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Therapeutic value of EGFR inhibition in CRC and NSCLC: 15 years of clinical evidence

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ABSTRACT

Epidermal growth factor receptor (EGFR) plays a key role in tumour evolution, proliferation and immune evasion, and is one of the most important targets for biological therapy, especially for non-small-cell lung cancer (NSCLC) and colorectal cancer (CRC). In the past 15 years, several EGFR antagonists have been approved for the treatment of NSCLC and metastatic CRC (mCRC). To optimise the use of anti-EGFR agents in clinical practice, various clinical and molecular biomarkers have been investigated, thus moving their indication from unselected to selected populations. Nowadays, anti-EGFR drugs represent a gold-standard therapy for metastatic NSCLC harbouring *EGFR* activating mutation and for *RAS* wild-type mCRC. Their clinical efficacy is limited by the presence of intrinsic resistance or the onset of acquired resistance. In this review, we provide an overview of the antitumour activity of EGFR inhibitors in NSCLC and CRC and of mechanisms of resistance, focusing on the development of a personalised approach through 15 years of preclinical and clinical research.

INTRODUCTION

Cancer cells may acquire the capacity for autonomous proliferation through the uncontrolled production of specific molecules that promote cell growth (growth factors) or through abnormal, enhanced expression of specific proteins (growth factor receptors) on the cell membranes to which growth factors selectively bind. These processes lead to different intracellular signals that ultimately trigger the proliferation of cancer cells, induction of angiogenesis and metastasis.[1] In this scenario, epidermal growth factor receptor (EGFR) family plays a key role in tumour growth and progression by promoting a variety of functions including proliferation, survival, invasion or immune evasion.[1]

Colorectal cancer (CRC) and non-small-cell lung cancer (NSCLC) remain the most common causes of cancer-related mortality.[2] The majority of patients are diagnosed with metastatic disease, meaning that chemotherapy is the treatment of choice. However, despite advances, treatment with chemotherapy offers an overall survival (OS) benefit usually restricted to only a few months.[2–4] In the past decade, the introduction of targeted therapies has radically changed the median survival of these patients, providing more treatment options and better results.[1] Nowadays, several EGFR antagonists are used for the treatment of metastatic NSCLC and metastatic CRC (mCRC). Despite the progress achieved, the clinical efficacy of these agents is limited by the presence of intrinsic (primary) or the development of acquired (secondary) resistance.

In this review, we provide an overview of EGFR signalling pathway and antitumour activity of EGFR inhibitors, focusing on different approaches to overcome the resistance to anti-EGFR therapies and potential future directions for more tailored cancer therapies.

EGFR SIGNALLING PATHWAY IN CANCER

The EGFR signalling pathway is the first oncogenic driver recognised in human epithelial cancer.[1] The *EGFR* gene is located on chromosome 7p12–13 and belongs to a family of cell membrane receptor tyrosine kinases, including EGFR (ERBB1), HER2/c-neu (ERBB2), HER3 (ERBB3) and HER4 (ERBB4). They are composed of single amino acid chain protein structure with an extracellular ligand binding domain, a transmembrane domain for homodimerisation or heterodimerisation and a tyrosine kinase intracellular portion. Major ligands are as follows: epidermal growth factor (EGF), transforming growth factor α (TGF-α), heparin binding EGF (HB-EGF), β-cellulin, amphiregulin and heregulin.[1] The interaction between ligands and receptor induces

conformational change of receptor leading to homodimerisation or heterodimerisation, thereby resulting in activation of EGFR kinase activity and subsequent activation of several signalling transduction cascades involved in cellular proliferation, survival, differentiation and migration. The two principal downstream effectors of EGFR activation are the retrovirus-associated DNA sequences (RAS)/v-RAF 1 murine leukaemia viral oncogene homologue 1(RAF)/mitogen-activated protein kinase (MAPK) pathway, which regulates cell cycle progression, and phospho-inositide-3 kinase (PI3K)/protein kinase B (AKT) pathway, which controls antiapoptotic signal.[1]

DEVELOPMENT OF EGFR ANTAGONISTS IN CANCER TREATMENT: STATE OF THE ART

In 1980, Drs John Mendelsohn and Gordon Sato postulated that a monoclonal antibody (mAb) against the EGFR could prevent ligand binding and inhibits activation of the receptor's tyrosine kinase and cancer cell proliferation. Based on this hypothesis, interest on anti-EGFR treatments for specific tumours such as CRC and NSCLC has led to the development of two classes of drugs: mAbs and tyrosine kinase inhibitors (TKIs).[5]

In 1995, the first preclinical results of efficacy of anti-EGFR mAb C225/cetuximab were published.[5]

Cetuximab is an immunoglobulin (Ig) G1 human–murine chimeric counterpart of the murine mAb M225. It binds to the external domain of EGFR with high affinity and promotes receptor internalisation and subsequent degradation, determining receptor downregulation.[1] Since cetuximab is immunogenic in about 5% of patients, a full human antibody (and not a human–mouse chimaera) against EGFR, panitumumab, has been developed (table 1).[6–9]

TKIs are small molecules that compete with and prevent binding of ATP to the intracellular tyrosine kinase region (table 1).

Table 1 Anti-EGFR drugs in mCRC and NSCLC treatment

Drug name	Type	Target specificity
Cetuximab	Chimeric mAb	EGFR ECD
Panitumumab	Humanized mAb	EGFR ECD, included EGFR S492R mutated (resistant to cetuximab)
SYM004	Oligoclonal, mixture of two recombinant chimeric mAbs	EGFR ECD, including mutations of the EGFR; directed against non-overlapping epitopes of the EGFR
MM151	Oligoclonal, mixture of three mAbs	EGFR ECD, including mutations of the EGFR, directed towards three different, non-overlapping epitopes of the EGFR
Gefitinib	Reversible TKI	EGFR intracellular domain with activating mutations
Erlotinib	Reversible TKI	EGFR intracellular domain activating mutations
Afatinib	Irreversible TKI	Pan-HER intracellular domain
Osimertinib	Irreversible TKI	EGFR intracellular domain with activating mutations and T790M mutation

ECD, extracellular domain; EGFR, epidermal growth factor receptor; mAb, monoclonal antibody; mCRC, metastatic colorectal cancer; NSCLC, non-small-cell lung cancer; TKI, tyrosine kinase inhibitor.

