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Targeting EGFR and IGF 1R: a promising combination therapy for metastatic cancer

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1. ABSTRACT

Acute drug resistance, intolerable side effects and non-specific target activation are the crucial barriers for efficient translational outcome of target directed cancer drug discovery. In the last five years, many of the bull's eye drugs failed to obtain FDA approval because of highly complicated mechanisms of the targeting receptors. These receptors include epidermal growth factor receptor (EGFR) and Insulin-like growth factor receptor 1 (IGF 1R), and are considered as pivotal signaling routes in highly transformed metastatic cancers. IGF 1R and EGFR families show homology in their structure and both the receptors share considerable crosstalk in their functions. An aberrant activation of these two pathways is often diagnosed among many cancer patients. Therefore, target based monoclonal antibodies and small molecule tyrosine kinase inhibitors, either in combination or co-targeting these two receptors may provide a new era of promising therapy and can help in remarkable progress among cancer patients.

2. INTRODUCTION

Epidermal growth factor receptor (EGFR) and Insulin-like growth factor receptor (IGF1R) are essential for normal cellular functions. Both are hyperactivated in cancer cells, and help in cellular proliferation, survival, metastasis and invasion (1, 2, 3). On the other hand EGFR mediated signal transduction pathway has been one of the most explored pathway in carcinogenesis. The phosphorylation of EGFR activates a chain of biological cascades including cellular proliferation, differentiation, motility, invasion, adhesion, DNA repair and survival (4).Mounting evidences have shown the tremendous signaling interferences and functional crosstalk between these two receptor tyrosine kinases from distinct families. Thus, simultaneous blocking of EGFR and IGF 1R abrogate anchorage-independent growth, survival, oncogenic transformation and metastasis (4, 5). Many of the target directed monotherapies have shown less sensitivity due to ATP binding clefts of the receptors.

Again in many cases drug resistance and acquisition of new mutations occur. The major kinase inhibitors exert its cytotoxic effects by blocking a selective kinase and promoting a strong selective pressure among cells to overcome this resistance through mutation in the kinase gene that reduce drug binding. Additionally, resistances due to mutations in the important survival kinases [(BCR–Abl 1 (T315I), PDGFR alpha (T674I), EGFR (T790M) and KIT (T670I)) have hindered drug efficiency (30). Among the survival kinases, Akt activation is highly predominant and Akt promotes the activation of EGFR and IGF 1R (15). During disease progression, mutations in the gatekeeper amino acids are likely to be a common occurrence in clinical kinase inhibitors' resistance. Thus, many of the single therapies failed in the early phase of clinical trials because of drug resistance, acquisition of new mutations and nonspecific drug-target interaction. As for example, EGFR targeting erlotinib resistance acquired by the cancer cells due to activation of IGF 1R and physiologically higher doses of erlotinib/gefitinib could not alter this issue (6, 7). Moreover, IGF 1R targeting therapies developed undesirable hyperglycemic condition because of lack of specificity of the inhibitors (8, 9). Therefore, the justification of using combination of inhibitors to overcome this drug resistance issue, illuminate a new hopeful path for the treatment. It would also help to attenuate the expressions of major survival kinases. In recent years, both IGF 1R and EGFR signaling have been explored in details for development of novel therapeutic agents through targeting growth factor pathways to improve patients' outcome. Such targeted monotherapy and/or combination therapy elevated the specificity of traditional cytotoxic agents, differentiating between malignant and non-malignant cells, produce a higher therapeutic windows and significant toxicity profile other than the conventional therapies already available (5).

Moreover, ample evidences have shown that the IGF-IR regulates functional crosstalk with other growth factor tyrosine kinase receptors, such as the EGFR, VEGFR, HER-2 and helps to coordinate the malignant behaviors of cancer cells (2, 3). A bispecific antibody, Di-diabody (that can selectively target both EGFR and IGF 1R) has been accomplished to exert promising *in vivo* anti-tumor effects in colorectal and pancreatic carcinoma xenografts (10). Given the importance to EGFR-IGF 1R signaling, Lu *et al* demonstrated that incubation with bispecific antibody completely abolished both EGF and IGF 1-stimulated receptor activation as the combination of anti-EGFR IMC-11F8 and anti-IGF 1R IMC-A12 did. That bispecific antibody too abrogated the downstream signal transduction molecules, including both Akt and p44/p42, which were stimulated by EGF and IGF (10). In a nut shell, number of recent studies has clearly revealed that the anti-neoplastic and anti-metastatic potential could be improved by co-parallel inhibition of EGFR and IGF 1R pathways by rationally designed combination of drugs (11, 12, 13). The primary objective of this review is to summarize the published clinical studies on EGFR and IGF 1R inhibitors (monoclonal antibodies and TKIs) alone and in combination to dissect the previous failures and share results to facilitate discussion about the future perspective of these combinations to control metastatic cancer progression.

3. WHAT IS EGFR?

The EGFR is a transmembrane glycoprotein receptor tyrosine kinase belongs to the ErbB subfamily of receptors. Other members include HER-2/c-neu (Erb-2), HER-3 (ErbB-3) and HER-4 (ErbB-4). Structurally, EGFR consists of an extracellular ligand binding domain, a hydrophobic transmembrane domain and an intracellular tyrosine kinase activity regulatory domain controlling the cellular signal transduction mechanism. The extracellular portion of EGFR consists of four domains, two known as Beta –solenoid, where the ligand binds and interacts; and other two domains have large loop helping in receptor dimerization (3). The intracellular region consists of three domains and they involve in protein interaction. The activation of EGFR occurs through its association with specific ligands such as EGF, TGF-alpha, epiregulin etc. Upon binding to specific ligands, the conformational changes in EGFR lead to a dimerization either with same family member (homodimerization) or with other member of the EGFR family (heterodimerization) (3). Following receptor dimerization, activated intrinsic protein tyrosine kinase initiates a cascade of events that includes intracellular mitogenic signaling and other cellular activities (4). Receptor activation also triggers the recycling or degradation fate of dimmer molecule internalization into the clathrin pits through the endocytosis.

