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Anti-Tumour Treatment

EGFR-directed monoclonal antibodies in combination with chemotherapy for treatment of non-small-cell lung cancer: an updated review of clinical trials and new perspectives in biomarkers analysis

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ABSTRACT

Lung cancer still represents one of the most common and fatal neoplasm, accounting for nearly 30% of all cancer-related deaths. Targeted therapies based on molecular tumor features and programmed death-1 (PD-1)/programmed death ligand-1 (PDL-1) blockade immunotherapy have offered new therapeutic options for patients with advanced non-small-cell lung cancer (NSCLC). Activation of the epidermal growth factor receptor (EGFR)-pathway promotes tumor growth and progression, including angiogenesis, invasion, metastasis and inhibition of apoptosis, providing a strong rationale for targeting this pathway. EGFR expression is detected in up to 85% of NSCLC and has been demonstrated to be associated with poor prognosis. Two approaches for blocking EGFR signaling are available: prevention of ligand binding to the extracellular domain with monoclonal antibodies (mAbs) and inhibition of the intracellular tyrosine kinase activity with small molecules. There is a strong rationale to consider the tumor's level of EGFR expression as one of the most significant predictive biomarkers in this setting. In this paper we provide an update focusing on the current status of EGFR-directed mAbs use for the treatment of patients with advanced NSCLC, through a review of all clinical trials involving anti-EGFR mAbs in combination with chemotherapy (CT) for advanced disease and with chemo-radiotherapy for stage III disease. Here we also discuss the current status of predictive biomarkers for anti-EGFR mAbs when added to first-line CT in patients with advanced NSCLC. Finally, we focused on the relevance of EGFR fluorescence in situ hybridization (FISH)+ and immunohistochemistry (IHC)-Score ≥ 200 as predictive biomarkers for the selection of patients who would be most likely to derive a clinical benefit from treatment with CT in combination with anti-EGFR mAbs, with particular reference also to histology.

Introduction

Although significant improvements have been observed in the last decades regarding new treatment options, lung cancer continues to represent one of the most common and fatal neoplasms, accounting for nearly 30% of all cancer-related deaths [1]. Approximately 80% of lung cancers are histologically defined as non-small-cell lung cancer (NSCLC), including adenocarcinomas, squamous cell carcinomas (SCC) and large cell carcinomas. NSCLC is diagnosed at a late stage in up to 70% of cases and has a 5-year survival rate of around 15% in patients without targetable mutations [2]. The efficacy of chemotherapy (CT) has reached a therapeutic plateau with a median overall survival (OS) around 8–11 months. Immunotherapy has recently emerged as a promising therapeutic strategy for NSCLC. To evade the host immune surveillance, tumor cells can inactivate T-cell function by expressing ligands that act on immune checkpoints, including programmed cell death 1 (PD-1) [3]. Thus immunotherapy targeting PD-1 or its ligand (PD-L1) has emerged as a new therapeutic strategy for NSCLC, with recent applications also in the first-line setting, whether alone or in combination with chemotherapy (CT) [4–6]. Targeted therapies based on molecular tumor features have offered new treatment options for patients with advanced NSCLC [7]. Furthermore, growth factor receptor system and angiogenesis have been studied as potential therapeutic targets, either as single agent or in combination with CT.

Epidermal growth factor receptor (EGFR) is a member of the ErbB family of trans-membrane tyrosine kinase receptors. Epidermal growth

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factor (EGF) and transforming growth factor-alpha (TGF-α) are ligands that bind to the extracellular domain of EGFR, inducing a conformational change and dimerization of the receptor and leading to the activation of the intracellular tyrosine kinase [8]. Once activated, EGFR transduces its numerous cellular responses through three primary signaling cascades: the mitogen-activated protein kinase (MAPK)-pathway, the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT)-pathway and the Janus kinase/signal transducer and activator of transcription (JAK/STAT)-pathway [9]. Activation of the EGFR-pathway promotes tumor growth and progression, including angiogenesis, invasion, metastasis, promotion of proliferation and inhibition of apoptosis, providing a strong rationale for targeting this pathway. EGFR-protein expression is detected in up to 85% of NSCLC and has been demonstrated to be associated with poor prognosis [10]. There are two approaches for inhibiting EGFR signaling: prevent ligand binding to the extracellular domain with a monoclonal antibody (mAb) and inhibit the intracellular tyrosine kinase activity with a small molecule. Anti-EGFR mAbs bind to the EGFR on the surface of tumor cells and competitively block the binding of the ligand EGF; antibody receptor complexes are then internalized and degraded, leading to EGFR downregulation on the surface of tumor cells and inhibition of EGFR signaling. mAbs may also act via immunological mechanism such as antibody-dependent cellular toxicity (ADCC) [11].

In this article we provide an update of the current status of EGFR-directed mAbs and their use in treatment of patients with NSCLC, through a review of all clinical trials involving anti-EGFR mAbs in combination with CT for advanced disease and with chemo-radiotherapy (CT/RT) for stage III disease. Here we also discuss the current status of predictive biomarkers analysis for anti-EGFR mAbs when added to first-line CT in patients with advanced NSCLC.

EGFR-directed monoclonal antibodies in association with chemotherapy

Cetuximab

Cetuximab is a chimeric human-murine monoclonal IgG1 antibody [12]. It inhibits EGFR signaling through binding to the external domain of EGFR and may also act via ADCC and complement-dependent cytotoxicity [11]. Cetuximab's administration consists of an intravenous infusion with a loading dose of 400 mg/m^2, followed by weekly doses of 250 mg/m^2.

First-line treatment trials

Cetuximab combined with first-line CT has been studied in 20 phase II trials and 3 phase III trials in patients with advanced NSCLC (Table 1).

Two randomized phase II trials suggested improved efficacy of CT plus cetuximab compared to CT alone; the BMS 100 trial [16] compared cetuximab in combination with gemcitabine and cisplatin or carboplatin with CT alone for EGFR-unselected patients. Partial responses (PR) were observed in 27.7% of patients in the cetuximab arm and 18.2% in the CT alone arm; median PFS was 5.09 months in the cetuximab arm (95% CI, 4.17–5.98) *vs* 4.21 in the CT alone arm (95% CI, 3.81–5.49), median OS was 11.99 months (95% CI, 8.80–15.18) *vs* 9.26 (95% CI, 7.43–11.79) respectively.

The Lung Cancer Cetuximab Study (LUCAS) [26] compared cetuximab plus CT (cisplatin-vinorelbine) with CT alone in patients with EGFR-expressing NSCLC. The response rate (RR) was 35% *vs* 28% for cetuximab arm and CT alone arm respectively. The median PFS was 5.0 months in the cetuximab arm (95% CI, 4.5–5.8) *vs* 4.6 in the CT alone arm (95% CI, 2.5–6.0), median OS was 8.3 months (95% CI, 6.1–9.9) *vs* 7.3 (95% CI, 5.6–9.5) respectively.

In the South West Oncology Group (SWOG) S0342 trial [28] EGFR-unselected patients were randomized to receive carboplatin and paclitaxel with concurrent cetuximab or the same CT regimen followed by sequential cetuximab. The median PFS was 4.3 months in the concurrent cetuximab arm (95% CI, 3.7–4.7) *vs* 4.4 in the sequential cetuximab arm (95% CI, 4.0–5.3). Median OS was 10.9 months (95% CI, 9.2–13.0) *vs* 10.7 (95% CI, 8.5–12.8) respectively and the RR was similar in both arms, but sensory neuropathy was higher in the concurrent arm compared to the sequential arm (15% *vs* 5% respectively, p = 0.036).

Based on the encouraging results of the phase II trials, 3 phase III randomized trials were designed in order to confirm and validate the efficacy of cetuximab in combination with CT as first-line treatment of patients with advanced NSCLC.

The BMS 099 trial [27] compared cetuximab plus carboplatin and taxane with CT alone for EGFR-unselected patients. The primary endpoint was PFS assessed by a blinded Independent Radiology Review Committee (IRRC). When assessed by the investigators PFS was significantly longer in the cetuximab arm (median 4.3 *vs* 3.78 months, HR: 0.77, p = 0.0015), but there wasn't a difference between the two arms when assessed by the IRRC (median 4.4 *vs* 4.2 months, HR: 0.90, p = 0.24). The reasons for discrepancy between the IRRC and the investigators results are not clear. The overall agreement rate between IRRC and investigators was 72.5% and 69.5% for the cetuximab arm and CT arm alone respectively. For unknown reasons in the IRRC data set the percentage of censored events was higher in the CT arm alone than in the cetuximab arm (28.9% *vs* 29.0% respectively).

