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Recent Advances in Molecular Targeted Therapy for advanced Colorectal Cancer and Non – Small Cell Lung Cancer

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Abstract

Cancer remains one of the most life-threatening diseases to date. Traditionally, chemotherapy treatments for locally advanced or metastatic cancer had little or no efficacy. For example, colon and lung cancers were associated with poor clinical outcomes as recently as a decade ago. However, increased understanding of the molecular mechanisms underlying carcinogenesis has spurred focus on the development and incorporation of molecular targeted agents in current therapeutic options for these difficult-to-treat diseases. Such agents have the ability to target a variety of cancer relevant molecules, including epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) and its receptor. In addition, protein tyrosine kinases have been proven to be good targets to develop small molecule inhibitors that compete with ATP and inhibit kinase activity. These inhibitors have clinically effective responses. In this review, we describe the current status of targeted therapies in the treatment of advanced colon and lung cancers focusing on clinical data based on experience with in monoclonal antibodies and tyrosine kinase inhibitors acting in these pathways.

Keywords: Molecular-targeted drug; Chemotherapy; CRC; NSCLC; Review

Introduction

The World Health Organization announced that cancer is a leading cause of death worldwide, accounting for 7.6 million deaths, around 13% of all deaths in 2008. Deaths from cancer are expected to continue rising worldwide, with an estimated 13.1 million deaths in 2030. In Japanese vital statistics of 2010, lung cancer was the leading cause of death (23.8%) for males, followed by stomach (15.6%) and colorectal cancer (11%). Similarly, lung cancer was the 2nd cause of death in females (13.7%; colorectal was 14.4%). Thus, lung and colorectal cancer are two leading causes of death in Japan.

Cancer treatment consists of surgery, radiotherapy, and chemotherapy and one or more interventions are carefully selected depending on the tumor and stage of the disease. The goal of treatment is to cure the disease for leukemias, lymphomas, and testicular seminoma, if appropriate chemotherapy is provided. Although we have not improved mortality rates or prolonged survival times for metastatic cancer such as colon or lung cancer until recently, we have now identified the driving genes and pathways of various tumors. According to a statement by the NCI (National Cancer Institute), targeted cancer therapy includes drugs or other substances that block the growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression. By focusing on molecular and cellular changes that are specific to cancer, targeted cancer therapy is expected to be more effective than other types of treatment, i.e. cytotoxic antineoplastic agents and radiotherapy, and less harmful to normal cells. Targeted cancer therapy is being studied as a single treatment, in combination with other targeted therapy, and/or cytotoxic drugs. Most targeted therapy includes either small-molecule drugs or monoclonal antibodies. Small-molecule drugs are typically able to diffuse into cells and can act on targets that are found inside the cell. Most monoclonal antibodies cannot penetrate the cell's plasma membrane and are directed against targets that are expressed outside cells or on the cell surface. This review will focus on the recent development of standardized treatment including new molecular target agents against advanced cancers that have no longer satisfactory treatment until recently, especially colon and lung cancers.

Advances in cytotoxic chemotherapy

Metastatic colorectal cancer (mCRC): Since the 1960s, 5-fluorouracil (5-FU) was the only effective chemotherapeutic agent in the treatment of mCRC [1]. Since the 1990s, there have been several attempts to improve the therapeutic effectiveness of 5-FU using a modulating drug such as leucovorin (LV). Prior to 2000, bolus 5-FU and LV were the accepted standard treatment for mCRC (Figure 1). However, this treatment was shown to extend patient survival to only 5 months longer than that with the best supportive care (BSC) [2,3]. There has been an increased understanding of the optimal method and schedule of administration of 5-FU. The rationale for continuous infusion of 5-FU is based on its very short half-life of 10 minutes with bolus delivery. Tumor response rate (RR) was significantly higher in patients assigned to infusional 5-FU than that in patients assigned to bolus 5-FU (22% vs. 14%). Overall survival (OS) was also significantly longer in patients assigned to infusional 5-FU, although median survival times (MSTs) were similar [4].

Over the last decade, the chemotherapeutic agents oxaliplatin (OX) and irinotecan (IRI) have been approved. Both LV and 5-FU can be combined with IRI or OX to make a treatment termed FOLFIRI or FOLFOX, respectively. These treatments consist of administration of a bolus of 5-FU, LV, and either OX or IRI followed by an infusion of 5-FU over 46 hours (Figure 2). Two new drugs, IRI and OX, have demonstrated survival improvements when given either alone or in combination with LV plus 5-FU, in first- or second-line therapy [5-8].

