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Critical Review

Molecular Profiling to Optimize Treatment in Non-Small Cell Lung Cancer: A Review of Potential Molecular Targets for Radiation Therapy by the Translational Research Program of the Radiation Therapy Oncology Group

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Summary

The Translational Research Program of the Radiation Therapy Oncology Group provides a review of molecular pathways that can be targeted in conjunction with radiation therapy in patients with advanced non-small cell lung cancer and strategies for

Therapeutic decisions in non-small cell lung cancer (NSCLC) have been mainly based on disease stage, performance status, and co-morbidities, and rarely on histological or molecular classification. Rather than applying broad treatments to unselected patients that may result in survival increase of only weeks to months, research efforts should be, and are being, focused on identifying predictive markers for molecularly targeted therapy and determining genomic signatures that predict survival and response to specific therapies. The availability of such targeted biologics requires their use to be matched to tumors of corresponding molecular vulnerability for maximum efficacy. Molecular markers such as epidermal growth factor receptor (EGFR), *K-ras,* vascular endothelial growth factor (VEGF), mammalian target of rapamycin (mTOR), and anaplastic lymphoma kinase (ALK) represent potential parameters guide treatment decisions. Ultimately, identifying patients who will respond to specific therapies will allow optimal efficacy with minimal toxicity, which will result in more judicious and effective

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their selection. Only a tailored approach based on tumor genetic make-up will likely result in significantly improved survival in these patients.

application of expensive targeted therapy as the new paradigm of personalized medicine develops.

Keywords: Non-small cell lung cancer, Genomic signatures, Molecular markers, Radiation therapy, Chemotherapy

Introduction

Currently, therapeutic decisions in non-small cell lung cancer (NSCLC) are based on disease stage, performance status, and co-morbidities, not on molecular classification. Although treatment is generalized, NSCLC is a heterogeneous disease. Even in tumors bearing similar clinical and/or histologic features, different treatment outcomes are observed. Thus, there is strong interest in developing new molecular tools to predict survival and to identify patients who may benefit from targeted therapies. However, for these molecular assays to be useful, they need to be well integrated into the current work flow of the clinic; the team would need to obtain relevant molecular data on each patient's tumor in a timely manner without undue treatment delay, and design appropriate treatment regimen based on results of these genomic tests (1). In this review, we discuss molecular pathways that can be targeted in conjunction with radiation therapy (RT) in patients with advanced NSCLC and strategies for their selection (Fig.1).

Molecular Targets

EGFR

One of the most important cellular targets overexpressed in NSCLC is epidermal growth factor receptor (EGFR). The prototypical patient with *EGFR*-mutant NSCLC is a young, Asian, female, nonsmoking patient with adenocarcinoma. Aberrant EGFR expression can stem from mutation L858R (exon 21), in-frame deletions (exon 19), or gene amplification (2). Abnormalities in the EGFR signaling pathway thus provide a tangible target for customizing treatment with EGFR tyrosine kinase inhibitors (TKIs), which are now approved first-line treatment for these tumors. Although responses to first-generation TKIs have been impressive, emergence of new mutations, such as T790M (exon 20), ultimately result in treatment resistance (2). This resistance has led to the development of second-generation TKIs.

Second-generation TKIs

The second-generation TKIs are irreversible inhibitors of other Human Epidermal growth factor Receptor (HER) family members in addition to EGFR. BIBW2992 inhibits EGFR and HER2, whereas PF-00299804 is a pan-HER inhibitor. In a BIBW2992 phase I trial, 3 NSCLC patients with exon 19 deletions experienced sustained partial responses up to 34 months (3). PF-00299804 inhibits both *EGFR*-activating mutations as well as the T790M resistance mutation (4). A phase I study of PF-00299804 found that adverse effects were mild. Four of 57 NSCLC patients, all were previously treated with first-generation TKIs, had a partial response (5). Preliminary results of a phase II trial of PF-00299804 in *K-ras* wild-type NSCLC after erlotinib failure demonstrate stable disease in 9 of 18 adenocarcinoma patients and in 1 of 2 non-adenocarcinoma patients (6).

Second-generation TKI trials focus mainly on patients who developed resistance to first-generation TKIs. Phase II/III trials of BIBW2992 in selected NSCLC patients are currently underway, one of which is investigating T790M development suppression (7). LUX-Lung 5 and LUX-Lung 6 are investigating BIBW 2992 in patients who failed erlotinib/gefitinib and *EGFR* mutation-positive adenocarcinoma, respectively. Although the focus of second-generation TKIs is on preventing or overcoming resistance, they should also be evaluated in conjunction with tumors harboring *K-ras* mutations, which have also been correlated with first-generation TKI resistance (8).

Increased EGFR expression has been associated with radioresistance (9). Combination RT and BIBW2992 therapy was investigated in FaDu head-and-neck squamous cell carcinoma (SCC) cells (10). Radiosensitivity of FaDu cells after preincubation with BIBW2992 was marginally, although statistically significantly, increased. Enhancement ratios were smaller for irradiated than for nonirradiated tumors, suggesting an additive rather than synergistic effect for BIBW2992 combination with RT.

