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Dual epidermal growth factor receptor (EGFR)/insulin-like growth factor-1 receptor (IGF-1R) inhibitor: A novel approach for overcoming resistance in anticancer treatment

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ABSTRACT

Small molecule inhibitors of epidermal growth factor receptors (EGFR) have been found to show a good initial response in cancer patients but during the course of treatment, patients develop resistance after a few weeks of time. Development of secondary mutations or over-activation of insulin like growth factor (IGF-1R) pathway are a few of the several mechanisms proposed to explain the resistance. To study the effect of dual inhibition of EGFR and IGF-1R in overcoming the resistance, three strategies were envisaged and are reported in this manuscript: 1) a virtual predictive tumor model, 2) *in vitro* experimental data using a combination of EGFR and IGF-1R inhibitors and 3) *in vitro* experimental data using in house dual inhibitors.
Findings reported in this manuscript suggest that simultaneous inhibition of IGF-1R and EGFR either by combination of two inhibitors or by dual kinase inhibitors is more efficacious compared to single agents. *In vitro* cell based experiments conducted using epidermoid cancer cell line, A431 and an EGFR mutant cell line, H1975 along with virtual predictions reported here suggests that dual inhibition of EGFR and IGF-1R is a viable approach to overcome EGFR resistance.

1. Introduction

Members of tyrosine kinase family play an important role in cancer progression (Ghoreschi et al., 2009; Krause and Van Etten, 2005) and some of them have been exploited for anti-cancer therapy (Arora and Scholar, 2005). Among these, members of the epidermal growth factor receptor (EGFR) family like Her-1 and 2 have so far been the most successful ones as anti-cancer targets (Baselga, 2000). EGFR inhibitors like lapatinib, cetuximab, gefitinib and erlotinib are in clinic for anti-cancer therapy. Despite initial positive response observed with each of these drugs, emergence of drug resistance (Yamasaki et al., 2007) and associated dose-limiting side effects (Heist and Christiani, 2009) has been observed in clinic. For more effective treatment response and to overcome resistance, the current trend for emerging therapies is now shifting towards the development of dual or pan kinase inhibitors (Ghoreschi et al., 2009). One such attractive approach is dual targeting of EGFR and insulin-like growth factor receptor (IGF-1R) kinases.

EGFR and IGF-1R both belong to the receptor tyrosine kinase family of enzymes and play important roles in cell growth and survival (Wilsbacher et al., 2008). While EGFR inhibition is a well precedented approach, IGF-1R inhibitors are still in the early stages of development. There are several lines of evidences (Arteaga, 1992; Baserga, 1995; Baserga et al., 2003; Chernicky et al., 2000; Dunn et al., 1998; Sachdev et al., 2003), suggesting a role of IGF-1R in tumor progression and existence of a functional cross talk between IGF-1R and members of EGFR family (Camirand et al., 2005; Chakravarti et al., 2002; Coppola et al., 1994; Hurbin et al., 2002; KaulfuB et al., 2009; Rowinsky et al., 2007). This suggests that targeting both these kinases together could be a better approach over inhibiting individual enzyme's activity.

In order to support our hypothesis, in-vitro study was designed using a combination of EGFR inhibitor(erlotinib) and IGF-1R inhibitor (AEW-541) and our novel small molecule dual inhibitors which were synthesized in-house (structures of these given in Table 4) . It was observed that when the erlotinib and AEW-541 were used in combination, they synergistically inhibit the cell proliferation of A431 cells. This was further confirmed using a virtual predictive

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systems biology approach and by generating *in-vitro* experimental data, in parallel and blinded fashion. Methods were developed to computationally design an EGFR and IGF-1R over-expressing system mimicking the diseased states in cancer patients. Virtual experiments were conducted on systems with different levels of dominance of EGFR and IGF-1R pathways and the predictions were prospectively validated at the experimental level by generating *in vitro* data at the cellular and biochemical level. In addition to the standard EGFR and IGF-1R inhibitors, we also used our in-house dual EGFR and IGF-1R inhibitors, RBx 32D7E8EC (N*2*-Benzothiazol-6-yl-N*4*-(5-cyclopropyl-1H-pyrazol-3-yl)-6-methyl-pyrimidine-2,4-diamine and RBx 52E98E1(N*4*-(5-Cyclopropyl-1H-pyrazol-3-yl)-N*2*-(1H-indazol-5-yl)-5-methyl-pyrimidine-2,4-diamine) as tool molecules to validate the concept (Table 4).

