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Targeting the Epidermal Growth Factor Receptor in Bladder Cancer

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Introduction

The Epidermal Growth Factor Receptor (EGFR), or ErbB1, is a member of the ErbB family of tyrosine kinase receptors. It is a 170 kDa transmembrane glycoprotein that can bind a variety of ligands on its extracellular domain, most notably Epidermal Growth Factor (EGF). Binding of ligand induces homo- or heterodimerization with a second EGFR molecule or another member of the ErbB family, respectively. Once dimerized, the molecule undergoes auto-phosphorylation on intracellular tyrosine residues. Phosphorylation of these tyrosine residues allows for recruitment of ATP to the catalytic kinase domain of EGFR, which allows for phosphorylation of effector molecules. Thus, a phosphorylation cascade is set off, leading to activation of various intracellular signaling pathways that have been implicated in tumorigenesis and cancer progression, including the RAS/MAPK (mitogen-activated protein kinase), PI3K (phosphoinositide 3-kinase)/AKT, and STAT3 (signal transducer and activator of transcription 3) pathways [1,2].

Activated EGFR directly interacts with the SH2 (src homology 2) domain-containing molecule Grb2 (growth factor receptor-bound protein 2), which leads to downstream activation of RAS. This is followed by subsequent phosphorylation of members of the MAPK/ERK Pathway (Raf, MEK, and ERK). ERK (extracellular signal-regulated kinase) then translocates into the nucleus and activates transcription factors such as fos and jun that promote proliferation, cell cycle progression, and an invasive phenotype. Concurrent activation of the MAPK signaling pathway also occurs by EGFR-dependent activation of phospholipase C (PLC). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). DAG activates protein kinase C (PKC) which in turn activates Raf-1, an important intermediate in the MAPK pathway [3]. In a parallel signaling pathway, EGFR activates phosphotidylinositol 3-kinase (PI3K). PI3K phosphorylates phosphatidylinositol-2-phosphate (PIP2) to form PIP3. PIP3 activates the protein kinase Akt, which phosphorylates the mammalian target of rapamycin (mTOR). Signaling through mTOR induces cell proliferation and inhibits apoptosis. There are also interactions between EGFR and the Jak (Janus kinase)/Stat pathway, which have also been implicated in tumorigenesis. It has been shown that Jak2, which is activated by cytokine signaling, can phosphorylate EGFR, thus promoting signaling via the MAPK pathway [4]. In addition, Stat3, which is a transcription factor promoting cell growth, can be activated by ErbB2. While the above discussion has highlighted some of the key EGFR-mediated pathways, it must be kept in mind that there is cross-talk between these pathways, which exist as part of a complex network. As each homo- or heterodimer of ErbB proteins preferentially activates a different set of pathways, the complexity of this network is compounded further. In addition, there exists cross-talk between the ErbB-activated pathways and pathways activated by non-ErbB signaling such as cytokine and growth factor receptors [2]. One such example is the Jak/EGFR interaction described above.

Derangements of EGFR have been described in cancer *in vitro* and *in vivo* and are believed to be a driving force for many cancers. These alterations include gene amplification (leading to cell surface overexpression), mutation of the intracellular kinase domain (leading to constitutive EGFR expression), and in-frame deletions in the extracellular domain [5]. It seems to be the case that different cancers will predominantly demonstrate one of these alterations, but not the others. EGFR overexpression was originally detected in a subset of breast cancers, and has since been shown in a number of other cancers including ovarian, gastric, and salivary, as well as in bladder [1].

Activating mutations of the intracellular kinase domain that cause constitutive receptor activation most notably occur in non-small cell lung cancer (NSCLC). The presence of these mutations, which occur in 10% of patients with NSCLC, are predictive of response to targeted therapy with gefitinib [6]. Other cancers in which mutations of the kinase domain have been reported include colorectal [7] and head and neck [8]. To date, these mutations have not been detected in bladder cancer. Truncating mutations of the extracellular domain occur in 40% of glioblastomas [9]. The protein product expressed in these tumors is called EGFR variant III (EGFRvIII). This variant has also been found expressed in other cancers, including breast, ovarian, and lung but absent in healthy tissues [10]. The expression of EGFRvIII has been associated with resistance to gefitinib in lung cancer [11,12].

