

EGFR-targeting therapy as an evolving concept: learning from nimotuzumab clinical development

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Abstract: Epidermal growth factor receptor (EGFR)-targeted therapies have been extensively evaluated in the clinic for different tumor localizations and using different EGFR-targeting products, either registered or still in clinical development. Nonetheless, there still is a long way to go to optimize the clinical benefit from EGFR-targeted therapies. In this article we briefly discuss on current paradigms guiding the use of EGFR-targeting agents in the clinic, and on new emergent concepts. The discussion is largely based on experiences from the clinical development of the monoclonal antibody nimotuzumab, which has shown a quite particular clinical profile, characterized by a very low toxicity. In order to optimize the design of EGFR-targeting therapies, clinical researchers should take into account the interconnection between the EGFR pathway and other cellular pathways. Thus, clinical trials need to incorporate more translational research.

Keywords: Epidermal growth factor receptor (EGFR); nimotuzumab; targeted therapy; oncogene addiction



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Introduction

Basic research on the epidermal growth factor receptor (EGFR) has been connected to oncology from its very beginnings, three decades ago, when important scientific discoveries laid the basis for a new therapeutic approach in cancer—molecular targeting of the EGFR. Among these discoveries were the findings that the EGFR shares a high sequence homology with the retroviral oncogenic protein v-ERBB (1) and that it shows an increased expression in human squamous cell lung cancers (2).

In parallel with these discoveries our group found that the EGFR is expressed in human breast tumors, showing an inverse correlation with the expression of the estrogen receptor (3). These studies prompted us to extend the concept of tumor hormone-dependence to growth factors such as the EGF (3), which in turn led to the idea of blocking EGF binding to its receptor using antagonistic antibodies, in order to inhibit tumor growth. Following this therapeutic approach, two different projects were initiated by our group in the subsequent years. One of them led to the development of the antibody nimotuzumab (4-6), while the second project resulted in CimaVax, a recently

registered (in Cuba) EGF-based cancer vaccine (7).

Today, the available repertoire of EGFR-targeted agents comprises several monoclonal antibodies (mAbs) and small tyrosine kinase inhibitors (STKIs), some of them already registered and others under clinical investigation (8-10). Nonetheless, there still is a long way to go to optimize the clinical benefit from EGFR-targeted therapies. In this article we briefly discuss on current paradigms guiding the use of EGFR-targeting agents in the clinic, and on new emergent concepts. The discussion is largely based on experiences from the clinical development of nimotuzumab, which has shown a quite particular clinical profile.

A clinical look at EGFR-targeted therapies

Clinical results from phase III clinical trials have been modest

EGFR-targeted therapies have been extensively evaluated in the clinic for different tumor localizations and using different EGFR-targeting products, either registered or still in clinical development (8-10). The clinical benefit achieved with these products in advanced cancer patients, however,

has been limited in terms of median overall survival. Taking cetuximab (a monoclonal antibody) and erlotinib (a tyrosine kinase inhibitor) as examples of successful anti-EGFR drugs, we see that in spite of marketing approvals, their impact in terms of clinical benefit has been in general limited when evaluated for the intent-to-treat populations in different clinical trials.

For cetuximab, although encouraging results were obtained in locoregionally advanced head and neck cancer [median survival time (MST): 49 months for patients treated with cetuximab and radiotherapy (RT), *vs.* 29.3 months with RT alone] (11), the survival advantage was dramatically reduced in patients with recurrent or metastatic carcinomas of the head and neck that were treated with cetuximab and chemotherapy (CTP) (MST: 10.1 *vs.* 7.4 months) (12). In KRAS wild-type metastatic colorectal cancer, cetuximab plus CTP resulted in a modest MST improvement (23.5 *vs.* 20.0 months) (13). Similarly modest results were obtained in another clinical study in patients with colorectal cancer (14,15). In advanced non-small cell lung cancer (NSCLC), the small increase in overall survival precluded marketing approval (16). In pancreatic cancer, the encouraging results from preclinical and early clinical studies with cetuximab were not confirmed in a phase III trial (17). With erlotinib, the clinical trials conducted in NSCLC patients (18-20) and in pancreatic cancer (21) also evidenced a limited impact.

