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The Role of EGFR Family Inhibitors in Muscle Invasive Bladder Cancer: A Review of Clinical Data and Molecular Evidence

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Purpose: Conventional platinum based chemotherapy for advanced urothelial carcinoma is plagued by common resistance to this regimen. Several studies implicate the EGFR family of RTKs in urothelial carcinoma progression and chemoresistance. Many groups have investigated the effects of inhibitors of this family in patients with urothelial carcinoma. This review focuses on the underlying molecular pathways that lead to urothelial carcinoma resistance to EGFR family inhibitors.

Materials and Methods: We performed a PubMed® search for peer reviewed literature on bladder cancer development, EGFR family expression, clinical trials of EGFR family inhibitors and molecular bypass pathways. Research articles deemed to be relevant were examined and a summary of original data was created. Meta-analysis of expression profiles was also performed for each EGFR family member based on data sets accessible via Oncomine®.

Results: Many clinical trials using inhibitors of EGFR family RTKs have been done or are under way. Those that have concluded with results published to date do not show an added benefit over standard of care chemotherapy in an adjuvant or second line setting. However, a neoadjuvant study using erlotinib before radical cystectomy demonstrated promising results.

Conclusions: Clinical and preclinical studies show that for reasons not currently clear prior treatment with chemotherapeutic agents rendered patients with urothelial carcinoma with muscle invasive bladder cancer resistant to EGFR family inhibitors as well. However, EGFR family inhibitors may be of use in patients with no prior chemotherapy in whom EGFR or ERBB2 is over expressed.

Key Words: urinary bladder neoplasms, drug resistance, neoplasm invasiveness, EGFR tyrosine kinase inhibitor 324674, antineoplastic agents

Abbreviations and Acronyms

BCG = bacillus Calmette-Guérin
EGFR = epidermal growth factor receptor
GC = gemcitabine and cisplatin
HB-EGF = heparin-binding epidermal growth factor-like growth factor
HER = human EGFR
IHC = immunohistochemistry
MIBC = muscle invasive bladder cancer
NMIBC = nonMIBC
OS = overall survival
PI3K = phosphatidylinosital-4, 5-bisphosphate 3-kinase
RC = radical cystectomy
RTK = receptor tyrosine kinase
TCC = transitional cell carcinoma
TCGA = The Cancer Genome Atlas
TURBT = transurethral resection of bladder tumor
UC = urothelial carcinoma

CURRENT TREATMENT OPTIONS IN BLADDER CANCER

Of all urothelial malignancies 90% arise from the transitional epithelium and are classified as TCC (fig. 1).[1] MIBC comprises 33% of initial cases of TCC while the remaining cases are classified as NMIBC.[2] NMIBC is more easily treated and managed than MIBC. Standard of care treatment for NMIBC is TURBT followed by a single dose of intravesical chemotherapy or intravesical BCG.[3] While this regimen yields a 5-year survival rate of 82% to 100%, the 2-year recurrence rate in these patients

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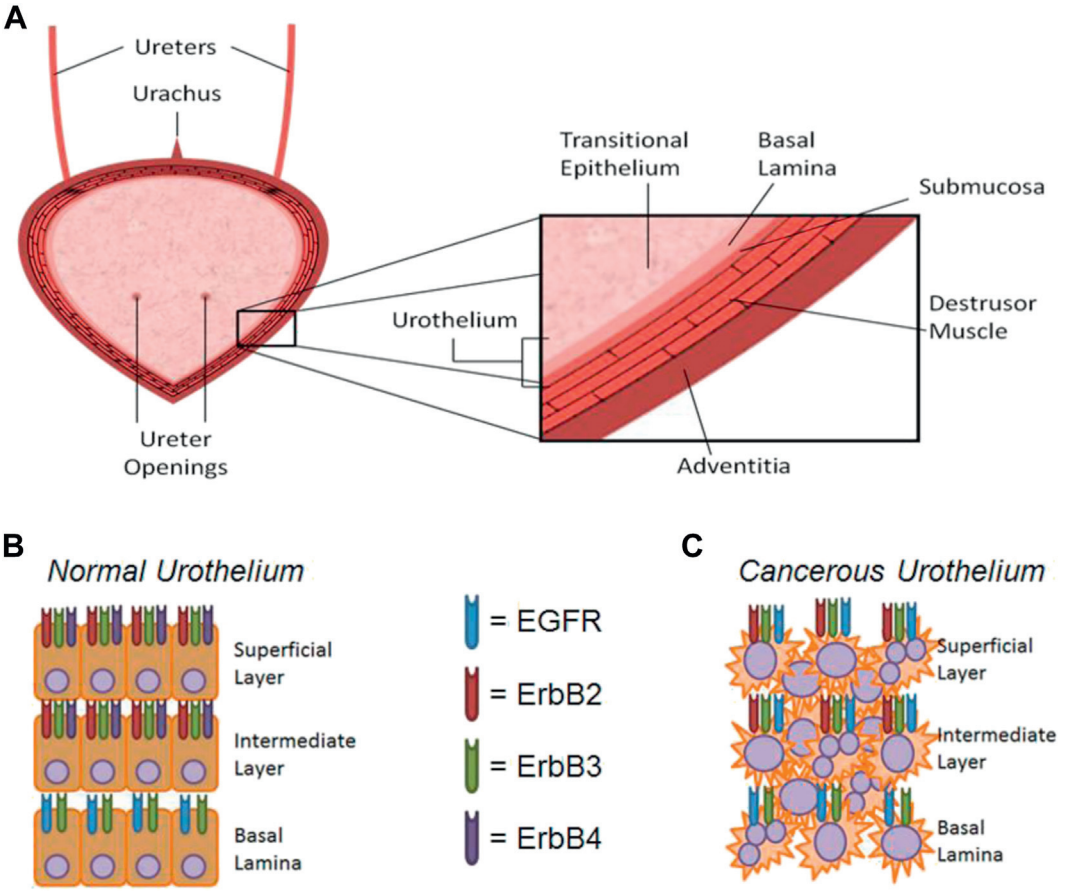