The FLEX trial [20] compared cetuximab added to first-line CT (cisplatin-vinorelbine) with CT alone in patients with advanced EGFR-protein positive NSCLC (in at least one positively stained tumor cells). A total of 1.861 patients were screened, of them 1.688 (90.7%) with tumor specimens suitable for assessment of EGFR expression and 1.442 (77.5%) with EGFR-expressing tumors. This trial met its primary endpoint (OS), demonstrating superior survival for CT plus cetuximab compared to the CT alone population (HR: 0.87 [95% CI 0.762–0.996; p = 0.044], median 11.3 *vs* 10.1 months). While PFS was not significantly different (HR: 0.94 [95% CI 0.825–1.077; p = 0.39], median 4.8 *vs* 4.8 months), the time-to-treatment failure favored the cetuximab arm (HR: 0.86, 95% CI 0.76–0.97, p = 0.015).

The SWOG S0819 trial [51] was an open-label, phase III study, designed to evaluate the effectiveness of cetuximab in addition to carboplatin-paclitaxel or carboplatin-paclitaxel-bevacizumab as first-line treatment for EGFR-unselected patients affected by advanced NSCLC. This study also assessed EGFR fluorescence in situ hybridization (FISH) as a potentially predictive biomarker for cetuximab in this population [53]. There was no difference in the survival distribution between the arms in the entire study population (HR: 0.93 [95% CI 0.83–1.04; p = 0.22], median 10.9 *vs* 9.2 months for cetuximab arm and control arm respectively). PFS in the EGFR FISH-positive (FISH+) sub-population was also not significantly different between the treatment arms (HR: 0.92 [95% CI 0.75–1.12; p = 0.40], median 5.4 *vs* 4.8 months for cetuximab arm and control arm respectively).

Maintenance treatment trials

A recent meta-analysis [54] evaluated efficacy of first-line therapies with maintenance regimens for advanced NSCLC in EGFR-selected patients. In EGFR FISH+ patients with squamous cell carcinoma (SCC), first-line CT plus cetuximab with cetuximab maintenance demonstrated a combined clinically meaningful OS and PFS benefit, with OS HR 0.56 (95% CI, 0.35–0.89) and 99% probabilities of outperforming standard chemotherapy with no maintenance. On the contrary, for EGFR FISH-positive and unselected histology patients, first-line CT plus cetuximab with cetuximab maintenance did not demonstrate a clinically meaningful OS or PFS benefits.

Phase IIIb NEXT trial [40] explored the efficacy and safety of two different cetuximab maintenance therapy schedules in EGFR-unselected progression-free patients after 4–6 cycle of first-line CT plus cetuximab: these schedules consisted of weekly administration at 250 mg/m^2 and every 2 weeks administration at 500 mg/m^2. The 1-year survival rate

Table 1
Clinical studies of combined anti-EGFR antibodies and chemotherapy for non-small-cell lung cancer (EGFR: epidermal growth factor receptor; OS: overall survival; PFS: progression-free survival; NA: not applicable; MTD: maximum-tolerated dose; DE: dose-escalation: PK: pharmacokinetics; WT: wild-type; RR: response rate; ECOG PS: eastern cooperative oncology group performance status; TTP: time-to-progression; ORR: overall response rate; CT: chemotherapy; FISH: fluorescence in situ hybridization).

Trial	First author, year	Study phase	Setting	Sample size	Population	Regimen	Primary outcome	OS (months)	PFS (months)
NA	Robert, 2005 [13]	I/IIa	1st line	35	EGFR+	Cetuximab + carboplatin-gemcitabine	Safety, toxicities	10.3	5.3
NA	Thienelt, 2005 [14]	I/II	1st line	31	EGFR+	Cetuximab + carboplatin-paclitaxel	Safety	11.0	5.0
NA	Kollmannsberger, 2006 [15]	I	All lines	18	EGFR+	Matuzumab + paclitaxel	MTD, safety, tolerability	NA	NA
BMS 100	Butts, 2007 [16]	II	1st line	131	Unselected	Arm A: cetuximab + cisplatin/carboplatin-gemcitabine Arm B: cisplatin/carboplatin-gemcitabine	RR	11.99 *vs* 9.26	5.09 *vs* 4.21
NA	Belani, 2008 [17]	II	1st line	80	Unselected	Cetuximab + carboplatin-docetaxel	RR	10.3	4.6
OPN-017	Borghaei, 2008 [18]	II	1st line	53	Unselected	Cetuximab + carboplatin-paclitaxel	RR	13.8	5.53
NA	Blumenschein, 2009 [19]	Ib	1st, 2nd line	45	Unselected	Arm A: motesanib DE + carboplatin-paclitaxel Arm B: motesanib DE + panitumumab + carboplatin-paclitaxel Arm C: motesanib 125 mg/m^2 + panitumumab + carboplatin-paclitaxel	MTD, safety, PK	NA	NA
FLEX	Pirker, 2009 [20]	III	1st line	1.125	EGFR+	Arm A: cetuximab + cisplatin-vinorelbine Arm B: cisplatin-vinorelbine	OS	11.3 *vs* 10.1	4.8 *vs* 4.8
CALGB 30402	Lilenbaum, 2009 [21]	II	1st line	59	ECOG PS: 2	Arm A: cetuximab + docetaxel Arm B: bortezomib + docetaxel	PFS	5.0 *vs* 3.9	3.4 *vs* 1.9
NA	Stinchcombe, 2009 [22]	II	1st line	57	Unselected	Cetuximab + carboplatin	RR	8.2	2.9
NA	Socinski, 2009 [23]	II	1st line	168	Unselected	Arm A: cetuximab + carboplatin AUC6 every 3 weeks-paclitaxel 225 mg/m^2 every 3 weeks Arm B: cetuximab + carboplatin AUC6 every 4 weeks-paclitaxel 100 mg/m^2 days 1, 8, 15 every 3 weeks	PFS	11.4 *vs* 9.8	4.7 *vs* 4.3
NA	Jalal, 2009 [24]	I/II	< 2nd+ line	36	Unselected	Cetuximab + pemetrexed	TTP	9.7	3.4
NA	Kim, 2009[25]	II	2nd line	55	EGFR+	Cetuximab + docetaxel	RR	7.5	2.7
LUCAS	Rosell, 2008[26]	II	1st line	86	EGFR+	Arm A: cisplatin-vinorelbine Arm B: cetuximab + cisplatin-vinorelbine	ORR	7.3 *vs* 8.3	4.6 *vs* 5.0
BMS 099	Lynch TJ, 2010 [27]	III	1st line	676	Unselected	Arm A: cetuximab + carboplatin-paclitaxel/docetaxel Arm B: carboplatin-paclitaxel/docetaxel	PFS	9.7 *vs* 8.4	4.4 *vs* 4.2
SWOG S0342	Herbst, 2010 [28]	II	1st line	224	Unselected	Arm A: carboplatin-paclitaxel + concurrent cetuximab Arm B: carboplatin-paclitaxel + sequential cetuximab	OS	10.9 *vs* 10.7	4.3 *vs* 4.4
NA	Schiller, 2010 [29]	II	2nd line	150	Unselected	Arm A: pemetrexed Arm B: pemetrexed + matuzumab 800 mg every week Arm C: pemetrexed + matuzumab 1.600 mg every 3 weeks	RR	7.9 *vs* 12.4 *vs* 5.9	2.7 *vs* 2.3 *vs* 2.5
CALC1- E	Gridelli, 2010 [30]	II	1st line	58	Age ≥ 70, ECOG PS < 2	Arm A: gemcitabine + concurrent cetuximab Arm B: gemcitabine + sequential cetuximab	1 year-survival rate	5.5 *vs* 8.3	2.8 *vs* 3.7
CALC1-PS2	Gridelli, 2010 [30]	II	1st line	42	ECOG PS :2, Age < 70	Arm A: gemcitabine + concurrent cetuximab Arm B: gemcitabine + sequential cetuximab	1 year-survival rate	9.2 *vs* 6.0	5.3 *vs* 2.1
NA	Spigel, 2010 [31]	II	1st line	69	Unselected	Cetuximab + docetaxel-gemcitabine	ORR	9.4	4.0
TaxErb	Fischer, 2012 [32]	II	1st line	75	Unselected	Cetuximab + carboplatin-docetaxel	ORR	12.9	4.8
NA	Qi, 2012 [33]	II	1st line	41	Unselected	Arm A: nimotuzumab + carboplatin-paclitaxel liposome Arm B: carboplatin-paclitaxel liposome	ORR	NA	6.9 *vs* 5.7
SWOG S0536	Kim, 2013 [34]	II	1st line	102	Non-squamous histology	Cetuximab + bevacizumab + carboplatin-paclitaxel × 6, then maintenance with cetuximab + bevacizumab	Safety	15.0	7.0
NA	Schmid-Bindert, 2013 [35]	II	1st line	113	Non-squamous histology	Cetuximab + cisplatin-pemetrexed × 4–6, then maintenance with cetuximab + pemetrexed	ORR	11.3	5.8
NA	Bonomi, 2013 [36]	II	1st line	121	Non-squamous histology	Arm A: cetuximab + bevacizumab × 6 + carboplatin-paclitaxel × 6 Arm B: cetuximab + bevacizumab × 6 + carboplatin-paclitaxel × 3	PFS	12.06 *vs* 11.63	6.05 *vs* 4.50