In bladder carcinoma, EGFR overexpression is an independent predictor of poor survival and stage progression. It was found to be 80% sensitive in predicting stage progression in non-muscle invasive high grade tumors (T1, grade 3) [13]. Moreover, while in 95% of healthy urothelium EGFR expression is limited to the basal cell layer, EGFR is expressed in both deep and superficial cell layers in 92.3% of low grade UC specimens and 100% of high-grade UC specimens [14]. However, in a study of 75 specimens from patients with UC and a panel of 12 bladder cancer cell lines, there was a failure to detect either activating mutations of the kinase domain or truncating mutations of the extracellular domain (EGFRvIII). However, 31-48% of these bladder tumors overexpressed EGFR [11]. A recent case-control study did identify single nucleotide polymorphisms (SNPs) that are associated with an increased risk of bladder cancer as well as survival [15].

There will have been an estimated 72,570 (54,610 in men and 17,960 in women) new cases of bladder cancer and 15,210 bladder cancer-related deaths in the United States in 2013 [16]. The majority of these tumors are Urothelial Carcinomas (UC). While radical cystectomy is the treatment of choice for muscle-invasive bladder

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cancer, 50% of patients will develop recurrent disease following surgery [17]. Moreover, 10-30% of patients presenting with non-muscle invasive disease will progress to muscle-invasive disease [18]. While neoadjuvant combination chemotherapy has shown a survival benefit in patients with muscle-invasive disease [19], bladder cancer remains a highly aggressive entity and new treatments that slow down progression or prevent recurrence are highly desirable. The standard of care perioperative chemotherapy regimens used are GC (gemcitabine and cisplatin), MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin), dose dense MVAC, or CMV (cisplatin, methotrexate, and vinblastine). In the setting of surgical management, either adjuvant or neoadjuvant chemotherapy may be given. A meta-analysis demonstrated that the improvement in overall survival with neoadjuvant platinum-based chemotherapy is approximately 5% [20]. Clearly, there is a need for better therapeutic agents that can improve upon the outcomes of patients with muscle-invasive disease.

Because derangements in EGFR are so ubiquitous in cancer, EGFR has emerged as an attractive therapeutic target. Targeted therapy in bladder cancer has been the topic of a number of reviews [21,22]. Two major drug classes have been developed targeting EGFR. The first are the monoclonal antibodies (moAb) that target the EGFR extracellular domain. Antibody binding to EGFR induces endocytosis followed by lysosomal degradation of the receptor [23]. The Fc domain of bound antibody also induces antibody-dependent cell-mediated cytotoxicity (ADCC), enhancing the antitumoral response of the immune system [24]. The second class of biologic agents targeting EGFR is the tyrosine kinase inhibitors (TKIs). These are small peptide molecules that bind to the catalytic site on the receptor's intracellular domain, preventing binding of ATP and subsequent phosphorylation of the receptor's target proteins. Gefitinib (Iressa) and erlotinib (Tarceva) are the two molecules in this class which are specific for EGFR. A number of multikinase inhibitors are being studied, which target multiple receptors including EGFR.

Monoclonal Antibodies

Cetuximab (Erbitux) is a chimeric human/mouse IgG1 moAb that binds the extracellular domain of EGFR, preventing dimerization. It has been approved in the treatment of colon and head and neck cancers. Cetuximab administration in nude mice with UC inhibited tumor growth, at least partly through an antiangiogenic mechanism [25]. The combination of cetuximab and paclitaxel slowed tumor growth in mice implanted with UC more than either drug alone [26]. The antitumorigenic effect of this combination was thought to be mediated primarily by inhibiting angiogenesis and promoting apoptosis. Cetuximab has been successfully combined with the VEGF antagonist bevacizumab (Avastin) in the treatment of colon and head and neck cancers [27,28]. This combination has been investigated in bladder cancer in combination with photodynamic therapy (see "Other therapies targeting EGFR" section).