3.1. Role of EGFR signaling in metastatic cancer development

EGFR was initially proposed to be an anti cancer target by Mendelson (14).There are some key signaling mechanisms prevalent downstream of EGFR mediating it's effects in cancer cells. One such mechanism involves the PI3K-Akt pathway that plays pivotal role in cell survival and growth. During tumorigenesis, Akt phosphorylates and inhibits downstream pro survival protein BAD and transcription factor FOXO. Other Akt functions include activation of MDM2 which negatively regulates p53 and inhibits GSK3-beta that activates pro-survival MLC-1 (15). Cell growth in cancer cells is attributed to mTOR activation via the PI3K-Akt pathway. In response to constitutive EGFR signaling, the NF-kB and JAK/STAT survival pathways get activated. Another imperative signaling downstream of EGFR involving cancer cell metastasis is the Ras-Raf-MAPK pathway. EGFR upon ligand interaction dimerizes and autophosphorylates itself on tyrosine residues present on its cytoplasmic domain (16). This autophosphorylation, in turn provides docking sites for adaptor proteins like Grb which further interacts with another protein Sos. Formation of a receptor-GRB-Sos complex activates GTPase Ras which initiates a cascade of Ser-Thr phosphorylation events converging on MAP-Kinase. MAPK in turn phosphorylates various target proteins including transcription factors, components that mediate cytoskeletal function and cell-cell adhesion mechanisms (16). Thus, activation of EGFR confers metastatic potential of tumor cells.

The *EGFR* gene is located at chromosomal locus 7p12.3-p12.1. In an oncogenic state *EGFR* can either be over expressed, amplified, and/or mutated (16). The single nucleotide polymorphism in the promoter region (-216) of *EGFR* is associated with its increased production (16). Overexpression of EGF and EGFR has been implicated in dephoshorylation and down regulation of focal adhesion kinase (FAK) leading to metastasis (17).Evidences suggest that patients suffering from pancreatic ductal carcinoma, possess a strong correlation among EGF, EGFR expression and tumor invasiveness (18).Moreover, higher levels of EGF have been documented to increase the metastatic potential of squamous cell and primary renal cell carcinomas (19, 20). EGF signaling also stimulates bone metastasis in kidney, prostate and breast cancers (21), whereas EGF induced MMP's (Matrix metalloproteases) are reported to play a role in tumor invasiveness of squamous cell carcinoma (22). Overexpression of EGFR has been implicated in 60% of the patients suffering from non-small-cell lung cancer (NSCLC) and amplified in 30% of NSCLC patients (23, 24). EGFR also compensates cell functions via phosphoinositide signaling involving phospholipase C. Phospholipase C and PI3K corroborate in EGF induced migration capability of breast cancer cells (25).

Apart from overexpression, mutations in the EGFR gene lead to the development of various types of cancers. Mutations of *EGFR* are heterozygous and the mutated alleles exhibit gene amplification (26). The kinase domain mutations of the receptor render it constitutively active and are mainly located within exons 18-21 (16). It has been postulated that 10% to 40% of patients with adenocarcinomas harbor EGFR activating mutations resulting a robust signal transduction cascade (27, 23). EGFR over expression in Triple Negative Breast Cancer (TNBC) cells could lead to epithelial mesenchymal transition (EMT), in which the cells loose their epithelial characteristics and acquire mesenchymal phenotype with fibroblast like morphology, cytoskeleton reorganization, increased invasiveness and metastatic capability (28).

3.2. EGFR targeted monotherapy

As our understanding of the molecular pathways that drive proliferation and growth of cancer cells evolves, inhibition or modulation of these signaling pathways by molecular-directed agents has opened up a new avenue of therapeutic intervention. Several classes of EGFR-targeted candidates, including anti-EGFR monoclonal antibodies and the small molecule TK inhibitors, have been illustrated to bind to the EGFR/or prevent functioning of the receptor and downstream signaling of tumor cell growth.(29,30).Plenty of drugs have been discovered to control metastasis, caused by constitutive EGFR activation and other ErbB receptor signaling (29). Most of these drugs (like erlotinib and gefitinib) specifically targeted EGFR and other members of this family. However, EGFR directed single drug therapy (monotherapy) primarily failed due to development of resistances in the *EGFR*. These drugs either developed secondary mutations in the *EGFR* or conferred resistance through continuing activation of PI3K network (30).