(*continued on next page*)

Table 1 (*continued*)

Trial	First author, year	Study phase	Setting	Sample size	Population	Regimen	Primary outcome	OS (months)	PFS (months)
SELECT	Kim, 2013 [37]	III	2nd line	938	Unselected	Arm A: pemetrexed + cetuximab Arm B: pemetrexed	PFS	6.9 *vs* 7.8	2.9 *vs* 2.8
NA	Crawford, 2013 [38]	II	1st line	194	EGFR+	Induction therapy with carboplatin-paclitaxel + panitumumab × 6, then: Arm A: carboplatin-paclitaxel + panitumumab × 6, then maintenance with panitumumab Arm B: carboplatin-paclitaxel × 6	Toxicities, TTP	8.5 *vs* 8.1	4.1 *vs* 4.2
NA	Babu, 2014 [39]	II	1st line	110	Unselected	Arm A: nimotuzumab + carboplatin-paclitaxel Arm B: carboplatin-paclitaxel	ORR	10.1 *vs* 10.4	4.9 *vs* 4.8
NEXT	Heigener, 2014 [40]	IIIb	Maintenance	311	Unselected	Induction therapy with cetuximab + platinum-based CT × 4–6, then: Arm A: maintenance cetuximab every 2 weeks Arm B: maintenance weekly cetuximab	OS	12.6 *vs* 12.6	2.6 *vs* 2.8
CHAMP	Schuette, 2015 [41]	II	1st line	98	Non-squamous, KRAS WT	Arm A: panitumumab + cisplatin-pemetrexed Arm B: cisplatin-pemetrexed	PFS at 6 months	NA	NA
NA	Qi, 2015 [42]	II	1st line	59	Unselected	Arm A: nimotuzumab + carboplatin-docetaxel Arm B: carboplatin-docetaxel	ORR	NA	7.9 *vs* 6.3
INSPIRE	Paz-Ares, 2015 [43]	III	1st line	633	Non-squamous histology	Arm A: necitumumab + cisplatin-pemetrexed Arm B: cisplatin-pemetrexed	OS	11.3 *vs* 11.5	5.6 *vs* 5.6
SQUIRE	Thatcher, 2015 [44]	III	1st line	1.093	Squamous histology	Arm A: necitumumab + cisplatin-gemcitabine Arm B: cisplatin-gemcitabine	OS	11.5 *vs* 9.9	5.7 *vs* 5.5
CERTO	Vansteenkiste, 2015 [45]	II	1st line	169	EGFR+	Arm A: cetuximab + platinum-based CT Arm B: cetuximab + platinum-based CT + cilengitide	PFS	13.2 *vs* 11.8	6.8 *vs* 5.6
ECOG E4508	Hanna, 2015 [46]	II	1st line	140	Unselected	Arm A: cetuximab + carboplatin-paclitaxel Arm B: cixutumumab + carboplatin-paclitaxel Arm C: cetuximab + cixutumumab + carboplatin-paclitaxel	PFS	9.8 *vs* 7.7 *vs* 8.8	3.4 *vs* 4.2 *vs* 4.0
INN06	Hilbe, 2015 [47]	II	Induction CT	41	Unselected	Cetuximab + cisplatin-docetaxel × 2	Radiological response	NA	22.5
NA	Spigel, 2017 [48]	II	1st line	167	Squamous histology	Arm A: necitumumab + carboplatin-paclitaxel Arm B: carboplatin-paclitaxel	ORR	5.4 *vs* 5.6	13.2 *vs* 11.2
NA	Spigel 2017 [49]	II	1st line	66	Non-squamous, KRAS WT	Panitumumab + carboplatin-pemetrexed	ORR	6.0	NA
NA	Thomas 2017 [50]	II	1st line	90	Unselected	Arm A: BTH1677 + cetuximab + carboplatin-paclitaxel Arm B: cetuximab + carboplatin-paclitaxel	ORR	10.3 *vs* 12.4	4.3 *vs* 4.4
SWOG S0819	Herbst, 2017 [51]	III	1st line	1.313	Unselected	Arm A: cetuximab + carboplatin-paclitaxel (± bevacizumab) Arm B: carboplatin-paclitaxel (± bevacizumab)	PFS (FISH + patients), OS	10.9 *vs* 9.2	4.6 *vs* 4.5

was 62.8% (95% CI, 54.7–70.0) for every 2 weeks cetuximab and 64.4% (95% CI, 56.2–71.4) for weekly cetuximab; OS was 12.6 months (95% CI, 10.1–14.8) for every 2 weeks cetuximab and 12.6 months (95% CI, 9.3–16.0) for weekly cetuximab; PFS was 2.6 months (95% CI, 2.1–2.8) for every 2 weeks cetuximab and 2.8 months (95% CI, 2.6–4.0) for weekly cetuximab. Safety profiles were similar, manageable and in line with expectations. Authors concluded that both weekly and every 2 weeks cetuximab maintenance therapy were associated with similar survival outcomes and safety profiles.

Second-line and beyond treatment trials

Cetuximab in combination with CT has been studied also in the setting of second-line and beyond treatment. In particular, 2 phase II trials and 1 phase III trial assessed the efficacy of cetuximab in combination with mono-chemotherapy with docetaxel or pemetrexed.

A phase II study of cetuximab in combination with docetaxel was performed by Kim and Colleagues [25] in patients with evidence of EGFR expression (≥1+). ORR was 20% (95% CI, 10.4–33.0%) and median time-to progression (TTP) was 104 days. Median OS was 7.5 months (95% CI, 6.7–12.0) with a 1-year survival of 35%; median PFS was 2.7 months (95% CI, 1.8–4.4).

The combinations of cetuximab and pemetrexed or docetaxel was then assessed in the phase III SELECT-trial [37]. In this unmasked, open-label, randomized trial EGFR-unselected patients with NSCLC previously treated with one line of platinum-based CT were randomly assigned to receive either pemetrexed or docetaxel and then randomly assigned within each group to receive their CT with or without cetuximab until disease progression or unacceptable toxicity. Median PFS was 2.9 (95% CI, 2.7–3.2) *vs* 2.8 months (95% CI, 2.5–3.3) for cetuximab plus pemetrexed arm and pemetrexed arm respectively (HR: 1.03 [95% CI 0.87–1.21; p = 0.76]); median OS was 6.9 (95% CI, 6.3–7.9) *vs* 7.8 months (95% CI, 6.8–8.4) for cetuximab plus pemetrexed arm and pemetrexed arm respectively (HR: 1.01 [95% CI 0.86–1.20; p = 0.86]). Median PFS was 2.4 (95% CI, 1.6–2.9) *vs* 1.5 months (95% CI, 1.5–2.5) for cetuximab plus docetaxel arm and docetaxel arm respectively (HR: 0.91 [95% CI 0.73–1.13; p = 0.39]); median OS was 5.8 (95% CI, 4.7–8.3) *vs* 7.8 months (95% CI, 6.1–9.2) respectively (HR: 1.13 [95% CI 0.90–1.41; p = 0.31]). A significantly higher proportion of patients in the cetuximab plus pemetrexed group experienced at least one serious adverse event than those patients in the pemetrexed group (41 *vs* 29% respectively, p = 0.0054). Authors concluded that the use of cetuximab is not recommended in combination with CT in previously treated patients.

Induction treatment trials

Since the addition of cetuximab to standard platinum-based CT significantly improved efficacy in advanced stage NSCLC, it seemed reasonable to also evaluate a combination therapy with cetuximab and chemotherapy in earlier stages.

The INN06-study [47] was a multicenter phase II trial evaluating 2 cycles of combination therapy with cetuximab and cisplatin-docetaxel as induction regimen prior to surgery in CT-naïve patients with NSCLC stage IB-IIIA, without selection for EGFR-expression status. 20 patients (51.3%) achieved a PR, 17 (43.6%) a stable disease (SD), 2 (5.1%) were not evaluable. 37 patients (94.9%) underwent surgery; 29 (78.4%) showed a stage-shift between initial evaluation and pathological staging and 8 patients (21.6%) remained stable; in 22 patients (59.4%) a pathological down-staging was achieved, in 7 patients (18.9%) the pathological stage was higher as originally defined by comprehensive staging. In 3 patients pathological complete response (CR) was observed (8%). At the time of final analysis (2013, December), median PFS was 22.5 months (95% CI, 10.3–34.7), median OS has not been reached and 5-years OS was 58%.

