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Radiosensitizers in Pancreatic Cancer – Preclinical and Clinical Exploits with Molecularly Targeted Agents

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Abstract

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Conflict of Interest Statement

The authors declare no conflict of interest.

There has been an explosion in the number of molecularly targeted agents engineered to inhibit specific molecular pathways driving the tumorigenic phenotype in cancer cells. Some of these molecularly targeted agents have demonstrated robust clinical effects, but few result in meaningful durable responses. Therapeutic radiation is used to treat a majority of cancer patients with recent technologic and pharmacologic enhancements, leading to improvements in the therapeutic ratio for cancer care. Radiotherapy has a very specific role in select cases of postoperative and locally advanced pancreatic cancer patients, but control of metastatic disease still appears to be the major limiting factor behind improvements in cure. Recent rapid autopsy pathologic findings suggest a sub-group of advanced pancreatic cancer patients where death is caused from local disease progression and who would thus benefit from improved local control. One promising approach is to combine molecularly targeted agents with radiotherapy to improve tumor response rates and likelihood of durable local control. We review suggested recommendations on the investigation of molecularly targeted agents as radiosensitizers from preclinical studies to implementation in phase I–II clinical trials. We then discuss a select set of molecularly targeted therapies that we believe show promise as radiosensitizers in the treatment of pancreatic cancer.

Keywords

pancreatic cancer; radiotherapy; radiosensitizer; molecularly targeted agents

Introduction to Molecularly Targeted Radiosensitizers

Radiotherapy (RT) is an integral part of both definitive and palliative cancer management, estimated to be indicated in the treatment of 52% of cancer patients [1]. While RT can afford local control, the addition of systemic therapy may manage occult distant disease and in some cases, also offer radiosensitization benefit [2]. Yet the use of conventional cytotoxic chemotherapy as a means of radiosensitization may lead to increased toxicity due to lack of specificity for tumor cells. Therefore, there has been evolving interest in identifying agents that selectively target tumor-specific pathways important in RT-induced cell death with the goal of augmenting the effects of RT while minimizing sensitization of normal tissues. Many of the targeted agents currently studied as potential radiosensitizers are cytostatic [3]; unlike conventional cytotoxic chemotherapies, these agents may avoid or reduce normal tissue toxicity by exploiting molecular differences between malignant and nonmalignant cells.

Yet, despite the potential benefit of using novel targeted agents as radiosensitizers, there have been relatively few clinical trials involving the combination of such agents and RT. Although there are an estimated 400 phase I non-RT oncology trials per year³, there were only approximately 30 phase I and I/II trials utilizing RT in 2009 [4], which may be due in part to several limitations specific to combination radiosensitizer and RT trials. Moreover, despite success in phase III clinical trials, agents such as the hypoxic tumor cell radiosensitizer nimorazole [5] may fail to become adopted into standard clinical practice; Overgaard and colleagues provide a review of multiple factors contributing to lack of implementation of this and other hypoxic radiosensitizers [6]. To address the lack of formal guidelines for the development of such agents, recommendations and strategies for radiosensitizer development in preclinical and clinical trials have been suggested. This review provides a brief summary of these recommendations [3,7–11].

Recommendations for Preclinical Studies with Radiosensitizers

Many novel targeted agents have mechanisms of action that are well-positioned to serve as RT enhancers—some of which have a promising role in the management of pancreatic

cancer and will be reviewed in this manuscript. Although *in vitro* and *in vivo* studies are inadequate to address all of the complexities of cancer biology, they are a necessary starting point for discovery of novel molecularly-targeted radiosensitizing agents and are required prior to moving forward with large-scale clinical trials where patients may be exposed to potentially toxic therapy. Through biomarker discovery and establishing proof-of-concept principles, such preclinical studies also lay the framework for incorporation of translational endpoints into trial design.