Is clinical efficacy necessarily bound to toxicity?

Clinical researchers are more and more pointing out the need of revising the clinical trial endpoints that are being used for EGFR-targeted agents, since neither toxicity nor tumor shrinkage have resulted adequate surrogates to evaluate their clinical efficacy (22,23). But in spite of the increasing understanding on this problem, clinical studies are still being guided by the classical cytotoxic paradigm that correlates clinical efficacy with objective clinical responses. Moreover, clinical efficacy has been linked to skin rash toxicity. This applies both to EGFR-targeting mAbs, like cetuximab (15) and panitumumab (24), and to STKIs. Overall, more than a dozen phase II and III clinical trials using EGFR inhibitors have shown an association between rash incidence, severity and survival (25). This association implies also a drawback since cumulative toxicity may impair the chronic use of the anti-EGFR agents and their combination with other therapies (26). A relevant question here is whether the clinical efficacy of EGFR-targeting drugs (in terms of overall survival) is unavoidably

bound to toxicity, or not necessarily. The clinical experience with the monoclonal antibody nimotuzumab suggests that there are alternatives to the classical cytotoxic paradigm and that clinical efficacy may be accompanied with a low toxicity profile.

Nimotuzumab: diverging from the cytotoxic paradigm

In this section we briefly review the development of nimotuzumab and discuss the main lessons we have extracted from its application in the clinic and from mechanistic studies.

The development of the humanized anti-EGFR antibody known today as nimotuzumab (also as Taixinsheng, in China) started in the late 80s at the Cuban Institute of Oncology in Havana (see the timeline in *Figure 1*). The parent murine antibody, called R3, was generated in Balb/c mice immunized with purified human EGFR, and selected for its ability to block EGF binding to the receptor (27). Subsequently, the murine mAb was shown to have antitumor effects *in vitro* (28). Aiming forward to the clinic, R3 was humanized by CDR grafting as early as in 1994 (29), becoming into a human IgG1/kappa antibody that retained practically the same antigen-binding affinity of the original murine mAb (30). The humanized antibody was initially called as h-R3, and later (in 2004) given the non-proprietary name nimotuzumab (31).

In early diagnostic clinical studies with ^{99m}Tc-radiolabelled R3, involving 148 patients with different types of cancer of epithelial origin, the antibody accumulated in primary tumors and metastases previously identified by biopsy, showing an overall sensitivity of 84% and 100% specificity (32). These and other results from preclinical studies (performed with the murine and/or the humanized mAbs) (33) opened the door to therapeutic clinical studies with nimotuzumab in patients with head and neck tumors, which started in Cuba in 1998 (34).

Since then, nimotuzumab has been extensively tested in about 30 completed clinical trials conducted in different countries, and a similar number of clinical studies are currently ongoing in a dozen countries, including several phase III and phase IV trials. A large part of the clinical development has been taking place in China, where the antibody (called as Taixinsheng) has been assayed in several phase I/II clinical trials in different types of cancer: esophageal tumors (35-38), nasopharyngeal carcinoma (39,40) and other head and neck tumors (41,42), pancreatic cancer (43), glioma