Other combinations

Although this article is essentially focused on combination between CT and EGFR-directed monoclonal antibodies, it's worth highlighting other possible drug combinations. In particular, the simultaneous use of cetuximab plus afatinib has recently emerged as a new possibility for the treatment of patients with advanced NSCLC harboring mutated EGFR after progression on first-generation EGFR tyrosine-kinase inhibitors (erlotinib or gefitinib), with consequently acquired resistance to TKIs [55]. A study conducted in transgenic mice with $EGFR^{L858R+T790M}$-induced lung adenocarcinomas demonstrated that that this combination of cetuximab and afatinib could overcome T790M-mediated resistance [56]. Based on these data, a phase IB/II was conducted evaluating the same association between cetuximab and afatinib in patients affected by EGFR-mutant advanced NSCLC, who progressed on erlotinib or gefitinib [57]. The rate of disease control was 94%, with objective response rate in 40%; median PFS was 4.7 months and the median duration of response was 7.7 months; response occurred in patients with or without T790M mutations. In the same study, a separate cohort exploring afatinib plus cetuximab after progression with afatinib is reported [58]: 36 patients transitioned to the combination therapy; 4 patients (11%) responded, median PFS was 2.9 months. Median PFS with afatinib plus cetuximab for patients who received afatinib monotherapy for ≥12 *vs* < 12 weeks was 4.9 *vs* 1.8 months (p = 0.0354) and for patients with T790M-positive *vs* T790M-negative tumors was 4.8 *vs* 1.8 months (p = 0.1306). In another study conducted using mice with TKI-naïve $EGFR^{L858R}$-induced lung adenocarcinoma, it was demonstrated that afatinib plus cetuximab delayed the time to relapse and the incidence of drug-resistance compared to single agent erlotinib or afatinib [59]. Antitumor efficacy of dual blockade of EGFR signaling by osimertinib in combination with cetuximab or selumetinib was evaluated in activated EGFR human NSCLC tumor models [60]: the addition of cetuximab or selumetinib to osimertinib in second-line treatment reverted the sensibility to osimertininb in the majority of mice, with a RR of 50% to 80% and a median PFS of first- plus second-line of therapy of 28 weeks; the early use of combinations in first-line therapy increased the RR to 90%. Given these promising results, two randomized phase II/III trials of afatinib plus cetuximab *vs* afatinib alone in treatment-naïve patients with advanced EGFR-mutant NSCLC are ongoing by the South West Oncology Group (SWOG S1403) and the Intergroupe Francophone de Cancerologie Thoracique (IFCT-1503 ACE-Lung study).

Necitumumab

Necitumumab (IMC-11F8) is a fully humanized IgG1 monoclonal antibody directed against EGFR. Whereas the mechanisms of inhibition of EGFR activation are close between necitumumab and cetuximab, the major difference concerns the variable domains of the antibody. The variable region of cetuximab is of mouse origin, while the variable domains of necitumumab are of human origin; consequently, treatment with necitumumab is expected to be associated with less hypersensitivity reactions and toxicity [61]. Necitumumab, as well as cetuximab, is fully human IgG1 and can induce ADCC.

First-line treatment trials

Two large parallel phase III trials with similar designs have been conducted in patients with NSCLC, according to histological subtype and include the INSPIRE trial [43] for non-SCC NSCLC and the SQUIRE trial [44] for SCC.

The INSPIRE-trial [43] was a randomized, open-label, controlled phase III study in patients with previously untreated, stage IV non-SCC NSCLC, without any selection for EGFR expression status. Patients received CT with cisplatin + pemetrexed for a maximum of 6 cycles alone, or in combination with necitumumab. Enrollment in this trial was stopped after 633 patients enrolled following an Independent Data Monitoring Committee (IDMC) recommendation based on an observed higher recurrence rate of fatal thromboembolic events in the necitumumab-containing arm. There was no significant difference in OS

between treatment groups, with a median OS of 11.3 months (95% CI, 9.5–13.4) in the necitumumab plus cisplatin-pemetrexed group *vs* 11.5 months (95% CI, 10.1–13.1) in the cisplatin-pemetrexed group (HR: 1.01 [95% CI 0.84–1.21; p = 0.96]). Median PFS was 5.6 months (95% CI, 5.1–6.0) in the necitumumab plus cisplatin-pemetrexed group *vs* 5.6 months (95% CI, 4.8–5.7) in the cisplatin-pemetrexed group (HR: 0.96 [95% CI 0.80–1.16; p = 0.66]).

The second study (SQUIRE) [44] was a randomized, open-label, controlled phase III study with patients with previously untreated, stage IV squamous NSCLC, without any selection for EGFR expression status. Patients received CT with cisplatin + gemcitabine for a maximum of 6 cycles alone, or in combination with necitumumab. In contrast to the INSPIRE-trial, in this study, the primary end-point was met: median OS was of 11.5 months (95% CI, 10.4–12.6) in the necitumumab plus CT group *vs* 9.9 months (95% CI, 8.9–11.1) in the CT alone group (HR: 0.84 [95% CI 0.74–0.96; p = 0.01]). Median PFS was 5.7 months (95% CI, 5.6–6.0) in the necitumumab plus CT group *vs* 5.5 months (95% CI, 4.8–5.6) in the CT alone group (HR: 0.85 [95% CI 0.74–0.98; p = 0.02]). Whereas the ORR didn't statistically differ between the two arms, the disease control rate (DCR) was significantly higher in patients treated with necitumumab (82% *vs* 77%, p = 0.04).

A recent retrospective analysis [52] of the SQUIRE trial investigated the efficacy and safety of single-agent necitumumab continuation therapy until progressive disease, comparing patients treated with necitumumab monotherapy after completion of ≥4 cycles of CT (necitumumab continuation) with those in the CT arm who were progression-free and did not discontinue because of adverse events after completion of ≥4 cycles of CT (cisplatin + gemcitabine non-progressors). Median OS from randomization in the necitumumab continuation *vs* cisplatin + gemcitabine non-progressors arm was 15.9 *vs* 15.0 months (HR: 0.82 [CI 95% 0.69–1.05]) and median PFS from randomization was 7.4 *vs* 6.9 months (HR: 0.86 [CI 95% 0.70–1.06]). OS and PFS benefits were similar when assessed from the post-induction period and in EGFR-expressing patients.

Other EGFR-directed monoclonal antibodies

Panitumumab

Panitumumab is a fully human IgG2 monoclonal antibody against EGFR, which has been shown to have activity as a single agent and in combination with CT for the treatment of metastatic colorectal cancer, but is not recommended for use in patients whose tumors have mutations in codons 12 or 13 of KRAS [62]. The use of panitumumab in NSCLC was evaluated in a two-part phase II study [38] assessing the efficacy and safety of carboplatin-paclitaxel with or without panitumumab in previously untreated patients with advanced NSCLC and with 10% or more cells expressing EGFR. TTP was 18.1 weeks (95% CI, 13.6–23.3) in panitumumab plus CT arm *vs* 23.0 weeks (95% CI, 15.9–24.1) in CT arm (HR: 0.90 [95% CI 0.66–1.21; p = 0.555]). Median PFS was 17.6 weeks (95% CI, 11.7–22.4) for panitumumab plus CT arm *vs* 18.3 weeks (95% CI, 13.4–23.7) for CT; median OS was 37.0 weeks (95% CI, 30.9–52.1) *vs* 35.0 weeks (95% CI, 29.3–51.4) respectively; ORR was 15.2% *vs* 11.1%. Authors concluded that although toxicity was predictable and manageable, the addition of panitumumab to CT did not improve TTP in patients with previously untreated advanced NSCLC.

The randomized phase II CHAMP-trial [41] evaluated the activity of panitumumab in combination with cisplatin and pemetrexed in the first-line treatment of patients with advanced non-SCC KRAS-wild-type (WT). Patient enrollment had to be stopped prematurely because of the incidence of unacceptable side effects that occurred during treatment with panitumumab and chemotherapy; they included hematologic events such as anemia, leukopenia and thrombocytopenia; the most frequently occurring non-hematologic adverse events in the panitumumab arm were nausea, vomiting, dyspnea and skin toxicity.

Same results in terms of toxicity were obtained in another trial with panitumumab added to standard combination CT as first-line treatment for patients with advanced KRAS-WT non-SCC NSCLC [48]. 29 of 66 patients (44%) had objective responses, median TTP was 6 months, median OS was 17 months. Panitumumab increased treatment-related toxicity, notably skin rah. Authors concluded that the addition of panitumumab increased toxicity and had no discernible impact of efficacy.

Matuzumab

Matuzumab is a humanized monoclonal antibody of the immunoglobulin IgG1-subclass that binds selectively to EGFR and inhibits ligand-mediated activation. In contrast to the chimeric antibody cetuximab, matuzumab has a prolonged half-life of 6–8 days and doesn't induce auto-antibody. Besides blocking ligand-binding and subsequently inhibiting signal transduction, in xenograft models matuzumab has also shown the ability to attract immunocompetent cells by ADCC [63]. Matuzumab was evaluated in lung cancer in one phase I trial in combination with paclitaxel and in one phase II trial.