In Vitro Studies

In vitro studies are conducted to demonstrate the anti-cancer activity, target knockdown, tumor selectivity, mechanism of action, and resistance pathways of the targeted agent and typically include the use of cell lines (cancer cells and/or stromal cells) grown in tissue culture. Molecularly targeted agents can be broadly classified into tumor-specific and tumor non-specific groups. For those agents that are hypothesized to interact with non-specific targets that are aberrantly expressed in a wide range of cancers, investigators should select cell lines based on knowledge of expression of the target with consideration of tumor types that will be studied in clinical trials [3,7,9]. For targeted agents with a more limited scope, it is appropriate to focus on at least two cell lines that overexpress the target of interest [7]. One of the main objectives of preclinical studies is to allow derivation of the dose enhancement ratio (DER), defined as the surviving fraction at an indicated radiation dose divided by the surviving fraction at the same dose of radiation plus the potential sensitizer [12,13]. It is recommended that cell death be measured with the gold standard clonogenic survival assay [14]. In rare situations, colorimetric or optical viability assays may be reasonable alternatives [7].

In Vivo Studies

In vivo studies are necessary in order to examine agents that act on the tumor microenvironment or other non-cell autonomous cancer cell processes, such as anti-angiogenic agents. Prior to performing therapeutic efficacy studies *in vivo*, it is recommended that a suitable pharmacokinetic profile of the drug be established in the appropriate animal. Furthermore, it is preferred that active concentration levels within the tumor as well as downstream modulation of the target can be verified. The majority of *in vivo* studies involve immunocompromised mice with mutations in DNA response and repair pathways, including athymic, severe combined immune-deficiency (SCID) or NOD-SCID mice. The abnormal DNA repair mechanisms in these mice limit the applicability of results with radiosensitizers given the integral role of DNA damage to the biologic effect of radiation therapy [7]. Furthermore, anti-tumor effects of RT may be mediated by the immune system. Therefore, immunocompromised mice are not optimal in this regard given that they lack a functional immune system. As a result of these limitations, genetically engineered mouse models (GEMMs) are becoming more widely used in preclinical studies with and without RT [15,16]. “Co-clinical trials” that use GEMMs that faithfully replicate the mutational events observed in human cancers to conduct preclinical trials that parallel ongoing human phase I/II clinical trials have shown great promise in lung and prostate cancer [17–19]. In addition, more sophisticated animal studies with RT are now possible with the advent of technologies that integrate treatment planning, imaging, and RT delivery capabilities such as the microRT small animal conformal irradiator and the small-animal radiation platform (SARRP) [20,21]. For preclinical studies, abbreviated courses of radiation therapy with hypofractionated regimens are reasonable for proof-of-principal studies, especially given the recent trend towards more conformal therapy and stereotactic body radiotherapy (SBRT)/stereotactic ablative body radiation (SABR).

Recommendations for Clinical Trials with Radiosensitizers

Identification of Patient Populations

Selection of the optimal patient population is critical for clinical trials with novel radiosensitizers. Clinical trials with single systemic agents often target patients with metastatic and refractory cancer. Despite allowing for assessment of toxicity with relative ease, these trials are unlikely to afford high response rates or meet cost-benefit analysis thresholds for approval by regulatory agencies such as the FDA [7]. The logical alternative is to conduct radiosensitizer trials in patients with potentially curable disease; however, this raises ethical considerations, especially when toxicity from the radiosensitizer may lead to delay or interruption of curative RT [7,22]. One solution to overcome the above ethical issues is to study cancers with a poor prognosis but for which definitive management may still be attempted, such as pancreatic cancer.

Specific Considerations for Early Stage Clinical Trials

There are many challenges inherent in clinical trial design with targeted radiosensitizers. First of all, the extent to which normal tissues are exposed to RT is directly related to the site of treatment. Different tumor sites and histologies are often included in trials with single agent systemic therapies. In trials with radiosensitizers, a similar approach complicates the estimation of toxicity and decision for dose-escalation [7]. In addition, the maximum tolerated dose determined from traditional phase I studies as a single agent may be different from the biologically active dose as a radiosensitizer [23].

