Am J Transl Res 2011;3(4):362-373
www.ajtr.org /ISSN:1943-8141/AJTR1106004

Review Article

Urothelial carcinomas: a focus on human epidermal receptors signaling

Petros D. Grivas[1], Mark Day[2], Maha Hussain[1,2]

[1]Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan, Ann Arbor, MI 48109, USA; [2]Department of Urology, University of Michigan, Ann Arbor, MI 48109, USA.

Received June 21, 2011; accepted July 15, 2011; Epub July 25, 2011; Published August 15, 2011

Abstract: Bladder cancer is a common malignancy and a frequent cause of cancer-related death worldwide. The benefit from current chemotherapy has reached a relative plateau, thus identification of molecular targets for better therapy is a high priority. Human epidermal receptors constitute a family of receptor tyrosine kinases, which appear to be implicated in cellular transformation and can be over-expressed in a variety of solid tumors. There is preclinical and clinical data suggesting the role of EGFR and HER2 in urothelial carcinoma, thus prompting clinical investigation of anti-HER targeted therapies attempting to inhibit HER-induced tumor-promoting signaling. There is significant and dynamic cross-talk between HER and other signaling pathways and the identification of the structure and function of such cellular networks in the setting of urothelial cancer is a complex and difficult task. The development of prognostic and predictive biomarkers is needed in order to improve the personalized management of patients with urothelial cancer.

Keywords: Bladder cancer, urothelial carcinoma, human epidermal receptors, EGFR, HER2, trastuzumab

Introduction

Bladder cancer is the fourth most common malignancy, and the eighth leading cause of cancer death in men [1]. The estimated number of new cases in men worldwide is 297,300 with the estimated death rate being 112,300 [2]. In women, the cumulative risk of developing bladder cancer by age 75 is 0.2-0.4% with the cumulative risk of dying from this disease by that age is 0.1% [2]. Based on NCI data, the estimated new cases and deaths from bladder cancer in the United States in 2010 was 70,530 and 14,680, respectively (http://www.cancer.gov/cancertopics/types/bladder). Urothelial carcinoma is the most common histology, accounting for about 90% of cases. At the time of diagnosis, 75-80% of bladder carcinomas are superficial and approximately 20% of those eventually become invasive. A significant number of patients present with or develop advanced/metastatic disease, which can be fatal.

Urothelial carcinoma is considered chemotherapy-sensitive; however, overall survival for patients with advanced disease has not been significantly impacted over the past 3 decades since the introduction of combination methotrexate, vinblastine, doxorubicin and cisplatin (MVAC) in the early 1980s. Since then, several regimens have been tested against MVAC including gemcitabine plus cisplatin (GC), which had comparable efficacy with relatively less toxicity [3-6]. However, irrespective of treatment arm, less than 10% of these patients achieved a long-term disease-free survival [7,8]. The addition of paclitaxel to the GC regimen resulted in higher response rates, but at the expense of increased hematological toxicity, and did not provide significant overall survival benefit [9].

Given the dismal prognosis of advanced urothelial carcinoma, more effective systemic therapy is needed. Bladder cancer has several molecular alterations that regulate cellular processes, such as proliferation, differentiation, angiogenesis, metastasis and apoptosis. Some of these molecular alterations may serve as potential

targets for systemic therapy, either as monotherapy or in combination. Therefore, a thorough understanding of the molecular mechanisms that underlie urothelial cell malignant transformation and progression is critical for the optimization of treatment.

This review will focus on the role that human epidermal receptor (HER) family appears to play in the pathogenesis and prognosis of urothelial carcinomas, and it will summarize preclinical and clinical data regarding HER targeting approaches.

Human epidermal receptor (HER) family

The human epidermal receptor (HER) family of receptor tyrosine kinases consists of 4 receptors; HER1 (EGFR, erb-B1), HER2 (neu, erb-B2), HER3 (erb-B3), HER4 (erb-B4), and is implicated in several cellular processes, such as proliferation, growth, and survival. Epidermal Growth Factor Receptor (EGFR) is a 170 Kd membrane–spanning glycoprotein consisting of an extracellular ligand-binding domain, a trans-membrane domain, and an intracellular cytoplasmic domain with tyrosine kinase activity [10]. These receptor proteins belong to subclass I of the super-family of receptor tyrosine kinases (RTKs), classified based on their sequence homology and domain organization. They are expressed in many tissues of epithelial, mesenchymal, and neuronal origin and are critical for cell proliferation and tissue differentiation [11-14]. These receptors are usually monomers, but ligand binding induces the formation of homo- and hetero-dimers, activating the intracellular kinase domain, leading to phosphorylation of tyrosine residues. This can cause stereo-chemical conformational changes in the receptor structure and increases its affinity with downstream adaptors and transducers, which bind to the receptor and form functional complexes in the cytoplasmic milieu, initiating a "marathon" of signal transduction in the nucleus, thus regulating gene transcription [10]. The target genes and the encoded proteins are essential for cell proliferation, differentiation, apoptosis, invasion, metastasis and angiogenesis, and under certain circumstances can contribute to tumor initiation and progression. The activity of signaling pathways and the genomic effects depend on the relative micro-concentrations of adaptors, transducers and effectors that influence intracellular signal transduction and trafficking, at a certain point in time in the sub-cellular microenvironment. Receptor–ligand complexes can be internalized, resulting in signal termination, while the receptor itself can be either recycled in the membrane or degraded, depending on the biochemical micro-context. Intervention in these processes can influence the receptor expression, modulating its role in cell signaling.

Table 1. Endogenous HER ligands

EGF, TGF-α, Amphiregulin, Heparin – binding EGF, Epiregulin, Betacellulin	EGFR
	HER2
Neuregulins 1 and 2	HER3
Neuregulins 1-4, Amphiregulin, Betacellulin, Epiregulin, Heparin-binding EGF	HER4

The HER family is stimulated by several growth factors (**Table 1**), while epigen can act as a ligand that promotes hetero-dimerization [15]. Over-expression of these ligands is considered to promote tumor development via EGFR in *vitro* [16, 17], and can be produced by a variety of tumors, which also express EGFR, suggesting that autocrine stimulatory mechanisms might participate in EGFR–driven tumor development [17]. It is worth mentioning that HER2 has no known ligand, while HER-3 does not have a functional tyrosine kinase domain, but rather depends upon neuregulin binding and subsequent hetero-dimer formation, particularly with HER2, to be trans-phosphorylated and acquire signaling competence [18]. Human epidermal receptors co-expression can activate cell signaling and tumor cell invasion pathways, not activated by single receptors [19]. Interestingly, human epidermal receptors also appear to interact with cell adhesion proteins. We have previously shown that soluble E-cadherin fragment can form a complex with HER2 and HER3 in breast cancer cells, resulting in stabilization of HER2/HER3 hetero-dimer, induced receptor activation and signaling via ERK pathway, supporting cell migration and proliferation [20].