Colorectal cancer

The EGFR-directed mAbs cetuximab and panitumumab were approved for the treatment of chemorefractory mCRC patients in 2004 and 2006, respectively (figure 1). Both drugs have very similar efficacy, achieving objective response rates (ORRs) of ~10% when used as monotherapy in chemorefractory mCRC.[10 11] Cetuximab was initially approved on the basis of the BOND clinical trial, which compared cetuximab plus irinotecan with cetuximab alone in mCRC patients who did not respond to irinotecan monotherapy.[12] Combination arm showed improvement in response rate (RR) and progression-free survival (PFS). Subsequently, the NCI-CO17 (Cetuximab and Best Supportive Care Compared with Best Supportive Care Alone in Treating Patients with Metastatic EGFR-Positive Colorectal Cancer) study confirmed the superiority of cetuximab in patients who have received all available chemotherapies.[13]

In second-line treatment of chemorefractory mCRC, three large randomised phase III clinical trials confirmed the value of adding EGFR agents to conventional chemotherapy in terms of RR and PFS, but unfortunately not in OS.[14–16] The lack of meaningful result regarding median OS may be affected by subsequent treatments. In particular, exploratory analyses have suggested that poststudy anti-EGFR treatment may have reduced any potential difference across treatment arms, prolonging survival in the patients who received it.[14–16] In first-line setting, the phase III CRYSTAL trial (Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) demonstrated that cetuximab improved the standard chemotherapy regimen, in particular reduced the risk of progression (8.9 vs 8 months, HR 0.85; p=0.048), enhanced tumour response (46.9% vs 38.7%, OR 1.40; p=0.004) and radical resection (R0) of metastasis with curative intent (p=0.002).[17] OS analysis did not appear to be statistically significant different between treatments groups (19.9 vs 18.6; HR 0.93, p=0.31). Cetuximab in combination with

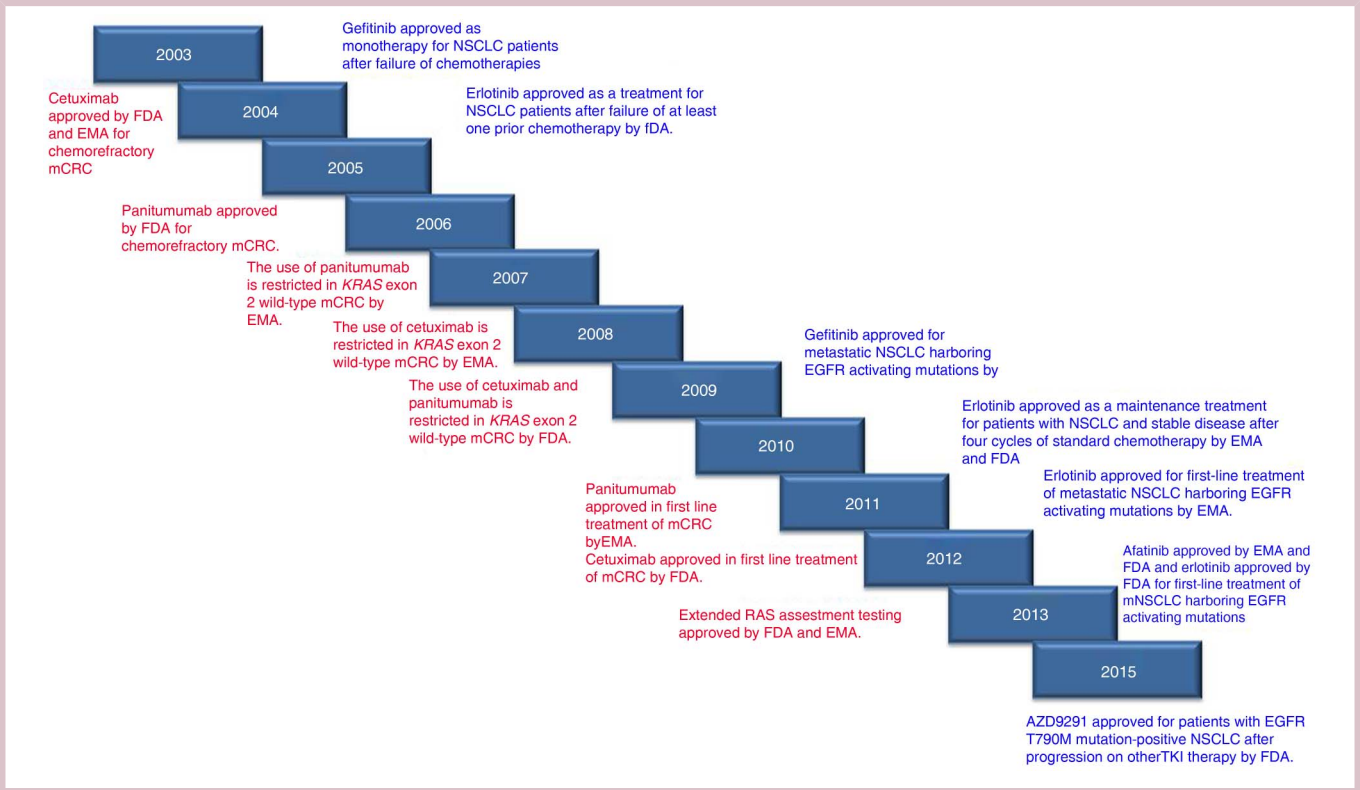

Figure 1 Development of anti-EGFR agents in mCRC and NSCLC.