Figure 1. Bladder structural layers and EGFR family member expression. *A*, various structures of bladder and tissue layers from transitional epithelium, which is innermost layer, to adventitia, which is outermost layer. Urothelium is composed of transitional epithelium, basal lamina and submucosa. *B* and *C*, pattern of EGFR family member expression. *B*, in normal urothelium. *C*, in cancerous urothelium.

is 28% to 40%[4,5] and as high as 80% in the subset of patients who initially present with high grade tumors (table 1).

Approximately 15% to 30% of high grade NMIBC cases develop into MIBC,[1] in addition to 30% with de novo MIBC presentation. MIBC is highly aggressive compared to NMIBC, correlating with a high rate of metastasis and mortality. Most patients who present with MIBC undergo RC alone. However, level 1 evidence supports the use of platinum based neoadjuvant chemotherapy followed by RC and urinary diversion or radiation therapy with accompanying chemotherapy.[1,6] A multicenter, randomized, controlled clinical trial showed 77-month median survival (57% 5-year survival rate) in patients with nonmetastatic MIBC treated with neoadjuvant chemotherapy combined with cystectomy compared to 46-month median survival (43% 5-year survival rate) in those treated with cystectomy alone.[7] The patient prognosis after surgery depends on the extent of invasion and whether lymph node metastases are present.[8]

Upon the development of metastasis cytotoxic chemotherapy with GC as a first line treatment is gaining acceptance.[8] Although it is initially effective, average survival on this treatment is only 15 months with a 5-year survival rate of between 5% and 20%.[8,9] Therefore, treatment to prevent the dissemination of bladder tumors to distant sites and treatments that sensitize patients with MIBC to chemotherapy are required at this time.

EGFR FAMILY IN BLADDER CANCER

The 4 receptors that comprise the EGFR family are EGFR (ErbB1/Her1), human EGFR 2 (ERBB2/HER2/NEU), human EGFR 3 (ERBB3/HER3) and human EGFR 4 (ERBB4/HER4). A search of publications revealed 421 that discussed the EGFR family or bladder cancer (fig. 2). These receptors are stimulated by a number of growth factors, including EGF, transforming growth factor-α and amphiregulin for EGFR, the heregulins for ERBB3 and ERBB4, and HB-EGF for EGFR and ErbB4

Table 1. *UC current staging, TNM classification and treatment options*

Stage	TNM Classification	Treatment Options		% Recurrence Risk
		Common	Other	
0	Ta, N0, M0 or Tis, N0, M0	TUR + fulguration, segmental cystectomy if aggressive	Intravesical thiotepa, mitomycin, doxorubicin or BCG	28–40
I	T1, N0, M0	TUR + fulguration, segmental cystectomy if aggressive	Intravesical thiotepa, mitomycin, doxorubicin or BCG	80
II	T2a, N0, M0 or T2b, N0, M0	RC, neoadjuvant chemotherapy for MIBC	Definitive radiation therapy with systemic chemotherapy	50
III	T3a, N0, M0, or T3b, N0, M0, or T4a, N0, M0	RC, neoadjuvant chemotherapy for MIBC	Definitive radiation therapy with systemic chemotherapy	50
IV	T4b, N0, M0, or any T, N1–N3, M0, or any T, any N, M1	Palliative care + clinical trials (most cases)	Radical cystectomy with pelvic lymph node dissection (some cases)	–
Recurrence	Any T, any N, any M	TUR + fulguration, segmental cystectomy if aggressive (low stage)	Radical cystectomy, neoadjuvant chemotherapy for MIBC (high stage)	Not applicable

(fig. 3).[10] Interestingly HB-EGF may have different characteristics based on whether it is present in its membrane-anchored or soluble form.[11] In contrast, ERBB2 is an orphan receptor that is believed to exist in a constitutively primed state,[12] which binds to other activated EGFR family members. Each EGFR member consists of a ligand binding extracellular domain, a transmembrane domain and an intracellular tyrosine-kinase domain (fig. 4).