HER family over-expression has been reported in a number of solid tumors, such as colorectal, breast, lung, head and neck, pancreatic, urothelial carcinoma, and gliomas [21-23]. Enhanced ligand levels, hetero-dimerization, cross-phosphorylation, and cross–talk with other surface receptors commonly contribute to tumor aggressive behavior. Co-expression of either EGF or TGF-α and EGFR has been associated

with poor prognosis in pancreatic cancer [24]. Apart from the known role in breast and gastric cancer, data also suggest that HER2 is an unfavorable prognostic factor in prostate cancer [25,26].

EGFR mutations have been reported in several cancer types [27]. For example, tumor specific EGFRvIII, an EGFR mutant, has a constitutively activated tyrosine kinase domain that can be implicated in cellular transformation. Preclinical studies have implied that EGFR expression is correlated with tumor cell motility, invasiveness, angiogenesis, and metastatic potential [28-31]. The relationship between EGFR expression and survival of patients with several cancer types has been examined in a meta-analysis of data derived from more than 200 studies, involving more than 20,000 patients [32].

EGFR expression and role in bladder cancer

In normal urothelium EGFR is expressed only by the basal cells, and EGF is physiologically excreted in the urine, but a layer of EGFR-negative cells prevents its binding to EGFR. The disruption of this barrier may allow ligand-receptor binding, which may play a role in tumorigenesis. The urinary concentration of EGF in patients with urothelial carcinoma was significantly lower than that of controls, which implies EGF uptake by the tumor receptors [33].

The level of EGFR expression has been correlated with higher tumor grade and stage, disease progression, and worse prognosis in bladder carcinomas [33-40]. In two multivariate analyses, EGFR over-expression has shown to be an independent predictor of survival [39, 41]. In a third multivariate analysis, there was a significant correlation between EGFR expression and survival [26]. However, EGFR expression was not predictive of survival independent of stage. EGFR expression has also been associated with disease-specific mortality [40]. The estimated 5-year cancer-specific survival in a group of 121 patients who underwent radical cystectomy with curative intent was 60% in 47 patients with weak or moderate EGFR expression compared to 41% for 45 patients with strong EGFR expression (p=.039) [40].

HER3 and HER4 expression and role in bladder cancer

EGFR and HER2 are often co-expressed and form hetero-dimers with the other two members of HER family, HER3 and HER4. Data from 88 bladder cancer patient biopsies showed that the outcome of patients with EGFR- and HER2- expressing tumors is dependent on the expression of HER3 and HER4 [42]. A recent literature review on prognostic indicators of recurrence, progression, treatment response, and mortality in urothelial carcinoma concluded that EGFR and HER2 expression appears to indicate poor prognosis, while HER4 and Fibroblast Growth Factor Receptor 3 (FGFR3) appear to be favorable prognostic indicators [43]. Another study using urothelial carcinoma tissue arrays, stained for EGFR, HER2, HER3 and HER4, revealed that high EGFR or low HER4 expression was associated with non-papillary, high grade and invasive tumors, as well as with significantly lower recurrence-free and overall survival (p<0.002, p=0.028, p=0.047, respectively). HER2 and HER3 expression was not associated with overall or recurrence-free survival [44].

EGFR targeting in bladder cancer

Preclinical studies

Supportive data for the potential role of EGFR blockade in urothelial cancer comes from preclinical studies. Utilizing bladder cancer cell lines, it was demonstrated that the addition of gefitinib (an EGFR tyrosine kinase inhibitor) to radiation therapy resulted in a significant radiosensitization effect [45]. Only a modest induction of apoptosis with single agent gefitinib was observed, but there was a marked induction of apoptosis with gefitinib in combination with ionizing radiation. A recent study on bladder cancer cell lines suggested that activation of the EGFR induced a cell-survival function when bladder cancer cells were treated with the DNA-damaging drug etoposide, and that combined treatment with etoposide and the EGFR inhibitor gefitinib might improve the efficacy of treatment [46]. Moreover, dual EGFR and VEGF inhibition with vandetanib was found to sensitize bladder cancer cells to cisplatin in a dose- and sequence-dependent manner [47]. The same dual approach also increased epithelial characteristics and chemotherapy sensitivity in mesenchymal bladder cancer cells [48]. In addition, members of the microRNA-200 family were reported to control the epithelial-mesenchymal process and sensitivity to anti-EGFR therapy in bladder cancer cells [49]. The expression of microRNA-200 was found to be sufficient to restore EGFR-

dependency at least in a number of mesenchymal bladder cancer cells. One of microRNA-200 targets includes ERRFI-1, which appears to be a novel regulator of EGFR-independent growth.

The combination therapy of photodynamic therapy and cetuximab, a monoclonal antibody against the extracellular domain of EGFR, inhibited effectively tumor growth in a bladder tumor xenograft model, and can be considered a promising therapeutic strategy [50]. Additionally, an interesting study attempted to define molecular biomarkers of response to cetuximab in a panel of urothelial carcinoma cell lines [51]. The results suggested that expression of intact HER-4 (p=0.008), E-cadherin (p=0.015), beta-catenin (p=0.015) and loss of expression of platelet-derived growth factor receptor beta (p = 0.167) were associated with response to cetuximab therapy.

Clinical trials

Despite the potential biologic role for EGFR, clinical trials evaluating EGFR targeted therapy have surprisingly been limited. SWOG evaluated the role of gefitinib in 31 patients with metastatic transitional cell carcinoma, who had failed one chemotherapeutic regimen [52]. Patients were required to have a pre-treatment biopsy to assess EGFR expression. The median progression-free survival was 2 months; 2 patients survived past 6 months without disease progression. Grade 4 cerebrovascular ischemia and an increase in creatinine level were reported. There was one confirmed partial response in a patient with pulmonary metastases. Recently, a randomized, non-comparative phase II trial evaluated the efficacy of cetuximab combined with paclitaxel in 39 patients with previously treated (with platinum-based chemotherapy) metastatic urothelial carcinoma [53]. Patients were randomized to cetuximab 250mg/m^2 (after 400 mg/m^2 loading dose) with or without paclitaxel 80 mg/m^2 every week. The cetuximab arm closed when 9 of the first 11 patients progressed by 8 weeks. In the combination arm, 35.7% of patients were progression-free for more than 16 weeks. The overall response rate was 28.5%, with 2 complete and 6 partial responses; 4 additional patients had unconfirmed partial responses. The median progression-free survival for the combination arm was 115 days, while the median number of administered cycles was 3. Grade 3 toxicity occurring in more than 2 patients included rash, fatigue, anemia, hypomagnesemia. The authors concluded that this combination merits further evaluation.