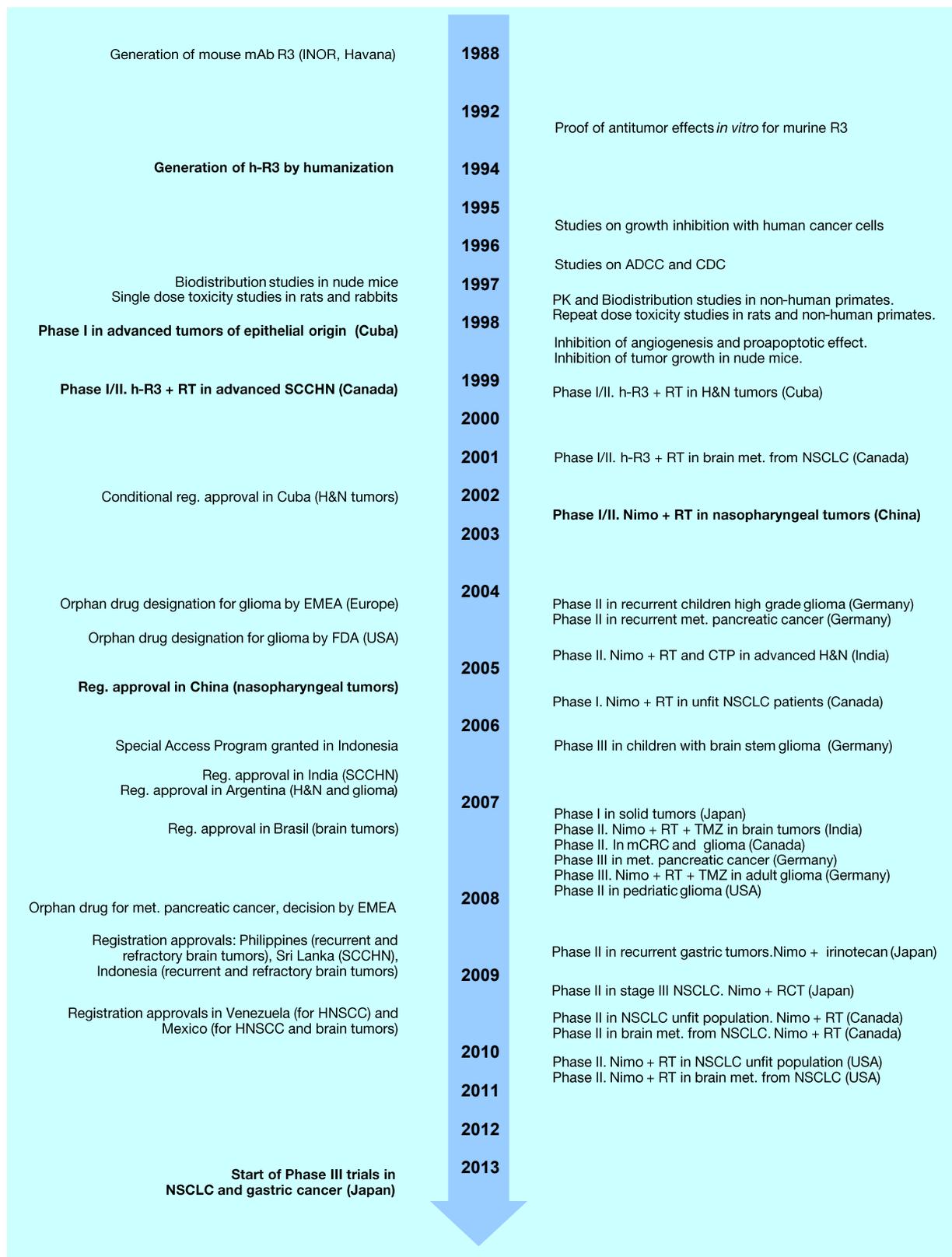


Figure 1 Timeline showing a selection of main events in the preclinical and clinical development of nimotuzumab.

(44,45) and NSCLC (46,47).

At present, nimotuzumab is indicated for the treatment of patients with nasopharyngeal cancer and other advanced head and neck tumors, adult high grade glioma, children glioma, and advanced esophageal cancer. The antibody has been registered in more than 30 developing countries, including Brazil, India and China. Altogether, about 30,000 patients have been treated with nimotuzumab worldwide.

Nimotuzumab shows a low toxicity profile, allowing long-term treatments

Clinical studies conducted in patients with head and neck tumors (48,49), NSCLC (50), pancreatic cancer (51) glioblastomas (52,53) and esophageal tumors (37,54) have shown that the combination of nimotuzumab with either RT or chemotherapy provides clinical benefits. More statistically sound results may come from the currently ongoing phase III trials.

Notably, the clinical effects obtained with nimotuzumab have been accompanied with a very low toxicity profile (55), which in turn has made possible the use of this antibody in prolonged treatments, lasting several months or even years, that have been characterized by the induction of long-term stable disease (6). A recent example of this was a randomized, double blind trial conducted in patients with high grade glioma (56). In this study nimotuzumab was administered during one year up to a maximum cumulative dose of 3,600 mg, which is probably the highest cumulative antibody dose ever administered to glioma patients. Nonetheless, no severe adverse events attributable to the antibody were observed. Although nimotuzumab did not significantly improve the rates of objective response or disease control, it did increase the progression free survival (median PFS: 15.7 vs. 6.5 months for nimotuzumab and control arms, respectively) and overall survival (median OS: 17.8 vs. 12.6 months) (56).