Patients with acquired resistance to gefitinib / erlotinib, developed a secondary mutation in exon 20 leading to substitution of metheonine by threonine at position 790 (T790M) in the kinase domain (7), analogous to those observed in BCR-Abl and KIT in imatinib-resistant chronic myelogenous leukemia and gastrointestinal stromal cell carcinoma respectively (43). Additionally, insertions in exon 20 of the EGFR gene were reported to occur at 4% frequency in EGFR mutant lung tumor (31). Even though receiving a geftinib/erlotinib treatment, patients' case history reflected insertion mutations at exon 20 with progression of the disease (32). Other mutations like G719A, G719C and G719S have also been highlighted to cause drug resistances (6, 31). Cetuximab, the most promising candidate as EGFR targeted monoclonal antibodies, has been considered potential on the basis of improvement of overall survival (OS) and progression free survival (PFS), but still the candidate has messed up to qualify the disease recurring issue due to acquisition of secondary mutations (33).Functional crosstalk between EGFR and IGF 1R appeared to be particularly sensitive in EGFR positive tamofexin resistant variants of MCF-7 (Tam-R) and T47D-R breast cancer cell lines (34). Tam-R cells were highly dependent on EGFR for cell growth through an interaction between IRS-1 and EGFR and subsequent phosphorylation of IRS-1 at Y896. Treatment of these cells with gefitinib showed reduced IRS-1/EGFR association but promoted IRS-1/IGF 1R association (leading to IRS-1 Y612 and Akt phosphorylation) suggesting the limited efficacy of this drug due to EGFR/IGF 1R crosstalk (35). EGFR/IGF 1R crosstalk in hepatocellular carcinoma was also recommended a limited anti-tumor effect of gefitinib (36). In prostate epithelial cells Akt was co-operatively being controlled by EGFR and IGF 1R, whereas the inhibition of EGFR pathway shifted the regulation of Akt to IGF 1R (37). Switching between EGFR and IGF 1R signaling was assumed to affect the efficiency of anti-EGFR (gefitinib / erlotinib) drugs (39).Also, gefitinib resistant breast and prostate cancer cell lines documented an increased IGF 1R signaling which contributed towards invasive capacity of these cell lines (38).

3.3. EGFR targeted combination therapy

In the recent years, new combination drugs have emerged (more than 25 rational combinations in clinical trial) to provide better opportunities for the treatment of patients bearing EGFR mutations. The EGFR pathway therefore presents a feasible target for
pharmacologic intervention in solid tumors, and several agents have demonstrated encouraging antitumor activity. Combinations of pan-inhibitors like HKI-272, HKI-357 and HKI-569 have shared some promises in countering these mutations (16). The EGFR and ErbB2 dual inhibitor HKI-272 has been reported to inhibit metastatic tumor growth transformed with EGFRvIII, L858R and L858R-T790M mutated cells (40). Another cutting edge approach to counter T790M drug resistance is a combination therapy of HKI-272 with Erbitux (cetuximab) illustrating reduced proliferation of erlotinib-resistant tumors having T790M mutation (41) (Table 1). Cetuximab based combinatorial

Table 1. List of leading egfr inhibitors in clinical trial

Therapeutic agent	Type	manufacturer	Phase	Indications	Comments
Cetuximab (*Erbitux*)	mAb	ImClone, Bristol-Myers Squibb	I, II	NSCLC, CC, Metastatic Squamous Cell Carcinoma of the Head and Neck	Chimeric, (mouse/human) monoclonal antibody
Erlotinib	TKI	(*Tarceva*) OSI Pharmaceutical	I, II, III	Solid tumors, Lung Cancer, NSCLC, Glioblastoma; Gliosarcoma	It binds in a reversible fashion to the adenosine triphosphate (ATP) binding site of the receptor
Lapatinib	TKI	(*Tykerb*) SmithKline Beecham	I, II	Advanced NSCLC, Head and Neck ,CC, Metastatic Cervical Cancer, Breast Cancer	An orally active drug for breast cancer and other solid tumours
Gefitinib	TKI	*(Iressa)* AstraZeneca	I, II	Head and Neck Cancer, Ovarian Cancer; Peritoneal Neoplasms, Fallopian Tube Cancer, Myelogenous Leukemia ,Breast Cancer	Tyrosine kinase inhibitor
Panitumumab	mAb	*(Vectibix*) Amgen	I, II, III	Colon Cancer; Colorectal Cancer; Gastrointestinal Cancer; Metastatic Colorectal Cancer; Rectal Cancer, NSCLC, Squamous Cell Carcinoma of the Head and Neck	Fully human monoclonal antibody
PKI166 (CGP 75166);	TKI	Novartis Pharma	I	Advanced solid malignancies	Potently inhibits the EGFR and HER-2 tyrosine kinase activities

Data adopted from http://clinicaltrials.gov

strategies are currently in broad use for the treatment of colorectal (27), head & neck (42) and lung (23) metastasis. Several phase I/II studies with cetuximab in combination with cytotoxic agents (e.g. gemcitabine etc.) or with other targeted antibodies, such as trastuzumab have translated into encouraging results (30, 43, 33). Dual targeting of EGFR with first and second generation TKIs has provided important clues to engage researchers for the development of rational combination of inhibitors. Apart from the encouraging breakthrough in current EGFR research, failure of EGFR-TKI and chemotherapy combination to improve the overall survival of NSCLC patients in large scale randomized clinical trials, regardless of concurrent (44) or sequential (45) administration of the drugs, is still not clearly understood. Several pharmaceuticals and academics have initiated new dimension of combination approaches to resist refractory conditions (Table-1). A new study has been undertaken by Seattle Cancer Care Alliance [(SCCA)/ www.seattlecca.org/clinicaltrials] targeting EGFR in NSCLC patients with a combination of erlotinib and IGF 1R inhibitor OSI 906, and the scientific world is awaiting for the result of this trial.