The EGFR family of RTKs relies on dimerization among the 4 family members to transmit signal from the extracellular space into the cytoplasm, where downstream signaling cascades are activated (fig. 3). Upon ligand binding a conformational change is observed in the extracellular domain of the receptor, which enables dimerization with the other EGFR family members.[13] Dimerization induces the dimer partners to undergo transactivation, causing phosphorylation of specific sites. These sites serve as docking sites for various adapter proteins that activate a host of pathways, including PKC, PI3K, RAS, SRC, ABL, PAK and STAT5 (fig. 3).[14] Not surprisingly the recent TCGA study of 131 MIBC samples, 118 peripheral blood samples and 23 tumor adjacent, normal-appearing bladder samples revealed that changes that affected the PI3K/Akt/mTOR pathway and the RTK/RAS pathway occurred in 42% and 44% of bladder tumors, respectively.[15]

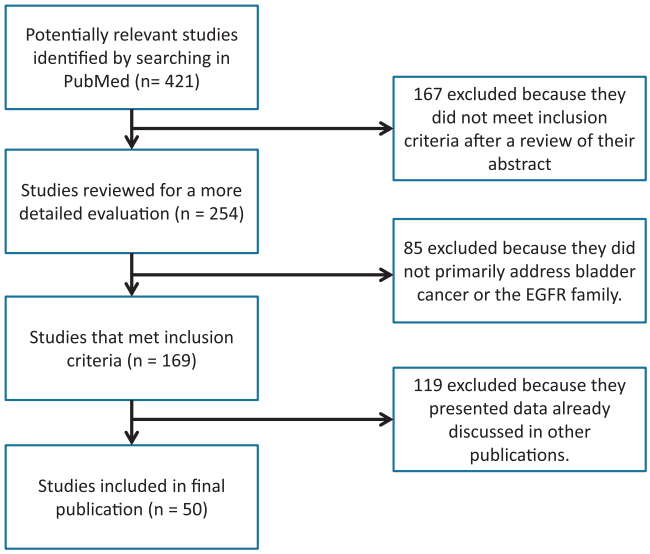

Figure 2. For literature search strategy PubMed was initially queried for relevant publications meriting inclusion in bladder cancer discussion. After initial search 167 publications were removed because they did not meet inclusion criteria for review. During more thorough review of remaining 254 publications 85 were excluded because they did not primarily address bladder cancer or EGFR family of RTKs. Another 119 publications were excluded because they did not add new data to understanding of EGFR family role in MIBC.

Epidermal Growth Factor Receptor

In normal urothelium EGFR is expressed in the basal layer and correlates with a less differentiated state of these cells.[16] Due to the apparent role of EGFR in bladder cell dedifferentiation it is not surprising that EGFR over expression in UC has been reported frequently in the literature (table 2).[17–19] A study of 56 samples of NMIBC or MIBC demonstrated that while EGFR over expression was modest in NMIBC (3 of 25 cases or 12%), it was more evident in MIBC (10 of 28 or 35%) as determined by IHC.[19] A similar study used a cohort of 175 NMIBC and 70 MIBC cases.[18] In accord with the rates in the previous study EGFR over expression was observed in 67 of 245 cases (27%).[18] Furthermore, a study of 21 patients with NMIBC (3 or 14%) or MIBC (18 or 86%) showed that 14 (74%) were positive for EGFR staining by IHC while EGFR over expression was noted in 10 (53%).[17] Lastly, HB-EGF has a prognostic role in patient survival. In a study of 121 NMIBC and MIBC specimens patients with predominantly nuclear expression of HB-EGF had 30% lower 5-year cancer specific survival than patients

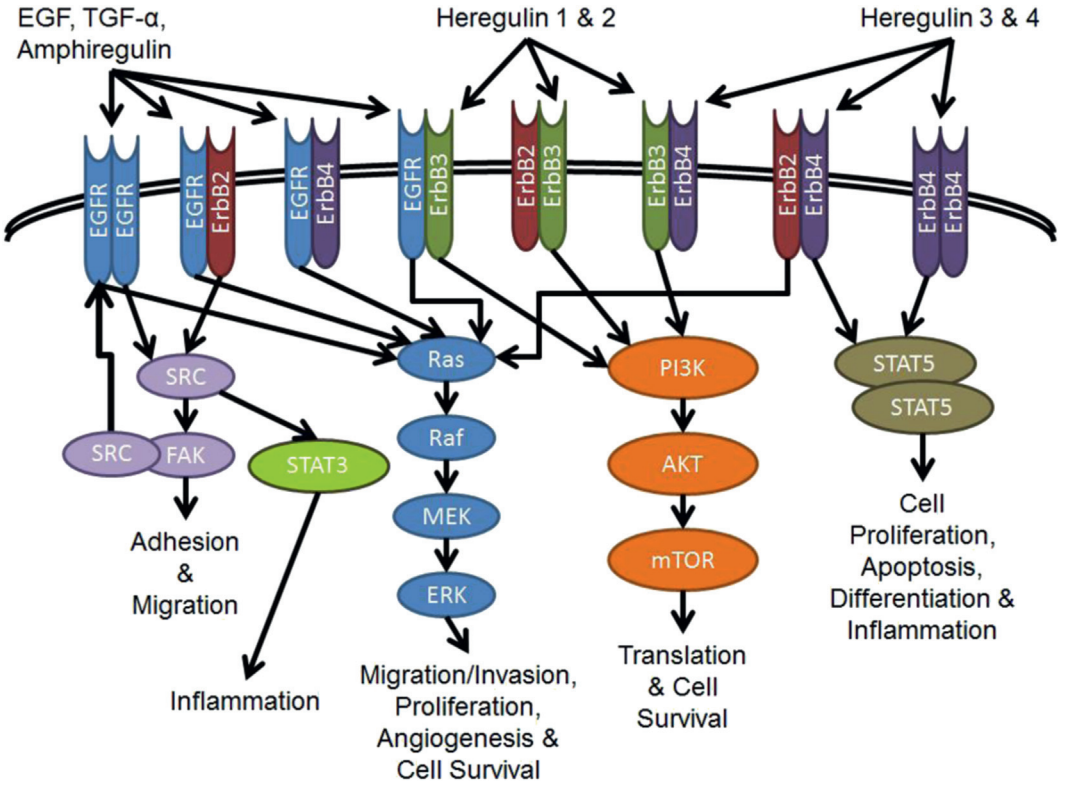

Figure 3. Pathway of all possible EGFR family dimer combinations showing various growth factors known to activate EGFR family and all possible dimer combinations. EGFR primarily signals through canonical SRC and RAS pathways leading to migration, invasion, adhesion, angiogenesis and survival of cancer cells. ERBB4 signals through STAT5 pathway, which leads to proliferation, apoptosis, differentiation and inflammation. *EGF*, epidermal growth factor. *TGF-α*, transforming growth factor-α.