Traditional trial designs, such as the classic cohort-of-three design, requires each patient or cohort of patients to be fully evaluated for the dose-limiting toxicity (DLT) before new patients can enroll [7]. Cohort-of-three trials that include radiation therapy may be prohibitively long because acute toxicity can occur even 8 to 12 weeks following treatment rather than within a few days of administration as is often the case with systemic agents alone [3]. As a result, spinoffs of the classic cohort-of-three trial aimed to reduce how often patient accrual is suspended include the Rolling Six Design (RSD) and the Continual Reassessment Method (CRM). A trial design that specifically addresses the late toxicity issues inherent in RT is the Time-to-Event Continual Reassessment Method (TITE-CRM), which allows staggered accrual without the need for complete DLT follow-up of previously treated patients [24,25]. In addition to the general concepts behind dose escalation in phase I trials described above, various novel phase I trial designs have been introduced and proposed as models for use in radiosensitizer trials in order to ensure timely recruitment and completion of phase I studies. Phase 0 or window-of-opportunity trial design is viewed as low risk and allows the patients to receive the study drug during the 1 or 2 weeks prior to RT [26,27]. Drug duration escalation studies escalate the total number of fractions of RT that are given in conjunction with the systemic agent. Lastly, the ping-pong or flip-flop design allows multiple systemic agents to be studied in the same clinical trial and is particularly useful in studies with RT because it simultaneously allows for prolonged observation of a single cohort of patients without delaying accrual [7,8,28]. A current example of a ping-pong trial design is the UK “DREAM” study investigating the addition of cediranib (AZD2171) and a MEK inhibitor (AZD6244) to standard chemoRT in patients with rectal cancer [29].

Phase II Trial Designs with Radiosensitizers

In order to maximize evaluation of true clinical activity and obviate the need to rely on historical control data when deciding if the radiosensitizer with RT is superior to RT alone, it is recommended that randomized phase II trials be performed in place of single-arm phase II studies [30–32]. In addition to tumor response criteria, clinical trials may include

surrogate endpoints to enable promising molecularly targeted agents to advance more quickly to phase III trials. Other useful strategies include investigating multiple agents compared to a standard therapy control group. This allows pilot efficacy testing of each targeted agent against the control. Additional recommendations regarding phase II trial design can be found in the NCI-RTOG Translational Program Strategic Guidelines published by Lawrence and colleagues [3].

Pancreatic Cancer: Overview and Rationale for Targeted Radiosensitizer Development

Pancreatic adenocarcinoma (PDA) is the tenth most frequently diagnosed cancer but the fourth leading cause of cancer death in the United States, accounting for an estimated 37,390 deaths per year [33]. The standard of care for resectable PDA is surgical resection, generally with pancreaticoduodenectomy. Adjuvant management options include gemcitabine- or fluoropyrimidine-based chemotherapy, with or without the addition of fluoropyrimidine-based chemoRT. For borderline resectable and initially unresectable disease, neoadjuvant therapy followed by possible resection may be attempted [34]. Although there is no phase III data to support this approach, a meta-analysis by Lawrence and colleagues showed that neoadjuvant chemoRT in patients with initially unresectable disease resulted in similar survival outcomes as those seen in patients with initially resectable disease [35]. The standard approach for unresectable disease is upfront chemotherapy, generally with FOLFIRINOX, gemcitabine as a single or combined agent, or capecitabine, followed by consolidative chemoRT in select patients [34].

Yet, despite a variety of standard surgical, chemo- and radiotherapeutic regimens investigated, the 5-year survival for PDA remains at approximately 6% [33]. Although surgical resection is thought to be the only means for potential cure, historic 5-year overall survival rates following resection still range from 10 to 36% [36–39]. Trials randomizing cases of resectable PDA to adjuvant chemoRT versus observation alone showed at least a trend toward a survival benefit from chemoRT, but the 5-year overall survival rates remained at 7–29% [40–43]. Retrospective data comparing adjuvant chemoRT to observation showed similarly improved but bleak 5-year overall survival at 20–28% with adjuvant therapy [44–46]. Moreover, greater than 80% of patients have locally advanced or metastatic disease at the time of diagnosis [47]. In the unresectable setting, median survival from the best-performing arms of large prospective CRT trials ranged from 8.4 to 15 months [48–52]. Furthermore, physical RT dose escalation and beam conformality may be approaching its limits; although ongoing investigations shows fractionated SBRT to be a promising strategy for relative dose-escalation [53–56]. Earlier phase I/II studies with single fraction SBRT (25 Gy $\times$ 1 fraction) showed excellent local progression free survival greater than 90%, but significant late gastrointestinal toxicity [54]. Recently, our group reported the results of a phase II trial of gemcitabine and fractionated SBRT (6.6 Gy $\times$ 5 fractions), which demonstrated excellent tumor response and local control with minimal grade 3 toxicity [57]. Despite, these technological improvements, it is unclear at this point if further improvement to local control will decrease the risk of metastatic seeding.