A clinical trial evaluated the efficacy of adding cetuximab to paclitaxel as salvage chemotherapy in metastatic bladder cancer patients who had failed first-line platinum-based treatment [29]. The paclitaxel alone arm of the study closed after 9 of 11 patients progressed after 8 weeks. The cetuximab-paclitaxel combination arm completed accrual of 28 patients, and 12 of these had progression-free survival greater than 16 weeks. The authors concluded the cetuximab-paclitaxel combination merits further study as salvage chemotherapy in UC. A Phase II clinical trial is looking at the effect of gemcitabine and cisplatin chemotherapy with and without cetuximab in patients with locally advanced or metastatic UC (stage>T4b). The results are pending. (Clinicaltrials.gov, ID: NCT00645593).

Panitumumab (Vectibix) is a fully humanized IgG2 moAb. It is approved for the treatment of colorectal cancer. Phase III trials with panitumumab are also underway in NSCLC. A multicenter phase II study is currently underway in Germany comparing first-line chemotherapy with GC alone versus GC with panitumumab in patients with locally advanced or metastatic disease and wild-type *HRAS* [30]. A phase I trial evaluated the combination of panitumumab with motesanib (AMG 706), a multikinase inhibitor primarily targeting VEGFR, alongside gemcitabine and cisplatin, in patients with advanced solid tumors (Clinical trials.gov, ID: NCT00101907/20040206). Two patients with bladder cancer were enrolled in this study. However, this study was terminated due to high toxicity of the combination therapy [31]. Other moAbs that specifically target EGFR and which have been tested in other cancers include matuzumab, zalutumumab, and nimotuzumab, but to our knowledge these have not been evaluated in bladder cancer.

Tyrosine Kinase Inhibitors

Gefitinib was initially approved by the FDA for treatment of NSCLC in 2003. It was shown to be especially effective in a subset of patients (25 among the 275 treated) with metastatic NSCLC harboring a particular set of mutations in the intracellular kinase domain of EGFR [6] A Phase 3 trial looked at the molecular predictors of outcome with gefitinib treatment in 1,692 patients with refractory advanced NSCLC [32]. The authors found that EGFR copy number and protein expression level most significantly correlated with outcome. Patients with mutations in *EGFR* also had higher response rates. Other studies have also shown Japanese origin and history of never smoking to be independent predictors of response to gefitinib in NSCLC [11,33,34].

Gefitinib has shown antitumor activity in preclinical studies in UC cell lines [35,36]. When gefitinib was evaluated in a Phase II trial as part of combination treatment with GC in chemotherapy-naïve patients with locally advanced or metastatic cancer, the treatment group showed an overall response rate of 42.6% with a median survival time of 15.1 months and median time to progression of 7.4 months [37]. This study did not find any improved efficacy over standard treatment with GC alone. A Phase II trial evaluated gefitinib in patients with metastatic UC who had failed first-line chemotherapy. The median progression-free survival was two months. The authors concluded that gefitinib is ineffective as second-line therapy in patients with UC [38]. A Phase II trial is underway comparing GC with and without gefitinib in the treatment of chemotherapy-naïve patients with locally invasive or metastatic disease (Clinicaltrials.gov, ID: NCT00246974). Gefitinib is also being studied in combination therapy with docetaxel (Taxotere) as consolidation chemotherapy in a Phase II trial in patients with locally unresectable or metastatic UC (Clinicaltrials.gov, ID: NCT00479089). A trial looking at the efficacy of gefitinib monotherapy on non-resectable locally invasive or metastatic UC has been completed (Clinicaltrials.gov, ID: NCT00014144). Finally, a Phase III trial is underway comparing intravesical BCG therapy alone or combined with gefitinib in the treatment of high-risk superficial UC (stages Ta, TIS, T1) (Clinicaltrials.gov, ID: NCT00352079).

Erlotinib is an oral TKI indicated in the treatment of metastatic colorectal cancer, metastatic NSCLC, and (in combination with bevacizumab) renal cancer. In a Phase 3 clinical trial comparing erlotinib to placebo in patients who had failed first or second-line chemotherapy in NSCLC, erlotinib improved survival by two months

and decreased symptoms [39]. The authors noted that independent predictors of survival were Asian origin, history of non-smoking, and adenocarcinoma histology. Interestingly, in their study, *EGFR* mutations were not predictive of survival.