with predominantly nuclear expression of HB-EGF ($p = 0.027$).[20]

Overall meta-analysis of 5 studies in Oncomine of superficial TCC[21–25] and 6 of MIBC[21–26] comprising a total of 566 samples revealed that EGFR expression did not significantly differ in normal tissue compared to superficial TCC ($p = 0.525$). However, it was over expressed in MIBC compared to normal tissue (1.49-fold change, $p = 0.034$, table 2). While these studies indicate that EGFR is over expressed in bladder cancer, by looking closely at the large cohort series it can be determined that EGFR over expression is more common and occurs more frequently in MIBC than in NMIBC.

Despite the presence of EGFR mutations in many other cancers a survey of 11 UC cell lines and 75 primary tumors demonstrated no mutations when analyzed by automated sequencing.[27] A specific probe of exons 19 and 21 via quantitative polymerase chain reaction of formalin fixed, paraffin embedded primary tumors from 21 patients, primarily MIBC, revealed the same result.[17] Furthermore, a study of 28 urothelial primary adenocarcinomas[28] and another study in 8 cell lines[29] showed no mutations in EGFR, indicating that mutations in EGFR are rare in primary UC. However, in the TCGA study there was a 9% incidence of *EGFR* amplification in 131 MIBC samples.[15]

Although to our knowledge the EGFR mutation rate in distant metastases is unknown, a study of 17 patients with MIBC (total of 22 primary tumors and 24 associated metastases) showed strong concordance (mean 75%) between the chromosomal aberrations in the primary tumors and their associated metastases.[30] Since mutations and gene amplifications of EGFR are a rare event in UC, it was hypothesized that EGFR over expression is due to deregulation of the protein recycling and degradation pathways.[17,29] Specifically, endophilin A1, which regulates EGFR endocytosis, was commonly down-regulated in bladder tumors, providing another plausible mechanism of EGFR over expression.[31] Despite these findings EGFR expression has not been determined to be an independent predictor of disease progression or mortality.

ERBB2

Although ERBB2 is an orphan receptor with no identified ligand and, thus, it cannot form active homodimers, ERBB2 transmits signals by forming