Our group is currently conducting a randomized, open-label, phase II clinical trial investigating the potential benefit of adding cetuximab to the standard gemcitabine/cisplatin chemotherapy in patients with locally advanced or metastatic urothelial carcinoma (Clinical Trials.gov identifier: NCT00645593). The trial has completed accrual, and the results are pending.

HER2 expression and role in bladder cancer

HER2 expression in bladder carcinoma is variable between different studies, ranging between 9 and 81% [54-58]. We have previously reported that 28% of primary bladder cancers over-express HER2 by immunohistochemistry (IHC) and that primary tumor over-expression consistently predicts over-expression in a distant or regional metastatic site [59]. However, 45% of HER2-negative primary tumors may show over-expression in their corresponding metastasis. These data suggested that HER2 might play a role in the biological progression of bladder cancer and the development of metastatic disease. The median survival for HER2-positive primary cancers was 33 months compared to 50 months for HER2-negative cancers (p=0.46). HER2 over-expression in the metastatic lymph nodes did not have prognostic value.

In a recent study with 1,005 patients, HER2 protein over-expression was found in 9.2%, while HER2 gene amplification was found in 5.1% of tumor specimens [60]. Variability in IHC assays as well as tumor staining heterogeneity might account for, at least partially, the discordant results among different studies. In a clinical trial, HER2-positive staining was associated with a lower complete response rate after chemotherapy/irradiation (50 *vs* 81%, p = 0.03), but not with overall survival [61]. This study suggested that HER2 could be more related to the resistance to combined chemo-radiotherapy and thus may be more important in the local control of the disease. Moderate and heavy HER2 expression correlated significantly with aneuploidy, higher grade, and shorter overall survival, in a study with over 14 years of follow-up, while HER2 gene amplification correlated with grade, stage and survival in approximately 25% of patients in a different study [62, 63].

However, in another report, EGFR and HER2 expression was inversely related to tumor invasion in grade III tumors [64]. HER2 status has not consistently correlated with EGFR expression, stage, grade and survival [65-66]. The higher rate of HER2 gene amplification in T1 compared to more invasive tumors that was reported implied a role in the development of early disease, but not in further invasion [67].

A series of studies have reported a negative prognostic value of HER2 expression in urothelial carcinoma (**Table 2**). Three retrospective analyses of patients with urothelial carcinoma revealed an association between HER2 expression and poor outcome [68-70]. A study of patients with locally advanced urothelial carcinoma, receiving surgery alone or with adjuvant MVEC chemotherapy reported that bladder carcinoma had significantly higher HER2 staining compared to the upper urinary tract disease [71]. In the adjuvant MVEC arm only, HER2 immunoreactivity correlated with shorter progression-free and disease-specific overall survival in the univariate analysis. In another study, HER2 protein was over-expressed in 41 out of 80 tumors, corresponding to advanced stage and grade, shorter specific and overall survival, but not to disease recurrence [72]. A cohort of 198 patients undergoing radical cystectomy with lymphadenectomy reported HER2 staining in a 27.8% of primary tumors compared to 44.2% of metastatic lymph nodes; HER2 expression correlated with lymphovascular invasion and higher risk for recurrence and cancer-specific mortality [73].

In another study with 59 cases, normal urothelium and the neighboring renal parenchyma were HER2-negative [74]. HER2-negative tumors were for the most part well-differentiated, low grade, papillary, or rarely infiltrative. HER2 over-expression did not correlate with stage or lymph node status; however, high staining intensity was associated with high grade. Another study did not identify a strong association between HER2 protein over-expression and gene amplification in high grade invasive urothelial carcinomas; polysomy of chromosome 17 was reported in 9 out of 27 tumors [75]. A similar study suggested that although HER2 gene amplification was detected in high grade and invasive tumors, it was a rare event, and that polysomy of chromosome 17 was associated with tumor stage and grade and thus could be considered a biomarker of tumor progression [76]. Co-amplification of HER2 and MYC was reported in a subset of patients with metastatic urothelial cancer, while in a different study, invasive micropapillary carcinoma showed higher immunoreactivity for MUC1, CA125, and HER2 compared to invasive urothelial carcinoma with retraction artifact [77, 78]. A tissue microarray study with 100 upper urinary tract urothelial

Table 2. Studies reporting negative prognostic value of HER2 protein expression or gene amplification in urothelial carcinoma

Author/reference	Correlative outcome/HER2 marker	Patients/ Cases
Chakravarti et al [61]	lower complete response rate to chemoradiation / protein expression	73
Lönn et al [63]	aneuploidy, higher grade, shorter overall survival / protein expression	91
Lipponen et al [62]	higher stage and grade, shorter overall survival / gene amplification	178
Masliukova et al [68]	shorter relapse-free survival/ protein expression / protein expression	63
Kolla et al [69]	higher stage and grade, positive lymph node status, shorter disease-free and disease-related survival / protein expression	90
Krüger et al [70]	higher grade, shorter disease-free and disease-related survival / protein expression	138
Tsai et al [71]	site (bladder *vs* upper urinary tract), shorter progression-free and disease-related survival / protein expression	114
Skagias et al [72]	higher stage, grade, shorter disease-specific and overall survival / protein expression	80
Bolenz et al [73]	lymph nodes (vs primary site), lymphovascular invasion, higher recurrence risk, shorter disease-specific survival / protein expression	198
Alexa et al [74]	higher grade/ protein expression	59

Table 3. Clinical trials with lapatinib in bladder cancer

Wülfing et al [85]	Phase II, single arm, multicenter, open-label, lapatinib monotherapy in urothelial carcinoma progressed on platinum-containing chemotherapy (results reported)
NCT00447226	Phase II, placebo controlled, double-blind, randomized, discontinuation study in HER2 positive solid tumors (terminated)
NCT00623064	Phase I, cisplatin, gemcitabine and lapatinib as first line therapy in advanced/metastatic urothelial cancer (unknown recruitment status)
NCT00949455	Phase II/III, randomized comparison of maintenance lapatinib *vs* placebo after first line chemotherapy in EGFR- and/or HER2- over-expressing locally advanced or metastatic bladder cancer (recruiting)
NCT00313599	Phase I dose-escalation of a 2-day lapatinib chemo-sensitization pulse prior to weekly IV Abraxane in advanced solid tumors (ongoing, not recruiting)
NCT01245660	Pilot (phase 0), lapatinib as neo-adjuvant treatment in local bladder cancer (open, not recruiting yet)

carcinomas revealed 10 cases with HER2 expression, 84 cases with cytoplasmic phospho-AKT and 6 cases with nuclear phospho-AKT; the latter was found to be an independent prognostic factor [79].

HER2 targeting in bladder cancer

A study evaluated the efficacy of trastuzumab in 6 patients with HER2 positive metastatic urothelial carcinoma; all patients achieved a partial response, with 30-80% reduction in the size of the metastatic lesions [80].