FOLFOX-4 (oxaliplatin, folinic acid and 5-fluorouracil) was investigated in the phase II randomised OPUS trial (Oxaliplatin and Cetuximab in First-Line Treatment of mCRC). The primary objective was ORR. The results of this trial demonstrated a higher ORR in cetuximab arm (46% vs 36%, p=0.0064), but not statistically significant improvement in terms of PFS or OS, in unselected population.[18] It is now generally recognised that the large differences in treatment response among mCRC patients are due to the fact that tumours differ at the molecular level. Retrospective subgroups analyses of these trials have shown that the benefit of anti-EGFR treatment is limited to molecularly selected population.[19]

The efficacy of cetuximab in combination with an oxaliplatin-based first-line chemotherapy has been evaluated also in the COIN trial.[20] In this randomised controlled trial, patients were randomised to oxaliplatin and fluoropyrimidine chemotherapy (arm A), the same combination plus cetuximab (arm B) or intermittent chemotherapy (arm C). Unfortunately, even if the addition of cetuximab to oxaliplatin-based chemotherapy increases RR, there was no evidence of benefit in terms of PFS or OS.[20] Similar results have been obtained in the NORDIC-VII trial. In fact, there was no significant trend towards a higher ORR, PFS or OS in the patients receiving FLOX plus cetuximab (arm B) as compared with FLOX alone (arm A) in the first-line treatment of mCRC patients.[21] In these two trials, the backbone chemotherapy was peculiar and different if compared to previous study. In particular, the use of capecitabine, in the COIN trial, and the use of bolus 5-FU, in the NORDIC trial, enhanced the gastrointestinal toxicity resulting in a higher dose reduction for patients.

Regarding panitumumab, the PRIME trial (Panitumumab Randomized Trial in Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy) was the first study to evaluate the addition of panitumumab to FOLFOX4 in first-line mCRC patients.[22] The study confirmed the efficacy of panitumumab in combination with oxaliplatin-based chemotherapy.[22] Similarly to cetuximab, the lack of efficacy in terms of OS is due to the absence of patients' selection.[23]

Non-small-cell lung cancer

Two reversible EGFR TKIs (gefitinib and erlotinib, first-generation TKIs) and one irreversible EGFR TKI (afatinib, second-generation inhibitor) are currently used for treatment of NSCLC with *EGFR* activating mutation in first-line setting (table 1). Based on the results of BR21 study, only erlotinib has received approval for second-line/third-line treatment in NSCLC patients unselected for *EGFR* mutations.[24] More recently, on November 2015, Food and Drug Administration (FDA) approved AZD9291 (osimertinib), a third-generation EGFR TKI, for the treatment of NSCLC patients with documented positivity to EGFR resistance mutation T790M after progression to a first-line therapy with TKI (table 1 and figure 1).[25] *EGFR* activating mutations are mostly located

within exons 18–21, which encode the kinase domain, leading to receptor constitutive activation;[26] although 188 *EGFR* mutations are known, only two, the deletion of 5 amino acids from exon 19 and the missense mutation in exon 21, resulting in a substitution of arginine for leucine at position 858 (L858R), account for about 80–90% of the cases.[27] Other less common mutations are G719X, L861X and insertions at exon 19. Initially, gefitinib and erlotinib were tested in combination with chemotherapy, but no differences were observed in OS between treatment arms.[28–31] Gefitinib and erlotinib have also been tested in lines of treatment subsequent to the first. Among all trials, BR 21 was the only one demonstrating activity of erlotinib versus placebo in second or third line of therapy in terms of RR, PFS and OS, leading to the approval in 2004 of erlotinib in this setting.[32] In this study, NSCLC patients were randomised 2:1 to erlotinib or placebo in second or third line of therapy. In erlotinib group, RR was 9%, with a median duration of response of 7.9 months and a disease control rate of 45%, with higher responses in women (p=0.006), adenocarcinoma (p<0.001) and never smokers (p<0.001). PFS was 2.2 and 1.8 months for placebo (HR 0.61, p<0.001), and OS was 6.7 vs 4.7 months (HR 0.70, p<0.001) in favour of erlotinib.[32]

The role of EGFR TKI versus chemotherapy as first line of therapy in NSCLC patients has been explored in several trials in clinically or molecularly selected population.[33–41] All these trials demonstrated superiority of EGFR TKIs, as compared to chemotherapy, in terms of RR, PFS and quality of life, in NSCLC patients whose tumours harbour activating EGFR mutations. Only afatinib demonstrated a benefit in OS in a pooled analysis.[33–41]

TOWARDS OPTIMISING THE USE OF EGFR INHIBITORS: FROM UNSELECTED TO SELECTED PATIENTS

The well-known model of carcinogenesis has hypothesised that germline or somatic mutations are required for malignant transformation, and the accumulation of multiple mutations determines the biological behaviour of the tumour. Understanding the underlying biology of tumours form is necessary to develop effective personalised therapies. CRC and NSCLC are complex and heterogeneous diseases, with distinct genotypes and phenotypes, whose knowledge is mandatory to select patients who can benefit from targeted therapy. Mainly, in the development of anti-EGFR agents over the last 15 years, several biomarkers and molecular pathways have been investigated, moving from unselected to selected population (tables 2 and 3).