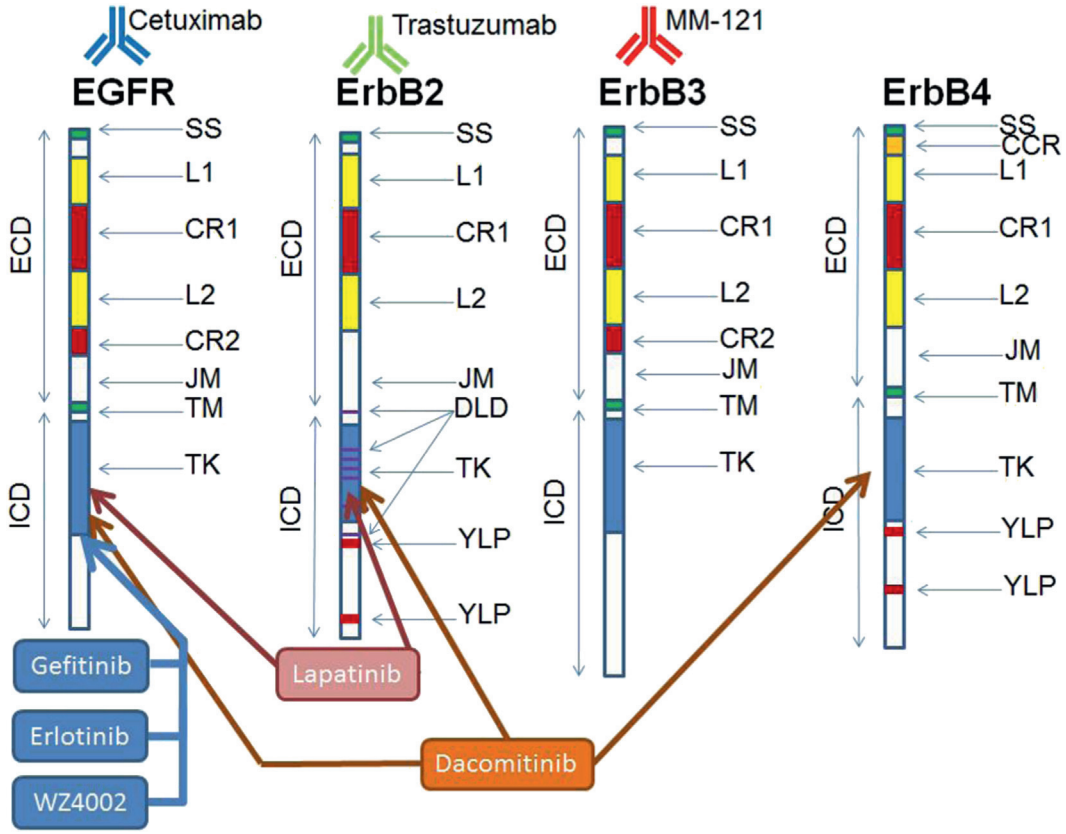

Figure 4. Structure of ErbB RTKs and inhibitors of each family member, representing most common isoform as described in AceView (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/). Each family member also contains number of spliced variants not discussed in this review. Extracellular domain contains cleavable signaling sequence (*SS*) in N-terminal end followed by 2 ligand interacting domains, L1 and L2, separated by 1 or 2 furin-like cysteine rich regions CR1 and CR2. In ERBB4 but not in other receptors coiled-coil region (*CCR*) is located between signaling sequence and L1 domains. Juxtamembrane domain (*JM*) is region of many spliced alternative isoforms, especially in ERBB4. Tyrosine kinase (*TK*) domain is present in all family members but in ERBB2 it is marked by dileucine domain (*DLD*) not found in others. ERBB2 and ERBB4 each contain 2 YLP motifs in C-terminal end that is not seen in other 2 members. Also shown are various EGFR family inhibitors. Antibodies cetuximab, trastuzumab and MM-121 work by binding to extracellular domain and inhibiting ligand binding, respectively. Note small molecule inhibitors gefitinib, erlotinib, lapatinib and dacomitinib. Arrows from each inhibitor indicate where these inhibitors bind on respective molecules.

heterodimers with other members of the EGFR family.[32] ERBB2 is normally expressed on the superficial and intermediate layers of the urothelium.[19] During wound healing ERBB2 promotes migration and re-epithelialization of the damaged tissue.[10] ERBB2 expression correlates with metastatic MIBC as well as tumor recurrence[18] while co-expression of ERBB2 and P53 increased the probability of nodal metastases (table 2).[33] However, a study of 70 primary TCCs revealed that ERBB2 over expression is indiscriminate of disease with or without metastasis.[32] Of these 70 cases 9 of 19 nonmetastatic tumors (47%) and 18 of 51 metastatic tumors (35%) over expressed ERBB2 as determined by moderate (2+) or high (3+) staining by IHC.[32] Meta-analysis of 5 studies of superficial TCC[21–25] and 6 of MIBC[21–26] reported in Oncomine, comprising a total of 566 samples, showed that ERBB2 expression did not significantly differ in normal tissue compared to superficial TCC ($p = 0.586$). However, it was significantly over expressed in MIBC compared to normal tissue (1.55-fold change, $p < 0.0001$).

While *ERBB2* amplification is a common occurrence in other cancers, in a study of 73 primary UCs gene amplification was present in only 3% to 9% of cases, including 53 NMIBC and 20 MIBC samples.[2] However, unlike EGFR, ERBB2 mRNA levels were highly up-regulated in 18 NMIBC and MIBC samples compared to normal tissue.[34] A TCGA study of 131 MIBC bladder tumors corroborated this finding, indicating that *ERBB2* mutation or amplification was present in 9% of samples, similar to levels in breast cancer, but with more mutations and fewer amplifications in bladder than in breast tumors.[15] A study seeking to correlate the EGFR family expression profile with the patient prognosis demonstrated that the predictive value of ERBB2 when co-expressed with EGFR or ERBB3

Table 2. *Roles of EGFR receptor family in UC progression*

	Presence vs Normal Tissue			
Receptor	NMIBC	MIBC	Differentiation Role	Disease Progression Correlations
EGFR:			Causes cell dedifferentiation	EGFR protein responds to high epidermal growth factor in urine[27] + drives cells proliferation + growth[39]
No. pts	230	336		
Fold change	0.73	1.49		
p Value	0.525	0.034		
ERBB2:			Forms heterodimers with other ErbB family members.	Correlates with muscle invasive metastases, + first + second recurrences,[21] may increase tumor response to growth factors in urine[32]
No. pts	230	336		
Fold change	1.94	1.55		
p Value	0.586	<0.001		
ERBB3:			Forms heterodimers which primarily signal the PI3K pathway	ErbB3 protein responds to heregulins in urine,[27] correlates with tumor size, No. + histological grade[21]
No. pts	204	283		
Fold change	1.94	1.53		
p Value	<0.001	0.004		
ERBB4:			Causes cell differentiation	Correlated with stage in 1 study[32]
No. pts	204	283		
Fold change	0.06	0.71		
p Value	0.065	<0.001		

may be due to its ability to increase the response of tumors to growth factors in urine.[18] Indeed, a previous study showed that ERBB2 could slow the degradation of EGFR molecules that were bound to ligand.[35] *HER2* amplification was reported to be more common in associated metastases than in their corresponding primary tumors. Specifically in a study of 150 MIBC cases the *HER2* amplification rate in metastases was 15.3% compared to 8.7% in primary tumors ($p = 0.0003$).[36]