We prospectively evaluated the rate of HER2 expression and feasibility of anti-HER2 targeting in a multicenter phase II trial in patients with urothelial cancer [81]. In this study, 109 patients were evaluated for HER2 status by immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH); 57 were considered HER2 positive at least by one method (either IHC or FISH), and 44/57 received combination of carboplatin, paclitaxel, gemcitabine, and trastuzumab. Five patients (11%) achieved a complete response, 26 (59%) a partial response, 5 (11%) had stable disease, and 5 (11%) had no response assessment, with an overall response rate of 70%. Median time to progression was 9.3 months, and median survival 14.1 months. Patients with HER2 positive tumors had a higher rate of liver/bone metastases, higher median number of metastatic sites and a higher incidence of two or more metastatic sites, implying a negative prognostic role of the receptor. Additionally, there are 3 recruiting and 4 completed clinical trials investigating an anti-HER2 strategy in various settings and stages in patients with bladder cancer, as reported in the U.S. NIH Clinical Trial Registry (http://clinicaltrials.gov).

Dual EGFR and HER2 targeting in bladder cancer

The expression of EGFR and HER2 in malignant urothelial cells is biologically attractive for combined targeting. This can be achieved by either the combined use of agents such as the antibodies cetuximab and trastuzumab that target each receptor separately, or an agent like lapatinib, which is an oral reversible non-covalent dual inhibitor of the EGFR and HER2 tyrosine kinases. The latter approach has the advantage of a single oral drug that could, at least in theory, be easier and less toxic.

Preclinical data with lapatinib showed promising activity in transitional carcinoma cell lines, enhancing the activity of concomitant chemotherapy in a dose-dependent fashion [82, 83]. Specifically, synergistic effects have been demonstrated in bladder cancer cell lines treated with lapatinib in combination with gemcitabine and cisplatin. Lapatinib was also shown to reverse multidrug resistance in cancer cell lines by inhibiting the activity of ATP-binding cassette proteins, suggesting that it may reverse chemoresistance in the clinical setting [84].

Lapatinib has been evaluated in the clinical setting (Table 3). A recent phase II trial tested lapatinib monotherapy at the dose of 1,250 mg daily in 59 patients with urothelial carcinoma who have progressed on a prior platinum-

containing chemotherapy schedule [85]. Of the 34 patients evaluable for response, only 1 patient achieved an objective response, and 18 had stable disease, with a median time to progression of 8.6 weeks and a median overall survival of 17.9 weeks. Lapatinib was well tolerated. Although this study was initially considered negative, further analysis showed that clinical benefit (response and stable disease rate) was found to correlate with EGFR over-expression (p=.029), and, to some extent, HER2 over-expression. EGFR and HER2 were evaluated by immunostaining and positivity was defined as any membranous staining above the background level of the cell in >10% of tumor cells; intensity staining of 2+ or 3+ was characterized as over-expression. Of all samples, 52% over-expressed EGFR and 44% HER2. Of the 19 patients with clinical benefit, 17 patients had EGFR and/or HER-2 over-expressing tumors. Patients with EGFR and/or HER-2 over-expressing tumors had a median survival of 30.3 weeks compared to 10.6 weeks in patients with tumors with negative/low expression (p=.0001), implying that this subset of patients may benefit from the inhibitor.

Although a phase II, placebo-controlled, double-blind, randomized, discontinuation study of lapatinib in patients with HER2 positive solid tumors, including bladder, failed to meet the primary objective of tumor response rate at 12 weeks from first dose and was closed (Clinical Trials.gov identifier: NCT00447226), several trials are evaluating the efficacy of lapatinib in different settings. A phase I trial is evaluating lapatinib with cisplatin and gemcitabine as first-line therapy in locally advanced or metastatic urothelial cancer patients in Europe (Clinical Trials.gov identifier: NCT00623064); a randomized, multicenter, phase II/III clinical trial is comparing maintenance lapatinib monotherapy to placebo, after first-line chemotherapy that resulted in no disease progression, in patients with EGFR and/or HER2 over-expressing locally advanced or metastatic bladder cancer (Clinical Trials.gov identifier: NCT00949455). A phase I dose-escalation study of a 2-day oral lapatinib chemosensitization pulse, given prior to weekly abraxane chemotherapy in patients with advanced solid tumors, including bladder, completed accrual (Clinical Trials.gov identifier: NCT00313599). Moreover, a pilot study of lapatinib as neoadjuvant treatment in patients with local bladder carcinoma before cystectomy was just launched (Clinical Trials.gov identifier: NCT01245660).

A recent study compared global genome-wide microarray to EGFR-pathway microarray profiling to identify predictive models of lapatinib sensitivity in bladder cancer [86]. The top-performing combination model included the phosphorylated EGFR (pTyr-1173), with a mean predictive accuracy for response to lapatinib of 98.3%. Two of the three phosphoproteomic models in this study included phosphorylated HER2 (pY1248), in a site associated with activation, poor prognosis, and therapeutic response to trastuzumab in breast cancer patients [87-89]. This phosphorylation site was also found to be regulated *in vitro* and *in vivo* by lapatinib [90, 91].

HER signaling cross-talk with other pathways

As discussed above based on available data, HER family appears to play a role in the pathogenesis and prognosis of urothelial carcinoma. However, there is significant multiplicity, complexity and dynamic cross-talk in the biochemical pathways involved in the initiation and progression of cancer in general. For example, there is evidence of significant cross-talk between the hepatocyte growth factor (HGF)/mesenchymal-epithelial transition factor (MET) pathway and HER signaling proteins. HGF/MET axis can potentially substitute HER components activity, thus conferring resistance to EGFR-targeting drugs [92]. Insulin growth factor-1 (IGF1) pathway also appears to interact with HER signaling activity. Specifically, IGF1-mediated "trans-activation" of EGFR mediates the IGF1-stimulated phosphorylation of Src homology-2 domain (Sh2) and thus the subsequent activation of the extracellular signal-regulated kinase (Erk) cascade [93]. Additionally, EGFR interaction with adhesion molecules, such as integrins, can influence the activity of transcription factors. EGFR/beta1-integrin complex interaction can drive integrin-dependent PI3K/Akt activation, Akt translocation into the nucleus and phosphorylation of FoxO1, which is a Forkhead transcription factor; FoxO1 inactivation results in increased levels of the transcription factor Egr-1, thus influencing gene transcription [94]. Overall, there appears to be a "chaotic model' of molecular interactions that is difficult to be precisely determined and predicted with conventional informatics approaches.