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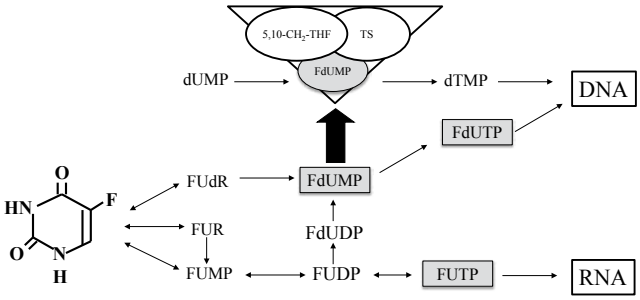

Figure 1: The mechanism of action of 5-FU. 5-FU is converted to three main active metabolites: fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). FUMP is phosphorylated to fluorouridine triphosphate (FUTP), or converted to fluorodeoxyuridine diphosphate (FdUDP). In turn, FdUDP can either be phosphorylated or dephosphorylated to generate the active metabolites FdUTP and FdUMP, respectively. FdUMP acts by forming a complex with thymidylate synthase (TS), an integral component of DNA synthesis. The other active metabolite, FUTP, is integrated into cellular RNA and may alter RNA processing and function. Leucovorin (LV) increases the intracellular pool of 5,10-methylene tetrahydrofolate (CH2THF), thereby enhancing TS inhibition by FdUMP.

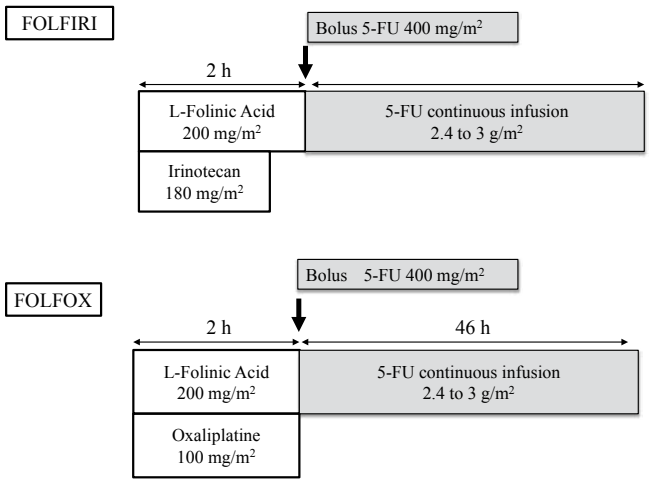

Figure 2: Design of FOLFIRI and FOLFOX regimens.

Tournigand et al. performed a randomized study to evaluate these two improved regimens (FOLFOX or FOLFIRI) and to determine the best sequence to treat patients with mCRC (GERCOR study) [9]. Median survival was 21.5 months in patients allocated to FOLFIRI followed by FOLFOX6 versus 20.6 months allocated to FOLFOX6 followed by FOLFIRI (P=0.99). Median progression-free survival (PFS) was 14.2 months in arm FOLFIRI - FOLFOX6 sequence versus 10.9 months in arm FOLFOX6 - FOLFIRI sequence (P=0.64). In first-line therapy, FOLFIRI achieved 56% RR and 8.5 months of median PFS, while FOLFOX6 achieved 54% RR and 8.0 months of median PFS (P=0.26). Second-line FOLFIRI achieved 4% RR and 2.5 months median PFS, while FOLFOX6 achieved 15% RR and 4.2 months PFS. These results concluded that both regimens had similar efficacy when used as first-line therapy. Therefore, either FOLFOX or FOLFIRI can be considered a standard option for first-line treatment of mCRC. Recently, XELOX includes a combination of oral 5-FU derivatives known as capecitabine (XEL) plus OX. XEL is an oral fluoropyrimidine with similar efficacy as 5-FU.