4. WHAT IS IGF 1R?

The insulin-like growth factor 1 (IGF-1) receptor is a transmembrane tyrosine kinase receptor which is activated by IGF-1 (structurally similar to insulin) and related growth factor IGF-2. IGF 1R belongs to a family of receptor consisting of insulin receptor (IR) and the IGF-2R (and their respective cognate ligands IGF-1 and IGF-2) respectively and along with several IGF-binding proteins (47, 48). IGF 1R and IR both have ATP binding sites requiring phosphates for autophosphorylation. The mature cell membrane-bound IGF 1R consists of two 130 to 135 kDa alpha-chains and two 90-95 kDa beta-chains, with several alpha–alpha and alpha–beta disulfide bridges (48). The alpha and beta subunits are synthesized from a single mRNA precursor. The alpha-subunits are entirely extracellular and form the ligand-binding domain (49) that binds to ligand molecule. After ligand binding, the alpha chains induce the tyrosine autophosphorylation on intracellular portion of beta chain which consists of a binding site for phosphorylated substrate at Y950. Phosphorylated Y950 then serves as docking sites for several receptor substrates, including the insulin receptor substrates (IRS1–4) and Shc (50, 51). These substrates eventually initiate phosphorylation cascades transmitting the IGF 1R signal.

4.1. Role of IGF 1R signaling in metastatic cancer development

During embryogenesis and development (in human and animal species) IGF 1R and its ligands contribute a critical role. Functions of growth hormones (GH) are controlled by IGF-I during postnatal development and longitudinal growth. Elevated sex steroid level stimulates GH production during puberty leading to activation of the GH/IGF-I axis (52). Increased expression of IGF-I, IGF-II, IGF 1R or combinations has been reported in malignancies like glioblastomas, neuroblastomas, meningiomas, medulloblastomas (53, 54), carcinomas of the breast (55, 56) and prostate (57), and transformations of the gastrointestinal tract, such as colorectal and pancreatic carcinomas (58, 59).

Cancer metastasis is a multistep process driven by complex molecular interaction between the cancer cells and changing microenvironment. Tumor-induced neovascularization, extracellular matrix (ECM) degradation, cell movement through tissue barriers and survival/proliferation within new organ microenvironments are the key steps in regulating cancer metastasis (60). IGF's role in metastasis has been established in several human cancers and multiple studies have demonstrated that high concentration of serum IGF and/or lower level of IGFBPs are associated with increased risk for several cancers, including pre-menopausal breast carcinoma (61, 62, 63), prostate carcinoma (57, 64), lung cancer (65), colorectal carcinoma (58, 59, 66), endometrial cancer (67), and bladder cancer (68).This indicates a possible paracrine role for IGF in the development of tumor microenvironment. A

recent study based on colorectal carcinoma revealed that IGF 1R and p53 expression levels were not significantly associated with long-term survival (69). Again substantial comparison between IGF 1R expression in human synovial sarcomas and different levels of lung metastasis has implicated that IGF 1R overexpression rendered lung metastasis (70). Interestingly, in uveal melanoma patients, there is a positive correlation between high IGF 1R expression and the risk of liver metastasis (71). Similar findings in gastric cancer have revealed that IGF 1R overexpression in the primary tumor correlates with increased lymph node metastasis (72).Analyses of gallbladder carcinomas has clearly documented IGF 1R expression in 52 out of 55 primary tumors, and 17 corresponded to metastasis (73).

One of the major rate-limiting factors in tumor growth is nutrient and oxygen availability. Expanding tumors overcome lack of nutrient supply and hypoxia by instigating a process of neovascularization, i.e., angiogenesis. Although hypoxia is a major trigger for tumor-dependent angiogenesis, the IGFs and insulin play an early role in this process, preceding and/or augmenting the hypoxic stimulus (74).Induction of hypoxia-inducible factor 1alpha (HIF-1alpha) by IGF-I and IGF-II in cultured cells (74), leads to the formation of HIF-1alpha/arylhydrocarbon receptor nuclear translocator complex which is involved in transcriptional regulation of hypoxia response element-containing gene in VEGF (75). Inhibition of ubiquitination and degradation of HIF-1alpha by hypoxia induces its expression whereas IGF-I stimulates HIF-1alpha protein synthesis directly via MAPK and PI3K signaling (76). On the other hand, IGF-II, IGFBP-2, and IGFBP-3 synthesis are dependent on HIF-1alpha (77). Collectively, the IGF system is involved in angiogenesis through several other mechanisms and IGF-I and IGF-II stimulate the migration and morphological differentiation of endothelial cells to induce angiogenesis (78, 79). IGF-I is transported across the vascular endothelial cell lining through a paracellular route and binds to the sub-endothelial extra cellular matrix (ECM) indicating a fundamental role in stability and migration of endothelial cells (80).

4.2. IGF 1 R targeted monotherapy

4.2.1. IGF 1R antibodies

IGF 1R is an intricate and complex signaling pathway that provides opportunities to researchers for therapeutic intervention. Over the past years, number of novel therapeutics was launched targeting IGF 1R including monoclonal antibodies and small molecule tyrosine kinase inhibitors. Few of them are in phase I, II, and III clinical trials and couple of them have provided substantial new information about the clinical trials**,** particularly CP-751, 871, IMC-A12, R1507, AMG-479, AVE-1642, MK-0646, XL-228, OSI-906, and BMS-754807. The first IGF 1R targeting monoclonal antibody, aIR3, has evaluated anti-cancer activity and implicated in the inhibition of tumors growth *in vivo* (81). aIR3 has induced insulin receptor down regulation through a dimer formation with IGF 1R, or through endocytosis of insulin receptors (82).Concerning treatment regime, CP-751 871, a humanized monoclonal antibody which is currently in three phase II trials for metastatic breast cancer, NSCLC and prostate cancer, has shown significant IGF 1R inhibition and drug tolerance (83). AMG 479, another fully humanized monoclonal antibody against IGF 1R has exhibited anticancer/antimetastatic activity for solid tumors and sarcomas in phase I & II trial (84).Similar work suggests that Dalotuzumab (MK-0646), a monoclonal antibody, has exhibited a promising clinical activity with well-tolerated, low clearance rate and long terminal half-life. MK-0646 also revealed dose-proportional PK and inhibited IGF 1R signaling in treated tumors (85). Interestingly, a neutralizing monoclonal antibody (mAb) specifically directed against IGF 1R (MAB391) has been demonstrated in increased phosphorylation of the reciprocal receptor (8).