Colorectal cancer

Cetuximab and panitumumab are efficient only in a small percentage of patients. Retrospective analyses provided compelling evidence that patients with CRC carrying activating *KRAS* gene mutations do not benefit from anti-EGFR therapy.[19 23] This evidence has led the FDA and the European Medicines Agency (EMA) to restrict the use of panitumumab and cetuximab only in *KRAS* exon 2 wild-type (WT) mCRC patients (figure 1).[17 18] Recently, retrospective and prospective trials showed that other mutations in *KRAS* and *NRAS* are involved in the efficacy of anti-EGFR therapy, underlying the importance of a complete *RAS* mutational analysis (ie, *KRAS* and *NRAS* analysis of exon 2 (codons 12/13), exon 3 (codon 59/61) and exon 4 (codon 117/146)).[19 23 48] Based on these results, in 2013, the EMA restricts indication of anti-EGFR mAbs in mCRC patients from '*KRAS* WT', to, in a more extended way, 'all *RAS* WT' patients (figure 1).

Recently, the role of EGFR inhibition in second-line treatment of *KRAS* exon 2 WT mCRC patients after progression from first-line treatment with cetuximab has been explored in CAPRI-GOIM trial (table 2).[44] In particular, mCRC patients were treated with FOLFIRI plus cetuximab in first line and at progression were randomised to receive FOLFOX alone or FOLFOX plus cetuximab. In *KRAS* exon 2 WT population (Intention to Treat Population), no significant difference in PFS, RR and OS was observed among the two arms. However, a trend in favour of the FOLFOX plus cetuximab combination was recorded in all end points. More importantly, in 'all *RAS* WT' patients (WT for *KRAS/NRAS/BRAF/PIK3CA* genes), a significant prolonged PFS for the treatment with FOLFOX plus cetuximab when compared with FOLFOX alone with an HR of 0.56 and a p value of 0.025 was reported. OS was also increased in these patients with an HR of 0.57, approaching statistical significance (p=0.056) (table 2).[44 45] The results of this trial suggest that continuing cetuximab treatment in combination with chemotherapy is of potential therapeutic efficacy in molecularly selected patients and should be validated in randomised phase III trials.

The selection of patient based on *RAS* status is a fundamental step in the treatment of mCRC. In first-line setting, the antiangiogenic drug, bevacizumab, is available as an alternative therapy to anti-EGFR antibody. Nowadays, an open question is which of these two classes of drugs could be the best option in this scenario.

In this regard, the FIRE-3 (Irinotecan-Based Chemotherapy Plus Cetuximab or Bevacizumab in First Line Treatment mCRC) and Cancer and Leukemia Group B (CALGB) 80405 (Cetuximab and/or Bevacizumab Combined with Combination Chemotherapy in Treating Patients with Metastatic Colorectal Cancer), two phase III clinical trials, compared cetuximab with bevacizumab plus chemotherapy, whereas the PEAK (Panitumumab or Bevacizumab Efficacy in Combination with Oxaliplatin-Based Chemotherapy in mCRC Subjects with *KRAS*-WT Tumors), a randomised phase II clinical trial, compared panitumumab with bevacizumab in the first-line treatment of *KRAS* WT exon 2 mCRC (table 2).[42 43 49] Final results of FIRE-3 and PEAK clinical trials showed in

Table 2 EGFR targeting in mCRC

Study	*RAS* status	n	Treatment	RR %	RR p value	mPFS months	mPFS p value	OS months	OS p value
CALGB/SWOG 80405	*KRAS* WT exon 2	559	(FOLFOX OR FOLFIR)/Bevacizumab	NA	NA	10.84	0.55	29.0	0.34
ESMO 2014		578	FOLFOX OR FOLFIR)/Cetuximab			10.45		29.9	
Lenz *et al* 2014	*RAS* WT	256	FOLFOX OR FOLFIR)/Bevacizumab	53.6	<0.01	NA	NA	31.2	0.40
Venook *et al* 2014		270	FOLFOX OR FOLFIR)/Cetuximab	68.6		NA		32.0	
European Consortium	KRAS mut exon 2-3-4	253	Chemotherapy/Cetuximab	6.7		2.8		7.4	< 0.0001
De Rock *et al* 2010	KRAS WT exon 2-3-4	352		<0.0001		<0.0001		11.5	
	NRAS mut exon 2-3-4	13	Chemotherapy/Cetuximab	35.8		5.5		8.8	0.051
	NRAS WT exon 2-3-4	289		7.7	0.0013	3.5	0.055	11.5	
				38.1		6.5			
OPUS	KRAS WT exon 2	82	FOLFOX4/Cetuximab	57	0.0027	8.3		22.8	0.39
Bokemayer *et al* 2011,		97	FOLFOX4	34		0.0064		18.5	
Tejpar *et al* 2014	KRAS mut exon 2	77	FOLFOX4/Cetuximab	34	0.0290	7.2		13.4	0.20
		59	FOLFOX4	53		5.5	0.015	17.5	
	KRAS mut exon 3-4	17	FOLFOX4/Cetuximab	47.1	0.57	8.6		14.8	0.41
	NRAS mut exon 2-3-4	19	FOLFOX4	36.8		7.3	0.96	17.8	
	RAS muta at any exon	94	FOLFOX4/Cetuximab	36.2	0.11	7.4		13.4	0.089
		78	FOLFOX4	48.7		5.6	0.018	17.8	
	RAS WT	36	FOLFOX4/Cetuximab	61.1	0.00	7.8		20.7	0.50
		46	FOLFOX4	30.4		12	0.018	17.8	
						5.8			
CRYSTAL	KRAS mut exon 3-4	32	FOLFIRI/Cetuximab	34.4	0.97	7.2	0.56	18.2	0.50
Van Cutsem *et al* 2015	NRAS mut exon 2-3-4	31	FOLFIRI	35.5		6.9		20.7	
	RAS mut at any exon	246	FOLFIRI/Cetuximab	31.7	0.40	7.4	0.47	16.4	0.64
		214	FOLFIRI	36		7.5		17.7	
	RAS WT	178	FOLFIRI/Cetuximab	66.3	<0.0001	11.4		28.4	0.0024
		189	FOLFIRI	38.6		0.0002		20.2	
						8.4			
20050181	RAS mut at any loci	299	FOLFIRI/Panitumumab	NA	NA	4.8	0.14	11.8	0.34
Peeters *et al* 2014		294	FOLFIRI			4		11.1	
	RAS WT	208	FOLFIRI/Panitumumab	NA	NA	6.4	0.007	16.2	0.08
		213	FOLFIRI			4.6		13.9	
PRIME	KRAS WT exon 2, other RAS	51	FOLFOX/Panitumumab	NA	NA	7.3	0.33	17.1	0.12
Douillard *et al* 2013	mut	57	FOLFOX			8.0		17.8	
		272	FOLFOXI/Panitumumab	NA	NA	7.3		15.5	0.001
	RAS mut at any loci	276	FOLFOX			0.008		18.7	
		259	FOLFOX/Panitumumab	NA	NA	8.7		25.8	0.009
	RAS WT	253	FOLFOX			10.1		20.2	
						0.004			
						7.9			