K-ras

K-ras is considered one of the most commonly mutated proto-oncogenes in human cancers. Preclinical studies have illustrated oncogenic *K-ras* plays a role in tumor cell RT resistance, and genetic inactivation of *K-* or *N-ras* in tumor cell lines has been shown to increase radiosensitivity (11). However, only one specific Ras inhibitor, salirasib, is currently in clinical studies for NSCLC. A phase II trial in adenocarcinoma-type NSCLC has released preliminary results. Following 10 weeks of treatment, 28% of previously treated *K-ras* mutation-positive patients and 38% of previously untreated patients who were former smokers had stable disease (12).

Two distinct molecular pathways are involved in lung cancer: *K-ras* mutations in smokers and *EGFR* mutations in never-smokers. *K-ras* encodes a GTPase downstream of EGFR, which may provide a biological explanation for why *EGFR* and *K-ras* mutations appear mutually exclusive. The presence of somatic *K-ras* mutations appears to be associated with a lack of sensitivity to gefitinib and erlotinib (8). Therefore, *K-ras* mutational status (or lack thereof) may be an important predictive factor in defining patients who will benefit most from first-generation TKIs.

Angiogenesis

Angiogenesis has emerged as an anticancer target, as many believe that new vessel growth is necessary for tumor proliferation. Angiogenesis can be induced by various factors, including vascular

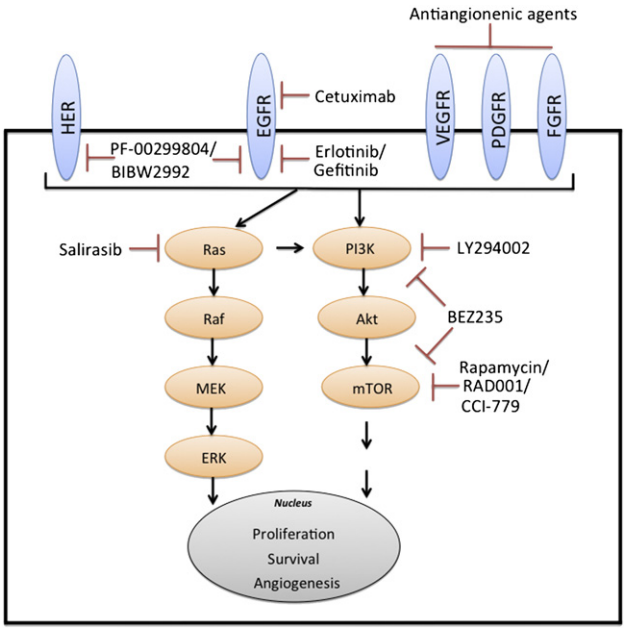

Fig. Summary of potential molecular targets and therapeutic agents. Potential molecular targets include receptor tyrosine kinases and intracellular signaling molecules, which promote tumor cell proliferation, survival, and angiogenesis. Therapeutic agents targeting these kinases and pathways are shown. *Abbreviations:* EGFR = epidermal growth factor receptor; ERK = extracellular signal-regulated kinase; FGFR = fibroblast growth factor receptor; HER = human epidermal growth factor receptor; mTOR = mammalian target of rapamycin; PDGFR = platelet-derived growth factor receptor; PI3K = phosphatidylinositol 3-kinase; VEGFR = vascular endothelial growth factor receptor.

endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). VEGF inhibition can be achieved with ligand antibody or TKI inhibition of VEGFR. However, a proposed mechanism of resistance is the ability of tumor cells to upregulate other angiogenesis pathways, such as PDGF and FGF (13). Therefore, TKIs with ability to block several receptors, such as PDGFR and FGFR, may be more efficacious.

Bevacizumab

Bevacizumab is a humanized VEGF monoclonal antibody. First-line bevacizumab use in advanced NSCLC is largely based on results of Eastern Cooperative Oncology Group (ECOG) trial 4599, which showed a 2-month survival increase when combined with platinum-based therapy (14). Preliminary phase I/II trial data demonstrate serious toxicities such as hemoptysis or fistulas (tracheal, bronchial, or esophageal) with concurrent bevacizumab and RT application (15). Similarly, a phase II trial of bevacizumab plus chemoradiation was ended prematurely because of trache-oesophageal fistula development (16). The Southwestern Oncology Group (SWOG) is sponsoring a phase I/II trial combining cisplatin, etoposide, RT, and bevacizumab with docetaxel and bevacizumab consolidation in three different NSCLC patient populations. This trial will provide valuable information on the safety of this multi-modality approach.

The temporal relationship between RT and bevacizumab administration may be important. Using ovarian and breast carcinoma and melanoma xenografts, Dings et al illustrated enhanced tumor growth inhibition when RT was given several days after bevacizumab administration. This period coincided with the "tumor oxygenation" window, during which tumor oxygen levels improve with pruning of microvessel density (17). It would be interesting to study this vascular normalization phenomenon in NSCLC to investigate optimal administration of bevacizumab and RT.

Axitinib

Axitinib is a TKI of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, and c-kit. A phase I trial of 36 solid tumor patients found an maximum tolerated dose (MTD) of 5 mg twice daily (18). The 2 enrolled NSCLC patients both had tumor cavitation, indicating an antiangiogenic effect, and subsequently died of hemoptysis. In a phase II trial of axitinib monotherapy in 32 NSCLC patients, 75% of whom had adenocarcinoma, the median overall survival (OS) was 14.8 months, and progression-free survival (PFS) was 4.9 months (19).