4.2.2. Small molecule inhibitors

IGF 1R shares 85% homology with the insulin receptor (IR) while the ATP binding cleft is 100% conserved. Therefore, designing of IGF 1R tyrosine kinase inhibitors became very complicated (86). Various laboratories are using these subtle differences to generate IGF 1R inhibitors with specificity and selectivity. Such as NVP-AEW541 and NVP-ADW742 (Novartis) are inhibitors of the IGF 1R kinase demonstrating greater selectivity over insulin receptor (87, 82 and 88). These agents prevented tumor growth in biliary tract cancer, GIST cell lines, fibrosarcoma, myeloma and Ewing's sarcoma, and also enhance tumor cell chemo sensitivity (88, 89). Inhibitors of substrate phosphorylation showed greater potential for specific IGF 1R inhibition than the inhibitors with ATP binding cleft (Table 2). Cyclolignan picropodophyllin (PPP), which inhibits tyrosine phosphorylation at Y1136 in the activation loop of the IGF 1R kinase domain, could not alter insulin receptor (90). Particular classes of catechols acted as substrate-competitive inhibitors of the IGF 1R in human breast and prostate cancer cells (91) and abrogated cell invasion. OSI-906 and A928605 are the potential second generation TKIs and showed promising anti-neoplastic property in preclinical trials (8).

4.3. IGF 1R targeted combination therapy

The IGF 1R signaling is a key adaptive survival pathway and mediator of resistance to cytotoxic chemotherapeutics, ionizing radiation, and certain targeted agents, including inhibitors of epidermal growth factor receptor (EGFR), HER-2, and mammalian target of rapamycin (92, 37). Due to resistances not in favor of conventional targeted therapies for particular pathways (EGFR, HER-2 etc.) have emerged against single targeted therapy, the rationale of employing combinational therapy has made strong ground to explore IGF 1R inhibitors in combination with other inhibitors that have generated resistance. This strategy has shown some optimistic results. AMG-479, an anti IGF 1R monoclonal antibody in combination with Cetuximab, gemcitabine and erlotinib and small molecule EGFR inhibitors respectively have shown effective regression of various tumor models(93). A-928605, an anti IGF 1R tyrosine kinase inhibitor has proven more efficacious when used in combination with

Table 2. List of leading IGF 1R inhibitors in preclinical/clinical trial

Therapeutic agent	Type	manufacturer	Phase	Indications	Comments
A-928605 [101,113]	Small molecule inhibitor	Abbott	Pre-clinical	Cancer	ATP- competitive kinase inhibitor.
AMG 479[114,43,115] AMG 655[116]	Monoclonal antibody	Amgen	I, II	Solid tumors, NHL, sarcoma	Fully human monoclonal antibodies.
Anti-IGF 1R/BIIBO22[117]	Monoclonal antibody	Biogen Idec	Pre-clinical	Solid tumors	Fully human monoclonal antibody.
ATL1101 [118]	Antisense	Antisense Therap. Ltd	I	Psoriasis, HRPC	Blocks translation of the IGF1R.
AVE1642 [119,120]	Monoclonal antibody	Immunogen, Sanofi-Aventis	I, II	Solid tumors	Humanized antibody.
CP-751,871 [121,122,123]	Monoclonal antibody	Pfizer	I, II, III	Breast and prostate cancers, NSCLC, Rheumatoid arthritis	Fully human monoclonal antibody
IMC-A12 [124,33]	Monoclonal antibody	Imclone Systems	I, II	Colorectal cancer, prostate cancer, solid tumors	Fully human monoclonal antibody. Also, exists as a Di-diabody with the EGFR antibody IMC-11F8.
MK-0646 [125,126]	Monoclonal antibody	Merck	I, II	Breast, pancreatic, and prostate cancers	Monoclonal antibody
R1507 [127,128129]	Monoclonal antibody	Genmab A/S, Roche Holdings Ltd	I, II	Lymphoma, NHL, sarcoma, solid tumors	Fully human IgG1 recombinant antibody
SCH-717454 (19D12)/Robatumumab [130]	Monoclonal antibody	Schering-Plough/Medarex	I	Osteosarcoma, Ewing's, sarcoma, colorectal cancer.	Fully human monoclonal antibody
NVP-AEW 54 [131], NVP-ADW742	Tyrosine kinase inhibitor	Novartis	Pre-clinical	biliary tract cancer, GIST cell lines	27-fold cellular selectivity against IGF1R over the IR
AVE 1642 [132]	Monoclonal antibody	Sanofi-Aventis	I	HCC, multiple myeloma	Humanized antibody of IGF 1R
MDI-573[133]	Monoclonal antibody	MedImmune	I	Solid tumors	Fully human monoclonal antibody of IGF-1 and IGF-2
OSI-906	Tyrosine kinase inhibitor	OSI	II/III	Adenocortical carcinoma, Ovarian	Small molecule inhibitor of IGF 1R

approved EGFR inhibitors in non-small cell lung and human pancreatic tumor models compared to monotherapy (94). Significant inhibition of cell proliferation and tumor growth has been observed when NVP-AEW-541 a tyrosine kinase inhibitor against IGF 1R was studied in combination with mTOR inhibitor Everlemous (89). These positive observations highlight the use of combination therapy for targeting IGF 1R signaling pathways.