Promising new pathologic data suggests advanced pancreatic cancer may be composed of distinct morphologic and genetic subtypes with different patterns of metastasis that seems to be correlated with DPC4 status [58]. Although still preliminary, these DPC4 data may suggest a sub-group of advanced pancreatic cancer patients that would benefit from improved local control. Regardless, novel strategies such as molecularly targeted radiosensitizers to improve control of both resectable and locally advanced PDA are needed. There are clear advantages to studying radiosensitizers in this patient population, including lack of significant improvement to survival outcomes in a variety of previously investigated

strategies as well as the opportunity to use tumor markers such as CEA and CA19-9 to assess disease response. Additionally, given constraints of RT dose escalation due to tumor location, tumor-targeted radiosensitizers may allow for further biological dose escalation with relative sparing of normal tissue.

Candidate Targeted Agents as Radiosensitizers in Pancreatic Cancer

In the remainder of this review, we will consider a select number of promising novel molecularly targeted agents that may serve as radiosensitizers amenable to clinical study for PDA, including tyrosine kinase inhibitors, TGF- β inhibitors, and heat shock protein 90 inhibitors.

Tyrosine kinase inhibitors

Overexpression of the epidermal growth factor receptor (EGFR) surface protein has been reported in up to 60% of pancreatic tumors and has been associated with disease progression and poor prognosis [59,60]. As such, there has been considerable interest in exploring EGFR inhibitors in PDA. Early preclinical models showed decreased growth and metastatic potential in pancreatic tumor xenografts treated with erlotinib, an EGFR tyrosine kinase inhibitor, when given alone or in combination with other anticancer agents [61]. These findings eventually prompted a phase III clinical trial of erlotinib/gemcitabine versus gemcitabine alone, which resulted in a statistically significant increase in survival with combination therapy [62].

Building on these results from the metastatic setting, a number of studies across multiple cancer cell lines have revealed potential mechanisms for radiosensitization with EGFR inhibitors. These include induction of apoptosis [63], blockade of RT-induced proliferation and accelerated repopulation [64], alteration of cell cycle distribution with a shift from S phase to G0/G1 [65], and inhibition of DNA damage repair [66]. Phase I and phase II clinical trials were therefore developed to study the combination of RT with erlotinib in pancreatic cancer in both the adjuvant and unresectable, locally advanced settings [67–71]. While safety outcomes have been satisfactory, only modest increases in efficacy have been seen. These limited results may stem from *de novo* or acquired resistance through activation of alternate pathways that bypass EGFR. For example, in lung cancer there have been multiple resistance mechanisms characterized for acquired resistance to EGFR TKIs that when co-targeted with EGFR inhibition, can re-sensitize cancer cells to EGFR TKIs [72]. Better characterization of bypass pathways and incorporation of additional agents that target these pathways may therefore prove fruitful going forward.

Indeed, mitogen-activated proliferation kinases (MAPK) and Akt are being increasingly recognized as important contributors to cell proliferation, tumorigenesis, and RT resistance across multiple tumor types. Their relevance to PDA stems from the 90% of pancreatic tumors harboring KRAS mutations, resulting in activation of the RAF/MEK/MAPK(ERK) signaling cascade [73]. Additionally, preclinical data in multiple pancreatic cell lines have shown transient activation of both Akt and ERK after administration of low-dose RT [74].