Fenton et al found that combined axitinib and RT in DU145 human prostate xenografts produced better tumor response than either monotherapy. Compared to the response in controls, vascular density progressively declined and overall tumor hypoxia increased. However, reductions in total and perfused vessels were proportionate, indicating combination therapy did not specifically target functional vasculature (20). Fenton et al further aimed to evaluate whether the sequencing of axitinib and RT would affect therapeutic response, and tumor growth inhibition was found to be equivalent for each treatment schedule, suggesting that post- or pre-irradiation drug administration serves equally well in supplementing RT (21).

BIBF 1120

BIBF 1120 is an inhibitor of VEGFR, FGFR, and PDGFR. A phase I study of BIBF 1120 and pemetrexed in 26 NSCLC patients found that all patients experienced an adverse event, although most adverse events were of low severity. One patient experienced complete response 44 days after initiating medication, and was still in complete response at the study conclusion (>3.5 years later). MTD of BIBF 1120 in combination with standard pemetrexed was 200 mg twice daily (22). In a phase II study, NSCLC patients treated with BIBF 1120 after failure of platinum therapy had a median PFS of 6.9 weeks and median OS of 21.9 weeks (23).

The effect of BIBF 1120 on tumor radiation response has also been studied. Zips et al treated poorly differentiated SCC FaDu in nude mice with BIBF 1120. Although BIBF 1120 significantly reduced vessel perfusion and tumor growth rate, tumor hypoxia, and radiation sensitivity of tumor stem cells were not affected. Concurrent BIBF 1120 also had no effect on local tumor control after RT (24). Further investigation regarding mechanisms of interaction between anti-angiogenic agents and RT is warranted.

mTOR/PI3K

The mammalian target of rapamycin (mTOR) pathway has been shown to be constitutively activated in an estimated 74% of resected NSCLC malignancies (25). Rapamycin analogues and dual PI3K/mTOR inhibitors inhibit signal transduction downstream of mTOR. Preclinical studies have demonstrated inhibition of tumor proliferation by rapamycin and its analogues in several cancer xenografts, including NSCLC (26). In addition, persistent PI3K/Akt/mTOR pathway activation has been associated with resistance to anti-EGFR drugs (27), suggesting a benefit of profiling for mTOR/PI3K pathway alterations to optimize EGFR therapy.

Ekshyyan et al compared cisplatin to the rapamycin analogue CCI-779 as a radiosensitizer in preclinical studies (28). Combined CCI-779 and RT inhibited head-and-neck SCC tumor growth by >90% and more than doubled survival compared with RT alone. Similarly, use of dual PI3K/mTOR inhibitor BEZ235 was shown to sensitize NSCLC cancer cells to radiation (29), and targeting PI3K with inhibitor LY294002 was found to synergistically enhance radiation efficacy in bladder cancer cells (30). This inhibition of the PI3K pathway, independent of mTOR inhibition, could be useful clinically. In the future, it will be important to determine cellular networks responsible for the antitumor effects induced by PI3K/mTOR blockade to optimize clinical response.

ALK

The anaplastic lymphoma kinase (ALK) fusion protein results in constitutive activation of the ALK tyrosine kinase and development of tumorigenic activity. Recently, Soda et al identified fusion of *ALK* with echinoderm microtubule-associated protein-like 4 (*EML4*) in 6.7% of Japanese NSCLC patients (31). The *EML4-ALK* fusion is detected more frequently in patients of a younger age population with limited smoking history, and *EML4-ALK*-positive NSCLC is more commonly classified as adenocarcinoma with signet ring cells, providing methods to stratify patients both clinically and molecularly for targeted ALK therapy (32). *EML4-ALK* has thus been proposed as a new diagnostic marker and therapeutic target in NSCLC.

Crizotinib

Small-molecule inhibitors such as crizotinib have been shown to inhibit ALK phosphorylation and signal transduction (33). A multicenter trial found that, at a mean treatment duration of 6.4 months with crizotinib, the overall response rate of NSCLC patients with ALK rearrangements was 57% (34). Several clinical trials are currently underway to further investigate the effects of crizotinib in treating EML4-ALK-positive NSCLC.

IPI-504

Heat shock protein 90 (hsp90) inhibitors have emerged as a class of promising drugs to target multiple oncogenic pathways, including EML4-ALK-positive NSCLC. Retrospective analysis of a phase II trial of IPI-540 (a hsp90 chaperone inhibitor) revealed ALK rearrangements in 2 of the 5 NSCLC patients who exhibited a partial response (35). Laboratory studies have subsequently confirmed the selectivity of cell lines with ALK rearrangements to hsp90 inhibition (36), pointing to another method to pharmacologically target EML4-ALK-positive NSCLC.