5. CO-TARGETING IGF 1R AND EGFR

The regulation of EGFR signal transduction in metastatic tumors has been adopted as a therapeutic strategy to reverse tumor growth and metastasis. Inhibition of constitutively activated EGFR signaling has been correlated with induction of apoptosis, abrogation of angiogenesis and invasion in various tumors (29, 95). Over expression and/or hyperactivation of EGFR lead to continued activation of PI3K/AKT and MAPK kinase pathways (25, 95)and thus render resistances to EGFR and IGF 1R therapies in preclinical trials in lung cancers and glioblastomas (6, 96, 97 and 98).Hence, dual inhibition of these two receptors is seemingly important for optimal suppression of solid tumor growth because both parallel and reciprocal pathways exist between the IGF 1R and EGFR signaling cascades and these pathways are closely linked in tumorigenesis (Figure 1) As IGF 1R mediates resistance through continued activation of PI3K in glioblastomas (99) IGF 1R inhibition in combination with EGFR (by co-targeting) has proven an effectual strategy to prevent the emergence of resistance in these cell lines. Accordingly, the adopted incremental strategies for combination therapy including bispecific antibodies against both IGF 1R and EGFR, and combination of monoclonal antibodies or small tyrosine kinase inhibitors are short listed below.

5.1. Monoclonal antibodies

Erbitux, an anti-EGFR mAb has revealed a significant anti-tumor activity in animal models and in the clinic. Treatment of BxPC-3 pancreatic carcinoma xenograft using Erbitux combination with anti-IGF 1R antibody IMC-A12 or anti-VEGFR2 antibody DC101 resulted in a greater inhibition of growth. Erbitux with A12 (IMC-A12: Erbitux dose ratio =1: 1) resulted in a combination index (CI) value of 0.02 while Erbitux and DC101 (DC101: Erbitux dose ratio of 8:1) produced the CI equal to 0.1. These CI <<1 values are positives of synergistic approach towards target-related signaling pathways to build substantial anti-tumor effects. In synergistic approach, the total dose required for each chemotherapeutic agent was less than administered alone (100). Similarly, IMC-A12 in combination with cetuximab has shown regression in pancreatic carcinoma xenograft growth significantly. When AMG479, an anti-IGF 1R mAB was administered in combination with cetuximab (an anti-EGFR mAB) evaluated effective inhibition in tumors of cetuximab refractory head and neck squamous cell carcinoma (101). A recombinant human IgG -like bispecific antibody, a Di-diabody (10), had been developed using the variable regions from two antagonistic antibodies: IMC-11F8 to EGFR and IMC-A12 to IGF 1R which was

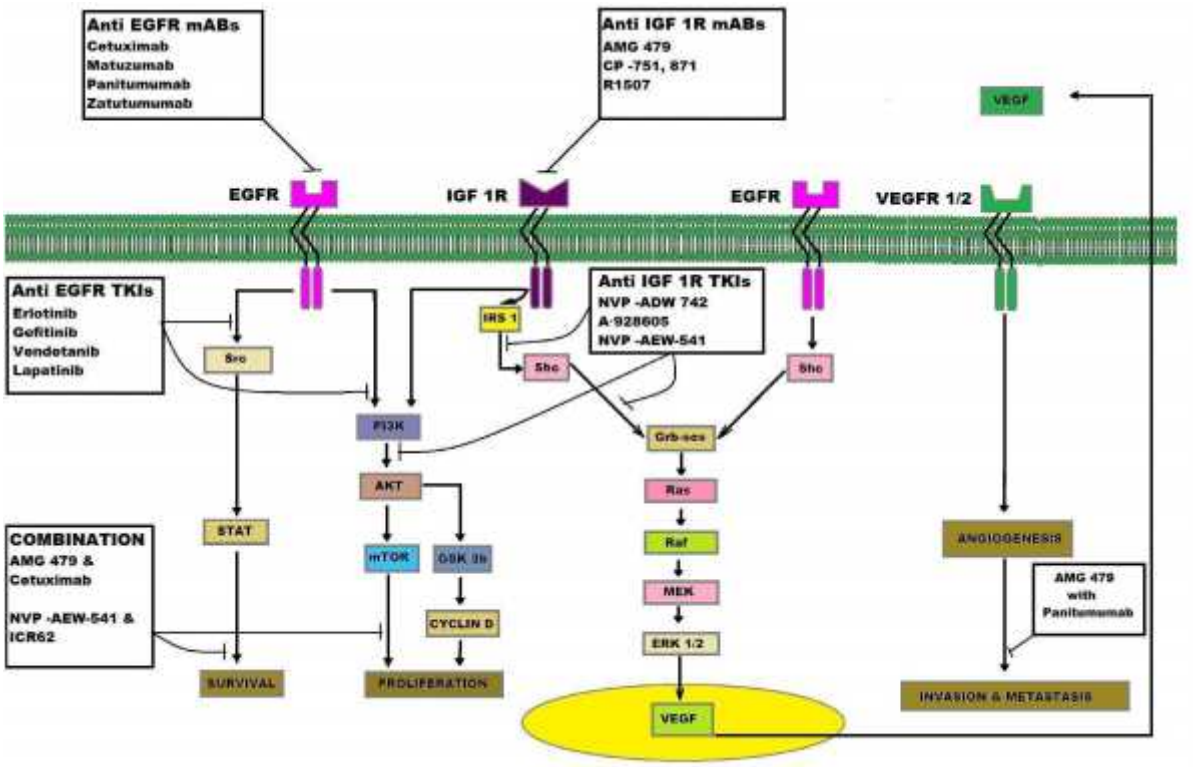

Figure 1. Schematic representation of leading TKIs and mAb's inhibiting important steps in IGF 1R and EGFR signaling pathways. Certain pathways confer resistance against existing therapies. Targeting such pathways (thick arrows) using combinational treatments (thin arrows) e.g. by combining IGF 1R-EGFR inhibitors, shows some promise in combating potential disease mechanisms. Therapeutic agents that specifically block proteins that activate downstream signals common to targets of existing therapies give an additional advantage in blocking these intermediates. Certain EGFR and IGF 1R tyrosine kinase inhibitors target common downstream signaling proteins required for progression and metastatic persistence of disease.