Erlotinib has been shown to inhibit cell proliferation, angiogenesis, and invasion in a number of preclinical trials. A phase II study evaluated the clinicopathologic efficacy of erlotinib in patients with stage T2 disease [40]. In this study 20 patients received neoadjuvant erlotinib. Complete pathological response (stage pT0) was seen in 25% of patients while 35% of patients were downstaged (stage </= pT1). Fifty percent of patients remained alive and with no evidence of disease at 24.8 months. It should be kept in mind that among patients undergoing cystectomy for T2 disease with negative lymph nodes, the rate of disease-free survival at 5 years is 78% [17,32]. Erlotinib is currently being evaluated in a Phase II trial as neo-adjuvant therapy in patients requiring cystectomy (Clinicaltrials.gov, ID: NCT00749892). Another Phase II study is looking at the role of erlotinib when given both as neoadjuvant treatment and as maintenance therapy in patients with muscle-invasive disease (stage T2) (Clinicaltrials.gov, ID: NCT00380029). A phase II trial is also underway comparing the effect of erlotinib and the green tea extract Polyphenon E on recurrence in former smokers who have undergone cystectomy for the treatment of bladder cancer (Clinicaltrials.gov, ID: NCT00088946). The results of these trials have not yet been reported.

Two agents which belong to the class of multikinase inhibitors are lapatinib (Tycerb), which acts on EGFR as well as Her2 and vandetanib (Caprelsa), which acts on VEGFR-2, VEGFR-3, EGFR, and RET. Lapatinib has been approved in combination with capecitabine for the treatment of metastatic breast cancer that has progressed despite standard therapy. In bladder, *in vitro* studies in bladder cancer cells showed that the addition of lapatinib to GCT (gemcitabine, paclitaxel, cisplatin)-treated cells enhanced cell killing as measured by flow cytometry [41]. Lapatinib has not yet been evaluated in clinical trials in the setting of bladder cancer. Vandetanib was recently approved for the treatment of progressive medullary thyroid cancer. It is under investigation in the treatment of refractory advanced lung cancer [42] . To our knowledge it has not been studied in bladder cancer.

Predictors of Response to Treatment in Bladder Cancer

While preclinical studies have demonstrated response of bladder tumors to gefitinib, the mechanism of this response remains unclear. For example, while activating mutations in the kinase domain have been found to correlate with clinical response to gefitinib in NSCLC patients, mutations in the exons 18-21, containing the kinase domain, were not found in any bladder cancer cell lines or patient samples [11]. In the same study, EGFRvIII was not detected in any of the cell lines or patient samples by PCR. This indicates that patient response to gefitinib cannot be predicted by the same set of mutations as those present in NSCLC responders to treatment.

A number of studies looked at predictors of response to gefitinib in multiple bladder cancer cell lines. In one study, activation of glycogen synthase kinase-3β (GSK-3β) predicted sensitivity to gefitinib [43]. Gefitinib-resistant cell lines, on the other hand, could be made sensitive to gefitinib by inhibition of platelet-derived growth factor receptor β (PDGFRβ). This study showed that the mechanism of resistance to gefitinib in a subset of bladder tumors is activation of PDGFRβ, which inactivates GSK-3β, thereby bypassing EGFR signaling. Another study showed that growth inhibition with gefitinib positively correlated with p27 protein expression and decreased cyclin-dependent kinase 2 (cdk2) activity [44]. The results also suggested that resistant cell lines expressed higher levels of vimentin and lower levels of E-cadherin, suggesting greater epithelial to mesenchymal transition, though this correlation did not hold for all of the cell lines studied.

It has also been noted that different bladder cancer cell lines differ with respect to their sensitivities to cetuximab. While this difference cannot be explained by mutations in *EGFR*, it is hypothesized that it may be due to differential expression of molecules that closely associate with EGFR and modulate its activity. In particular, cell lines that do not express E-cadherin have been shown to be resistant to cetuximab, while those that express E-cadherin are cetuximab-sensitive [45]. Moreover, knocking out E-cadherin in two of the sensitive cell lines rendered these lines resistant to cetuximab. Validating E-cadherin or other molecules as biomarkers of cetuximab sensitivity could enable physicians to pick out a subset of patients who are most likely to be responsive to therapy with cetuximab.