Mitotic molecular targets

Anticancer agents that disrupt mitotic spindle formation and induce apoptosis have been used successfully to treat a variety of cancers. However, significant DLTs have been associated with the antimicrotubule taxanes and Vinca alkaloids. To induce tumor apoptosis with fewer side effects, researchers have aimed to develop novel taxane formulations or to identify specific molecular targets of microtubule inhibition. One such molecular target is polo-like kinase 1 (PLK-1), which plays key roles in mitotic progression. *PLK-1* expression, although elevated in many tumors, is largely absent in surrounding non-proliferating healthy tissues, providing a specific molecular target of cancer cells (37).

Abraxane

Abraxane is an albumin-bound, nanoparticle formulation of paclitaxel. A phase I/II trial of Abraxane as initial therapy in advanced NSCLC found an MTD of 125 mg/m^2 (38). In a phase II trial, a combination of Abraxane, carboplatin, and bevacizumab in non-squamous NSCLC yielded a response rate of 31% and a stable disease rate of 54% (39).

Interestingly, a trial comparing Abraxane administered every 3 weeks (q3w) vs. every week in addition to q3w carboplatin found that weekly Abraxane dosing produced less serious side effects and a greater response rate (47% vs. 30%) compared with q3w dosing. A retrospective analysis revealed that patients with nonsquamous histology had a significantly improved response rate with weekly dosing compared with q3w dosing, whereas patients with squamous histology had longer PFS and OS with q3w dosing rather than weekly dosing (40), suggesting that histologic morphology may optimize patient selection.

Abraxane has been evaluated preclinically and clinically for radiation-modulating effects. Wiedenmann et al found that, when given before radiation, Abraxane produced supra-additive effects in ovarian and mammary carcinoma murine models, significantly increasing radiocurability. Conversely, Abraxane did not increase the radioresponse of normal tissue, suggesting a selective effect (41). Preliminary results from a phase I study of Abraxane with carboplatin and RT in NSCLC suggested weekly combination therapy at 40 mg/m^2 and found that Abraxane is well tolerated (42).

Eribulin

Eribulin mesylate, a synthetic analogue of halichondrin B, is a non-taxane microtubule dynamics inhibitor currently in phase II trials for advanced NSCLC (43). As a non-taxane inhibitor, it may be especially suited for conditions in which taxanes have not been effective. A phase II trial of eribulin in advanced NSCLC found an overall partial response rate of 9.7% and a 1-year survival rate of 46.4% (44). As a microtubule inhibitor, eribulin is likely to have a significant opportunity to enhance therapy when combined with RT and thus may be an ideal candidate for clinical trials of combined chemoradiation in NSCLC.

PLK-1 inhibitors

PLK-1 inhibition has been shown to induce apoptosis in cancer cell lines (45), and several PLK-1 inhibitors have completed clinical trials for solid tumors. The efficacy of targeting PLK-1 combined with RT has not yet been tested in NSCLC, but head-and-neck SCC studies show promising results. Gerster et al found that decreased PLK-1 expression resulted in increased cytotoxicity of the FaDu head-and-neck SCC cells, and cytotoxicity was enhanced by RT addition (46).

Apoptosis

In recent years, apoptosis has become an attractive target for cancer therapy. Apoptosis is regulated by the complex interaction of 2 groups of Bcl-2 family proteins: antiapoptotic proteins Bcl-2, Bcl-xL, Bcl-w, and Mcl-1, and proapoptotic proteins Bax, Bak, Bad, and Bim. Tumor cell survival can be induced by proapoptotic signal inactivation or antiapoptotic pathway activation. Defects in the apoptotic pathway correlate with treatment resistance and are frequently observed in NSCLC (47).

Table 1 Preclinical studies of molecular targets and radiation therapy in non-small cell lung cancer (NSCLC)

Agent	Molecular target	Study	Population	Treatment	Results
BIBW2992	EGFR, HER2	Pao et al (8)	FaDu human SCC *in vivo* and *in vitro*	BIBW2992 + RT	Radiosensitizing effect
Bevacizumab	VEGF-A	Socinski et al (15)	Xenograft models of ovarian and breast carcinoma, melanoma	Bevacizumab + RT several days after	Enhanced tumor growth inhibition that coincided with the "tumor oxygenation window"
Axitinib	VEGFR-1, -2, -3, PDGFR, c-kit	Fenton et al (20)	DU145 human prostate xenograft models	Axitinib + fractionated RT	Increased tumor response, decreased vascular density, increased overall tumor hypoxia
		Fenton et al (21)	Human prostate xenograft models	Axitinib + RT (before or after)	Tumor growth Inhibition was found to be equivalent for each treatment schedule
BIBF 1120	VEGFR-2, PDGFR, FGFR	Zips et al (24)	FaDu SCC in nude mice	BIBF 1120 + RT	Tumor hypoxia and radiation sensitivity of tumor stem cells were not affected
CCI-779	mTOR	Ekshyyan et al (28)	Head and neck SCC in mice xenograft models	CCI-779 + RT	Inhibited tumor growth by >90%; doubled the survival compared with RT alone
BEZ235	mTOR, PI3K	Konstantinidou et al (29)	Transgenic mice with oncogenic K-ras-induced NSCLC and K-ras mutation-positive NSCLC cell lines	BEZ235 + RT	Radiosensitizing effect both *in vivo* and *in vitro*
LY294002	PI3K	Gupta et al (30)	T24 human bladder cancer T24 cell xenografts	LY294002 + RT	Radiosensitizing effect
Abraxane	Microtubules	Wiedenmann et al (41)	Murine models of ovarian and mammary carcinoma	Abraxane + RT after	Increased radiocurability of carcinoma; did not increase radioresponse of normal tissue
AT-101	Bcl-2	Moretti et al (50)	NSCLC cell lines (A549, HCC2429)	AT-101 + RT	Radiosensitizing effect
YM155	Survivin	Iwasa et al (53)	NSCLC tumor xenograft	YM115 + RT	Radiosensitizing effect
Terameprocol	Survivin transcription	Sun et al (55)	NSCLC cell lines (HCC2429, H460)	Terameprocol + RT	Radiosensitizing effect
TG101209	JAK2, survivin expression	Sun et al (56)	NSCLC cell lines (HCC2429, H460) and xenografts	TG101209 + RT	Radiosensitizing effect; K-Ras-mutant cells were more resistant to radiation-induced and TG101209-induced apoptosis
ABT-888	PARP	Albert et al (57)	H460 NSCLC xenograft model	ABT-888 + RT	Increased tumor growth delay compared to either therapy alone