Advanced non-small cell lung cancer (NSCLC): NSCLC accounts for approximately 85% of all cases of lung cancer. Approximately 40% of patients with NSCLC present at an advanced stage. Patients with a PS score of 2 have a poor prognosis, with an MST of approximately 4 months [10]. Combination chemotherapy is considered to be standard care for patients with advanced NSCLC [11]. Ramalingam and Belani [12] did a review in "*The Oncologist*" about cytotoxic chemotherapy of advanced NSCLC. Both platinum-based two-drug regimens and non-platinum combinations have been shown to be efficacious as first-line treatment [13-15]. The benefits of platinum-based combination chemotherapy over BSC were first reported in a randomized clinical trial published in 1988 [16]. Further evidence for the efficacy of platinum-based chemotherapy was provided by a meta-analysis of all available randomized clinical trials [17]. The analysis demonstrated that cisplatin-based chemotherapy was associated with a 10% greater 1-year survival rate. Non-platinum newer agents such as taxanes, gemcitabine, and vinorelbine have been combined with platinum compounds. Several randomized clinical trials have been conducted to evaluate cisplatin as monotherapy or in combination with a taxane, gemcitabine, or vinorelbine [18-20].

Pemetrexed is a multi-targeted inhibitor of three key enzymes in the folate metabolic pathway: thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT) [21,22]. To compare the efficacy and toxicity of pemetrexed versus docetaxel in patients with advanced NSCLC previously treated with chemotherapy, a phase III study was carried out [23]. Treatment with pemetrexed resulted in clinically equivalent efficacy outcomes, but with significantly fewer side effects than that with docetaxel in second-line and should be considered a standard treatment option for second-line NSCLC when available. Intriguingly, in this study, OS and PFS of patients with squamous cell histology on the pemetrexed arm were significantly low (6.2 months vs. 7.4 months, 2.3 months vs. 2.7 months) [24]. In 2008, Scagliotti et al. [25] compared first-line pemetrexed/cisplatin to gemcitabine/cisplatin and found that pemetrexed was not inferior in terms of OS. As is the case with a second-line setting, results of subgroup analysis showed that pemetrexed improved OS in patients with non-squamous histology (adenocarcinoma and large cell carcinoma). Based on these results, pemetrexed has become a preferred treatment option either as single agent therapy in second-line or in combination with cisplatin in first line for patients with non-squamous cell histology.

Advances in molecular targeted therapy

Treatment targeted at VEGF: Angiogenesis is essential for tumor growth and metastasis [26]. Thus, controlling tumor-associated angiogenesis is a promising tactic in suppressing cancer progression. The tumor microenvironment comprises numerous signaling molecules and pathways that influence the angiogenic response. Several anti-angiogenic agents are currently approved by the Food and Drug Administration (FDA) for cancer, including the humanized antibody (bevacizumab) [27], which targets VEGF-A, the tyrosine kinase inhibitor sorafenib, which targets Raf and VEGF and PDGF receptors, and the tyrosine kinase inhibitor sunitinib, which targets VEGF and PDGF receptors [28] (Figure 3). Bevacizumab (BV) also has been shown to decrease interstitial pressure and to increase oxygenation in tumors, thereby potentially improving the ability of chemotherapy to reach and act within the tumor [29] (Figure 4).

BV was approved by the US FDA as first-line treatment for patients with mCRC in 2004 (Table 1). Hurwitz et al. designed a randomized trial (AVF2107g) [30] to compare IRI plus bolus 5-FU/LV (IFL) and

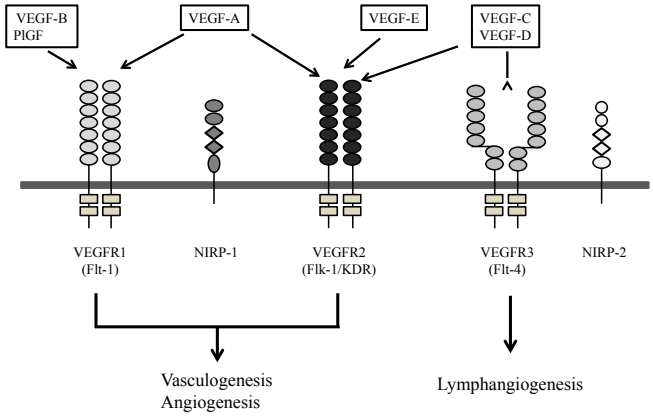

Figure 3: Binding specificity of VEGF family members and their receptors. VEGF ligands mediate their angiogenic effects by binding to specific VEGF receptors, leading to receptor dimerization and subsequent signal transduction. VEGFR-1 and VEGFR-2 are mainly associated with angiogenesis. VEGFR-3 is associated with lymphangiogenesis.

