136. Wang L, Mason KA, Ang KK, et al: MK-4827, a PARP-1/-2 inhibitor, strongly enhances response of human lung and breast cancer xenografts to radiation. Invest New Drugs 30(6):2113–2120, 2012.
137. RTOG: RTOG clinical trials protocol table, 2014. Available from: <http://www.rtog.org/ClinicalTrials/ProtocolTable.aspx>. May 20, 2014.
138. Ibrahim YH, García-García C, Serra V, et al: PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov 2(11):1036–1047, 2012.
139. Morgan MA, Parsels LA, Maybaum J, et al: Improving the efficacy of chemoradiation with targeted agents. Cancer Discov 4(3):280–291, 2014.
140. Vriend LE, De Witt Hamer PC, Van Noorden CJ, et al: WEE1 inhibition and genomic instability in cancer. Biochim Biophys Acta 1836(2):227–235, 2013.
141. Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12(4):252–264, 2012.
142. Rudd CE, Taylor A, Schneider H: CD28 and CTLA-4 coreceptor expression and signal transduction. Immunol Rev 229:12–26, 2009.
143. Parry RV, Chemnitz JM, Frauwirth KA, et al: CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 25(21):9543–9553, 2005.
144. Hirano F, Kaneko K, Tamura H, et al: Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res 65(3):1089–1096, 2005.
145. Hodi FS, O'Day SJ, McDermott DF, et al: Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363(8):711–723, 2010.
146. Robert C, Thomas L, Bondarenko I, et al: Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 364(26):2517–2526, 2011.
147. Ribas A, Kefford R, Marshall MA, et al: Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol 31(5):616–622, 2013.
148. Demaria S, Ng B, Devitt ML, et al: Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys 58(3):862–870, 2004.
149. Mole RH: Whole body irradiation—Radiobiology or medicine. Br J Radiol 26(305):234–241, 1953.
150. Postow MA, Callahan MK, Barker CA, et al: Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 366(10):925–931, 2012.
151. Stamell EF, Wolchok JD, Gnjatic S, et al: The abscopal effect associated with a systemic anti-melanoma immune response. Int J Radiat Oncol Biol Phys 85(2):293–295, 2013.
152. Hiniker SM, Chen DS, Knox SJ: Abscopal effect in a patient with melanoma. N Engl J Med 366(21):2035, 2012.
153. Freeman GJ, Long AJ, Iwai Y, et al: Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 192(7):1027–1034, 2000.
154. Day CL, Kaufman DE, Kiepiela P, et al: PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 443(7109):350–354, 2006.
155. Hirano F, Kaneko K, Tamura H, et al: Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res 65(3):1089–1096, 2005.
156. Taube JM, Anders RA, Young GD, et al: Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med 4(127):127ra37, 2012.
157. Brahmer JR, Tykodi SS, Chow LQ, et al: Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 366(26):2455–2465, 2012.
158. Hamid O, Robert C, Daud A, et al: Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 369(2):134–144, 2013.
159. Topalian SL, Hodi FS, Brahmer JR, et al: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366(26):2443–2454, 2013.
160. Lipson EJ, Sharfman WH, Drake CG, et al: Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clin Cancer Res 19(2):462–468, 2013.
161. Ma BB, Bristow RG, Kim J, et al: Combined-modality treatment of solid tumors using radiotherapy and molecular targeted agents. J Clin Oncol 21(14):2760–2776, 2003.
162. Maity A, Kao GD, Muschel RJ, et al: Potential molecular targets for manipulating the radiation response. Int J Radiat Oncol Biol Phys 37(3):639–653, 1997.

163. Bhardwaj V, Cascone T, Cortez MA, et al: Modulation of c-Met signaling and cellular sensitivity to radiation: Potential implications for therapy. Cancer 119(10):1768–1775, 2013.
164. Blumenschein GB, Jr, Mills GB, Gonzalez-Angulo AM: Targeting the hepatocyte growth factor-cMET axis in cancer therapy. J Clin Oncol 30(26):3287–3296, 2012.
165. Bhardwaj V, Zhan Y, Cortez MA, et al: C-Met inhibitor MK-8003 radiosensitizes c-Met-expressing non–small-cell lung cancer cells with radiation-induced c-Met-expression. J Thorac Oncol 7(8):1211–1217, 2012.
166. Qian LW, Mizumoto K, Inadome N, et al: Radiation stimulates HGF receptor/c-Met expression that leads to amplifying cellular response to HGF stimulation via upregulated receptor tyrosine phosphorylation and MAP kinase activity in pancreatic cancer cells. Int J Cancer 104(5):542–549, 2003.
167. Coussens LM, Zitvogel L, Palucka AK: Neutralizing tumor-promoting chronic inflammation: A magic bullet? Science 339(6117):286–291, 2013.
168. Shi Y, Massague J: Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113(6):685–700, 2013.
169. Chai Y, Lam RK, Calaf GM, et al: Radiation-induced non-targeted response in vivo: Role of the TGFbeta-TGFBR1-COX-2 signaling pathway. Br J Cancer 108(5):1106–1112, 2013.
170. Anscher MS, Kong FM, Murase T, et al: Short communication: Normal tissue injury after cancer therapy is a local response exacerbated by an endocrine effect of TGF beta. Br J Radiol 68(807):331–333, 1995.
171. Dancea H, Shareef M, Ahmed MM: Role of radiation-induced TGF-beta signaling in cancer therapy. Mol Cell Pharmacol 1(1):44–56, 2009.
172. Takebe N, Harris PJ, Warren RQ, et al: Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol 8(2):97–106, 2011.
173. Jimeno A, Weiss GJ, Miller WH, Jr, et al: Phase I study of the Hedgehog pathway inhibitor IPI-926 in adult patients with solid tumors. Clin Cancer Res 19(10):2766–2774, 2013.
174. Sekulic A, Migden MR, Oro AE, et al: Efficacy and safety of vismodegib in advanced basal-cell carcinoma. N Engl J Med 366(23):2171–2179, 2012.
175. Strosberg JR, Yeatman T, Weber J, et al: A phase II study of RO4929097 in metastatic colorectal cancer. Eur J Cancer 48(7):997–1003, 2012.
176. Chaudary N, Pintilie M, Jelveh S, et al: Hedgehog signaling in primary cervix xenograft (OCICx) models: A potential new therapeutic target in combination with chemoradiotherapy. J Clin Oncol 32(5s):Suppl; abstr 11120, 2014.
177. Chung CH, Dignam JJ, Hammond ME, et al: Glioma-associated oncogene family zinc finger 1 expression and metastasis in patients with head and neck squamous cell carcinoma treated with radiation therapy (RTOG 9003). J Clin Oncol 29(10):1326–1334, 2011.
178. Keysar SB, Le PN, Anderson RT, et al: Hedgehog signaling alters reliance on EGF receptor signaling and mediates anti-EGFR therapeutic resistance in head and neck cancer. Cancer Res 73(11):3381–3392, 2013.
179. Lawrence YR, Vikram B, Dignam JJ, et al: NCI-RTOG translational program strategic guidelines for the early-stage development of radiosensitizers. J Natl Cancer Inst 105(1):11–24, 2012.
180. Garnett MJ, Edelman EJ, Heidom SJ, et al: Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 483(7391):570–575, 2012.
181. ASCO: ASCO's 2013 choosing wisely: Top five list in oncology, 2014. Available from: <http://www.asco.org/practice-research/ascos-2013-top-five-list-oncology>. May 20, 2014.
182. Newman AM, Bratman SV, To J, et al: An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 20(5):548–554, 2014.