The phase II randomized trial [29] investigated matuzumab in combination with pemetrexed compared with pemetrexed alone as second-line therapy for EGFR-unselected patients with advanced NSCLC. In this study, matuzumab was administered at either 800 mg weekly dose or 1.600 mg every 3 weeks dose. ORR for the matuzumab-treated arms was 11% compared with 5% for pemetrexed alone (p = 0.332); apart from one patient in the pemetrexed alone group, all responses occurred in patients whose tumors expressed EGFR. The ORR for patients receiving weekly matuzumab was 16% (95% CI, 7–29) compared with 2% (95% CI, 0–11) for those receiving matuzumab every 3 weeks. DCR was similar in the different treatment groups (33% *vs* 36% *vs* 34%, respectively). Median PFS time was similar for all three treatment groups: 2.7 months (95% CI, 1.6–4.4) for the pemetrexed alone group, 2.3 (95% CI, 1.5–3.8) and 2.5 months (95% CI, 1.4–2.9) for those receiving matuzumab weekly or every 3 weeks, respectively. Median OS was 7.9 months (95% CI, 7.2–9.9) for the pemetrexed alone group, 12.4 (95% CI, 8.8, not evaluable) and 5.9 months (95% CI, 3.6–7.2) for those receiving matuzumab weekly or every 3 weeks, respectively.

Nimotuzumab

Nimotuzumab is a humanized anti-EGFR mouse monoclonal antibody designed to reduce immunoreactivity, with a slower rate of clearance from the body [64]. Nimotuzumab was evaluated in 3 phase II trials in NSCLC in combination with CT.

The trial of Babu and Colleagues [39] was a multicenter, open-label, randomized study evaluating the safety and efficacy of nimotuzumab in combination with carboplatin-docetaxel *vs* CT alone in EGFR-unselected patients with stage IIIB/IV NSCLC. CR was achieved in 2 patients each in the nimotuzumab (4%) and control groups (3.6%), while PR was achieved in 25 (50%) and 17 (30.9%) of patients in the nimotuzumab and control groups respectively. Statistically significant differences in ORR were observed between the nimotuzumab and control groups (54%, 95% CI 40.2–67.8 *vs* 34.5%, 95% CI 22–47.1, p = 0.04). The difference in OS was not statistically significant (10.1 months, 95% CI 8.1–13.6 *vs* 10.4 months, 95% CI 7.4–13.7, p = 0.48; HR: 0.844, 95% CI 0.529–1.35). Similarly, the difference in PFS was not statistically significant between the two arms (4.9 months, 95% CI 4.5–6.9 *vs* 4.8 months, 95% CI 0.533–1.222 for the nimotuzumab and control arm, respectively).

Another phase II trial[42] evaluated the same combination of nimotuzumab and carboplatin-docetaxel, with similar design and results: the total effective rate (ER) and DCR for nimotuzumab-containing arm were 36.7% and 86.7%, respectively. The ER and DCR for CT alone-arm were 27.6% and 82.8%, respectively. The difference between ER and DCR in the two groups was not statistically significant (p = 0.321 and p = 0.478, respectively). PFS was 7.9 *vs* 6.3 months for nimotuzumab-containing arm and CT alone-arm, respectively.

Meta-analysis

Results of previously described clinical trials have shown, with the exceptions of the INSPIRE [43] and CHAMP [41] trials, that the addition of EGFR-mAbs to CT is both tolerable and feasible; however, efficacy data were not clear and some trials didn't confirm a survival benefit for this kind of treatment. These conflicting results impeded the interpretation and translation of EGFR-mAbs to clinical practice. Therefore, a systemic review and meta-analysis was conducted to evaluate the efficacy and safety of addition of EGFR-mAbs to CT, compared with CT alone in patients with NSCLC [65]. Among the potentially eligible trials, 9 studies with 4.949 patients were included in the meta-analysis. In general, compared with CT alone, the addition of EGFR-mAbs significantly improved OS, with HR: 0.91 (95% CI, 0.86–0.97, p = 0.006), PFS, with HR: 0.83 (95% CI, 0.87–0.98, p = 0.01), RR, with odds radio (OR): 1.28 (95% CI, 1.12–1.47, p = 0.0003), DCR, with OR: 1.17 (95% CI, 1.01–1.36, p = 0.04). In contrast to first-line setting, combination of EGFR-mAbs with second-line CT failed to provide additional survival benefit, with HR: 1.03 (95% CI, 0.88–1.17, p = 0.66). In general, the toxicities of the combination strategy were tolerable and manageable.

In conclusion, the addition of EGFR-mAbs to CT provided superior clinical benefit along with acceptable toxicities to patients with advanced NSCLC. Following these results, in order to optimize the benefit effects of this kind of targeted therapy, research focused on the characterization of predictive biomarkers for the selection of those patients who will derive a substantial benefit from the addition of EGFR-mAbs to CT.

Predictors of response

Biomarker analysis in NSCLC is challenging because of several factors related to the heterogeneity of this kind of tumor, small sample availability and different prevalence of characteristic molecular patterns within a tumor. Several common histological subtypes exist and the effect of individual biomarkers in these subtypes can be different [66]. Because anti-EGFR mAb activity is mechanistically linked to direct interaction with the EGFR and because high levels of EGFR expression correlate with sensitivity to anti-EGFR mAbs *in vitro* [67], there is a strong rationale to consider the tumor's level of EGFR expression as one of the most significant predictive biomarkers in this setting. Furthermore, a high level of EGFR expression is likely to indicate tumor dependency on EGFR signaling and therefore sensitivity to anti-EGFR mAbs [68]. Tumors have to be considered as EGFR IHC+ when at least one membrane staining positive cell is identified [69]; in particular, the membrane staining may be scored in four different categories, including no staining (0), weak staining (1+, light brown membrane staining, visible only with high magnification), intermediate staining (2+, between 1+ and 3+) and strong staining (3+, dark-brown linear membrane staining, visible just with low magnification). The H-Score system is used to generate a semi-quantitative score, ranging from 0 to 300 and is calculated with the following formula: 1 × (percentage of 1+ cells) + 2 × (percentage of 2+ cells) + 3 × (percentage of 3+ cells). According to the "Colorado EGFR Scoring System" [70] (Table 2), tumors are considered as EGFR FISH+ if they harbor 4 or more copies of EGFR in ≥40% of cells, or if they show EGFR amplification (defined as gene-to-chromosome ratio ≥2 or presence of gene cluster or ≥15 gene copies in ≥10% of cells), all other tumors have to be classified as EGFR FISH.

In a retrospective analysis [71] of the FLEX trial's [20] results (Table 3), Authors investigated the predictive and prognostic use of four tumor-associated molecular characteristics (KRAS mutation, increased EGFR copy number, EGFR mutation and PTEN expression status) linked to the EGFR signaling pathway. Comparisons of treatment outcomes between the two groups (CT with or without cetuximab) according to KRAS tumor mutation status (mutant *vs* wild-type) provided no indication that this biomarker was predictive for the efficacy of CT plus cetuximab in relation to OS [HR: 1.00 (0.60–1.66), p = 1.00], PFS [HR: 0.84 (0.50–1.40), p = 0.5], or RR [HR: 2.11 (0.76–5.88), p = 0.15]. Also EGFR copy number assessed by FISH (positive *vs* negative) was not predictive for the efficacy of CT plus cetuximab in relation to OS [HR: 0.85 (0.56–1.29), p = 0.44], PFS [HR: 0.80 (0.51–1.25), p = 0.33], or RR [HR: 1.62 (0.70–3.76), p = 0.26]. EGFR mutation status (positive *vs* negative) was not predictive for the efficacy of CT plus cetuximab in relation to OS [HR: 1.48 (0.77–2.85), p = 0.24], PFS [HR: 0.92 (0.53–1.60), p = 0.76] and RR [HR: 1.36 (0.50–3.70), p = 0.55]. Finally, PTEN expression status (positive *vs* negative) was also not predictive for the efficacy of chemotherapy plus cetuximab in relation to OS [HR: 0.92 (0.61–1.39), p = 0.68], PFS [HR: 1.03 (0.66–1.61), p = 0.88] and RR [HR: 0.72 (0.31–1.68), p = 0.45]. Another retrospective analysis [72] of data from the FLEX study showed that high EGFR expression, as determined by IHC, could be considered a tumor biomarker that can predict survival benefit from the addition of cetuximab to first-line treatment: for patients in the high EGFR expression group (IHC-Score ≥ 200), OS was longer in the CT plus cetuximab arm than in the CT alone arm (HR: 0.73 [95% CI 0.58–0.93; p = 0.011], median 12.0 *vs* 9.6 months). No corresponding survival benefit was observed for patients in the low EGFR expression group (IHC-Score < 200) (HR: 0.99 [95% CI 0.84–1.16; p = 0.88], median 9.8 *vs* 10.3 months). A sensitivity analysis in the higher EGFR expression group using the Cox proportional hazards model with adjustment for selected baseline variables resulted in an HR of 0.67 (95% CI, 0.52–0.87, p = 0.002). In the low EGFR expression group, a similar sensitivity analysis resulted in an HR of 0.97 (95% CI, 0.82–1.15). A third analysis [73] assessed whether the activity of cetuximab in combination with CT was modulated by the EGFR mutation status of tumors. For patients with EGFR-WT tumors, a survival benefit associated with the addition of cetuximab to CT was apparent in the high EGFR expression group, with HR: 0.76 (95% CI, 0.57–1.00). However, no apparent benefit was observed in the low EGFR expression group for patient with EGFR-WT tumors, with HR: 0.98 (95% CI, 0.82–1.18); PFS and TTF were similar across treatment and EGFR expression groups for patients with EGFR WT tumors. In the high EGFR expression group, patients with tumor EGFR mutations may also have derived a survival benefit for the addition of cetuximab to CT. Conversely, in the low EGFR expression group of both mutation subgroups, the addition of cetuximab to CT seemed to be associated with shorter survival compared with chemotherapy alone. Authors concluded that the survival benefit from the addition of cetuximab to CT in the high EGFR expression group is regardless of EGFR mutation status.