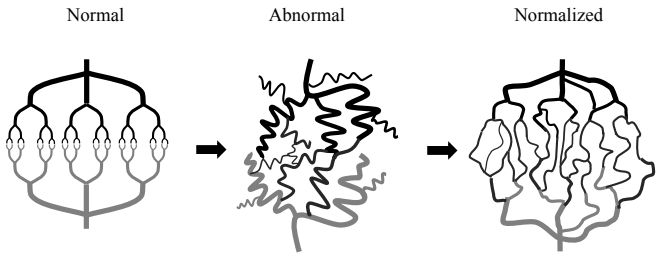

Figure 4: Effect of anti-VEGF on normalization of tumor vasculature. In tumors, VEGF-induced vessels are structurally immature and functionally abnormal. This abnormal vasculature leads to increased interstitial fluid pressure. It is proposed that anti-angiogenic therapies prune away these vessels, resulting in a vasculature that is both resistant to further treatment and inadequate for delivery of drugs or oxygen.

IFL with BV. The median duration of survival was 20.3 months in the IFL plus BV group, whereas it was 15.6 months in the control group, corresponding to a hazard ratio (HR) for death of 0.66 (P<0.001). The median duration of PFS was 10.6 months versus 6.2 months (HR for disease progression, 0.54; P<0.001); the corresponding rates of response were 44.8% and 34.8% (P=0.004). The median duration of the response was 10.4 months and 7.1 months (HR for progression, 0.62; P=0.001). Grade 3 hypertension was more common during treatment with IFL plus BV than with IFL plus placebo (11.0% vs. 2.3%) but was easily managed. BV can cause hypertension, bleeding, thrombosis, gastrointestinal perforation, and can delay wound healing [31]. Saltz et al. found that the addition of BV to OX-based chemotherapy significantly improved PFS from 8.0 to 9.3 months (N0169963 study) [32]. XELOX combination with or without BV was found to be non-inferior to FOLFOX with or without BV [33].

In NSCLC, high levels of VEGF expression are associated with a poor prognosis [34], suggesting that treatment targeting this pathway may be of significant therapeutic value. A randomized phase II trial showed that the addition of BV to carboplatin-paclitaxel improved RR (31.5% vs. 18.8%) and time to progression (7.4 months vs. 4.2 months) relative to chemotherapy alone in patients with advanced NSCLC [35]. There was also no significant improvement in OS. In this trial, major hemoptysis was associated with squamous cell histology, tumor necrosis and cavitation, and disease location close to major blood vessels. In order to do this major adverse event, only patients with predominantly non-squamous NSCLC were studied in subsequent trials. In 2006, the FDA approved BV for treating advanced NSCLC [36], based on data from a pivotal phase III trial (E4599) conducted by the Eastern Cooperative Oncology Group (ECOG) [37,38]. Patients with recurrent or advanced non-small-cell lung cancer (excluding squamous-cell tumors, brain metastases) were assigned to combination with paclitaxel and carboplatin or combination with paclitaxel and carboplatin plus BV. The median survival was 12.3 months in the group assigned to chemotherapy plus BV, while it was 10.3 months with chemotherapy alone. PFS in the two groups was 6.2 and 4.5 months, respectively, with corresponding RR of 35% and 15% (P<0.001). This was the first study to demonstrate a prolongation in the survival period of patients with NSCLC following administration of a molecule-targeted drug in combination with chemotherapy. A subsequent randomized phase III clinical study (AVAiL) [39] in which patients with no prior history of chemotherapy were divided into three treatment groups, a gemcitabine + cisplatin group (GC), and GC + BV 7.5 or 15 mg/kg groups. In this study, PFS differed significantly between any two of the three groups (6.1 months vs. 6.7 months *vs.* 6.5 months). This result suggested that the benefit of BV could be dependent on the chemotherapy regimen used.

To date, no predictive molecular marker for the activity of BV is available. In general, its identification is likely to be more difficult in angiogenesis targeting agents as host mechanisms play an important role in the anti-tumor effect.