specific to both receptors**.** This application has observed positive reduction in human tumor xenografts *in vivo* proliferation and controlling invasion (10). Dual treatment with monoclonal antibodies Cetuximab and IMC- A12 for EGFR and IGF 1R respectively induced apoptosis in squamous cell carcinoma cell line and enhanced survival in CSCC (Cutaneous Squamous Cell Carcinoma) nude mouse model (47). Co-targeting EGFR and IGF 1R in human malignant glioma cells has been found to sensitize CD95L-induced apoptosis (11). Thus, combination of inhibitors could simultaneously block both the receptors seeming plausible to prevent metastasis.

5.2. Tyrosine kinase inhibitors

Additional support for the strategy of dual inhibition was demonstrated when it was found that combined inhibition of multiple targets had the potential to overcome resistance to monotherapies [13]. A recent approach of NVP-ADW 742 and gefitinib combination in A431 cells synergistically blocked cell proliferation and increased cell death in clinical models (95). As a proof of principal, inhibition of proliferation was due to loss of mitogenic signaling with decrease in cyclin D1, where as decrease in Akt levels (after combination treatment to A431 cells) resulted in decelerated levels of Mcl-1 and Bax hyperactivation, suggesting that co inhibition of receptors augmented the intrinsic apoptosis pathway (95). The duo combination showed significant anti-proliferative property to overcome IGF-1 mediated resistance to chemotherapeutics. In addition, evidences suggest that EGFR inhibitors may sensitize some tumor cells to conventional chemotherapies by preventing salvage routes, dual inhibition of EGFR and IGF 1R signaling cascades may provide even greater chemosensitization. As for example, NVP-ADW 742 in combination with imatinib exhibited remarkable regression in tumor growth, metastasis and promoted apoptosis (102). Similarly, AMG 479 in triple combination with gemcitabine and erlotinib in pancreatic carcinoma xenograft model decreased Akt level and enhanced the anti-tumor effects along with approved therapies in invasive pancreatic carcinomas (103).

5.3. Combination of monoclonal antibodies and tyrosine kinase inhibitors

The last couple of years, a new approach of targeting particular receptor with combination of monoclonal antibodies and TKIs have motivated the researchers towards an optimistic era of new class of drugs (Table 3). A recent study has demonstrated that the activation of Akt by IGF-1 signaling was prevented by synergistic application of NVP-AEW541, a tyrosine kinase inhibitor against IGF1R in combination with ICR62, an

Table 3. IGF 1R and EGFR inhibitors combination in clinical trial

IGF 1R Inhibitor	Type	In Combination with	Type	Indications	Phase
MK-0646	Monoclonal antibody	Cetuximab and irinotecan	Monoclonal antibody	Colorectal Cancer	Phase I
MK-0646	Monoclonal antibody	Erlotinib	Tyrosine kinase inhibitor	Carcinoma, NSCL	Phase I
OSI-906	Tyrosine kinase inhibitor	Erlotinib	Tyrosine kinase inhibitor	NSCLC	Phase II
IMC-A12	Monoclonal antibody	Cetuximab	Monoclonal antibody	Head and Neck Cancer	Phase II
AMG 102 or AMG 479	Monoclonal antibody	Panitumumab	Monoclonal antibody	Colon Cancer Colorectal Cancer Gastrointestinal Cancer Metastatic Colorectal Cancer Rectal Cancer	Phase I,II.

anti-EGFR mAb in metastatic colorectal cancer cells. This combination has found to be additive in inhibiting the growth of Colo13, CCL235, CCL244 cells (29). ICR 62 is a potential agent to exert strong inhibitory effect against EGFRvIII expressing cells in the lung in athymic mice mediating through antibody-dependent cellular cytotoxicity (ADCC) (104). Thus, there must be supra-additive combination effect of NVP-AEW541 and ICR 62 in combating metastatic cancer growth through ADCC or blocking tumor blood supply (29). This combination is now on preclinical/clinical phase and generates a hope for the treatment of metastatic colorectal cancer. Interestingly, inhibition of one of these receptor signaling enhances the chemosensitivity of the other. Camarind *et al.* demonstrated that inhibition of insulin like growth factor-1 receptor signaling amplified growth inhibitory and proapoptotic effects of gefitinib (Iressa) in human breast cancer cells (9).Hyperglycemia is a mechanism-related toxicity obtained by IGF 1R inhibition because of similar receptor homology between IGF 1R and IR. Recent development of IGF 1R targeting monoclonal antibodies (OSI-906 and AMG 479) with erlotinib has shown evidences to minimize diabetic issue rather than combination of tyrosine kinase inhibitors (8).