Relationships between biomarker status, in particular KRAS and EGFR mutation by direct sequencing, EGFR protein expression by IHC and EGFR gene copy number by FISH, and clinical outcomes (OS, PFS and RR) were also assessed in a retrospective correlative analysis [74] of the results of the BMS 099 trial [27]. Adding cetuximab to CT did not significantly affect median OS in patients with KRAS WT tumors (HR:

Table 2
Interpretation of the EGFR FISH results according to the "Colorado Scoring Criteria" [70] (EGFR: epidermal growth factor receptor; FISH: fluorescence in situ hybridization; CEP7: centromeric protein 7).

Score	Observation	Features	Fish result
1	Disomy	< 40% of cells displaying ≥ 4 copies of the EGFR signal AND EGFR/CEP7 ratio < 2 AND < 10% of cells displaying ≥ copies of EGFR	NEGATIVE
2	Low trisomy		
3	High trisomy		
4	Low polysomy	≥ 40% of cells displaying ≥ 4 copies of the EGFR	
5	High polysomy	EGFR/CEP7 ratio ≥ 2 OR presence of gene clusters (≥ 4 spots) OR ≥ 10% of cells displaying ≥ 15 copies of EGFR	POSITIVE
6	Gene amplification		

Table 3
Efficacy results in the ITT population and EGFR expression groups for patients enrolled in FLEX-trial, BMS 099-trial, SQUIRE-trial and SWOG S0819-trial (ITT: intention-to-treat; EGFR: epidermal growth factor receptor; IHC: immunohistochemistry; FISH: fluorescence in situ hybridization; OS: overall survival; PFS: progression-free survival; HR: hazard ratio).

Trial	Treatment	N of patients	OS HR (95% CI)	OS (months)	p-Value	PFS HR (95% CI)	PFS (months)	p-Value
FLEX [20]								
ITT population	Cetuximab + cisplatin-vinorelbine	557	0.87 (0.76–1.00)	11.3	0.044	0.94 (0.82–1.08)	4.8	0.39
	Cisplatin-vinorelbine	568		10.1			4.8	
High EGFR	Cetuximab + cisplatin-vinorelbine	178	0.73 (0.58–0.93)	12.0	0.011	0.86 (0.68–1.09)	5.0	0.22
(IHC-Score ≥ 200)	Cisplatin-vinorelbine	167		9.6			4.6	
Low EGFR	Cetuximab + cisplatin-vinorelbine	377	0.99 (0.84–1.16)	9.8	0.88	0.98 (0.83–1.15)	4.6	0.80
(IHC-Score < 200)	Cisplatin-vinorelbine	399		10.3			4.9	
BMS 099 [27]								
ITT population	Cetuximab + carboplatin-paclitaxel/docetaxel	338	0.89 (0.75–1.05)	9.7	0.17	0.90 (0.76–1.07)	4.4	0.236
	Carboplatin-paclitaxel/docetaxel	338		8.4			4.2	
EGFR IHC+	Cetuximab + carboplatin-paclitaxel/docetaxel	66	1.02 (0.71–1.48)	8.3	0.91	1.15 (0.78–1.68)	4.6	0.48
(IHC-Score ≥ 1)	Carboplatin-paclitaxel/docetaxel	65		9.7			4.5	
EGFR IHC-	Cetuximab + carboplatin-paclitaxel/docetaxel	11	1.86 (0.57–6.11)	11.2	0.30	1.17 (0.37–3.72)	4.1	0.79
(IHC-Score: 0)	Carboplatin-paclitaxel/docetaxel	6		17.6			6.4	
EGFR FISH+	Cetuximab + carboplatin-paclitaxel/docetaxel	27	1.92 (1.05–3.54)	8.6	0.03	1.54 (0.81–2.93)	5.4	0.18
	Carboplatin-paclitaxel/docetaxel	27		12.5			5.4	
EGFR FISH-	Cetuximab + carboplatin-paclitaxel/docetaxel	26	0.84 (0.47–1.52)	7.4	0.57	0.65 (0.35–1.18)	4.3	0.15
	Carboplatin-paclitaxel/docetaxel	24		7.4			3.8	
SQUIRE [44]								
ITT population	Necitumumab + cisplatin-gemcitabine	545	0.84 (0.74–0.96)	11.5	0.01	0.85 (0.74–0.98)	5.7	0.02
	Cisplatin-gemcitabine	548		9.9			5.5	
EGFR IHC+	Necitumumab + cisplatin-gemcitabine	462	0.79 (0.69–0.92)	11.7	0.002	0.84 (0.72–0.97)	5.7	0.018
(IHC-Score ≥ 1)	Cisplatin-gemcitabine	473		10.0			5.5	
EGFR IHC-	Necitumumab + cisplatin-gemcitabine	24	1.52 (0.74–3.17)	6.5	0.015	1.33 (0.70–2.80)	4.2	0.25
(IHC-Score: 0)	Cisplatin-gemcitabine	23		17.4			5.6	
EGFR FISH+	Necitumumab + cisplatin-gemcitabine	111	0.70 (0.52–0.96)	12.6	0.066	0.71 (0.52–0.97)	6.1	0.057
	Cisplatin-gemcitabine	97		9.2			5.1	
EGFR FISH-	Necitumumab + cisplatin-gemcitabine	171	1.02 (0.80–1.30)	11.1	0.066	1.04 (0.82–1.33)	5.6	0.057
	Cisplatin-gemcitabine	178		10.7			5.5	
SWOG S0819 [51]								
ITT population	Cetuximab + carboplatin-paclitaxel ± bevacizumab	656	0.93 (0.83–1.04)	10.9	0.22	0.99(0.88–1.10)	4.6	0.83
	Carboplatin-paclitaxel ± bevacizumab	657		9.2			4.5	
High EGFR	Cetuximab + carboplatin-paclitaxel ± bevacizumab	159	0.78 (0.60–1.01)	12.1	0.06	0.92 (0.72–1.18)	4.9	0.52
(IHC-Score ≥ 200)	Carboplatin-paclitaxel ± bevacizumab	136		9.0			4.1	
Low EGFR	Cetuximab + carboplatin-paclitaxel ± bevacizumab	497	1.01 (0.85–1.20)	10.9	0.90	1.03 (0.88–1.21)	4.5	0.70
(IHC-Score < 200)	Carboplatin-paclitaxel ± bevacizumab	521		9.4			4.8	
EGFR FISH+	Cetuximab + carboplatin-paclitaxel ± bevacizumab	199	0.81 (0.66–1.00)	13.4	0.0048	0.92 (0.75–1.12)	5.4	0.40
	Carboplatin-paclitaxel ± bevacizumab	201		9.8			4.8	
EGFR IHC+/FISH+	Cetuximab + carboplatin-paclitaxel ± bevacizumab	72	0.61 (0.42–0.90)	12.9	0.06	0.64 (0.44–0.91)	5.5	0.01
	Carboplatin-paclitaxel ± bevacizumab	70		7.5			3.7	

0.93, 95% CI, 0.67–1.30, p = 0.68) or in a group with mutated KRAS (HR: 0.97, 95% CI, 0.45–2.07, p = 0.93). The same results were applicable on PFS, with no significant difference in group with KRAS WT (HR: 1.07, 95% CI, 0.77–1.50, p = 0.69) or mutated KRAS (HR: 0.64, 95% CI, 0.27–1.50, p = 0.30). Adding cetuximab to CT did not significantly affect OS in patients with EGFR WT tumors (HR: 0.91, 95% CI, 0.64–1.29, p = 0.61) or in the group with mutated EGFR (HR: 1.62, 95% CI, 0.54–4.88, p = 0.38). Also for PFS there was no significant difference in groups with EGFR WT (HR: 0.95, 95% CI, 0.66–1.35, p = 0.76) or mutated EGFR (HR: 1.17, 95% CI, 0.36–3.77, p = 0.79). With regard to EGFR protein expression, as assessed by IHC, adding cetuximab to CT did not significantly affect OS in patients with EGFR + tumors (HR: 1.02, 95% CI, 0.71–1.48, p = 0.91) or in the subset of patients with EGFR- tumors (HR: 1.86, 95% CI, 0.57–6.11, p = 0.30). The same results were applicable on PFS, with no significant difference in group with EGFR + (HR: 1.15, 95% CI, 0.78–1.68, p = 0.69) or EGFR- (HR: 1.17, 95% CI, 0.37–3.72, p = 0.79). Finally, regarding evaluation of EGFR gene copy number by FISH, the addition of cetuximab to CT did not significantly affect PFS in the FISH + subset (HR: 1.54, 95% CI, 0.81–2.93, p = 0.18) or in the FISH- subset (HR: 0.65, 95% CI, 0.35–1.18, p = 0.15). Patients with EGFR FISH + tumors had significantly shorter OS with cetuximab plus CT than with CT alone (HR: 1.92, 95% CI, 1.05–3.54, p = 0.03). In addition, OS did not differ by treatment in patients with FISH- tumors (HR: 0.84, 95% CI, 0.47–1.52, p = 0.57).

