Emerging Trends in Combinations of Gefitinib and Cytotoxic Agents: New Opportunities

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In the last 10 years, many pharmacological and biological approaches have been tested to develop novel targeted agents that are able to selectively inhibit important pathways that control cancer cell proliferation and growth [1]. The blockade of the epidermal growth factor receptor (EGFR) signal transduction pathway has emerged as one of the most successful strategies in the treatment of cancer with a wide preclinical and clinical evaluation [2]. The EGFR is a transmembrane glycoprotein with three different parts: an external ligand-binding domain, a transmembrane domain and an intracellular tyrosine kinase domain with catalytic activity [3]. Once the ligands – mainly TGF-α and EGF – bind to the receptor, a dimerisation process is induced. It results in the formation of either homodimers or heterodimers with other receptors of the HER family, producing autophosphorylation of different tyrosine residues located in the catalytic domain of the receptor, triggering different signalling transduction cascades that lead, at the end, to critical cellular processes such as proliferation, survival, angiogenesis and production of metastasis (fig. 1). The EGFR plays a critical role in most of human carcinomas. Enhanced expression of the ligands – mainly TGF-α – and the EGFR itself has been observed in these tumours and has been shown to negatively correlate with prognosis in many tumour types. EGFR overexpression has been observed in cell lines that are resistant to different cytotoxic drugs. For these and other reasons the EGFR signalling pathway has been in the forefront of the development of targeted therapies directed to control tumour growth. Several pharmacological approaches have been developed to block the EGFR itself. However, only two strategies have reached advanced clinical development: monoclonal antibodies directed to the extracellular domain that compete with the natural ligands [4–6], and low-weight molecules with tyrosine kinase inhibition capacity directed to the enddomain of the receptor [6, 7]. Several tyrosine kinase inhibitors (TKIs) directed to the EGFR are in clinical development (table 1). Among these inhibitors, gefitinib (ZD1839, Iressa®) is one of the compounds with the largest preclinical and clinical development.

Gefitinib is an oral anilinoquinazoline, a selective and reversible EGFR-TKI that blocks signal transduction pathways implicated in cancer cell proliferation and other processes connected with cancer growth. Early limited preclinical studies – with in vitro and in vivo evaluation – showed high antitumour activity against different tumour types [8–11]. Moreover, this activity was shown not only when this compound was administered as a single agent but also in combination with several cytotoxic drugs and with radiation therapy. These encouraging results led to the development of a vast clinical programme of gefitinib in many tumour types, with special emphasis on patients with non-small cell lung cancer (NSCLC).

The initial phase I studies showed gefitinib to have a good safety profile, with clear clinical activity in some tumour types including NSCLC [12]. Two large randomised phase II studies demonstrated that gefitinib was active in patients with advanced NSCLC refractory to, at least, one previous chemotherapy treatment [13, 14]. The efficacy results of these two studies were consistent, leading to a fast-track approval by the FDA with the indication of treatment for patients with advanced NSCLC refractory to standard chemotherapy treatment. The next step was to demonstrate that gefitinib could improve the efficacy results of first-line treatment when combined to standard chemotherapy schedules. Some preclinical studies favoured this approach although the experiments were limited with only few tumour cell lines evaluated. The initial phase I studies combining standard chemotherapy schedules with gefitinib showed that this approach was feasible. Two well-designed phase III studies evaluated the role of the addition of gefitinib to two different first-line schedules – cisplatin/gemcitabine and carboplatin/paclitaxel. Unfortunately, these two studies failed to demonstrate any advantage with...
the addition of gefitinib to standard chemotherapy in NSCLC [15, 16]. Erlotinib (OSI-774, Tarceva®), the other EGFR-TKI with advanced clinical development in patients with advanced NSCLC, also failed to show any advantage when combined with the same first-line chemotherapy schedules in the same tumour setting [17, 18].

These disappointing results of the combination of EGFR-TKIs with standard chemotherapy led to several different hypotheses trying to explain this lack of synergistic effect. Firstly, the objection was raised that the preclinical models that had evaluated the effect of this combination were biased as only a few lung tumour cell lines were analyzed [8, 9]. Indeed, these lung cancer preclinical models may only reflect a molecular subtype of cancer whose prevalence in the NSCLC type at large is unknown and unpredictable. Secondly, the group at MSKCC showed that gefitinib could have a preclinical syner-
gistic effect when combined with chemotherapy provided that gefitinib was administered at high pulse doses [19]. Thirdly, in the last 18 months it has been shown that those NSCLC patients that achieve the best efficacy with gefitinib as a single agent bear some selected mutations in the catalytic domain of the EGFR [20–22]. Therefore, the effect of gefitinib combined with standard chemotherapy could be diluted due to the low proportion of patients having EGFR mutations. And finally, an antagonistic effect between gefitinib and chemotherapy with the sequence administered in these studies could not be excluded [23].