Continued

Table 2 Continued

Study	*RAS* status	n	Treatment	RR		mPFS		OS	
				%	p value	months	p value	months	p value
PLANET	KRAS WT exon 2	38	FOLFOX/Panitumumab	73.7	NA	12.5	0.943	32.5	0.848
Abad *et al* 2014		39	FOLFIRI/Panitumumab	66.7		12.6		42.4	
	RAS mut at any loci	NA	FOLFOX/Panitumumab	50.0	NA	NA	NA	NA	NA
		NA	FOLFIRI/Panitumumab	57.1					
	RAS WT	NA	FOLFOX/Panitumumab	77.8	NA	12.8	0.621	39.0	0.935
		NA	FOLFIRI/Panitumumab	73.1		14.8		45.8	
PEAK	KRAS WT exon 2	142	mFOLFOX6/Panitumumab	NA	NA	10.9	0.353	34.2	0.009
Schwartzberg *et al* 2014		143	mFOLFOX6/Bevacizumab			10.1		24.3	
	RAS WT	88	mFOLFOX6/Panitumumab	NA	NA	13	0.029	41.3	0.058
		82	mFOLFOX6/Bevacizumab			9.5		28.9	
CAPRI I line	KRAS WT exon 2	340	FOLFIRI/Cetuximab	56.4		9.9			
Ciardiello *et al* 2014	RAS WT	124		62		11.1			
	QUDRUPLE WT (KRAS, NRAS,BRAF,PI3K)	104		64.4		11.3			
CAPRI II line	RAS WT	74	FOLFOX/cetuximab	21.6		6.4	0.19	17.6	0.86
Ciardiello *et al* 2016		79	FOLFOX	12.7		4.5		14	
	QUDRUPLE WT (KRAS, NRAS,BRAF,PI3K)	34	FOLFOX/cetuximab	24.9		6.9	0.025	27.3	0.056
		32	FOLFOX	9.4		5.3		19.8	

Table 3 EGFR targeting in NSCLC

Study	*EGFR* status	N	Treatment	RR		Median PFS		OS	
				Per cent	p Value	Months	p Value	Months	p Value
IPASS	Clinically selected	609 (261 pt EGFR mutation positive)	Gefitinib	43	<0.001	5.7	<0.001	18.8	0.109
Mok *et al* (2009)			Carboplatin–paclitaxel	32.2		5.8		17.7	
NEJM002	EGFR activating mutation	115	Gefitinib	73.7	<0.001	10.8	<0.001	27.7	0.483
Inoue *et al* (2011)		115	Carboplatin–paclitaxel	30.7		5.4		26.6	
WJTOG 3405	EGFR activating mutation	86	Gefitinib	62.1	<0.0001	9.2	<0.0001	36	
Mitsudomi *et al*[34]		86	Cisplatin–docetaxel	32.2		6.3		39	
OPTIMAL	EGFR activating mutation	83	Erlotinib	83	<0.0001	13.1	<0.0001	24	0.6849
Zhou *et al* (2011)		82	Carboplatin–gemcitabine	36		4.6		24	
EURTAC	EGFR activating mutation	86	Erlotinib	54.6	<0.0001	9.7	<0.0001	19.3	0.87
Rosell *et al* (2012)		87	Cisplatin–carboplatin plus docetaxel–gemcitabine	14.9		5.2		19.5	
LL3	EGFR activating mutation: del19 (49%)	230	Afatinib	56	<0.0001	11.1	0.0004	28.2	0.39
Yang *et al*[37]	858R (40%)	115	Cisplatin–pemetrexed	23		13.9 (del19) 6.9		33.3 (del19) 27.6 (L858R) 0.0015(del19) 0.29 (L858R)	
LL6	EGFR activating mutation	242	Afatinib	66.9	<0.0001	11	<0.0001	28.2 21.1 (del19+) 40.3(L858R) 23.1	0.61
Wu *et al* (2013)	del19 (51%) L858R (38%)	122	Cisplatin–gemcitabine	23		5.6		31.4 (del19) 19.6 (L858R) 0.023 (del19) 0.34 (L858R) 23.5 18.4 (del19) 24.3 (L858R)	
Pooled analysis OS LL3–LL6	EGFR activating mutation		Afatinib					25.8	0.37
Yang *et al*[46]			Chemotherapy					31.7 (del19) 22.1 (L858R) 0.0001 (del19) 0.16 (L858R) 24.5	