As such, inhibition of MAPK and Akt has garnered interest as potential targets for radiosensitization in PDA. Williams and colleagues performed *in vitro* clonogenic assays in multiple pancreatic cancer cell lines using a MEK inhibitor (PD0325901) to suppress ERK activation, resulting in significantly increased cell kill [75]. *In vivo* testing of MEK inhibition with concurrent RT in pancreatic cancer mouse xenografts showed significant therapeutic response when compared with RT or MEK inhibition alone. Furthermore, MEK inhibition resulted in up-regulation of Akt, highlighting the crosstalk between these pathways. As such, this group examined dual inhibition of MEK and Akt using two small

molecule inhibitors and found further enhanced therapeutic efficacy when combined with RT, suggesting that ERK and Akt activation play an important role in mediating RT resistance. Interestingly, sunitinib malate, a potent inhibitor of multiple tyrosine kinase receptors, may have activity against both of these pathways [76]. As an example, preclinical data examining the combination of RT and sunitinib in pancreatic cancer cell lines have shown significant attenuation of ERK and Akt pathways following RT [74]. Radiosensitization with sunitinib was thereafter confirmed in an *in vivo* pancreatic cancer xenograft model. Based on these results, further characterization of the inhibitory action of sunitinib on the ERK and Akt pathways and exploration of the role of sunitinib as a radiosensitizer in pancreatic cancer is certainly warranted.

TGF- β inhibitors

Transforming growth factor- β (TGF- β) is a well-known, yet incompletely understood pro-inflammatory cytokine that is secreted by cells in response to ionizing RT. TGF- β is thought to be a major player in the deleterious cytokine cascade responsible for inflammation and extracellular matrix remodeling that occurs following exposure to RT [77,78]. Despite the fact that TGF- β is known to be a player in radiation toxicity and is also recognized as a canonical tumor suppressor, there is substantial evidence that this inhibitory pathway becomes deranged in tumor cells and acts via a variety of mechanisms to promote tumor progression. Overexpression of TGF- β has been reported in PDA, and increased expression of TGF- β isoforms has been associated with decreased survival [79]. Overexpression of TGF- β has also been demonstrated in other solid malignancies such as glioblastoma, breast, prostate, non-small cell lung, renal, and bladder cancers [80]. When highly secreted from tumor cells, TGF- β acts to promote the tumorigenic microenvironment via collagen formation and angiogenesis. Furthermore, it has been shown that TGF- β inhibition increases the sensitivity of cells to cytotoxic therapy, including RT, via modulation of ATM [81], BRCA [82], and Rad51 [83]. When TGF- β inhibition was studied in the context of PDA *in vitro*, there was evidence of decreased cellular proliferation, migration, and invasion [84]. Thus, TGF- β inhibition appears uniquely poised to improve the therapeutic ratio of RT in PDA by increasing the sensitivity of tumor cells to RT, reversing the pro-tumorigenic microenvironment, and modulating the degree of normal tissue toxicity.

LY2157299 is one of many promising small molecule inhibitors of the TGF- β receptor 1 (TGF- β R1) that has been shown to decrease endothelial cell proliferation, migration, and tube formation *in vitro* [84] and is currently being studied in early clinical trials. A phase I study with LY2157299 in combination with gemcitabine in patients with advanced cancer was performed, with no dose limiting toxicities observed and all safety objectives achieved. The recommended dose of 150 mg twice per day in combination with gemcitabine has been advanced into a randomized phase II trial as first-line therapy in advanced stage cancer [85]. Preclinical studies are currently underway to investigate the combination of TGF- β receptor inhibition and RT in the management of PDA.

A promising treatment modality for locally advanced PDA is SBRT using high-dose, highly conformal RT delivered in 1–5 fractions as described above. Positive preclinical results with TGF- β receptor inhibition would set the stage for investigating LY2157299 as a radiosensitizer and adjunct in clinical trials with SBRT for locally advanced pancreatic cancer [86].