(*continued on next page*)

Table 1 (*continued*)

Agent	Molecular target	Study	Population	Treatment	Results
AZD2281	PARP	Senra et al (59)	NSCLC cell lines (Calu-6, A549) and Calu-6 xenograft	AZD2281 + RT	AZD2281 enhanced cytotoxic effects of RT; AZD2281 alone and in combination with RT increased perfusions of tumor blood vessels
9H10	CTLA-4	Dewan et al (64)	*In vivo* model of TSA mouse breast cancer cells and MCA38 colon cancer cells	9H10 + fractionated or single-dose RT	Fractionated RT enhanced tumor response at both primary site of tumor growth and secondary tumor sites outside radiation field
		Demaria et al (65)	Metastatic mouse mammary carcinoma 4T1	9H10 + fractionated RT	Significant survival advantage that correlated with inhibition of lung metastases formation

Abbreviation: RT = radiation therapy.

Bcl-2 inhibitors

Therapeutic targeting of Bcl-2 family members is a possible strategy to ameliorate drug resistance in NSCLC. Wesarg et al found that the Bcl-2 inhibitor ABT-737 is effective at chemosensitizing drug-resistant NSCLC cell lines that do not express high levels of endogenous Mcl-1 (48). Resistance to ABT-737 is determined by expression of Mcl-1, and detection of Mcl-1 may therefore help guide clinical application of ABT-737 (48). Qian et al showed that in Bcl-xL-overexpressing lung adenocarcinoma cells (which are resistant to apoptosis induction by the PI3K inhibitor LY294002), simultaneous inhibition of the PI3K/Akt pathway with LY294002 and Bcl-xL function with ABT-737-enhanced apoptotic response (49). These data suggest that these two pathways control cell death in a synergistic manner, and that inhibition of Bcl-xL could improve efficacy of PI3K/Akt inhibitors.

Exploitation of pro-apoptotic pathways to promote RT effectiveness has shown promising results. The pan-Bcl-2 inhibitor AT-101 increases apoptosis in a concentration-dependent manner in NSCLC cells. AT-101 also increased radiosensitivity, warranting the need for clinical studies to explore the potential of AT-101 as a radiosensitizing agent (50).

Survivin inhibition

Nuclear expression of the anti-apoptosis protein survivin increases the risk of disease recurrence and death in NSCLC patients (51), and cells with higher survivin levels have markedly less apoptosis in response to RT (52). Inhibition of survivin by antisense oligonucleotides has increased apoptosis of H460 lung cancer cells after RT (52). Preclinical data have shown that the survivin suppressor YM155 has a radiosensitizing effect in NSCLC cell lines (53). A phase II trial using YM155 in NSCLC found that YM155 monotherapy has modest antitumor activity, yielding stable disease in 37.8% of patients (54). These data suggest the need for further study of YM155 in multimodality therapy.

Terameprocol, an inhibitor of Sp-1-mediated survivin transcription, has been shown to increase radiosensitization in NSCLC cells, although the mechanism remains unclear, as decreases in survivin expression did not correlate with an increase in apoptosis (55). Gene expression of survivin is regulated by STAT3. Sun et al demonstrated that TG101209, a small-molecule inhibitor of JAK2 (a STAT3-activating tyrosine kinase), inhibited STAT3 activation and survivin expression and sensitized lung cancer cells to RT. Interestingly, *K-ras*-mutant cells were more resistant to radiation- and TG101209-induced apoptosis, suggesting the utility of selecting patients according to *K-ras* mutation status for future trials investigating combined TG101209 and RT (56).