HER2 Overexpression and Therapy in Bladder Cancer

HER2 is another member of the EGFR/ErbB family. Amplification or over-expression of this gene has been shown to play an important role in the pathogenesis of many solid tumors. The HER2 protein is a prognostic factor and a therapeutic target when overexpressed in other solid tumor such as breast cancer. Studies in UC have found mixed results regarding the prognostic value of HER2 overexpression. Some studied suggest HER2 to be a poor prognostic factor in UC, while others do not. Published data report a large range of expression rates of HER2 by Immunohistochemistry (IHC) and Fluorescence In Situ Hybridisation (FISH) at different stages of UC.

A study examining the Her2/neu status in non-muscle invasive bladder cancer (NMIBC) using FISH found gene amplification in 9% (16/178) of high grade UC and none (0/193) in low malignant potential and low grade UC [46]. The incidences of recurrence and progression in HER2-amplified high grade UC were significantly higher than in those without amplification. Immunohistochemistry and FISH results were in closest agreement when overexpression was defined as 50% of tumor cells showing immunoreactivity. In another study of NMIBC, HER2 protein was overexpressed in 68.2% (30/44) of pT1GIII specimens and also predicted recurrence [47].

In muscle invasive bladder cancer a study evaluating 80 cystectomy and lymph node dissection specimens found 28% (22/80) of cystectomy cases were HER2 positive by IHC and 53% (17/32)were positive in the lymph nodes.) [48]. In this study HER2 overexpression was not predictive of survival. In a large retrospective study of 1005 muscle-invasive tumors 9.2% of specimens were either 2+ or 3+ HER2 positive by IHC. All 3+ protein overexpression tumors also contained gene amplification by FISH but none of the samples with 2+ overexpression were FISH positive [49]. In a study of archival tumor tissues from patients with advanced urothelial carcinoma enrolled on two clinical chemotherapy trials the HER2 expression was analyzed by IHC and correlated with clinical outcomes. All 39 tumors were high grade. Strong HER2 expression (2+/3+) was seen in 28 patients (71%). Univariate analysis showed that increased HER2 expression predicted an improvement in progression free and overall survival. When HER2 status was used as a dichotomous variable, tumors with positive HER2 expression did not have any association with response or with progression free survival; however, positive HER2 status was associated significantly with a decreased risk of death [50].

In UC patients the majority of HER2 overexpression of the

urothelium seems to occur without underlying genetic amplification, in contrast to other tumors such as breast cancer [51]. HER2 targeting agents include the monoclonal antibody trastuzumab and the tyrosine kinase inhibitor lapatinib. A phase II trial in advanced urothelial cancer tested trastuzumab in combination with carboplatin, paclitaxel, and gemcitabine. Eligible patients were required to demonstrate overexpression of HER2 by IHC, FISH, or serum HER2 extracellular domain testing. Of the patients screened 52% had HER2 overexpression predominantly by IHC (> 90%), 25% of patients tested positive by FISH or serum testing. The median survival was 14.1 months. Response rates were higher in patients with high levels of IHC expression and in those with FISH-positive versus FISH-negative HER2 overexpression. A second-line phase II study of patients with advanced UC studied the efficacy of single agent lapatinib in 59 patients. An objective response rate was observed in 1 patient with 18 patients achieving stable disease. The median time to disease progression and overall survival were 8.6 weeks and 17.9 weeks, respectively. Clinical benefit was found to be correlated with EGFR overexpression, and HER-2 overexpression. The median OS was significantly prolonged in patients with tumors that overexpressed EGFR and/or HER-2 [52]. These data suggest that HER2 either alone or in combination with EGFR may be a viable target in UC.