Conclusion

HER family appears to be biologically implicated in urothelial carcinoma. The expression pattern of these receptors in a specific tumor as well their dynamic interactions with multiple signaling pathways might dictate cancer-related events. However, limited data is available on the potential efficacy of this approach hence therapeutic targeting of EGFR and HER2 has not been incorporated into the standard oncologist's armamentarium in this disease. Critical to such approach is tumor profiling for better patient selection. Recently, the International Consensus Panel on Bladder Tumor Markers concluded that, based on the current evidence, none of the current prognostic molecular markers, including microsatellite-associated markers, oncogenes, tumor suppressor genes, cell-cycle regulators and extracellular matrix-adhesion molecules, are sufficiently validated to be implemented in the management of patients with urothelial carcinoma [95]. Therefore, there is an unmet need for robust prospective validation of candidate biomarkers and investigation of their prognostic value and predictive role in response to molecular targeted therapies.

Address correspondence to: Maha Hussain, MD, Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan, Ann Arbor, MI 48109, USA. E-mail: mahahuss@med.umich.edu

References

[1] Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, Jemal A, Schymura MJ, Lansdorp-Vogelaar I, Seeff LC, van Ballegooijen M, Goede SL, Ries LA. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer 2010; 116(3): 544-573.

[2] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61(2): 69-90.

[3] Moore MJ, Iscoe N, Tannock IF.A phase II study of methotrexate, vinblastine, doxorubicin and cisplatin plus recombinant human granulocyte-macrophage colony stimulating factors in patients with advanced transitional cell carcinoma. J Urol 1993; 150(4): 1131-1134.

[4] Adamo V, Magno C, Spitaleri G, Garipoli C, Maisano C, Alafaci E, Adamo B, Rossello R, Scandurra G, Scimone A. Phase II study of gemcitabine and cisplatin in patients with advanced or metastatic bladder cancer: long-term follow-up of a 3-week regimen. Oncology 2005; 69(5): 391-398.

[5] Kaufman D, Raghavan D, Carducci M, Levine EG, Murphy B, Aisner J, Kuzel T, Nicol S, Oh W, Stadler W. Phase II trial of gemcitabine plus cisplatin in patients with metastatic urothelial cancer. J Clin Oncol 2000; 18(9): 1921-1927.

[6] Moore MJ, Winquist EW, Murray N, Tannock IF, Huan S, Bennett K, Walsh W, Seymour L. Gemcitabine plus cisplatin, an active regimen in advanced urothelial cancer: a phase II trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 1999; 17(9): 2876-2881.

[7] von der Maase H, Hansen SW, Roberts JT, Dogliotti L, Oliver T, Moore MJ, Bodrogi I, Albers P, Knuth A, Lippert CM, Kerbrat P, Sanchez Rovira P, Wersall P, Cleall SP, Roychowdhury DF, Tomlin I, Visseren-Grul CM, Conte PF. Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, phase III study. J Clin Oncol 2000; 18(17): 3068-3077.

[8] Stadler WM, Hayden A, von der Maase H, Roychowdhury D, Dogliotti L, Seymour L, Kaufmann D, Moore M. Long-term survival in phase II trials of gemcitabine plus cisplatin for advanced transitional cell cancer. Urol Oncol 2002; 7(4): 153-157.

[9] Bellmunt J, von der Maase H, Mead GM, Heyer J, Houede N, Paz-Ares LG, Winquist E, Laufman LR, de Wit R, Sylvester R. Randomized phase III study comparing paclitaxel/cisplatin/gemcitabine (PCG) and gemcitabine/cisplatin (GC) in patients with locally advanced (LA) or metastatic (M) urothelial cancer without prior systemic therapy; EORTC30987/Intergroup Study (abstract #LBA 5030). J Clin Oncol 2007; 25: 965s.

[10] Rowinsky EK. The erbB family: targets for therapeutic development against cancer and therapeutic strategies using monoclonal antibodies and tyrosine kinase inhibitors. Annu Rev Med 2004; 55: 433-457.

[11] Simon MA. Receptor tyrosine kinases: specific outcomes from general signals. Cell 2000; 103: 13-15.

[12] Walker RA. The erbB/HER type1 tyrosine kinase receptor family. J Pathol 1998; 185: 234-35.

[13] Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000; 103: 211-225.

[14] Daly RJ. Take your partners, please: signal diversification by the erbB family of receptor tyrosine kinases. Growth Factors 1999; 16: 255-263.

[15] Shepard HM, Brdlik CM, Schreiber H. Signal integration: a framework for understanding the efficacy of therapeutics targeting the hu-