ERBB3

The third member of the EGFR family, ERBB3, lacks intrinsic kinase activity, although it has a kinase domain. Upon binding to its ligands, Heregulin 1 and 2, ERBB3 forms heterodimers and homodimers but only the former are capable of transmitting signals, predominantly through the PI3K/AKT pathway (fig. 3).[37] In normal urothelium ERBB3 is expressed primarily on superficial cells but several studies demonstrated low grade expression of ERBB3 throughout the urothelium.[19] ERBB3 over expression may have a more inclusive effect on UC. A correlative IHC study in 245 patients, including 47 with NMIBC (19%), 118 with local MIBC (48%) and 80 with metastatic MIBC (33%), showed a positive association of ERBB3 with tumor size, number of tumors and histological grade while EGFR correlated only with tumor size and ERBB2 correlated only with tumor grade.[18] Furthermore, ERBB3 and ERBB2 were good predictors of first tumor recurrence.[18] In contrast, in a study in 73 patients with NMIBC or MIBC ERBB3 was under expressed in MIBC compared to NMIBC and it correlated strongly with ERBB2 expression (table 2).[2] However, ERBB3 was not significantly over or under expressed in NMIBC. Despite this last report an Oncomine meta-analysis of 4 studies of superficial TCC[22–25] and 5 of MIBC[22–26] comprising a total of 487 samples revealed that ERBB3 expression was significantly up-regulated in superficial TCC (1.94-fold change, $p < 0.0001$) and in MIBC (1.53-fold change, $p = 0.004$) compared to normal tissue (table 2). Significantly a study of 131 MIBC samples demonstrated that mutations in *ERBB3* were present in 6% of bladder tumors with similar levels of mutation having been previously reported.[15]

ERBB4

In contrast to the other members of this family, ERBB4 mediates differentiation in epithelial tissues, including the mammary gland.[38] A number of alternately spliced forms of ERBB4 were identified[38] that have unique roles in mammary gland development and differentiation as well as growth inhibition.[39,40] The 2 sites in ERBB4 where variations are introduced by alternative splicing are the juxtamembrane domain and the cytoplasmic domain.[38] The 2 juxtamembrane isoforms are identified as JM-a and JM-b, which differ by the insertion of 23 (JM-a) or 13 (JM-b) alternative amino acids in the proximal extracellular domain N-terminal to the transmembrane domain, while the cytoplasmic isoforms are CYT-1 and CYT-2, of which the latter has a 16 amino acid deletion containing a PI3K binding motif.[41,42] Since these variations are in different ERBB4 domains, there are 4 possible combinations, including JMa-/CYT-1, JM-a/CYT-2, JM-b/CYT-1 and JM-b/CYT-2 (fig. 4).[38] The JM-a ERBB4 isoform juxtamembrane domain is cleaved in regulated fashion by metalloproteases.[38,43] The

membrane bound 80 kDa cytoplasmic domain can be further cleaved by γ-secretase, which then allows the cytoplasmic domain to translocate to the nucleus, where it is believed to affect transcription.[43] Additionally, while the CYT-1 and CYT-2 isoforms can bind the adapter protein Shc, only the CYT-1 isoform can activate the PI3K/AKT signaling cascade.[38]

In the bladder ERBB4 is normally expressed in the superficial layer of the urothelium and it correlates with the more differentiated phenotype (table 2).[19,32] It was reported that most bladder tumors, NMIBC as well as MIBC, under express ERBB4 as a whole with this under expression becoming more frequent with disease progression.[2,32] In agreement with this finding an Oncomine meta-analysis of 4 studies of superficial TCC[22–25] and 5 of MIBC[22–26] comprising a total of 487 samples showed that ERBB4 expression was unchanged in superficial TCC compared to normal tissue ($p = 0.065$). However, it was significantly under expressed in MIBC compared to normal tissue (−0.71 fold change, $p < 0.0001$, table 2). A study of 18 samples from patients with NMIBC or MIBC indicated that the JM-a/CYT-1 and JM-a/CYT-2 splice variants of ERBB4 were over expressed in tumor tissues compared with samples of normal urothelium.[34] Interestingly it was suggested that the ability of the JM-a extracellular isoform to be cleaved by metalloproteases enables the cytoplasmic domain to function in a ligand independent manner.[38] This could then allow for unregulated activation of the Shc/RAS/MAPK pathway and, for the CYT-1 isoform, the PI3K/AKT pathway.

Therefore, overall EGFR and ERBB2 can be significantly over expressed in MIBC but not in NMIBC compared to normal urothelium while ERBB3 is over expressed in each. In contrast, ERBB4 is significantly under expressed in MIBC but not in NMIBC compared to normal tissue. While these results indicate the significance of the RTKs in UC progression, it is important to keep in mind that data sets such as Oncomine only report mRNA data, which only correlates to protein levels by approximately 40%.[44]

EGFR AND ERBB2 INHIBITOR CLINICAL TRIALS IN BLADDER CANCER

More recently clinical trials of bladder cancer have used EGFR inhibitors alone or combined with cytotoxic chemotherapy to explore new therapeutic strategies in patients with recurrent and metastatic MIBC. This has included using the inhibitors as neoadjuvant therapy in patients with MIBC treated with RC, that is those with localized disease, as well as for first and second line therapy for recurrent disease (table 3). However, only a few studies described are discussed in this review because many are ongoing and the data collected from some that are complete are as yet unreleased.