Nimotuzumab's low toxicity profile has been explained at the molecular level on the basis of its medium or "intermediate" affinity ($KD \sim 10^{-8}$ M for the Fab fragment, which represents a 10-fold lower affinity as compared with the cetuximab Fab) (30). According to this hypothesis, which was initially supported by mathematical modeling, antibodies with such medium affinities need bivalent binding for stable attachment to cells and, therefore, would accumulate mostly on those tumors that over-express the EGFR and to a lesser degree in normal epithelial tissues having lower expression levels of the receptor. Recent

studies have given further support to this hypothesis. In *in vitro* experiments with different tumor cell lines, Garrido and coworkers (57) showed that nimotuzumab binding and inhibition of EGFR phosphorylation occurs only for tumor cell lines with medium or high levels of EGFR expression (10^4 receptors per cell or higher). Furthermore, while nimotuzumab Fab fragments bound only to A431 cells (having the highest EGFR expression level), the Fab of cetuximab was able to bind to tumor cells with lower EGFR expression levels.

The idea that an antibody with a somewhat lower affinity may have advantages in the clinic is itself a deviation from the widely accepted paradigm that the highest the affinity, the better. We are tempted to speculate that the therapeutic ratio of some of the high-affinity anti-EGFR antibodies currently in the clinic might be improved by "optimizing" (lowering) their affinity, although the expected increase in clinical benefit might be seen in particular in patients bearing EGFR-overexpressing tumors. For nimotuzumab, several studies suggest that this antibody has a better clinical effect in tumors with medium or high levels of EGFR expression (48,49,54), which in turn should have become more "EGFR-addicted", as we discuss in the next section.

Oncogene addiction as ground for EGFR-targeted therapies

As introduced by I. Bernard Weinstein, the term "oncogene addiction" refers to the manifested dependency of some cancers on one or a few genes for maintenance of the malignant phenotype (58). It is such a dependency on a particular gene (and its associated signaling pathway) what sustains the targeted therapy approach in cancer treatment.

The clinical evidences of the EGFR oncogene addiction phenomenon are provided by those patients that show high responses to EGFR-targeted agents (59). High responses, on the other hand, have been associated with high expression levels of the receptor, as in the FLEX trial conducted in EGFR-expressing NSCLC patients, which were treated with cetuximab in combination with chemotherapy (16). Better clinical outcomes from treatment with nimotuzumab have been also associated with EGFR over-expression, as mentioned above. For erlotinib, better response and PFS rates in advanced NSCLC patients have been correlated with activating mutations in the tyrosine kinase domain of the EGFR (19,20). Activating mutations are an alternative (to EGFR over-expression) mechanism to increase downstream signaling in EGFR-addicted cells.

“Naïve” versus “adaptive” oncogene addiction

EGFR oncogene addiction may arise during tumor progression (by gene amplification and/or aberrant expression), in which case we would be in the presence of a “naïve” oncogene addiction, or it may develop as a resistant mechanism driven by chemo-therapy or RT (for example, deletion mutants and activating kinase domain mutations), being thus an “adaptive” addiction. A distinct medical positioning is required in each case regarding the use of an EGFR-targeted therapy as first or second line treatment. Naïve EGFR addiction reveals in tumors having EGFR over expression, as evidenced by the clinical responses observed in patients with locally advanced squamous cell carcinomas of the head and neck (11), NSCLC (60) and esophageal tumors (54) upon treatment with anti-EGFR agents. Adaptive EGFR addiction, on the other hand, is observed for tumors that become refractory to chemotherapeutic agents, which then respond to the combination of chemotherapy with an anti-EGFR agent, as is the case for refractory ADC tumors having low to medium EGFR expression, such as colorectal carcinomas, gastric, pancreatic and lung tumors.