Continued

Table 3 Continued

Study	*EGFR* status	N	Treatment	RR		Median PFS		OS	
				Per cent	p Value	Months	p Value	Months	p Value
								20.7 (del19) 26.9 (L858R)	
LL7	EGFR activating	160	Afatinib	70	0.008	11	0.0165	OS data are still	
Park *et al*, ESMO ASIA[47]	mutation	159	Gefitinib	56		12.7 (del19) 10.9 (L858R)		immature (actual HR 0.87)	
						0.1071 (del19) 0.0856 (L858R)			
						10.9 11 (del19) 10.8 (L858R)			
AURA	Pretreated T790M+	188	AZD9291	51		9.6			
Phase I Janne NEJM (2015)		127 centrally confirmed T790M+		61					
Phase II		61 centrally T790M –		21		2.8			
		210		61		8.6			
Phase I	Pretreated T790M+	46	CO1686	59		13.1			
Phase I/II	Pretreated T790M+	34	HM61713	58.8		Not reached			

EGFR, epidermal growth factor receptor; Lux Lung 3, LL3; Lux Lung 6, LL6; Lux Lung 7, LL7; mCRC, metastatic colorectal cancer; NSCLC, non-small-cell lung cancer; OS, overall survival; RR, response rate.

RAS WT population the superiority of EGFR mAbs arm in terms of OS, RR, depth of response and early tumour shrinkage. In contrast, CALGB 80405 did not support the superiority of anti-EGFR therapy. This discrepancy could be due to higher exposure rates to anti-EGFR therapy postprogression in the control arm of CALGB 80405 study and to the retrospective identification of *RAS* WT patients in this trials (table 2).[42 43 49]

Non-small-cell lung cancer

In the development of EGFR TKIs in NSCLC treatment, three populations of patients can be considered according to the presence or not of activating EGFR mutation: unselected, clinically selected and molecularly selected. Initially, clinical trials conducted in unselected populations did not show any significant benefit by TKIs treatment, alone or in combination with chemotherapy in first-line setting. From retrospective analysis, some clinical features such as Asian ethnicity, adenocarcinoma histology, female sex, smoking status or age seemed to be predictive of a major benefit from TKI treatment. Three studies were conducted in this setting, demonstrating that these clinical characteristics correlated with the presence of *EGFR* activating mutation (table 3).[33 50 51] In IPASS trial, gefitinib showed improvement in terms of RR (43% vs 32.2%, p<0.001), PFS (24.9% vs 6.7% 1-year progression-free patients, HR 0.74, p<0.001) and quality of life, without a significant improvement in OS (18.8 vs 17.4 months, HR 0.901, p=0.109) (table 3).[33] From 2009 to now, six randomised phase III trials have been conducted comparing EGFR TKIs to various platinum-based chemotherapy in the molecularly selected group, in Asian and Caucasian populations (table 3).[33–37 40 41] All these studies confirmed superior RR, PFS and quality of life with EGFR TKIs, without a significant advantage in OS (table 3). In all studies, PFS with TKI treatment reaches 8–10 vs 5–7 months with chemotherapy. In particular, in NEJ002 trial, gefitinib was compared to carboplatin–paclitaxel chemotherapy: PFS was 10.8 vs 5.4 months, HR 0.30, p<0.0001, along with a RR of 73.7% vs 30.1% (p<0.0001) in favour of gefitinib (table 3).[35] Similarly, EURTAC trial demonstrated the advantage of erlotinib versus platinum-based doublet chemotherapy in terms of PFS (9.7 vs 5.2 months, HR 0.37, p<0.0001) and RR (54.6% vs 14.9%) (table 3).[37] The lack of OS advantage by gefitinib or erlotinib therapy compared to chemotherapy is likely because of crossover to the other treatment arm. Results of Lux Lung 3 (LL3) and Lux Lung 6 (LL6) trials comparing Afatinib, irreversible pan-HER inhibitor, with first-line chemotherapy containing platinum plus pemetrexed or gemcitabine, respectively, showed an impressive superiority of TKI treatment in terms of ORR (58.1% vs 22.6% in LL3 and 66.9% vs 23% in LL6) and PFS (11.1 vs 6.9 months, HR 0.58, p=0.0004 in LL3; 11 vs 5.6 months, HR 0.28, p<0.0001 in LL6) (table 3).[40 41] In particular, in patients harbouring *EGFR* exon 19 deletion, PFS was 13.6 months in LL3 trial. A preplanned analysis of OS in the two trials confirmed a prolonged OS in *EGFR* deletion 19 positive patients (33.3 vs 21.1 months, HR 0.54, p=0.0015 in LL3; 31.4 vs 18.4 months, HR 0.64, p=0.023) (table 3).[46] Lux Lung 7 is the first trial comparing head-to-head two EGFR TKIs, afatinib and gefitinib, in first-line therapy of molecularly selected NSCLC patients. Preliminary data have been presented by Park at ESMO ASIA Congress in December 2015: as compared to gefitinib, afatinib treatment significantly reduced the risk of progression at 18 months (27% vs 15%, p=0.018) and at 24 months (18% vs 8%, p=0.018), showed higher ORR (70.0% vs 56.0%, p=0.008) and with a median duration of response of 10.1 months (95% CI 7.82 to 11.10) (table 3).[47] An equal low rate of treatment discontinuation due to inacceptable toxicities was reported in both arms (6.3%).[47] A trend through better PFS in NSCLC patients harbouring EGFR deletion 19 mutation compared to EGFR L858R mutation (12.7 vs 10.1 months) was evidenced, although OS data are not yet mature.[47]

PRIMARY AND ACQUIRED RESISTANCE TO EGFR BLOCKADE

Despite evidence of the efficacy of anti-EGFR in the treatment of mCRC and NSCLC patients, their use is limited by the presence of pre-existing intrinsic resistance mechanisms or by the ability of cancer cells to acquire resistance.[52 53] To this respect, main mechanisms of intrinsic resistance can be reconnected to a genetic alteration in EGFR, in other receptors tyrosine kinase (RTKs), or in the relative signalling pathway (figure 2).[52 53] On the other hand, the evolution of secondary resistance to anti-EGFR therapies can be defined as the consequence of a perturbation induced by EGFR blockade, causing the onset of independent clones. The overall scenario is complicated by the coexistence of different gene mutations in distinct tumour lesions (intermetastases heterogeneity), or even within different regions of the same lesion (intratumour heterogeneity).[54]