Neoadjuvant Therapy for Primary MIBC

A phase II study sought to determine whether 4 weeks of neoadjuvant erlotinib before RC would improve the survival of patients with MIBC.[45] The 20 patients enrolled in this study had clinical stage T2 disease and previously underwent TURBT but EGFR status was not a consideration. Significantly after erlotinib administration and at surgery it was found that 5 of the 20 patients (25%) had no detectable disease remaining (pT0) and 7 (35%) had experienced clinical down staging (pT1 or less). At a mean followup of 24.8 months 10 of the 20 patients (50%) were still alive and showed no evidence of disease. Therefore, as the investigators noted, EGFR inhibition in the neoadjuvant setting can have beneficial effects in patients undergoing RC for MIBC.

Efficacy

EGFR inhibitors as therapy for recurrent disease. A number of studies using the EGFR inhibitor gefitinib have been performed in combination with or after chemotherapy. A phase II study by SWOG using gefitinib as single agent salvage therapy was performed in 31 patients in whom conventional chemotherapy for metastatic TCC had previously failed.[46] Although EGFR status was not a condition of eligibility for this study, almost half of the pretreatment biopsies expressed strong EGFR staining. Despite this the median OS in patients in this study was 3 months and median progression-free survival was 2 months. In this group and at the dose used (500 mg) toxicity was high with grade 4 cardiovascular ischemia in 4 of 31 patients (13%).

In contrast, a phase II study using the same dose of gefinitib combined with GC treatment was performed in chemotherapy naïve patients by CALGB (Cancer and Leukemia Group B).[47] Patients were considered eligible for study if they had histologically confirmed metastatic MIBC and had not previously undergone any systemic therapies, including chemotherapy. Again EGFR status was not part of the eligibility criteria. Median survival in study patients was 15.1 months and median time to progression was 7.4 months. Although gefitinib was well tolerated in this patient group, there was no improvement in the response rate or survival compared to those in a historical control with GC alone.[8,9]

The results of these studies indicate that resistance to gefitinib develops after or in conjunction

Table 3. *Clinical trials of EGFR family targeted therapy alone or combined with platinum based chemotherapy*

ClinicalTrials.gov Identifier (disease)	Drug	Status	EGFR/ERBB2 Inclusion Requirement	Target No. Pts	Median OS	EGFR/ERBB2 Pos (%)	Therapy
Not applicable (MIBC)	GC or MVAC	Completed	None	405	GC 14 vs MVAC 15.2 mos	Unknown	1st Line systemic chemotherapy for no prior systemic therapy
NCT00380029 (MIBC)	Erlotinib	Closed	None	20	10 Survivors (50%) 24.8 mos vs standard of care 63%, 60 mos	Unknown	Neoadjuvant for T2 muscle invasive disease
NCT00014144 (MIBC)	Gefitinib	Closed	None	31	3 vs Standard of care 15.1 mos	EGFR (25), ERBB2 (18), EGFR/ERBB2 (10)	2nd Line for advanced TCC + failed previous chemotherapy
NCT00041106 (MIBC)	Gefitinib + GC	Completed	None	54	15.1 vs Standard of care 15.1 mos	Unknown	Initial for advanced TCC
NCT01374789 (MIBC)	Panitumumab + GC	Closed	None	124	Not reported	Not reported	1st Line for locally advanced or metastatic disease
NCT00949455 (MIBC)	Lapatinib	Unknown	Confirmed 2+ or 3+ IHC EGFR or ERBB2 staining	204*	Not reported	EGFR +/or ERBB2 (100)	Maintenance lapatinib after 1st line chemotherapy
NCT00005831 (MIBC)	Trastuzumab, carboplatin, paclitaxel + GC	Completed	ERBB2 over expression, gene amplification or elevated serum levels	109	14.1 vs Standard of care 15 mos	ERBB2 (100)	2nd Line for recurrent disease after local therapy or disease incurable by local therapy
Not applicable (MIBC)	Lapatinib	Completed	Confirmed 1+, 2+ or 3+ IHC EGFR or ERBB2 staining	59	All pts 17.9 wks, EGFR/ERBB2 over expression 30.3 wks, standard of care 15.1 mos	EGFR (51), ERBB2 (42), EGFR/ERBB2 (22)	2nd Line for advanced TCC + failed platinum based therapy
NCT01245660 (MIBC)	Lapatinib	Terminated due to low enrollment	None	3*	Not applicable	Not applicable	Neoadjuvant lapatinib before RC
NCT01828736 (MIBC)	Trastuzumab, GC + carboplatin/cisplatin	Closed	Confirmed 3+ IHC ERBB2 staining or IHC 2+ ERBB2 IHC staining + pos fluorescence in situ hybridization	61	Not reported	ERBB2 (100)	2nd Line for failed previous chemotherapy for nonmetastatic disease
NCT00004856 (MIBC)	Trastuzumab	Terminated	Confirmed 3+ IHC ERBB2 staining or *HER2* amplification on fluorescence in situ hybridization	40*	Not reported	ERBB2 (100)	2nd Line for failed previous chemotherapy
NCT01956253 (MIBC)	Neratinib	Closed	None	1	Not reported	Not reported	Efficacy evaluation of neratinib for metastatic bladder Ca harboring HER2-GRB7 fusion
NCT00238420 (MIBC)	Paclitaxel with/without trastuzumab	Closed	None	88	Not reported	Not reported	Local after TURBT + not RC candidate
NCT01353222 (MIBC or NMIBC)	DN24-02	Open	Confirmed 1+, 2+ or 3+ IHC ERBB2 staining	180*	Not reported	ERBB2 (100)	1st Line adjuvant for ERBB2 + UC requiring RC

*Number of recruited participants may differ.

with chemoresistance. It is also possible that chemotherapy naïve patients are better able to tolerate gefitinib, although a separate study may be required to test that hypothesis.