man EGFR family J Clin Invest 2008; 118(11): 3574–3581.
[16] Velu TJ, Beguinot L, Vass WC, Willingham MC, Merlino GT, Pastan I, Lowy DR. Epidermal-growth-factor-dependent transformation by a human EGF receptor proto-oncogene. Science 1987; 238: 1408-1410.
[17] Di Marco E, Pierce JH, Fleming TP, Kraus MH, Molloy CJ, Aaronson SA, Di Fiore PP. Autocrine interaction between TGF-a and the EGF – receptor: quantitative requirements for induction of the malignant phenotype. Oncogene 1989; 4: 831-838.
[18] Stern DF. ERBB3/HER3 and ERBB2/HER2 duet in mammary development and breast cancer. J Mammary Gland Biol Neoplasia 2008; 13: 215-223.
[19] Zhan L, Xiang B, Muthuswamy SK. Controlled activation of ErbB1/ErbB2 heterodimers promote invasion of three-dimensional organized epithelia in an ErbB1-dependent manner: implications for progression of ErbB2-overexpressing tumors. Cancer Res 2006; 66: 5201-5208.
[20] Najy AJ, Day KC, Day ML. The ectodomain shedding of E-cadherin by ADAM15 supports ErbB receptor activation. J Biol Chem 2008; 283(26): 18393-18401.
[21] Britsch S. The neuregulin-I/ErbB signaling system in development and disease. Adv Anat Embryol Cell Biol 2007; 190: 1-65.
[22] Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001; 2: 127-137.
[23] Revillion F, Lhotellier V, Hornez L, Bonneterre J, Peyrat JP. ErbB/HER ligands in human breast cancer, and relationships with their receptors, the bio-pathological features and prognosis. Ann Oncol 2008; 19: 73-80.
[24] Uegaki K, Nio Y, Inoue Y, Minari Y, Sato Y, Song MM, Dong M, Tamura K. Clinicopathological significance of epidermal growth factor and its receptor in human pancreatic cancer. Anticancer Res 1997; 17: 3841-3847.
[25] Neto AS, Tobias-Machado M, Wroclawski ML, Fonseca FL, Teixeira GK, Amarante RD, Wroclawski ER, Del Giglio A. Her-2/neu expression in prostate adenocarcinoma: a systematic review and meta-analysis. J Urol 2010; 184 (3): 842-850.
[26] Sadasivan R, Morgan R, Jennings S, Austenfeld M, Van Veldhuizen P, Stephens R, Noble M. Overexpression of Her-2/neu may be an indicator of poor prognosis in prostate cancer. J Urol 1993; 150(1): 126-131.
[27] Voldborg BR, Damstrup L, Spang-Thomsen M, Poulsen HS. Epidermal growth factor receptor (EGFR) and EGFR mutations, function and possible role in clinical trials. Ann Oncol 1997; 8: 1997-1206.
[28] Wells A. Tumor invasion: role of growth factor – induced cell motility. Adv Cancer Res 2000; 78: 31-101.
[29] Verbeek BS, Adriaansen-Slot SS, Vroom TM, Beckers T, Rijksen G. Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts. FEBS Lett 1998; 425: 145-150.
[30] Riedel F, Gotte K, Li M, Hormann K, Grandis JR. EGFR antisense treatment of human HNSCC cell lines down – regulates VEGF expression and endothelial cell migration. Int J Oncol 2002; 21: 11-16.
[31] Eccles SA. Cell biology of lymphatics metastasis. The potential role of c-erbB oncogene signaling. Recent Results Cancer Res 2000; 157: 41-54.
[32] Nicholson RI, Gee JMW, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001; 37: S9-S15.
[33] Chow NH, Liu HS, Lee EI, Chang CJ, Chan SH, Cheng HL, Tzai TS, Lin JS. Significance of urinary epidermal growth factor and its receptor expression in human bladder cancer. Anticancer Res 1997; 17: 1293-1296.
[34] Nguyen PL, Swanson PE, Jaszcz W, Aeppli DM, Zhang G, Singleton TP, Ward S, Dykoski D, Harvey J, Niehans GA. Expression of epidermal growth factor receptor in invasive transitional cell carcinoma of the urinary bladder. A multivariate survival analysis. Am J Clin Pathol 1994; 101: 166-176.
[35] Neal DE, Marsh C, Bennett MK, Abel PD, Hall RR, Sainsbury JR, Harris AL. Epidermal-growth-factor receptors in human bladder cancer: comparison of invasive and superficial tumours. Lancet 1985; 1: 366-368.
[36] Neal DE, Sharples L, Smith K, Fennelly J, Hall RR, Harris AL. The epidermal growth factor receptor and the prognosis of bladder cancer. Cancer 1990; 65: 1619-1625.
[37] Messing EM. Clinical implications of the expression of epidermal growth factor receptors in human transitional cell carcinoma. Cancer Res 1990; 50: 2530-2537.
[38] Bue P, Wester K, Sjöström A, Holmberg A, Nilsson S, Carlsson J, Westlin JE, Busch C, Malmström PU. Expression of epidermal growth factor receptor in urinary bladder cancer metastases. Int J Cancer 1998; 76: 189-193.
[39] Lipponen P, Eskelinen M. Expression of epidermal growth factor receptor in bladder cancer as related to established prognostic factors, oncoprotein (c-erbB-2, p53) expression and long-term prognosis. Br J Cancer 1994; 69: 1120-1125.
[40] Kramer C, Klasmeyer K, Bojar H, Schulz WA, Ackermann R, Grimm MO. Heparin-binding epidermal growth factor-like growth factor isoforms and epidermal growth factor receptor/ErbB1 expression in bladder cancer and their relation to clinical outcome. Cancer 2007; 109: 2016-2024.

[41] Mellon K, Wright C, Kelly P, Horne CH, Neal DE. Long-term outcome related to epidermal growth factor receptor status in bladder cancer. J Urol 1995; 153: 919-925.
[42] Memon AA, Sorensen BS, Meldgaard P, Fokdal L, Thykjaer T, Nexo E. The relation between survival and expression of HER1 and HER2 depends on the expression of HER3 and HER4: a study in bladder cancer patients. Br J Cancer 2006; 94: 1703-1709.
[43] Black PC, Dinney CP. Growth factors and receptors as prognostic markers in urothelial carcinoma. Curr Urol Rep 2008; 9(1): 55-61.
[44] Kassouf W, Black PC, Tuziak T, Bondaruk J, Lee S, Brown GA, Adam L, Wei C, Baggerly K, Bar-Eli M, McConkey D, Czerniak B, Dinney CP. Distinctive expression pattern of ErbB family receptors signifies an aggressive variant of bladder cancer. J Urol 2008; 179(1): 353-358.
[45] Maddineni SB, Sangar VK, Hendry JH, Margison GP, Clarke NW. Differential radiosensitisation by ZD1839 (Iressa), a highly selective epidermal growth factor receptor tyrosine kinase inhibitor in two related bladder cancer cell lines. Br J Cancer 2005; 92: 125-130.
[46] Munk M, Memon AA, Nexo E, Sorensen BS. Inhibition of the epidermal growth factor receptor in bladder cancer cells treated with the DNA-damaging drug etoposide markedly increases apoptosis. BJU Int 2007; 99(1): 196-201.
[47] Flaig TW, Su LJ, McCoach C, Li Y, Raben D, Varella-Garcia M, Bemis LT. Dual epidermal growth factor receptor and vascular endothelial growth factor receptor inhibition with vandetanib sensitizes bladder cancer cells to cisplatin in a dose- and sequence-dependent manner. BJU Int 2009; 103(12): 1729-1737.
[48] Li Y, Yang X, Su LJ, Flaig TW. VEGFR and EGFR inhibition increases epithelial cellular characteristics and chemotherapy sensitivity in mesenchymal bladder cancer cells. Oncol Rep 2010; 24(4): 1019-1028.
[49] Adam L, Zhong M, Choi W, Qi W, Nicoloso M, Arora A, Calin G, Wang H, Siefker-Radtke A, McConkey D, Bar-Eli M, Dinney C. miR-200 expression regulates epithelial-to-mesenchymal transition in bladder cancer cells and reverses resistance to epidermal growth factor receptor therapy. Clin Cancer Res 2009; 15(16): 5060-5072.
[50] Bhuvaneswari R, Gan YY, Soo KC, Olivo M. Targeting EGFR with photodynamic therapy in combination with Erbitux enhances in vivo bladder tumor response. Mol Cancer 2009; 8: 94.
[51] Black PC, Brown GA, Inamoto T, Shrader M, Arora A, Siefker-Radtke AO, Adam L, Theodorescu D, Wu X, Munsell MF, Bar-Eli M, McConkey DJ, Dinney CP. Sensitivity to epidermal growth factor receptor inhibitor requires E-cadherin expression in urothelial carcinoma cells. Clin Cancer Res 2008; 14(5): 1478-1486.
[52] Petrylak DP, Tangen CM, Van Veldhuizen PJ Jr, Goodwin JW, Twardowski PW, Atkins JN, Kakhil SR, Lange MK, Mansukhani M, Crawford ED. Results of the Southwest Oncology Group phase II evaluation (study S0031) of ZD1839 for advanced transitional cell carcinoma of the urothelium. BJU Int. 2010; 105 (3): 317-321.
[53] Wong Y, Litwin S, Plimack ER, Vaughn DJ, Song W, Cohen SM, Lee JW, Dabrow MB, Tuttle H, Hudes GR. Effect of EGFR inhibition with cetuximab on the efficacy of paclitaxel in previously treated metastatic urothelial cancer. J Clin Oncol 29: 2011 (suppl 7; abstr 243).
[54] Sato K, Moriyama M, Mori S, Saito M, Watanuki T, Terada K, Okuhara E, Akiyama T, Toyoshima K, Yamamoto T, Kato T. An immunohistologic evaluation of C-erb-B-2 gene product in patients with urinary bladder carcinoma. Cancer 1992; 70: 2493–2498.
[55] Mellon JK, Lunec J, Wright C, Horne CH, Kelly P, Neal DE. C-Erb-B2 in bladder cancer: molecular biology, correlation with epidermal growth factor receptors and prognostic value. J Urol 1996; 155(1): 321–326.
[56] Wester K, Sjöström A, de la Torre M, Carlsson J, Malmström PU. HER-2: a possible target for therapy of metastatic urinary bladder carcinoma. Acta Oncol 2002; 41: 282–288.
[57] Gandour-Edwards R, Lara PN Jr, Folkins AK, LaSalle JM, Beckett L, Li Y, Meyers FJ, DeVere-White R. Does HER2/ neu expression provide prognostic information in patients with advanced urothelial carcinoma? Cancer 2002; 95: 1009–1015.
[58] Chow NH, Chan SH, Tzai TS, Ho CL, Liu HS. Expression profiles of ErbB family receptors and prognosis in primary transitional cell carcinoma of the urinary bladder. Clin Cancer Res 2001; 7: 1957–1962.
[59] Jimenez RE, Hussain M, Bianco FJ Jr, Vaishampayan U, Tabazcka P, Sakr WA, Pontes JE, Wood DP Jr, Grignon DJ. Her-2/neu overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic significance and comparative analysis in primary and metastatic tumors. Clin Cancer Res 2001; 7(8): 2440-2447.
[60] Laé M, Couturier J, Oudard S, Radvanyi F, Beuzeboc P, Vieillefond A. Assessing Her-2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: results in 1005 patients. Ann Oncol 2010; 21(4): 815-819.
[61] Chakravarti A, Winter K, Wu CL, Kaufman D, Hammond E, Parliament M, Tester W, Hagan M, Grignon D, Heney N, Pollack A, Sandler H,