Cancer stem cells and oncogene addiction

The existence of EGFR addiction in cancer stem cells is supported by an increasing number of experimental evidences [reviewed in (59)]. For instance, modulation of EGFR expression in glioblastoma multiforme (GBM)-derived tumor initiating cells enhances or reduces their tumorigenic ability (61). On the other hand, GBM CD133+ tumor initiating cells are radio-resistant and most likely are the source of tumor recurrence after radiation (62). It has been shown that the combination of an anti-EGFR antibody (either cetuximab or nimotuzumab) with RT has a cytotoxic effect on CD133+ stem cells (63), which suggests that RT reinforces EGFR oncogene addiction in neural cancer stem cells and gives further support to the combined use of anti-EGFR antibodies and RT for treating brain tumors.

The relevance of the EGFR-signaling pathway in the tumorigenic and invasion capabilities of cancer stem cells, as well as the sensitivity of these cells to EGFR-targeted treatment, has been demonstrated for cells from head and neck (64) and breast tumors. In breast cancer, for instance, it was shown that EGFR activation can induce epithelial to mesenchymal transition (EMT), favoring invasion and metastasis (65). Furthermore, inhibition of the EGFR in aggressive inflammatory breast cancer reversed the

mesenchymal phenotype of cancer cells to a less aggressive and potentially more chemotherapy-sensitive epithelial phenotype (66). The capability of modulating the malignant cell phenotype has a particular translational relevance because it may imply that chronic use of EGFR-targeted therapy would have a controlling effect on EGFR-addicted metastases.

EGFR inhibition in addicted cells may induce immunogenic cell death

The antitumor effect of an anti-EGFR mAb can be mediated by T cells, as was demonstrated in a mouse model (67). In these experiments, depletion of CD4+ and CD8+ T cells abolished the anti-tumor effect of the antibody. In a subsequent study using a Lewis lung carcinoma model and the same anti-mouse EGFR antibody, it was demonstrated that this antibody, but not a STKI, promotes a CTL-activating immunogenic cell death (68). Remarkably, this immunogenic effect was independent of the antibody effector functions since removal of the Fc fragment did not prevent the induction of immunogenic apoptosis.

It has been argued that the “vaccinal effect” of anti-tumor monoclonal antibodies may be important to produce clinical benefit. In support of this hypothesis, a recent report shows that cetuximab activates NK cells and promotes dendritic cell maturation and CD8+ T-cell priming, leading to tumor antigen spreading and TH1 cytokine release. Moreover, cetuximab promoted an EGFR-specific cellular immunity (69). We believe that long-term responses require the involvement of the immune system, and in this regard, the fact that an anti-EGFR antibody may enhance the antitumor immunity gives support to the chronic use of these agents in the clinic.

Resistance mechanisms are driven by the need to satisfy oncogene addiction

The selection pressure created by treatment of an EGFR-addicted tumor with an anti-EGFR drug favors the survival of those cells that find an escape mechanism to satisfy their addiction. One of the evasion mechanisms is via mutations that impair drug binding or enhance receptor functioning. For example, the T790M mutation in the kinase domain confers resistance to gefitinib and erlotinib in lung adenocarcinomas by stabilizing the active tyrosine kinase conformation and enhancing ATP binding (70).

A second type of resistance mechanism consists in

making irrelevant the function of the EGFR itself, while ensuring downstream signaling via the PI3K/AKT or RAS/RAF/MEK/ERK pathways. This is the case of KRAS mutations that make downstream signaling independent of EGFR activation. Other members of the ErbB family also may play important roles in activating compensatory signals; for example, acquired resistance to cetuximab in colorectal cancer has been linked to activation of ERBB2 signaling (71). Interestingly, although EGFR-activating mutations and KRAS mutations have been reported to be mutually exclusive in colorectal cancer, a recent study using a mouse model of pancreatic ductal adenocarcinoma shows that upregulation and activation of the EGFR is required for the KRAS-driven tumorigenesis characteristic of this type of tumor (72).

A recent study in mice revealed a novel immunological mechanism of escape to treatment with an anti-EGFR antibody, resulting from the convergence of alterations in oncogenic and immunological pathways (73). Analyses of resistant tumor cell variants showed that EGFR inhibition produced *HER3* overexpression and *PTEN* deficiency, leading to hyperactivation of protumoral downstream signaling. Remarkably, concomitantly with these alterations MHC-I expression was downregulated as a consequence of transcriptional alterations in the IFN- γ pathway.