Primary resistance

The identification of genetic determinants of primary resistance to anti-EGFR therapies in CRC, in particular the activation of alternative pathway able to bypass the block of EGFR, is important to identify patients who should not be treated with EGFR mAbs. Beyond *RAS*, additional mechanisms of intrinsic resistance have been attributed to mutation in *BRAF* and *PI3KCA*;[55 56] amplification of *HER2*,[57] *MET*[58] and *KRAS*;[59 60] and loss of *PTEN* expression,[61] all of which are components of EGFR signalling transduction pathway or interact with. Overexpression of HER3[62] and IGF1[63] may also contribute to resistance.

Regarding NSCLC, the presence of activating mutation of *EGFR* gene is the predictor of response to EGFR TKIs, but the rate and the duration of response are

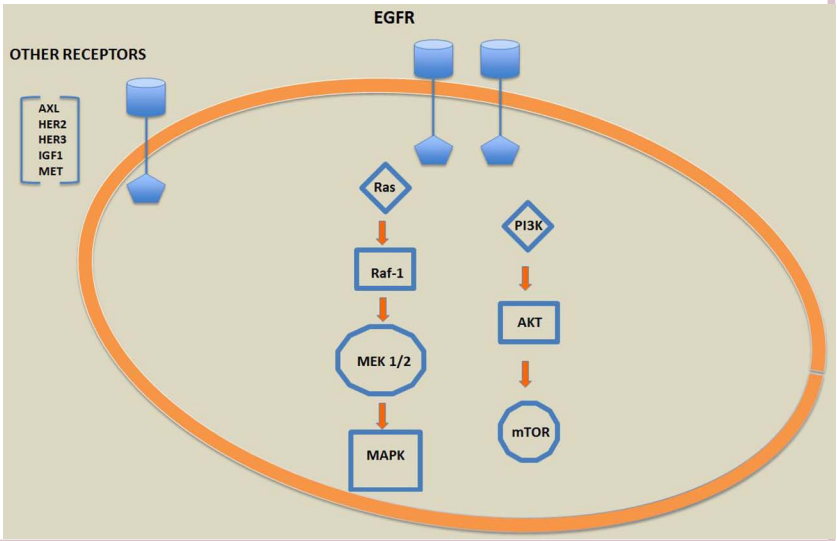

Figure 2 EGFR signalling pathways and major effectors involved in mechanisms of resistance.

different among patients harbouring common or uncommon mutations. The later ones seem to have a worse outcome.[64] The evidence of the resistance EGFR mutation T790M at baseline is associated with primary resistance since it is within the gatekeeper residue and restores the affinity for ATP to the level of *EGFR* WT, decreasing the effect of TKI.[65 66] Recent studies reported a prevalence of primary T790M in a percentage of patients from 35% to 79%.[67] When T790M is present together with sensitising mutation, their kinase activity is synergistic and the pressure of treatment with first-generation TKIs can select for rapid growth of the T790M positive subclones until acquisition of resistance. Recent data suggest that response to EGFR TKIs can also be influenced by high breast-related cancer antigens 1 (BRCA1) levels, leading to increased DNA damage repair ability[68] or activation of other signalling pathways such as NFkβ;[69] these finding needed further studies to be confirmed.

Mutations that contribute to secondary treatment resistance can also be responsible for the initial lack of response to a drug (primary or de novo resistance).

Secondary resistance

The activation of EGFR is mediated by ligands in CRC and by mutations in NSCLC. Despite this difference, mechanisms of acquired resistance to anti-EGFR treatment are similar and can be broadly categorised in three levels.[70] The first one: mutations in the receptor EGFR[71 72] or activation of parallel RTKs, such as HER2 and MET;[57 58 73 74] the second level involving mutation in bypass point of the pathway, such as RAS and BRAF;[59 60 70 75 76] the third level including the activation of downstream effectors, such as PIK3CA and MAPK-ERK (figure 2).[77–80] For the first level, anti-EGFR resistance resulted from a change in the epitope of EGFR that recognises the agent. In mCRC, the EGFR S492R mutation has been detected in patients that progressed after an initial response to cetuximab.[81] Interestingly, these resistant patients responded to panitumumab treatment. Other novel EGFR ectodomain mutations have been found and are under scrutiny.[72] Similarly, in NSCLC, a secondary mutation in EGFR exon 20 (T790M) occurred in more than 50% of cases of TKIs resistance.[82] T790M is a substitution of methionine for threonine in position 790 in kinase domain; this substitution altered the ATP-binding pocket conformation, enhancing the affinity for ATP with respect to TKIs.[65] This setting of patients can benefit from treatment with AZD9291.[83] Other known secondary resistance mutations are D761Y, L747S and T854A.[84]

HER2 amplification was found in 5% of mCRC patients (*KRAS*, *NRAS*, *BRAF*, *PIK3CA* WT) and was correlated with resistance to cetuximab,[85] thus together with xenopatients data[57] providing the rational for an ongoing clinical study with *HER2*-targeted therapies after failure of anti-EGFR.[86]

In the second level, despite the already known role of *RAS*, the negative prognostic impact of *BRAF* mutation (10–15% of mCRC patients) has been confirmed across several studies, but the debate still continues regarding its value as a predictive marker of response to anti-EGFR therapy.[19–21 87] Finally, at the third level, *PI3KCA* mutations in exon 20 were associated with lack of response to cetuximab in CRC (15–18%), whereas mutation in exon 9 was not, depending on the mutation in helical or kinase domain.[55 56]