Shipley W. Expression of the epidermal growth factor receptor and Her-2 are predictors of favorable outcome and reduced complete response rates, respectively, in patients with muscle-invading bladder cancers treated by concurrent radiation and cisplatin-based chemotherapy: a report from the Radiation Therapy Oncology Group. Int J Radiat Oncol Biol Phys 2005; 62(2): 309-317.

[62] Lipponen P, Eskelinen M, Syrjänen S, Tervahauta A, Syrjänen K. Use of inmunohistochemically demonstrated c-erb B-2 oncoprotein expression as a prognostic factor in transitional cell carcinoma of the urinary bladder. Eur Urol 1991; 20: 238–242.

[63] Lönn U, Lönn S, Friberg S, Nilsson B, Silfverswärd C, Stenkvist B. Prognostic value of the amplification of c-erb-B2 in bladder carcinoma. Clin Cancer Res 1995; 1: 1189–1194.

[64] Vollmer RT, Humphrey PA, Swanson PE, Wick MR, Hudson ML. Invasion of the bladder by transitional cell carcinoma: its relation to histologic grade and expression of p53, MIB-1, c-Erb-B2, epidermal growth factor receptor and bcl-2. Cancer 1998; 82: 715–723

[65] Mellon JK, Lunec J, Wright C, Horne CH, Kelly P, Neal DE. C-erbB-2 in bladder cancer: molecular biology, correlation with epidermal growth factor receptors and prognostic value. J Urol 1996; 155(1): 321-326.

[66] Liedberg F, Anderson H, Chebil G, Gudjonsson S, Höglund M, Lindgren D, Lundberg LM, Lövgren K, Fernö M, Månsson W. Tissue microarray based analysis of prognostic markers in invasive bladder cancer: much effort to no avail? Urol Oncol 2008; 26(1): 17–24.

[67] Rajjayabun PH, Keegan PE, Lunec J, Mellon JK. ErbB receptor expression patterns in human bladder cancer. Urology 2005; 66(1): 196–200.

[68] Masliukova EA, Pozharisskiĭ KM, Karelin MI, Startsev VIu, Ten VP. Role of Ki-67, mutated gene-suppressor p53 and HER-2neu oncoprotein in the prognosis for the clinical course of bladder cancer. Vopr Onkol 2006; 52(6): 643-648.

[69] Kolla SB, Seth A, Singh MK, Gupta NP, Hemal AK, Dogra PN, Kumar R. Prognostic significance of Her2/neu overexpression in patients with muscle invasive urinary bladder cancer treated with radical cystectomy. Int Urol Nephrol 2008; 40(2): 321-327.

[70] Krüger S, Weitsch G, Büttner H, Matthiensen A, Böhmer T, Marquardt T, Sayk F, Feller AC, Böhle A. HER2 overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic implications. Int J Cancer 2002; 102(5): 514-518.

[71] Tsai YS, Tzai TS, Chow NH. Does HER2 immunoreactivity provide prognostic information in locally advanced urothelial carcinoma patients receiving adjuvant M-VEC chemotherapy? Urol Int 2007; 79(3): 210-216.

[72] Skagias L, Politi E, Karameris A, Sambaziotis D, Archondakis A, Vasou O, Ntinis A, Michalopoulou F, Moreas I, Koutselini H, Patsouris E. Prognostic impact of HER2/neu protein in urothelial bladder cancer. Survival analysis of 80 cases and an overview of almost 20 years' research. J BUON 2009; 14(3): 457-462.

[73] Bolenz C, Shariat SF, Karakiewicz PI, Ashfaq R, Ho R, Sagalowsky AI, Lotan Y. Human epidermal growth factor receptor 2 expression status provides independent prognostic information in patients with urothelial carcinoma of the urinary bladder. BJU Int 2010; 106(8): 1216-1222.

[74] Alexa A, Baderca F, Zăhoi DE, Lighezan R, Izvernariu D, Raica M. Clinical significance of Her2/neu overexpression in urothelial carcinomas. Rom J Morphol Embryol 2010; 51(2): 277-282.

[75] Caner V, Turk NS, Duzcan F, Tufan NL, Kelten EC, Zencir S, Dodurga Y, Bagci H, Duzcan SE. No strong association between HER-2/neu protein overexpression and gene amplification in high-grade invasive urothelial carcinomas. Pathol Oncol Res 2008; 14(3): 261-266.