Connection between EGFR oncogene addiction and glucose metabolism

It has been shown that the EGFR prevents autophagic cell death by maintaining the intracellular glucose level, most likely through stabilizing interactions with the sodium/glucose co-transporter 1 (SGLT1) (74). Guaranteeing an active glucose transport is critical for tumor cell survival in the context of a tumor micro-environment characterized by hypoxia and nutrient starvation. Therefore, the role played by the EGFR in stabilizing the sodium/glucose co-transporters may reinforce the addiction to this oncogene.

Thinking of EGFR-targeting as a biological therapy

The classical approach of using EGFR-targeted agents as cytotoxic drugs has limited their actual potential. As has been widely documented, objective clinical responses often do not result in increase of survival time due to the rapid emergence of resistance, as occurs, for example, when using STKIs in NSCLC. We believe that targeted therapies have

the potential to stop disease progression and transform advanced cancer into a long-term controlled chronic disease based mostly on biological regulatory mechanisms.

Inhibition of EGFR activation may trigger several different mechanisms that would contribute to stop tumor progression. One of them is the induction of immunogenic cell death that then activates an anti-tumor T cell immunity. The existence and importance of this immune response have been recently demonstrated for cetuximab (69), as mentioned above. Another mechanism is the induction of anti-angiogenic effects, as has been shown for nimotuzumab (63,75).

The capability of targeting cancer stem cells and modulate their malignant cell phenotype is a third mechanism, which is particularly relevant because it may induce a long-term stable disease and have a controlling effect on metastases. The clinical experience using nimotuzumab in long-term treatments of advanced cancer patients gives certain support to this hypothesis. And last, but not least, the recently uncovered interconnection between the EGFR and glucose metabolism suggests that EGFR-targeted agents may control cell growth also by impairing the interaction between the EGFR and the glucose receptor SGLT1.

The interconnection of several cellular pathways in the oncogene addiction phenomenon opens a wide spectrum of possibilities for combinatorial therapies. In this regard, a better comprehension of the resistance mechanisms that emerge from the use of EGFR-targeted agents will provide important clues for the rational design of these therapies.

Concluding remarks

In our understanding, EGFR-targeted therapy is still an evolving concept. It is moving apart from the cytotoxic paradigm to become a biological therapy, with implications far beyond the control of tumor cell proliferation. In *Box 1* we summarize a few of the main ideas we have discussed here, together with our vision on the future progress of EGFR-targeted therapies. The design of EGFR-targeting therapies should take into account the convergence of different mechanisms and cellular pathways in the oncogene addiction phenomenon, and should be based on the concept of “personalized medicine”. Clinical trials need to incorporate more translational research, so the existing gap between basic research and clinical investigation has to be filled. One important milestone in the development of EGFR-targeted therapies is gaining a better understanding

Box 1 A vision on EGFR-targeted therapies

Oncospecific treatment such as radiotherapy and chemotherapy reinforce EGFR oncogene addiction, supporting the concept of combinatorial therapy

EGFR oncogene addiction goes beyond downstream signaling pathways, involving tumor metabolic remodeling and interconnection with the immune system

Activating mutations and protein overexpression are molecular prerequisites for EGFR oncogene addiction. Nonetheless, no single universal biomarker of this phenomenon is likely to exist

Identification of patient subpopulations likely to receive high clinical benefits from EGFR-targeted therapy would take into account tumor type, disease staging and previous treatments

Long-lasting clinical responses to EGFR-targeted therapies might require induction of immunogenic cell death and reversal of tumor-induced immunosuppression

Induction of stable disease with EGFR-targeted therapy might reflect a reversal of the malignant phenotype of cancer stem cells

Resistance mechanisms to EGFR-targeted therapies may provide important clues for the design of combinatorial therapies, which might include metabolic inhibitors, anti-inflammatory drugs and immunomodulators

Chronic use and treatment beyond early progression need to be evaluated in order to optimize the clinical benefit of EGFR-targeted therapies in advanced cancer patients

of the connection between the EGFR pathway and anti-tumor immunity.

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