Heat Shock Protein (HSP) 90 inhibitors

An additional factor that may contribute to resistance of PDA to therapies is the overexpression of proteotoxic response machinery, such as heat shock proteins (HSPs). PDA tumor cells are able to function under high levels of cellular stress such as hypoxia,

ischemia, increased levels of DNA damage, high levels of reactive oxygen species, and protein complex imbalances due to aneuploidy likely from the buffering capacity of HSP machinery [87–89]. HSPs belong to a group of proteins known as molecular chaperones, which assist in folding newly translated proteins, stabilize proteins in order to prevent aggregation, and protect against stress-induced protein denaturation [90]. HSPs are highly conserved and have a crucial role in cell cycle progression, apoptosis, and maintenance of homeostasis [91]. Mammalian HSPs have been classified into six families according to their molecular size: HSP100, HSP90, HSP70, HSP60, HSP40 and small HSPs (15 to 30 kDa) [92]. While HSPs with higher molecular weights are ATP-dependent, smaller HSPs are ATP-independent [93]. Studies have indicated that HSP90 is expressed at higher levels in tumor cells [94], including PDA [95]. Client proteins stabilized by HSP90 [96] include several proteins involved in tumor progression, tumor maintenance and RT resistance [97,98]. Thus, the stress response or non-oncogene addiction machinery presents an intriguing option for cancer therapeutics alone [96] or in combination with RT for PDA.

The first generation HSP90 inhibitors such as geldanamycin (GA) [99] showed promising anti-tumor activity *in vitro*, but *in vivo* studies revealed overall poor tolerability, with significant hepatotoxicity [100]. Several other GA analogues such as Tanespimycin (17-allylamino-17-desmethoxygeldanamycin, 17-AAG) and its N,N-dimethylethylamino analogue Alvospimycin (17-DMAG) [101] have been synthesized and were plagued by similar problems including poor solubility, difficulty in formulation, hepatotoxicity, inconsistent pharmacokinetics, susceptibility to P-glycoprotein efflux and polymorphic metabolism by NQO1/DT-diaphorase enzymes, thus thwarting translation into advanced phase clinical trial testing [102,103].

The limitations of the GA analogues gave rise to the development of next generation HSP90 inhibitors with improved water solubility and lower toxicity. Several synthetic small molecules have been developed, including AUY-922 and STA-9090. AUY-922, an isoxazole resorcinol, has a high affinity for the NH₂-terminal nucleotide binding site of HSP90 [104–106]. It is one of the most potent synthetic small molecule inhibitors of HSP90 [105]. AUY-922 demonstrated potent preclinical anti-cancer activity *in vitro* and *in vivo* against a range of histologic cell types including pancreas, prostate, lung, ovarian, and breast cancers, as well as myelomas, melanomas, and glioblastomas [105,107–110]. Ganetespib (STA-9090), a unique triazolone-containing small molecule inhibitor of HSP90, also has exhibited potent single agent activity in a broad range of preclinical models of human malignancies [111]. Moreover, ganetespib presents a more favorable toxicity profile and superior pharmacologic properties compared to other next-generation HSP90 inhibitors. Ganetespib alone was tested as second/third line therapy in a phase II clinical trial for metastatic pancreatic cancer that recently closed [112]. We, and others, have recently demonstrated profound radiosensitization of a limited range of epithelial cancer types with next-generation HSP90 inhibitors *in vitro* and *in vivo* [113,114]. An appealing treatment strategy for PDA may be the combination of RT with next-generation HSP90 inhibitors.

Conclusions and Future Directions

There has been relatively limited preclinical and clinical investigation of novel molecularly-targeted radiosensitizers despite their significant potential for improving cancer outcomes. This is likely due in part to several limitations unique to trials that test the combination of radiosensitizers and RT, including issues of funding, trial development, and identification of optimal patient populations, with lack of consensus among investigators and industry about how to address these issues. Yet for cancers such as PDA, with extremely poor outcomes despite utilization of a variety of traditional surgical and chemo- and radiotherapeutic approaches, targeted agents that maximize the therapeutic ratio of RT may provide a novel

means for significantly augmenting disease control. As such, we have summarized recommendations for preclinical and clinical studies of radiosensitizers published by a number of research groups and members of industry. We have additionally compiled a review of the rationale and evidence supporting the application of select targeted radiosensitizers including tyrosine kinase inhibitors, TGF- β inhibitors, and HSP90 inhibitors for management of pancreatic cancer.

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