ERBB2 inhibitor as chemosensitizing agent. A phase II trial using the humanized monoclonal ERBB2 antibody trastuzumab in combination with paclitaxel, carboplatin and gemcitabine enrolled 109 patients with local or metastatic MIBC and histologically proven transitional or squamous cell carcinomas that were incurable with local therapy and were chemotherapy naïve for advanced disease.[48] Patients were also required to have shown ERBB2 over expression to be eligible for the trial. Although the trial was careful to exclude those patients who would not benefit from the addition of trastuzumab and had an initial response rate of 70%, the median survival for those enrolled in the trial was 14.1 months[48] compared to 15 months in patients receiving standard of care for metastatic MIBC.[8,9] Therefore, it is possible that resistance to trastuzumab therapy sets in rapidly and nullifies the initial positive response. Another 2 phase II trials in patients with HER2 positive bladder cancer were initiated, including for trastuzumab alone and trastuzumab with chemotherapy (ClinicalTrials.gov NCT00004856 and NCT01828736, respectively). However, the results of these trials have not yet been reported.

Dual EGFR/ERBB2 inhibitor as second line therapy for MIBC. A phase II study in 59 patients with local or metastatic MIBC sought to determine the efficacy of the dual EGFR/ERBB2 inhibitor lapatinib as second line therapy after disease progression while on prior platinum based chemotherapy.[49] An objective response rate of greater than 10% was observed in only 1.7% of patients but 31% achieved stable disease. Median time to progression and OS in this post-chemotherapy population was 8.6 and 17.9 weeks, respectively. The objective response rate and stable disease correlated with EGFR over expression ($p = 0.029$). In addition, OS was significantly prolonged in patients who had EGFR or ERBB3 over expressing tumors ($p = 0.001$). Therefore, dual inhibition of EGFR/ERBB2 seems to be more effective for UC than single agents alone, even in chemotherapy resistant patients in whom EGFR or ERBB3 is over expressed. These results are in accord with those in tissue culture and animal models of bladder cancer progression, in which dual EGFR/ERBB2 inhibition appeared to be more effective than single kinase inhibition.[50]

Additional trials of diverse EGFR/ERBB2 inhibitors are ongoing or recently concluded. As these results become available the role of EGFR family inhibitors in MIBC will become more apparent.

POSSIBLE CAUSES OF MIBC RESISTANCE TO INHIBITORS OF EGFR FAMILY OF RTKS

Overall the clinical studies performed in patients with MIBC using EGFR family inhibitors demonstrate a lack of efficacy of this treatment combined with or after chemotherapy over the results of chemotherapy alone. However, notably this apparent inefficacy may be due to a lack of screening for the presence of an EGFR family member in the inclusion criteria rather than the inefficacy of the study drugs. Analysis of the results demonstrate that EGFR family inhibitors were fairly effective as neoadjuvant therapy in patients with MIBC undergoing RC as first line therapy for localized disease. They were partially effective in chemotherapy naïve patients with metastatic MIBC in whom EGFR or ERBB3 was over expressed. However, their effects in combination with chemotherapy or as salvage therapy in patients in whom chemotherapy had failed were far less obvious. It is important to note that EGFR and/or ERBB2 over expression/activation was not a criterion in some of the studies. In studies in which the status of these RTKs was determined the response rate was improved in patients with EGFR/ERBB2 over expressing tumors. Therefore, it is reasonable to assume that if the receptor was not over expressed, the corresponding inhibitors failed to have an effect. However, a lack of EGFR/ERBB2 expression alone may not be the only cause of resistance of patients to inhibitors of the EGFR family. As studies of other types of cancer have revealed, multiple other causes can lead to resistance to these inhibitors.

CONCLUSIONS

This analysis demonstrates that EGFR and ERBB2 inhibitors are not effective in all patients with bladder cancer. However, at the same time the literature supports the idea that a small cohort of patients with MIBC (those with EGFR and/or ERBB2 positive tumors) respond to EGFR and/or ERBB2 inhibitors initially. This stresses the importance of screening for the presence of EGFR family RTKs in any clinical trial of the role of EGFR family inhibitors. Recurrence is likely caused by the activation of bypass pathways that activate downstream targets or by mutations in downstream targets that decouple them from RTKs. As noted mutations in EGFR are rare in UC but mutations in related genes may drive the resistance of these tumors to EGFR/ERBB2 inhibitors.

Notably for reasons not currently clear clinical and preclinical studies show that prior treatment with chemotherapeutic agents rendered patients with UC (those with MIBC) resistant to EGFR family inhibitors as well. A likely cause is that chemotherapy

may suppress EGFR or ERBB2 expression, thereby rendering tumors resistant to EGFR family inhibitors. This speculation is supported by the observation that EGFR family inhibitors were particularly successful in causing MIBC down staging in a neoadjuvant setting in patients before they underwent RC for localized disease.

What this tells us is that EGFR family inhibitors will be particularly useful in patients with no prior chemotherapy in whom EGFR or ERBB2 is over expressed. While this limits the number of patients who may benefit from this treatment, these results assure us that EGFR family inhibitors will fill a niche that would serve a long-standing need in the treatment of UC. However, much work still must be done to fully understand the conditions under which EGFR family inhibitors would be effective in patients with MIBC.

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