Since EGFR and IGF-1R pathways are independently known to regulate the activation of a few biomarkers such as Akt, Erk1/2 and cyclin-D1 (Holbro and Hynes, 2004; Maiso et al., 2007), we chose two key markers of these signaling events pErk1/2 and cyclin-D1 and verified the effect of our drugs on these in A431 cell line in addition to the target receptors. In addition, the effectiveness of the dual inhibitors in an EGFR mutant cell line, H1975 was also tested. Data reported in this manuscript suggest that simultaneous inhibition of

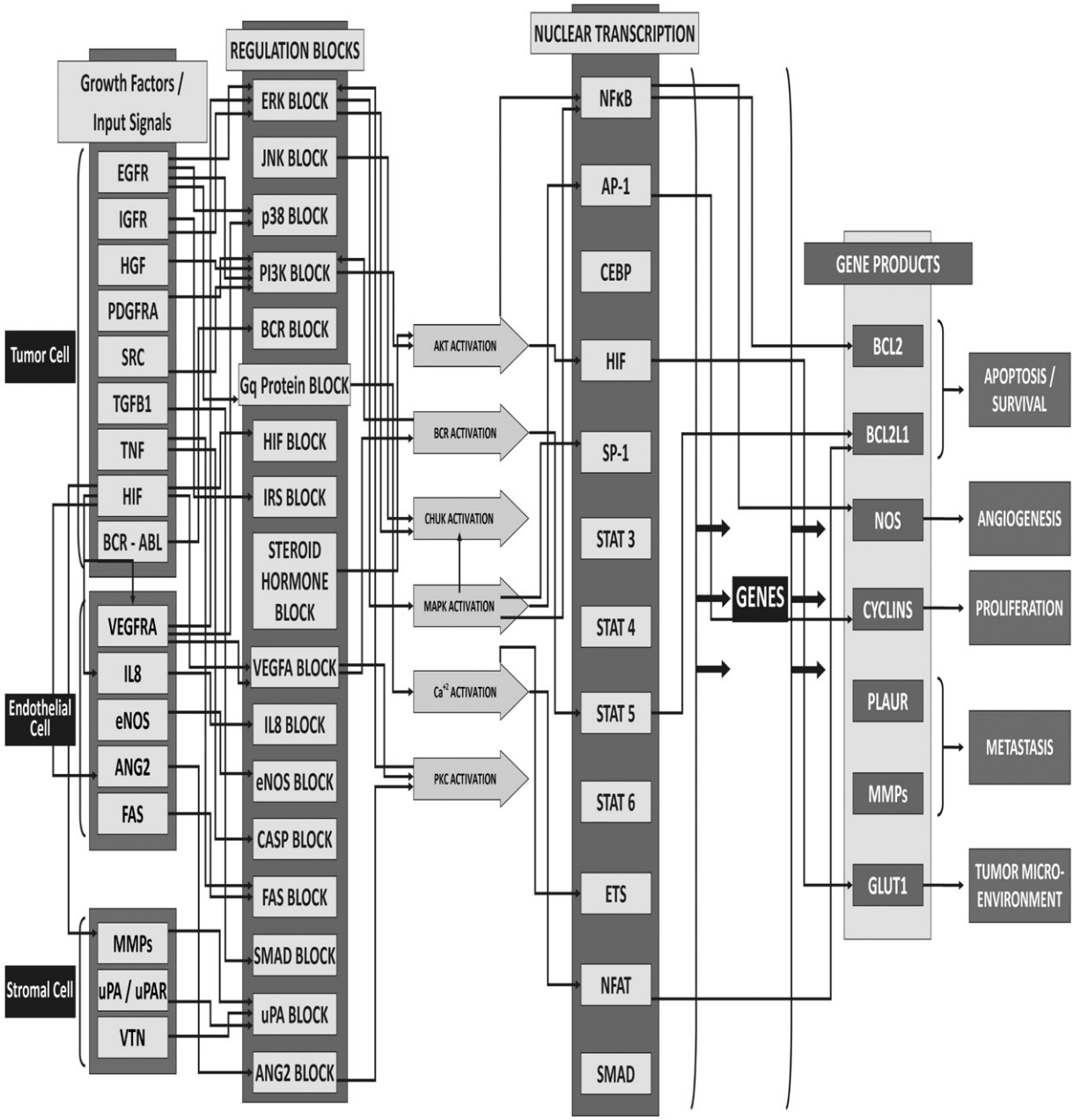

Fig. 1. Cross talk between various pathways in the Virtual Predictive Oncology Platform: the figure illustrates a high-level view for the cross-talk between the growth factor receptor pathways and major signaling cascades in Tumor cells. It highlights the presence of both autocrine and paracrine signaling within the tumor cell, as well as, paracrine inputs from the endothelial and stromal cell. The growth factor receptors like EGFR, IGF-1R and Hepatocyte growth factor (HGF) activate signaling cascades, resulting in the activation of key kinases like Akt, IKKa, and MAPKS — all of which converge at the activation of various transcription factors like NFKB, ETS1 and STATs. As a consequence of these events, downstream genes like BCL2, Cyclins and MMPs are activated which are associated with specific cancer phenotypes of proliferation, apoptosis, angiogenesis and metastasis. The cross-talk between all the above with the basic signaling represents our Control Oncology Platform. An increase in EGFR, PDGFRA and IGF-1R expression represents our Disease Stage.