In a retrospective analysis [75] of the results of the SWOG S0342 trial [28], it was reported that EGFR gene copy number detected by FISH predicted outcomes in patients with advanced-stage NSCLC receiving cetuximab plus CT. Median OS was 15 months compared with 7 months for patients with a positive *versus* negative FISH-test, respectively (HR: 0.58, p = 0.46). Median PFS was significantly longer (6 months) in the FISH + group compared with the FISH- group (3 months; HR: 0.45, p = 0.0011). A significant difference in PFS was also seen between FISH + and FISH − patients in each of the treatment arms (concurrent arm HR: 0.45, p = 0.02; sequential arm HR: 0.46, p = 0.03). These results suggest that the addition of cetuximab to CT may reverse the underlying poor prognosis associated with EGFR FISH positivity [10], supporting also the hypothesis that EGFR FISH may be broadly applicable for selection of patients for EGFR-target therapies.

Analysis of correlation of IHC-assessed EGFR-expression with outcomes in the SQUIRE trial [44] was performed in patients affected by advanced SCC treated with necitumumab [76]. OS for IHC + patients was significantly longer in the necitumumab plus CT group than in the CT group alone (HR: 0.79 [95% CI, 0.69–0.92; p = 0.002], median 11.7 *vs* 10.0 months). In the same study, while patients with high EGFR expression (H-Score ≥ 200) had a more favorable HR for OS, the trend was not observed for PFS and the treatment-by-marker interaction test p value was not significant. The subpopulation treatment effect pattern plot (STEPP) analysis across the ranges of EGFR protein expression for OS and PFS did not lead to the identification of a cut-point to differentiate those who benefit from the addition of necitumumab *versus* those who do not. These data suggested that patients whose tumors have detectable EGFR protein have survival benefit from the addition of EGFR-inhibitor to CT, regardless of the level of EGFR expression. Patients with no evidence of EGFR expression did not appear to benefit from the addition of necitumumab to CT (OS HR: 1.52 [95% CI, 0.74–3.17; p = 0.015], median 6.5 *vs* 17.4 months; PFS HR: 1.33 [95% CI, 0.66–2.75; p = 0.252], median 4.2 *vs* 5.6 months). The efficacy results of exploratory *post hoc* analysis of EGFR copy number gain by FISH showed a trend for a more favorable survival HR in the EGFR FISH + subpopulation but without statistically significant treatment-by-marker interaction tests (OS HR: 0.70 [95% CI, 0.52–0.96; p = 0.066], median 12.6 *vs* 9.2 months; PFS HR: 0.71 [95% CI, 0.52–0.97; p = 0.057], median 6.1 *vs* 5.1 months).

A recent retrospective analysis [77] from the results of the SWOG S0819 trial [51] was performed in order to determine whether a biomarker-enriched model based both on FISH and IHC might be an appropriate predictor of efficacy of cetuximab-based therapy in advanced NSCLC. In the sub-population of H-Score + patients there was not significant difference in OS (HR: 0.78 [95% CI, 0.60–1.01; p = 0.06], median 12.1 *vs* 9.0 months for cetuximab arm and control arm respectively) and PFS (HR: 0.92 [95% CI, 0.72–1.18; p = 0.52], median 4.9 *vs* 4.1 months for cetuximab arm and control arm respectively). When the evaluation of both biomarkers were positive (FISH + and H-Score +), a significant difference was observed in OS (HR: 0.61 [95% CI, 0.42–0.90; p = 0.01], median 12.9 *vs* 7.5 months for cetuximab arm and control arm respectively) and PFS (HR: 0.64 [95% CI, 0.44–0.91; p = 0.01], median 5.5 *vs* 3.7 months for cetuximab arm and control arm respectively). In the sub-population of EGFR FISH + patients with SCC, OS was significantly improved among patients randomized to the cetuximab-containing arm compared to control (HR: 0.58 [95% CI, 0.39–0.86; p = 0.01], median 11.8 *vs* 6.1 months). In this subpopulation PFS was marginally not significant between the arms (HR: 0.68 [95% CI, 0.46–1.01; p = 0.06], median 4.5 *vs* 2.8 months for cetuximab arm and control arm respectively). In the sub-population of H-Score + patients with SCC, OS was significantly improved among patients randomized to the cetuximab-containing arm compared to control (HR: 0.64 [95% CI, 0.43–0.95; p = 0.03], median 9.3 *vs* 6.9 months). Also for patients with SCC a significant difference in OS and PFS was observed when both biomarker's evaluations were positive; in particular OS HR: 0.32 (95% CI, 0.17–0.60; p = 0.0004, median 12.3 *vs* 4.9 months for cetuximab arm and control arm respectively), PFS HR: 0.50 (95% CI, 0.28–0.89; p = 0.02, median 5.2 *vs* 2.8 months for cetuximab arm and control arm respectively).

In summary, the predictive role of EGFR expression in patients with NSCLC treated with anti-EGFR mAbs was evaluated in many retrospective analyses with controversial results. However, the most recent data discussed above support the application of both EGFR FISH and IHC to identify specific sub-populations of patients which will benefit from the addition of an anti-EGFR mAb to first-line standard CT. Furthermore, this benefit seems to be more evident in the SCC population, with a significant improvement in OS and PFS for patients with EGFR FISH + or IHC + or both. Moreover, in accordance with previous analyses [77–79], a high level of EGFR expression is more common in SCC than in other histotypes of NSCLC. Patients selection based both on EGFR molecular assessment and histology has to be considered as cornerstone in the treatment's plan.

EGFR-directed monoclonal antibodies in association with chemo-radiotherapy

Many patients with inoperable stage III NSCLC are candidates for combined modality CT and radiotherapy (RT). Simultaneous administration of radiosensitizing agents that increase tumor cell kill might improve the therapeutic ratio. Based on the fact that EGFR is often overexpressed or mutated in NSCLC, the impact of EGFR-targeted therapies on cellular responses to ionizing radiation has been explored [80,81]. Stressors including irradiation can lead to autocrine secretion of the EGFR-ligand TGF-α and thereby to an activation of the receptor [82]. Furthermore, *in vitro*, irradiation leads to autophosphorylation of the EGFR, resulting in an increase in cell proliferation, suggesting that the EGFR may have a role in radiation-induced tumor repopulation [83]. Under experimental laboratory conditions in animal models cetuximab increased tumor radio-curability [84]. Clinically, a phase III trial in patients with squamous cancer of the head and neck revealed a significant improvement in OS with cetuximab and RT compared with RT alone [85]. Many clinical trials assessed the efficacy of cetuximab combined with RT, with and without concurrent CT in the treatment of stage III NSCLC (Table 4). In particular, we focus on 2 phase II trials and the most recent phase III trial assessing the efficacy of concurrent cetuximab and CT in combination with RT.

The Cancer and Leukemia Group B (CALGB)-trial 30,407 [90] was a

Table 4
Clinical studies of combined anti-EGFR antibodies, chemotherapy and radiotherapy for unresectable stage III non-small-cell lung cancer (EGFR: epidermal growth factor receptor; OS: overall survival; PFS: progression-free survival; NA: not applicable; CT: chemotherapy; RT: radiotherapy; DCR: disease control rate; IMRT: intensity modulated radiotherapy; OLCR: objective local control rate; MTD: maximum-tolerated dose).