All these alterations converge to activate the principal downstream effector of EGFR pathway: MAPK-ERK, whose EGFR-independent overactivation allows the tumour to survive in the presence of anti-EGFR drugs (figure 2).[88]

FUTURE DIRECTIONS

Targeting EGFR is an approved clinical strategy for the treatment of patients with *KRAS* and *NRAS* WT mCRC

and NSCLC with activating mutations of EGFR. Unfortunately, responses are transient, and acquired resistance inevitably emerges, which limits the clinical efficacy of these drugs. Several targeted therapeutic strategies designed to circumvent resistance driven by downstream pathway reactivation are being investigated in ongoing clinical trials that combine anti-EGFR drug with other targeting therapies.[77 78 86 88] However, there remains a significant unmet need for a therapeutic strategy to overcome resistance to anti-EGFR therapies.

Two new anti-EGFR drugs are under clinical investigation in mCRC: SYM004 and MM151. SYM004 is a 1:1 mixture of two recombinant human mouse chimeric mAbs directed against non-overlapping epitopes of the EGFR (table 1).[89] The binding site of the two antibodies is different from cetuximab, and therefore SYM004 could hypothetically be effective even in the presence of mutations in the extracellular domain (ECD) of the EGFR (table 1).[89 90] A unique feature of SYM004 is its ability to mediate rapid EGFR internalisation and subsequent degradation of internalised receptors via EGFR cross-linking.[89–91] The efficacy of this new agent is under investigations in phase II trial (CinicalTrials.gov Identifier: NCT02083653).

MM151 is a mixture of three different monoclonal IgG1 antibodies directed towards three different, non-overlapping epitopes of the EGFR. MM151 has demonstrated superiority to currently approved and investigated mAbs in preclinical models, displaying improvements in EGFR pathway inhibition and downstream signalling, as well as enhanced downregulation of the EGFR and engagement of innate immune responses (table 1).[92 93] Notably, MM151 targets regions of the EGFR distinct from those affected by ECD mutations (table 1).[94] Based on these preclinical studies, MM151 has advanced to clinical testing, and phase I results to date demonstrate an acceptable safety profile and objective clinical activity in refractory patients with cancer, including those failing cetuximab therapy (ClinicalTrials.gov Identifier: NCT01520389).

Similarly, in NSCLC, in over 50% of cases, acquired resistance to first-generation TKIs erlotinib or gefitinib is due to the onset of the second-site EGFR mutation T790M, substituting threonine for methionine at position 790 in exon 20.[83] In recent times, a great innovation in the treatment of EGFR T790M positive NSCLC is represented by the development of third-generation EGFR TKIs, which include the WZ4002, CO1686, AZD9291 and HM61713 inhibitors.[95] They demonstrated strong clinical activity in this setting of patients, with tumour responses in >50% of cases. Furthermore, since they are designed specifically for targeting mutant EGFR (including activating mutations such as L858R and/or T790M), they spare the WT receptor, thus inducing less toxicity than first-generation and second-generation inhibitors.[96] In particular, AZD9291 (osimertinib) has been just approved by FDA for the treatment of patients with documented T790M positive NSCLC at progression to a first-line therapy with TKI (table 1). AZD9291 in the AURA trial[84] demonstrated high efficacy in this setting of patients with an overall objective tumour RR of 61% in centrally confirmed T790M patients, with a median PFS of 9.6 months. Interestingly, no dose limiting toxicity occurred, and tolerability profile was good, making AZD9291 suitable for targeted therapy combinations. An ongoing multi-arm phase I study is evaluating combination of AZD9291 with anti-PD-L1 antibody, MET inhibitor or MEK inhibitor (CinicalTrials.gov Identifier: NCT02143466).

Some data are currently available regarding resistance to AZD9291, deriving from the analysis of resistance biopsy specimens and circulating DNA collected from patients during AURA study.[96] A new EGFR resistance mutation has been identified, EGFR C797S mutation, that confers resistance to all irreversible EGFR TKIs impairing the covalent binding to Cysteine 797 at the lip of the ATP-binding site, HER2 amplification, MET amplification and BRAF V600E mutation.

The availability of new EGFR TKIs will encourage the treatment of EGFR-mutant NSCLC patients with multiple lines of EGFR-targeted therapies; thus, future researches will identify new predictive biomarkers for selection of patients and identification of the best sequence of use of EGFR TKIs in clinical practice.

CONCLUSIONS

For decades, all patients with tumours originating from the same primary organ were treated as homogeneous population. Recent findings in the field of biomarkers for targeted therapy highlight the need for a new approach based on genomic analysis. Increasing knowledge of the mechanism of resistance to anti-EGFR suggests the importance of a deeper molecular characterisation of the primary tumour and of monitoring the molecular evolution of the tumour through repeated biopsies or analysis of circulating free tumour-derived DNA (liquid biopsy). The liquid biopsy is a rapid non-invasive method for tumour molecular profiling. It can allow tracking tumour clonal evolution and designing novel therapeutic strategies. Siravegna *et al*[97] reported that acquired resistance to anti-EGFR mAbs is associated with emergence of RAS pathway mutations that can be detected in the blood before disease progression is clinically manifest.

In the future, it is likely that new combination of therapies against known targets in EGFR pathways or in interacting pathways will be explored in preclinical and clinical studies. If a genetic driver cannot be found, a great promise could be reconducted all the different alterations, responsible of tumour progression, to one single target.

In this scenario, MAPK-ERK pathway is the ideal candidate, as a convergence point where several upstream signalling pathways can be blocked.

Thus, convergent evolution, such as the theory of everything, explains that one single force can govern and unify all of the aspects of the universe.

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