PARP

Poly(ADP-ribose) polymerase-1 (PARP1) is a key nuclear enzyme in DNA single-strand break repair. Therefore, PARP inhibition has been explored to increase the cytotoxicity of DNA-damaging RT. The PARP inhibitor ABT-888 has been shown to inhibit DNA repair and to increase apoptosis and autophagy in H460 lung cancer cells (57). In addition, combined ABT-888 and RT increased tumor growth delay compared with either therapy alone in a NSCLC xenograft (57). A phase I/II trial of ABT-888 vs placebo in combination with paclitaxel, carboplatin, and RT is currently underway (58). A study of AZD2281 by Senra et al suggests that PARP inhibition enhances RT not only by DNA repair inhibition but also by altering tumor vascular

Table 2 Clinical trials of molecular targets and radiation therapy in non-small cell lung cancer (NSCLC)

Agent	Molecular target	Trial	Population	Treatment	Results
Bevacizumab	VEGF-A	Phase II	Non-squamous, unresectable Stage III NSCLC, $n = 5$	Bevacizumab + Cb + pemetrexed + RT	Observed tracheoesophageal fistulae development and related morbidity and mortality; ended prematurely (14)
		Phase I/II	Stage III NSCLC patients, $n = 20$	Bevacizumab + Cb + pemetrexed + RT	Preliminary results; 44% objective responses, no progression, significantly decreased tumor volumes; esophagitis primary toxicity (13)
		Phase I/II	Stage III NSCLC patients	Bevacizumab + cisplatin + etoposide + RT	Not applicable; ongoing trial (95)
		Phase I/II	Stage III NSCLC patients	Bevacizumab + Cb + erlotinib + paclitaxel + RT	Not applicable; ongoing trial (96)
Abraxane	Microtubules	Phase II	Squamous NSCLC	Abraxane + Cb + RT	Not applicable; ongoing trial (97)
		Phase II	Stage III NSCLC patients	Abraxane + Cb + erlotinib + RT	Not applicable; ongoing trial (98)
		Phase I	Stage III unresectable NSCLC, $n = 8$	Abraxane + Cb + RT	Preliminary results; combination therapy well tolerated with expected adverse events (42)
ABT-888	PARP	Phase I/II	Stage III NSCLC patients	ABT-888 + Cb + paclitaxel + RT	Not applicable; ongoing trial (58)

Abbreviations: Cb = carboplatin; RT = radiation therapy.

hemodynamics (59). Further effects of PARP inhibition are apparent in a study by Gangopadhyay et al, which demonstrated caspase-dependent apoptosis in response to the PARP inhibitor PJ34 (60). The multiple mechanistic effects of PARP inhibition point to the utility of further exploring combination therapy with PARP inhibitors and RT.

Immune modulation

The goal of immune modulation has been to generate more effective host immune responses against neoplastic cells. Cytotoxic T lymphocytes (CTLs) recognize tumor-associated antigens (TAAs) and subsequently kill cells harboring TAAs. By exploiting the selectivity of host immune systems, immune-modulating drugs have the potential to cause fewer side effects than are associated with traditional chemoradiation. Two examples targets of immune modulation include CTL-antigen-4 (CTLA-4) and programmed-cell-death-1 (PD-1). CTLA-4 is upregulated in response to CTL activation and is responsible for blunting further CTL activation, leading to immune tolerance to TAAs (61). PD-1, a T-cell inhibitory receptor, also suppresses antitumor activity, and research suggests that CTLA-4 and PD-1 may work synergistically to induce CTL tolerance (62). Inhibition of these key immune checkpoint molecules can thus reduce immune tolerance to TAAs.

Ipilimumab

Ipilimumab is a human anti-CTLA-4 monoclonal antibody. Preliminary results of a phase II trial in 203 NSCLC patients found that ipilimumab, in addition to paclitaxel/carboplatin in concurrent and sequential regimens, extended immune-related PFS, although only the sequential regimen showed statistically significant results (63).

There is accumulating evidence that RT may complement anticancer immune modulation. In TSA mouse breast carcinoma cells, Dewan et al demonstrated that a combination of anti-CTLA-4 antibody 9H10 and fractionated RT significantly enhanced tumor response at both the primary site of tumor growth and the secondary tumor sites outside of the radiation field (64). Similarly, Demaria et al showed that combined 9H10 and RT resulted in a statistically significant survival advantage that correlated with lung metastasis inhibition (65).

MDX-1106

MDX-1106 is a fully human IgG4 PD-1 blocking antibody. A phase I trial of MDX-1106 as a single agent in solid tumors, including NSCLC, demonstrated anti-PD-1 was well tolerated, with the exception of one serious adverse event of inflammatory colitis. One NSCLC patient had significant tumor regression, although criteria were not met for partial response because of concomitant progression of tumor at other sites (66).

Summary of Targeted Therapy

Targeted therapy entails identification of molecular targets that function to promote not only cancer survival but also resistance to therapy. For example, screening a patient for *EGFR*-activating mutations can predict response to TKIs as first-line therapy (67), but further screening for resistance mutations can allow first-generation vs. second-generation TKI selection. Similarly, Mcl-1 overexpression can predict resistance to the Bcl-2 inhibitor

ABT-737 (48). Overexpression of specific molecular targets can also predict RT response, as increased EGFR expression and survivin levels have been associated with radioresistance (9, 52).

Other characteristics, such as tumor histology and patient smoking history, also play a role in optimizing therapy. For instance, *EGFR* amplification and increased gene copy number are more frequently associated with squamous than adenocarcinoma histology (68), whereas cancers expressing *ELM4-ALK* are more commonly classified as adenocarcinoma (32). Mutations in *K-ras* and the tyrosine kinase BRAF are more common in former smokers, whereas *EGFR* mutations and *ELM4-ALK* are more common in patients with limited smoking history (8, 69, 32), suggesting the ability to predict molecular targets from clinical history.