Other Therapies Targeting EGFR

A new EGFR-linked target for cancer therapy is the recently-characterized leucine-rich repeats and immunoglobulin domains 1 (LRIG1) protein [53]. LRIG1 is a natural ligand to ErbB receptor family members, and has been shown to interact with EGFR and target it for ubiquitylation and degradation [54]. This molecule was shown to act *in vitro* as a tumor suppressor in a superficial bladder cancer cell line [55]. Increased expression of LRIG1 correlated with decreased expression of EGFR, decreased cell proliferation, and decreased invasive capability. Experiments were carried out in which LRIG1 cDNA was delivered intratumorally via an adenoviral vector into nude mice bearing bladder tumor xenografts [56]. In these experiments, expression of LRIG1 led to decreased expression of EGFR, decreased proliferation, slower rate of tumor growth, and decreased microvessel density.

Photodynamic therapy (PDT) is another targeted therapeutic approach. PDT was first used in 1975 to diagnose and treat bladder cancer patients [57]. In PDT, a photosensitizer, often a hematoporphyrin derivative, induces tumor cell death when it is activated by a specific wavelength of light. It is thought that upon stimulation by light at specific wavelengths, energy is transferred from the photoexcited sensitizer to oxygen and reactive oxygen species are formed that cause cell damage. The precise location of cell damage varies according to photosensitizer localization. For example, damage can occur to the mitochondria, to the plasma membrane, or to lysosomes. The mechanism of cell death elicited may be either necrosis or apoptosis, and this may be explained by the subcellular compartment where initial damage occurs [58].

PDT with cetuximab was investigated in mice bearing bladder tumor xenografts. The combination of PDT with cetuximab strongly inhibited tumor growth, compared with PDT or cetuximab by itself [59]. Tumor inhibition was associated with increased apoptosis in the combination treatment group. The combination of PDT, cetuximab, and bevacizumab was also studied [60]. In *in vivo* studies, the combination therapy resulted in a more rapid tumor response than the dual therapy of PDT with either cetuximab or bevacizumab. The improved efficacy of the combination may come from combined effects of the two drugs on the angiogenic pathways in the tumor.

Antibodies to EGFR have been used to target toxins to tumor cells. Yang et al. constructed a fusion protein, DAB389EGF, that contains the catalytic and translocation domains of Diphtheria toxin fused to human EGF. As EGFR is overexpressed on the luminal surface of non-invasive bladder tumors but not on normal tumor epithelium, it was thought that intravesical delivery of such a construct would specifically target tumor cells and lead to their death, while the effect on healthy tissues would be minimized. *In vitro*, DAB389EGF inhibited tumor cell growth and suppressed clonogenicity in multiple cell lines. These effects positively correlated with the level of EGFR expression in a given cell line. Intravesical administration of DAB389EGF into nude mice bearing xenograft tumors inhibited tumor growth as measured by bioluminescence [61]. Moreover, intravesical infusion was less toxic to the mice than systemic treatment with DAB389EGF. The antitumor efficacy of DAB389EGF has also been demonstrated in *in vitro* breast [62] and pancreatic [63] cancer models, and in *in vivo* models of glioblastoma multiforme [64].

Finally, the therapeutic potential of microRNAs (miRNAs) has been explored with respect to EGFR. MiRNAs are 18-25 nucleotide-long RNA molecules which negatively regulate mRNA expression. They may do so either by promoting mRNA degradation or inhibiting translation of mRNA. It is estimated that in this way they regulate the expression of approximately 30% of the human genome [65]. Deregulation of miRNA activity is being studied for its role in tumorigenesis and cancer progession. Thus, miRNAs that regulate the expression of tumor suppressors or proto-oncogenes may themselves act as proto-oncogenes or tumor suppressors, respectively. It has been shown that expression of miRNA-133a (*miR-133a*) and *miR-133b*, two closely related molecules, is reduced in bladder cancer cells [66,67]. Transfection of cancer cells with mature *miR-133a* significantly inhibited cell proliferation, migration, and invasion [66]. This demonstrated that *miR-133a* does indeed act as a tumor suppressor. Expression profiling of patient tumor specimens revealed that low expression of *miR-133b* strongly correlated with progression in non-muscle invasive tumors [67]. Further investigation revealed that transfection of tumor cells with *miR-133a* or *miR-133b* led to decreased expression of EGFR and its downstream effector molecules including p-Erk and p-Akt. Bioinformatics studies identified EGFR as a direct target of *miR-133*, and two binding sites for *miR-133a/b* were subsequently identified on the 3' UTR of the *EGFR* transcript. While *in vivo* studies have yet to be conducted to evaluate the therapeutic value of *mi-R133* in inhibiting tumor growth, these findings open up a novel avenue for targeting EGFR and its downstream signaling pathways.