5.4. EGFR-IGF 1R combination in preclinical/clinical trials: new perspectives of discovery

It is now known that a matrix of interconnected, and in some cases redundant, signal transduction pathways is responsible for maintaining many solid tumors. For this reason, blockade of a single pathway may be in ineffective in the long term because activation of other pathways can serve as salvage or escape mechanisms for the tumor [60]. In the recent years, major clinical trials have been initiated targeting EGFR and IGF 1R signaling pathways controlling metastatic tumor growth (Table-3 & Fig.1). Several experimental studies have clearly revealed that co-expression of IGF-IR is associated with resistance to treatment with anti-EGFR and anti-HER-2 therapies; therefore, co-targeting the IGF- IR and EGFR or HER-2 with a combination with two inhibitory agents to respective targets may opt better therapeutic advantages over the single inhibitor alone (38, 105). Interestingly, NVPAEW541 in combination with ICR62 was found to be more effective on inhibition of growth in human colorectal cancer cell lines (e.g. Colo13, CCL235 and CCL244) than either agent solely. Similarly, others have reported enhanced antitumor activity in human breast and prostate cancer cell models employing IGF-IR TKI in combination with anti- EGFR or anti-HER-2-antibody (105, 106). More recently, Hopfner and colleagues have also reported that NVP-AEW541 in combination with anti-EGFR mAb cetuximab was more effective in preventing the growth of two human colorectal tumor cell lines, HCT 116 and HT 29 (107) than treating with either agent . A phase II study of anti-IGF1R mAb MK-0646 in combination with cetuximab and irinotecan was successfully persuaded (30). Eligible patients had previously failed to irinotecan as single therapy was grouped as per mode of administration. Ten patients were recruited as arm A, received MK-0646 10mg/kg/week, whereas 8 patients were recruited as arm B and received MK-0646 15mg/kg loading followed by 7.5 mg/kg/alternative weeks. These patients also received cetuximab at 400mg/m^2 loading followed by 250mg/m^2 weekly along with irinotecan as per it was previously given. This combination treatment was found to be tolerable without any concerning toxicities. This landmark study is in phase II/III clinical trial. An ongoing phase II clinical trial with advanced non-small cell lung carcinoma (NSCLC) has demonstrated encouraging antitumor activity utilizing the combination treatment of Erlotinib (Tarceva®) with OSI-906 (108). Additionally, another ongoing phase I / II clinical trial in patients with wild-type KRAS mCRC metastatic adenocarcinoma of the colon or rectum with AMG 102 or AMG 479 in combination with anti-EGFR mAb panitumumab is in development (109).

5.5. Rationality of metastasis inhibition by EGFR-IGF 1R combination therapy

Therapeutic approaches to individual inhibition of either of these pathways (EGFR/IGF 1R) have shown limited clinical efficacy, possibly because of incomplete suppression of metastasis and/or proliferative pathways resulting from intrinsic resistance, mutation, or salvage responses by the tumor. In addition, the lack of overlapping toxicities between inhibition of the IGF 1R (e.g., asthenia,constipation) and the EGFR (e.g., rash, diarrhea) pathways suggests that simultaneous inhibition of both of these pathways has the potential to be tolerated by patients (www.clinicaltrials.gov). From recent reports cited in this review it could be presumed that inhibition of EGFR or IGF 1R alone leads to hyper activation of the other pathway due to the existence of various crosstalk mechanisms. In such cases, monotherapy tends to develop resistance over a period of time and does not possess strong inhibitory effects on growth, proliferation or metastatic potentials of cancer cells. For example, transactivation of EGFR by IGF 1R signaling in breast cancers promoted invasion and metastasis (110) and Hepatoma cells showing HER-3 activation in an EGFR-dependent mechanism were reported to overcome IGF 1R inhibition by combination therapy suggesting the synergistic effects of the EGFR and IGF 1R inhibitors (111). Screening of a panel of cell lines with BMS-53624 (IGF 1R inhibitor), revealed that cell lines that

resistant to this molecule have increased EGFR signaling suggesting that a combination of BMS-536924 with EGFR inhibitor could have increased the antagonistic effect of this molecule (112). Given the potential crosstalk and well-established role of these pathways in metastasis and tumor growth, combined inhibition of both IGF 1R and EGFR signaling is a promising and active area of clinical investigation with approved and investigational agents being tested in a number of solid tumors.

6. CONCLUSION

From the illustrated discussion above it might be justified to comment on the judicial approach required for rational combination of drugs targeting specific pathway with significant tolerance level among patients and that should be translated into prime criteria for selection of typical amalgamation. In the last years, the incorporation of classical cytotoxic candidates in the treatment of metastatic cancer has improved survival in patients with advanced disease. Drugs targeting EGFR and IGF 1R axis have been considered as critical modus operandi because of their profound roles in survival, proliferation, migration of normal and cancer cells. There are lucid evidences showing that blockage in either of these evolutionary conserved pathways could hyperactivate the other (94), and cause serious disease resistance. Thus, in the current issue we have tried to focus only on those targeted monotherapy versus combination therapy for the treatment of metastatic cancer that eventually became important to overcome drug resistance and toxicity issue in this axis. Concerning huge efforts required to launch new trials in terms of expense and length of time, by dissecting the most recent studies of the clinical trials, the major results have been updated here. Incorporation of new examples of cellular signaling network materializing from proteomics and system biology based approach of combination treatment suggest that rationally predicted drug/drugs likely to yield incremental rather than transformative therapeutic gains. Cetuximab - irinotecan and AMG102 with panitumumab are potential candidates qualifying the criteria's. MK-0646 and irinotecan, AMG-102 with panitumumab, erlotinib & OSI 906 have been latently better qualified the toxicity and PK-PD profiles (http://clinicaltrials.gov). In the next years new upcoming data on EGFR-IGF 1R inhibitor combinations in current clinical development (Table 3) will hopefully provide newer opportunities for the treatment of metastatic cancers of big killers like lung, prostate, colorectal and breasts.

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