Treatment targeted at EGFR: Epidermal growth factor receptor (EGFR) and its downstream signaling pathways regulate key cellular events that drive the progression of many neoplasms. EGFR is expressed in a variety of human tumors, including carcinomas of the colon, lung, head and neck, pancreas, breast, ovary, bladder, and kidney. Mutations, gene amplification, and protein overexpression of various elements of this pathway not only contribute to carcinogenesis but also impact on prognosis and provide specific targets for therapeutic intervention. EGFR is a transmembrane tyrosine kinase receptor that belongs to the ErbB family of cell membrane receptors. Other receptors in this family include HER2/c-neu (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4) [40]. The EGFR signaling cascade begins with ligand activation of EGFR. Ligands can bind the ErbB family of receptors, including EGF and transforming growth factor-alpha [41]. Ligand binding induces dimerization of the receptor with formation of homodimers and heterodimers, which leads to the activation of tyrosine kinase. Two main intracellular pathways activated by EGFR are the mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT) pathway (Figure 5). These pathways lead to the activation of various transcription factors that then impact cellular responses such as proliferation, migration, differentiation, and apoptosis [42].

There are two classes of EGFR antagonists that are used in clinical practice for mCRC and NSCLC at this time: anti-EGFR monoclonal antibodies (cetuximab and panitumumab,) and small molecule EGFR tyrosine kinase inhibitors (TKIs) (gefitinib and erlotinib).

Anti-EGFR antibodies: Cetuximab (C-mab) is a recombinant, human/chimeric IgG_1 monoclonal antibody (mAb) that binds to the extracellular domain of human EGFR on both normal and tumor cells, and competitively inhibits the binding of epidermal growth factor and other ligands [43]. It has also been shown to mediate antibody-dependent cell cytotoxicity (ADCC) [44]. Likewise, panitumumab

(P-mab) is a fully human IgG_2 mAb. In 2004, the FDA approved C-mab for the treatment of mCRC with IRI-, and as a single agent for patients intolerant of IRI-based therapy (Table 2).

Cunningham et al. compared the efficacy of C-mab in combination with IRI with that of C-mab alone that was refractory to treatment with IRI (BOND trial) [45]. The rate of response in the combination-therapy group was significantly higher than that in the monotherapy group (22.9% vs. 10.8%). The median time to progression was significantly greater in the combination therapy group (4.1 months vs. 1.5 months, P<0.001). MST was 8.6 months in the combination therapy group and 6.9 months in the monotherapy group (P=0.48). As monotherapy, C-mab's benefit in terms of OS was confirmed in a large phase III study (NCIC-CTG CO.17 trial) [46] over that of C-mab to BSC in patients with EGFR expressing mCRC who had previously been treated with fluoropyrimidine, IRI, and OX, or had contraindications to these treatments. The partial RR with single-agent C-mab was 8.0% versus 0% for BSC; 29.6% of patients receiving C-mab achieved stable disease, versus 10.2% of those with BSC. The median OS time was significantly greater in patients treated with C-mab (6.1 months versus 4.6 months; p=0.005). In first-line therapy, C-mab combined with IRI (CRYSTAL) [47] and OX with C-mab (OPUS) [48] trials were the first two randomized trials to evaluate cytotoxic chemotherapy with or without C-mab in a front-line setting. In the CRYSTAL trial, C-mab plus FOLFIRI resulted in a longer median PFS interval than with FOLFIRI alone (8.9 months *vs.* 8.0 months) and a significantly higher RR. Although the CRYSTAL study met its primary endpoint (PFS), the magnitude of the benefit was considered underwhelming. In the randomized phase II OPUS trial, the addition of C-mab to FOLFOX4 resulted in a greater RR (45.6% VS. 35.7%), but no difference in terms of PFS (median, 7.2 months for both groups) was noted.

In the phase III FLEX trial [49] where C-mab with cisplatin/vinorelbine was compared with cisplatin/vinorelbine alone in advanced NSCLC patients with EGFR-detectable, significant improvements in OS for the C-mab group were reported (11.3 months *vs.* 10.1 months; P=0.0441). Based on this large phase III trial, current recommendations from the National Comprehensive Cancer Network, Inc. (NCCN) include C-mab /vinorelbine/cisplatin as a first-line therapy option in patients who meet criteria for therapy with C-mab. This benefit appeared to be consistent amongst patients with squamous histology and stands in contrast to data for other agents where a selective benefit was observed in patients with adenocarcinoma.