Trial	First author, year	Study phase	Sample size	Regimen	Primary outcome	OS (months)	PFS (months)
SCRATCH	Hughes, 2008 [86]	I	12	Platinum-based induction CT, then concurrent cetuximab/RT (64/2 Gy)	Toxicity	NA	NA
N0422	Jatoi, 2010 [87]	II	57	Concurrent cetuximab/RT (60/2 Gy)	11+ months-OS rate	15.1	7.2
NA	Choi, 2010 [88]	I	15	Concurrent nimotuzumab/RT (30–36/3 Gy)	Safety, feasibility	9.8	5.4
NA	Bebb, 2010 [89]	I	18	Concurrent nimotuzumab/RT (30 or 36/3 Gy)	Safety, feasibility	13.9	3.7
CALGB 30407	Govindan, 2011 [90]	II	101	Arm A: carboplatin-pemetrexed × 4, then concurrent CT/RT (70/2 Gy), then pemetrexed Arm B: carboplatin-pemetrexed × 4, then cetuximab + concurrent CT/RT (70/2 Gy), then pemetrexed	18+ months-OS rate	21.2 *vs* 25.2	12.6 *vs* 12.3
SATELLITE	Hallqvist, 2011 [91]	II	71	Induction cisplatin-docetaxel × 2, then concurrent cetuximab/RT (68/2 Gy)	DCR	17.0	NA
NEAR	Jensen AD, 2011 [92]	II	30	Concurrent cetuximab/IMRT (66/2 Gy), then 13 weekly maintenance cetuximab	Toxicity, feasibility	19.6	8.5
RTOG 0324	Blumenschein GB, 2011 [93]	II	87	Concurrent cetuximab, carboplatin-paclitaxel, RT (63/1.8 Gy), then carboplatin-paclitaxel × 2	Safety, compliance	22.7	NA
NA	Ramalingam, 2013[94]	II	40	Concurrent cetuximab/RT (73.5/2 Gy), then weekly cetuximab + carboplatin-paclitaxel × 3	OS	19.4	9.3
SWOG S0429	Chen, 2013 [95]	I	22	Concurrent cetuximab/RT (64.8/1.8 Gy), then weekly cetuximab till 2-years or disease progression	Toxicity, feasibility	14.0	8.0
NTR2230	Van den Heuvel, 2014 [96]	II	102	Arm A: concurrent daily cisplatin/RT (66/2.75 Gy) Arm B: concurrent daily cisplatin + weekly cetuximab/RT (66/2.75 Gy)	OLCR	NA	NA
NA	Dingemans, 2014 [97]	I	25	Induction carboplatin-gemcitabine × 2, then cetuximab + concurrent CT/RT (64/1.5 Gy)	MTD 3 months after treatment	NA	NA
NA	Dilling, 2014 [98]	II	27	Concurrent cetuximab/RT (70/2 Gy), then consolidation with cetuximab-docetaxel × 3	PFS	10.5	7.5
NA	Liu, 2015 [99]	I/II	27	Induction therapy with cetuximab + cisplatin-vinorelbine × 2, then concurrent cetuximab/RT (60–66/2 Gy)	Toxicities	26.7	13.5
RTOG 0617	Bradley, 2015 [101]	III	544	Arm A: concurrent carboplatin-paclitaxel, RT (60/2 Gy), then carboplatin-paclitaxel × 2 Arm B: concurrent carboplatin-paclitaxel, RT (74/2 Gy), then carboplatin-paclitaxel × 2 Arm C: concurrent cetuximab + carboplatin-paclitaxel, RT (60/2 Gy), then carboplatin-paclitaxel × 2 Arm D: concurrent cetuximab + carboplatin-paclitaxel, RT (74/2 Gy), then carboplatin-paclitaxel × 2	OS	28.7 *vs* 20.3 (60 Gy *vs* 74 Gy) 25.0 *vs* 24.0 (cetuximab *vs* no cetuximab)	NA

randomized phase II trial designed to investigate the activity of CT (carboplatin + pemetrexed) with or without cetuximab in combination with concurrent thoracic radiation therapy (TRT, 70 Gy in 35 fractions). Median OS was 25.2 *vs* 21.2 months for CT plus cetuximab arm and CT alone arm respectively; the 18-months OS rates observed were 54% (95% CI, 42–70) and 58% (95% CI, 46–74) respectively. An unplanned analysis of OS by histology revealed that among patients with squamous and non-squamous histology in both arms, the median OS was 22.4 months and 22.4 months for CT plus cetuximab arm and CT alone arm respectively, without any significant difference (p = 0.667).

A different combination CT-regimen was evaluated in the Radiation Therapy Oncology Group (RTOG) 0324 phase II trial [93]. In this study, patients with unresectable stage III NSCLC received a weekly dose of cetuximab and during week 2, patients started TRT (63 Gy in 35 fractions) with CT (carboplatin + paclitaxel). With a median follow-up of 21.6 months, the 24-months progression failure rate was 55.2% (95% CI, 44.6–65.7%); the 24-month survival rate was 49.3% (95% CI, 38.3–59.3) and median OS was 22.7 months (95% CI, 15.3–30.4). The unanswered question was whether EGFR expression status affects disease control or survival in patients receiving cetuximab with RT, with or without CT. To address this question, a secondary analysis of the RTOG 0324 trial investigated whether expression of EGFR was associated with survival and disease control [100]. Patients without IHC data had worse OS (HR: 1.63, p = 0.05), worse PFS (HR: 1.88, p = 0.008) and worse TTP (HR: 1.99, p = 0.01) than those with IHC data. In this study, no difference was found in OS, PFS, or TTP according to EGFR expression levels. Surprisingly, these outcomes differed not by EGFR expression but by the availability of samples for analysis.

On the basis of the encouraging results of the RTOG 0324 trial, the RTOG group designed a phase III trial (RTOG 0617) [101] to establish whether a 74 Gy dose was better than a 60 Gy dose and whether adding cetuximab to concurrent CT/RT would confer an OS benefit in patients with stage III NSCLC. Median OS was 28.7 months (95% CI, 24.1–36.9) for patients who received standard-dose RT and 20.3 months (95% CI, 17.7–25.5) for those who received high-dose RT (HR: 1.38, 95% CI, 1.09–1.76, p = 0.004). Median OS was 25.0 months (95% CI, 20.2–30.5) for patients who received cetuximab and 24.0 months (95% CI, 19.8–28.6) for those who did not (HR: 1.07, 95% CI, 0.84–1.35, p = 0.29). In a planned retrospective analysis, EGFR H-Score was evaluated in the sub-population with enough pathological material for central review; the data suggested that patients with an H-Score ≥ 200 might benefit from the addition of cetuximab to CT/RT, but that cetuximab might be detrimental for patients with an H-Score < 200.

In conclusion, given the strong preclinical rationale for combining EGFR-inhibitors and RT, results of many clinical trials remain controversial. A more complete understanding of underlying mechanisms is required in order to optimize EGFR targeting in the RT settings Correlative biomarker studies should be part of these research efforts as well. The question remains whether the efficacy of cetuximab and irradiation is comparable to concurrent CT/RT and if it is superior to RT alone in NSCLC.

Conclusion

Immunotherapy has recently demonstrated potential for applications in the first-line setting as a single agent for patients whose tumors have high PD-L1 expression (tumor proportion score TPS ≥ 50%)[4]. The addition of pembrolizumab to standard CT of pemetrexed and a platinum-based drug resulted in significantly longer OS and PFS than CT alone, regardless of PD-L1 expression levels [6]. First-line combination regimens including PD-1 or PD-L1 inhibitors may maximize the chance of response and lead to prolonged survival and are actually recommended in the international guidelines (pembrolizumab monotherapy for high PD-L1 expressors patients) or will be recommended in the next future (pembrolizumab in combination with CT regardless PD-L1 status). However, patients unsuitable for immunotherapy or targeted therapies alone, both for molecular and clinical reasons, are still candidates for traditional CT, potentially in combination with other drugs. For these patients further efforts to customize treatments are needed. Anti-EGFR mAbs in combination with CT have opened new opportunities in the treatment of patients affected by NSCLC. The characterization of biomarkers predicting benefit from EGFR-mAbs has been demonstrated as crucial to identify and select the sub-population that will receive the highest benefit from this kind of treatment. FISH and IHC are techniques already well-established and widely used in routine clinical practice, at relatively low costs. Therefore, logistical or technical reasons would not preclude the use of these EGFR expression assessments, with FISH+ and H-Score ≥ 200 as predictive biomarkers for the selection of patients who would be most likely to derive a clinical benefit from treatment with CT and anti-EGFR mAbs. In particular for squamous histology, based on the results of the SQUIRE and S0819 trials, the use of EGFR FISH evaluation could represent a valid tool for this selection. Perspective studies on EGFR FISH+ in SCC population should be performed.

In conclusion, we believe that in the era of personalized medicine in NSCLC, our analysis supports the possible role in clinical practice of a histology-driven and biomarker-enriched model based on EGFR FISH and IHC as appropriate predictors of efficacy of anti-EGFR mAbs-based therapy in advanced NSCLC. Recent results support the application of EGFR FISH and IHC to identify specific sub-populations of patients which will benefit from the addition of an EGFR-mAb to first-line standard CT.

Conflict of interest

All Authors of this manuscript declare no potential conflicts of interest.

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