Conclusion

The ErbB family of tyrosine kinase receptors is an important class of proteins involved in transducing extracellular signals that direct cells to divide and grow. EGFR, a member of this class, has been evaluated extensively as a target of antitumor medications because of its overexpression or mutation in a variety of tumor types and its central role in modulating carcinogenic properties of tumor cells. Monoclonal antibodies and tyrosine kinase inhibitors target different domains of the EGFR receptor and both have been investigated in the treatment of bladder cancer, either as monotherapy, in combination with standard chemotherapy, or in combination with other biologic agents. HER2 may also be a potential target. However, significant clinical efficacy has thus far been elusive with these agents. A number of next-generation TKIs and moAbs are being used in the laboratory and in clinical trials for a variety of cancers (Table 1), but their potential role in bladder cancer has not yet been explored.

Compound	Structure	Mechanism of Action	Currently FDA approved indications	Stage of Development in bladder cancer
Cetuximab (C225/Erbitux)	Human/mouse chimeric moAb	inhibits erbB1	H & N, mCRC	Phase II
Panitumumab (ABX-EGF/ Vectibix)	Human moAb	inhibits erbB1	mCRC	Preclinical
Nimotuzumab (Theracim)	Humanized moAb	inhibits erbB1	(being evaluated in various cancers including esophageal cancer, NSCLC, pancreatic cancer)	none
Matuzumab (EMD 7200)	Humanized moAb	inhibits erbB1	(being evaluated in NSCLC, EGC, ovarian cancer)	none
Zalutumumab	Human moAb	inhibits erbB1	(being evaluated in H & N, CRC, NSCLC)	none
Gefitinib (Iressa)	TKI	inhibits erbB1	NSCLC	Phase II, Phase III
Erlotinib (Tarceva)	TKI	inhibits erbB1	NSCLC	Phase II
Lapatinib (Tykerb)	MTKI	inhibits erbB1, erbB2	BRC	Preclinical
Vandetanib (Caprelsa)	MTKI	inhibits erbB1, VEGFR-2, VEGFR-3, and RET	MTC	none
AEE788	MTKI	inhibits erbB1, erbB2, VEGFR-2	(being evaluated in GBM)	none
AC 480 (BMS-599626)	MTKI	inhibits erbB1, erbB2	(being evaluated in BRC, GBM)	none
AZD 8931	MTKI	inhibits erbB1, erbB2, erbB3	(being evaluated in BRC, gastric cancer, other solid malignancies)	none
DAB389EGF	chimeric protein	conjugated toxin protein targeting erbB1	none	Preclinical
miR-133	microRNA	promotes *EGFR* mRNA degradation/ blocks *EGFR* translation	none	Preclinical
LRIG1 cDNA	cDNA	protein product inhibits erbB1	none	Preclinical

Acronyms
TKI: tyrosine kinase inhibitor; MTKI: multitargeted kinase inhibitor; moAb: monoclonal antibody; H & N: head and neck cancer; CRC: colorectal cancer; mCRC: metastatic colorectal cancer; NSCLC: non-small cell lung cancer; BRC: breast cancer; MTC: medullary thyroid cancer

Table 1: EGFR-targeted therapies in bladder cancer.

A number of other innovative treatment modalities are in development to target EGFR, but these treatment modalities are in experimental stages and it remains to be seen if they will make it to the clinical testing stage. It may turn out to be the case that the most effective treatment of bladder cancers will involve using a combination of treatment modalities against multiple targets. This could have the advantage of shutting off multiple parallel (and interacting) signaling pathways. At the same time, if a tumor develops resistance to therapy with a single agent by developing a mutation in the target protein or a downstream effector molecule, the addition of a second or third agent could overcome this resistance. Moreover, patients with different genetic disease signatures may benefit from different treatment combinations. To this end researchers are trying to establish biomarkers that would be of prognostic value. While many promising approaches are in development, it remains to be seen which therapies or combinations of therapies will show benefit in the treatment of patients.

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