At the same time it was shown that gefitinib as a single agent had no meaningful activity in patients with advanced colorec-
tal cancer (CRC) [24, 25]. However, gefitinib combined with oxaliplatin/5-flurouracil-based chemotherapy – both in the first-line setting and in a most refractory population – showed encouraging results in limited phase I/II studies [26, 27]. Actually, the efficacy results in terms of response rate achieved within these studies were clearly greater than the results obtained with the same oxaliplatin-based schedules in the same population in larger and pivotal phase III studies. In contrast, another phase I study with the combination of gefitinib and oxaliplatin did not show meaningful activity [28]. These inter-study comparisons only allow to generate hypotheses for the design and further development of controlled phase III studies to properly evaluate the role of gefitinib combined with oxaliplatin-based chemotherapy in advanced CRC. In this issue of the journal, Voigt et al. [29], present the results of a series of preclinical studies evaluating the effect of gefi-
tinib alone or combined with oxaliplatin in different tumour cell lines from CRC, testicular cancer, anaplastic thyroid carcinoma and vulvar carcinoma. Interestingly, the authors showed that gefitinib and oxaliplatin exhibited a synergistic effect in 4 out of 6 different CRC cell lines when these cells were exposed to the combination; in the other 2 cell lines gefitinib did not show any significant interaction with oxaliplatin. In con-trast, the combination of gefitinib with oxaliplatin did not ex-
hibit any synergistic effect in the experiments with the other tumour cell lines, including the highly EGFR-expressing vul-
var cell line A431. Indeed, in 4 out of 6 non-CRC cell lines this combination exerted a clear antagonistic effect. Moreover, the authors evaluated the cellular effects of gefitinib in the entire tumour cell lines, showing that gefitinib as a single agent only produced cellular changes in the EGFR overexpressing cell line A431, resulting in a marked G1/S accumulation, whereas no significant cell cycle effect could be detected in the cell

<table>
<thead>
<tr>
<th>Type of inhibition</th>
<th>Agent</th>
<th>EGFR IC50, µM</th>
<th>HER-2 IC50, µM</th>
<th>Irreversible</th>
<th>Development stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR specific and reversible</td>
<td>ZD1839 – gefitinib</td>
<td>0.02</td>
<td>3.7</td>
<td>no</td>
<td>phase II and III</td>
</tr>
<tr>
<td></td>
<td>OSI-774 – erlotinib</td>
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<td>3.5</td>
<td>no</td>
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</tr>
<tr>
<td></td>
<td>PKI-166</td>
<td>–</td>
<td>–</td>
<td>no</td>
<td>phase I</td>
</tr>
<tr>
<td>EGFR specific and irreversible</td>
<td>EKB-569</td>
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<td>1.2</td>
<td>yes</td>
<td>phase I and II</td>
</tr>
<tr>
<td>EGFR/HER-2 reversible</td>
<td>GW-2016 – lapatinib</td>
<td>0.011</td>
<td>0.009</td>
<td>no</td>
<td>phase I and II</td>
</tr>
<tr>
<td>Pan-HER irreversible</td>
<td>BMS-59626</td>
<td>–</td>
<td>–</td>
<td>no</td>
<td>phase I</td>
</tr>
<tr>
<td>EGFR/HER-2 and VEGFR reversible</td>
<td>CI-1033</td>
<td>0.0008</td>
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<td>yes</td>
<td>phase I</td>
</tr>
<tr>
<td></td>
<td>AEE788</td>
<td>0.002</td>
<td>0.006</td>
<td>no</td>
<td>phase I</td>
</tr>
</tbody>
</table>

![Fig. 1. Epidermal growth factor receptor (EGFR) signal transduction pathways.](image-url)
lines expressing low levels of EGFR. The authors concluded that mechanisms of action other than cell cycle perturbation would determine the interaction effect between gefitinib and oxaliplatin in the tumour cells showing synergistic effect. It would be of additional interest to evaluate the interaction effect between gefitinib and oxaliplatin as well as to assess the cellular effects in highly EGFR-overexpressing CRC cell lines, like the DiFi and the Caco-2 models. Any potential difference between these highly EGFR-expressing and the other CRC cell lines with low EGFR expression levels would help to address the mechanistic effects of this interaction.

Xu et al. [11] also evaluated the synergistic effects of gefitinib with oxaliplatin. These authors showed that the sequence of exposure has a critical role in the interaction between gefitinib and oxaliplatin, with a synergistic effect when oxaliplatin was followed by gefitinib, while the inverse sequence showed antagonism.

In summary, the present study, as well as other previously published studies, has provided instrumental knowledge to understand the positive interaction between gefitinib and oxaliplatin in CRC cell lines. All these preclinical studies offer a good rationale for the encouraging clinical effect observed in patients with advanced CRC treated with gefitinib combined with oxaliplatin-based chemotherapy in phase I/II studies. The next step would be to design and conduct randomized phase II studies or phase III studies with early stopping rules. All these studies should incorporate transcriptional profiling endpoints, like cDNA arrays and proteomic analysis, to define genomic signatures sensitive to these combinations [30]. The lessons that we have learned from the development of EGFR-TKIs, in addition to the instrumental knowledge that has been generated in the field of cancer cell-signalling, will translate into a more efficient development of these compounds, with a comprehensive interaction between the preclinical and the clinical setting.

References