[76] Simonetti S, Russo R, Ciancia G, Altieri V, De Rosa G, Insabato L. Role of polysomy 17 in transitional cell carcinoma of the bladder: immunohistochemical study of HER2/neu expression and fish analysis of c-erbB-2 gene and chromosome 17. Int J Surg Pathol 2009; 17(3): 198-205.

[77] Hansel DE, Swain E, Dreicer R, Tubbs RR. HER2 overexpression and amplification in urothelial carcinoma of the bladder is associated with MYC coamplification in a subset of cases. Am J Clin Pathol 2008; 130(2): 274-281.

[78] Sangoi AR, Higgins JP, Rouse RV, Schneider AG, McKenney JK. Immunohistochemical comparison of MUC1, CA125, and Her2Neu in invasive micropapillary carcinoma of the urinary tract and typical invasive urothelial carcinoma with retraction artifact. Mod Pathol 2009; 22(5): 660-667.

[79] Izquierdo L, Truan D, Mengual L, Mallofré C, Alcaraz A. HER-2/AKT expression in upper urinary tract urothelial carcinoma: prognostic implications. Anticancer Res 2010; 30(6): 2439-2445.

[80] Peyromaure M, Scotté F, Amsellem-Ouazana D, Vieillefond A, Oudard S, Beuzeboc P. Trastuzumab (Herceptin) in metastatic transitional cell carcinoma of the urinary tract: report on six patients. Eur Urol 2005; 48(5): 771-775; discussion 775-778.

[81] Hussain MH, MacVicar GR, Petrylak DP, Dunn RL, Vaishampayan U, Lara PN Jr, Chatta GS, Nanus DM, Glode LM, Trump DL, Chen H,

Smith DC. Trastuzumab, paclitaxel, carboplatin and gemcitabine in advanced human epidermal growth factor receptor-2/neu-positive urothelial carcinoma: results of a multicenter phase II National Cancer Institute trial. J Clin Oncol 2007; 25: 2218–2224.

[82] McHugh LA, Kriajevska M, Mellon JK, GriYths TR. Combined treatment of bladder cancer cell lines with lapatinib and varying chemotherapy regimens–evidence of scheduled-dependent synergy. Urology 2007;69(2):390–394.

[83] McHugh LA, Sayan AE, Mejlvang J, Griffiths TR, Sun Y, Manson MM, Tulchinsky E, Mellon JK, Kriajevska M. Lapatinib, a dual inhibitor of ErbB-1/-2 receptors, enhances effects of combination chemotherapy in bladder cancer cells. Int J Oncol 2009; 34(4): 1155–1163.

[84] Dai CL, Tiwari AK, Wu CP, Su XD, Wang SR, Liu DG, Ashby CR Jr, Huang Y, Robey RW, Liang YJ, Chen LM, Shi CJ, Ambudkar SV, Chen ZS, Fu LW. Lapatinib (Tykerb, GW572016) reverses multidrug resistance in cancer cells by inhibiting the activity of ATP-binding cassette subfamily B member 1 and G member 2. Cancer Res 2008; 68(19): 7905-7914.

[85] Wülfing C, Machiels JP, Richel DJ, Grimm MO, Treiber U, De Groot MR, Beuzeboc P, Parikh R, Pétavy F, El-Hariry IA. A single-arm, multicenter, open-label phase 2 study of lapatinib as the second-line treatment of patients with locally advanced or metastatic transitional cell carcinoma. Cancer 2009; 115(13): 2881–2890.

[86] Havaleshko DM, Smith SC, Cho H, Cheon S, Owens CR, Lee JK, Liotta LA, Espina V, Wulfkuhle JD, Petricoin EF, Theodorescu D. Comparison of global versus epidermal growth factor receptor pathway profiling for prediction of lapatinib sensitivity in bladder cancer. Neoplasia 2009; 11(11): 1185-1193.

[87] DiGiovanna MP, Carter D, Flynn SD, Stern DF. Functional assay for HER-2/neu demonstrates active signalling in a minority of HER-2/neu–overexpressing invasive human breast tumours. Br J Cancer 1996; 74: 802–806.

[88] Cicenas J,Urban P, KungW, Vuaroqueaux V, Labuhn M, Wight E, Eppenberger U, Eppenberger-Castori S. Phosphorylation of tyrosine 1248-ERBB2 measured by chemiluminescence-linked immunoassay is an independent predictor of poor prognosis in primary breast cancer patients. Eur J Cancer 2006; 42: 636–645.

[89] Hudelist G, Köstler WJ, Czerwenka K, Kubista E, Attems J, Müller R, Gschwantler-Kaulich D, Manavi M, Huber I, Hoschützky H, Zielinski CC, Singer CF. Her-2/neu and EGFR tyrosine kinase activation predict the efficacy of trastuzumab-based therapy in patients with metastatic breast cancer. Int J Cancer 2006; 118: 1126–1134.

[90] Zhang D, Pal A, Bornmann WG, Yamasaki F, Esteva FJ, Hortobagyi GN, Bartholomeusz C, Ueno NT. Activity of lapatinib is independent of EGFR expression level in HER2-overexpressing breast cancer cells. Mol Cancer Ther 2008; 7: 1846–1850.

[91] Spector NL, Xia W, Burris H 3rd, Hurwitz H, Dees EC, Dowlati A, O'Neil B, Overmoyer B, Marcom PK, Blackwell KL, Smith DA, Koch KM, Stead A, Mangum S, Ellis MJ, Liu L, Man AK, Bremer TM, Harris J, Bacus S. Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. J Clin Oncol 2005; 23: 2502–2512.

[92] Karamouzis MV, Konstantinopoulos PA, Papavassiliou AG. Targeting MET as a strategy to overcome crosstalk-related resistance to EGFR inhibitors. Lancet Oncol 2009; 10: 709–717.

[93] Roudabush FL, Pierce KL, Maudsley S, Khan KD, Luttrell LM. Transactivation of the EGF receptor mediates IGF-1-stimulated shc phosphorylation and ERK1/2 activation in COS-7 cells. J Biol Chem 2000; 275(29): 22583-22589.

[94] Cabodi S, Morello V, Masi A, Cicchi R, Broggio C, Distefano P, Brunelli E, Silengo L, Pavone F, Arcangeli A, Turco E, Tarone G, Moro L, Defilippi P. Convergence of integrins and EGF receptor signaling via PI3K/Akt/FoxO pathway in early gene Egr-1 expression. J Cell Physiol 2009; 218(2): 294-303.

[95] Habuchi T, Marberger M, Droller MJ, Hemstreet GP 3rd, Grossman HB, Schalken JA, Schmitz-Dräger BJ, Murphy WM, Bono AV, Goebell P, Getzenberg RH, Hautmann SH, Messing E, Fradet Y, Lokeshwar VB. Prognostic markers for bladder cancer: International Consensus Panel on bladder tumor markers. Urology 2005; 66(6 Suppl. 1): 64-74.