Yet, basing NSCLC treatment solely on histologic and clinical features may be problematic because of their unreliability. Furthermore, using these features to determine which molecular test to perform may be suboptimal, as highlighted by Bunn et al (70). Former smoking status is not a perfect indicator of *K-ras* or *BRAF* mutations, as *EGFR* mutations and *ALK* rearrangements have also been found in former smokers. Similarly, histologic subtype may not always be clear, as some tumors show mixed histology or are too poorly differentiated. Bunn et al, therefore, pointed to initial testing of multiple molecular markers as a more time-efficient method rather than choosing single selected tests based on clinical and histological features (70). With the rapid production of data currently within this area, questions related to optimizing strategies for molecular testing of tumor tissues are close at hand. It appears likely that *K-ras* mutation status will be shown to be of greatest value in predicting patients most likely to have tumors resistant to anti-EGFR therapies. Summaries of preclinical studies and clinical trials of molecular targets and RT in NSCLC are shown in Tables 1 and 2, respectively.

Genomic Signatures

Molecular technology can be used to identify and to quantify multiple gene patterns associated with specific conditions. These patterns are called the genomic signature of that condition, and they represent the cell phenotype at the simplest level. These signatures can help to differentiate subtle differences in the cell biology, which then provide us with new insights into the heterogeneous responses of these cells to different conditions (71, 72). In addition, genomic signatures assayed in a cell line can be used to study a larger specimen, such as a tumor, providing us with the tools required to further classify NSCLC. Here we focus on the use of genomic signatures for prognostication as well as for treatment selection (Table 3).

Signatures predicting survival

Genomic signatures have successfully been used to classify NSCLC patients into groups with significantly different survival outcomes (73–76). These signatures may be able to identify high-risk early-stage NSCLC that may require treatment in addition to standard surgical resection (77). A multi-site, blinded validation study of prognostic genomic signatures by the Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma found that, when combined with clinical data such as stage, age, and gender, prognostic signatures better correlate with survival (78). Several studies have found that prognostic genomic signatures that were developed for one histologic subtype may also be useful in estimating outcome in a different histology (79–81). These findings are vital for the future application of gene expression signatures in the clinic, suggesting that a simple universal signature may be used to determine outcome in all types of NSCLC, independent of histologic subtype. However, a review by Subramanian et al notes that there is still relatively little evidence that prognostic signatures are ready for clinical application, because of the nonreproducibility of some studies and the limited sample sizes (82). Prospective clinical trials are needed to validate these signatures and to determine whether they can be used to select for patients with stage I disease who would benefit from adjuvant therapy. One such trial currently underway is CALGB 30506, which aims to determine the survival difference between high-risk vs. low-risk stage I NSCLC patients whose risk is determined by prognostic signature (83).

Signatures predicting metastasis

At present, we do not have a well-established method of detecting micrometastasis in surgically treated early-stage NSCLC. A molecular signature associated with metastasis would allow identification of patients at high risk for tumor dissemination for more aggressive therapy. Several studies have shown that the tumor's ability to metastasize is an inherent property of its gene expression and can be detected before the actual metastasis occurs (84, 85). Although some the groundwork has been established, the ability to predict metastatic recurrence based on genomic signatures needs further refining before it can be clinically used.

Signatures predicting chemotherapy response

Although we have discussed the importance of developing genomic signatures to predict outcomes, it is also essential to use genomic signatures to optimize therapy. The BATTLE trial is the first completed prospective randomized study in NSCLC to mandate biopsy and biomarker-based, individualized treatment (86). A total of 255 pretreated NSCLC patients were randomized to erlotinib, vandetanib, erlotinib plus bexarotene, or sorafenib, based on molecular biomarkers. The 8-week disease control rate was 46%, and the 1-year survival was 35%.

Most previous research on genomic signatures for predicting chemosensitivity were conducted on cancer cell lines or xenografts. The NCI-60 human tumor cell lines have been tested for sensitivity to more than 100,000 agents, and genomic signatures were developed from these studies (87). This data may allow tailoring of patient treatments according to their tumor sensitivities. Because cisplatin is currently used as a first-line agent and pemetrexed as a second-line agent, differentiating between the two drugs based on data other than empirical assessment can result in more effective and judicious treatment for patients with advanced NSCLC.

Signatures predicting radiation therapy response

Similar to chemotherapy, there is a heterogeneous response of NSCLC to RT even in tumors with similar clinical and histological features, which has been attributed to genetic and epigenetic processes in tumor cells (88). Some of the pathways involved in radiation resistance have been discussed above. Here we focus on genomic signatures that can predict radiation sensitivity.

Currently, there is no validated genomic signature that can predict NSCLC radiation response. However, studies of the NCI-60

Table 3 Studies evaluating gene signatures for non-small cell lung cancer (NSCLC)

Study	Signature	Methodology	Results
Takada et al (73)	Analyzed expression profile of 1289 genes	cDNA microarray, $n = 92$ NSCLC samples	Stratified patients for lymph node metastasis and stage
Guo et al (74)	37-Gene signature and 12-gene signature	$n = 86$ Lung adenocarcinomas with previously reported gene expression profiles (microarray)	Predicted survival with 0.96 accuracy; 12 gene signature predicted stage of 94.2% of patients
Lu et al (75)	64-Gene signature	Microarray, RT-PCR, $n = 197$ stage I NSCLC patients	Significant prediction of overall survival
Endoh et al (77)	8-Gene signature	qRT-PCR, $n = 85$ lung adenocarcinoma patients	Stratified patients by prognosis
Beer et al (76)	Analyzed 4966 genes	Oligonucleotide arrays, $n = 86$ lung adenocarcinoma	Predicted survival
Sun et al (79)	Two 50-gene signatures	Oligonucleotide array, $n = 91$ NSCLC patients	Predicted survival for both adenocarcinoma and squamous NSCLC
Raponi et al (80)	50 mRNA transcripts	qRT-PCR and microarray, $n = 36$ squamous NSCLC	Predicted high-risk vs. low-risk patients ($P = .04$)
Roepman et al (81)	72-Gene signature	Microarray, $n = 172$ NSCLC patients	Associated with recurrence-free and overall survival
Ramaswamy et al (84)	128-Gene signature and reduced 17-gene signature	Microarray, $n = 279$ primary solid tumors	Distinguished primary from metastatic adenocarcinoma; tumors carrying signature more likely to be associated with metastasis and poor outcome ($P = .03$)
Seike et al (85)	11-Gene signature, CLASS-11 (Cytokine Lung Adenocarcinoma Survival Signature)	RT-PCR, $n = 80$ lung adenocarcinoma patients	Identified Stage I adenocarcinoma patients with poor prognosis ($P = .0002$)
Torres-Roca et al (89)		Microarray and qRT-PCR, $n = 35$ NCI60 cell lines	Predicted RT response in 22 of 35 cell lines ($P = .0002$)
Amundson et al (90)	22-Gene signature and 14-gene signature	Microarray, $n = 48$ human cancer cell lines	22-Gene signature differentiated cells with low survival after 2 Gy of RT; 14-gene signature identified cells lines more sensitive to 8 Gy of RT

Abbreviations: RT-PCR = reverse transcription polymerase chain reaction; q-RT-PCR = quantitative real-time polymerase chain reaction.

generated some genomic signatures that may reflect radiation sensitivity. A recent study using basal expression of 3 genes was able to correctly predict radiation response, as measured by survival after 2 Gy of ionizing radiation (SF2), in 22 of 35 cell lines (89). A more traditional genomic signature study was done using mRNA microarrays to profile radiation response (90). This study developed a 22-gene and 14-gene signature to reflect enhanced radiosensitivity at 2 Gy and 8 Gy, respectively. The study investigators also were able to identify an 18-gene signature that predicted radioresistance. Eschrich et al similarly generated a signature from 4 different clusters of genes that was predictive of radiosensitivity (91). This study was able to correctly estimate SF2 values for 3 of 4 NSCLC cell lines.

None of the aforementioned models have been tested clinically in tumors. However, it is important to be able to optimize RT dose and fields to achieve the best results without significant toxicities. Further research using patient tumors is required to develop useful signatures for RT sensitivity.

Next-Generation Sequencing

The advent of next-generation sequencing, a revolutionary platform for massive sequencing of the whole exome or genome that generates short base pair reads from millions of DNA fragments, is likely to have a significant impact on personalized medicine in NSCLC. Relative to cancer genetics, next-generation sequencing has the advantageous ability to detect novel chromosomal rearrangements, microbial infections, and copy number alterations (92). Through next-generation sequencing of 89 squamous NSCLC tumors, Belvedere et al derived a computational index related to the ratio of copy number states across the genome that was an independent prognostic indicator (93). Notably, whole-genome sequencing provides a single modality to identify the complete range of genomic alterations present within a tumor, even those within the noncoding regions not typically sequenced by other methods. For instance, Lee et al successfully sequenced a primary lung tumor and validated 530 somatic single nucleotide variants from >50,000 identified high-confidence single nucleotide variants (94). The study also found that mutations in expressed genes were selected against more than mutations in nonexpressed genes and promoter regions, providing evidence of selective pressures. Next-generation sequencing will likely prove to be pivotal in the discovery of new molecular targets for NSCLC therapy. However, the large volume of data produced that must be analyzed in regard to the biological and clinical implications presents a challenge in applying next-generation sequencing methods to clinical practice and patient care at present (92).

Conclusion

Current NSCLC treatment approaches are largely empiric, with minimal improvement in outcome over the last three decades. Only a tailored approach based on tumor genetic make-up will likely result in significantly improved survival in these patients. Therefore, prospective biomarker-driven therapy in selected patients is a research priority, with a number of important discoveries in the past few years. The development of useful genomic signatures in the laboratory setting provides an exciting new tool to cultivate novel treatments and to optimize present therapy. Although significant challenges remain, the pace and scale of progress in applying biomarkers and gene expression models is accelerating, especially in the current era of deep sequencing. The question now is how best to bring them through clinical trials and ultimately into routine patient management.

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