Predictive marker of anti-EGFR antibody therapy: The *K-ras* proto-oncogene encodes guanosine 5'-triphosphate (GTP) binding protein at the beginning of the MAPK signaling pathway. Somatic *K-ras* mutations have been found in many cancers, including 30%-40% of colorectal cancers, and are an early event in carcinogenesis. *K-ras* mutations, most commonly codon 12/13 missense mutations, lead to constitutive activation of the *K-ras* protein by abrogating GTPase activity. These mutations result in unregulated downstream signaling that will not be blocked by antibodies that target the EGFR receptor. The *BRAF* gene encodes a serine-threonine protein kinase that is downstream of K-ras in the MAPK signaling pathway. *BRAF* mutations occur in 5-22% of all colorectal cancers [50,51]. The most frequently reported *BRAF* mutation is a valine-to-glutamic acid amino acid (V600E) substitution [52]. *BRAF* mutations are mutually exclusive with *K-ras* mutations [50].

Subsequently, analysis of pooled data from CRYSTAL and OPUS studies confirmed the consistency of the benefit obtained across all efficacy end-points from adding C-mab to first-line chemotherapy in patients with the *K-ras* wild-type [53] (Table 3). Individual patient

Study name	Setting	Treatment	RR (CR+PR) (%)	mPFS (months)	mOS (months)
AVF2107g (phase III)	1st-line (n=923)	IFL+BV IFL 5-FU/LV+BV	44.8 (p=0.007) 34.8 40.0	10.6 (p<0.001) 6.2 8.8	20.3 15.6 18.3
N0 169963 (phase III)	1st-line (n=1400)	XELOX/FOLFOX+BV XELOX/FOLFOX	47 49	9.4 (p<0.001) 8.0	21.3 19.9
E4599 (phase III)	1st-line (n=850)	PTX/CBDCA+BV PTX/CBDCA	35 (p<0.001) 15	6.2 (p<0.001) 4.4	12.3 (p=0.003) 10.3
AVAiL (phase II)	1st-line (n=1043)	GEM/CDDP+BV(15mg/kg) GEM/CDDP+BV(7.5mg/kg) GEM/CDDP	30.4 (p= 0.0023) 34.1 (p<0.0001) 20	6.5 (p=0.03) 6.7 (p=0.003) 6.1	N.R. N.R. N.R.

RR: response rate, CR: complete response, PR: partial response, mPFS: median progression free survival, mOS: median overall survival, N.R.: not reported. BV: bevacizumab, IFL: irinotecan/5-FU/leucovorin, XELOX: oxaliplatine/capecitabine, FOLFOX: oxaliplatine/5-FU/leucovorin, PTX: paclitaxel, CBDCA: carboplatin, GEM: gemcitabine, CDDP: cisplatin

Table 1: Clinical trials of anti-VEGF antibody for mCRC and advanced NSCLC.

Study name	Setting	Treatment	RR (CR+PR) (%)	mPFS (months)	mOS (months)
BOND (phase II)	IRI failure (n=329)	IRI+C-mab C-mab	22.9 (p=0.007) 10.8	4.1 (p<0.001) 1.5	8.6 6.9
NCIC CO.17 (phase III)	5-FU, IRI, OX failure (n=572)	C-mab+BSC BSC	8.0 (p<0.001) 0	1.9 (p<0.0001) 1.8.	6.1 (p=0.005) 4.6
CRYSTAL (phase III)	1st-line (n=1217)	FOLFIRI+C-mab FOLFIRI	46.9 (p<0.004) 38.7	8.9 (p<0.048) 8.0	19.9 18.6
OPUS (phase II)	1st-line (n=337)	FOLFOX+C-mab FOLFOX	46 36	7.2 7.2	N.R. N.R.

RR: response rate, CR: complete response, PR: partial response, mPFS: median progression free survival, mOS: median overall survival, N.R.: not reported. C-mab: cetuximab, IRI: irinotecan, OX: oxaliplatine, FOLFIRI: irinotecan/5-FU/leucovorin, FOLFOX: oxaliplatine/5-FU/leucovorin

Table 2: Clinical trials of anti-EGFR antibody for Mcrc.

data from each study were analysed for OS, PFS, and ORR in patients evaluable for *K-ras* and *BRAF* mutation status. In patients with *K-ras* wild-type tumours adding C-mab to chemotherapy led to a significant improvement in OS, PFS and RR. *BRAF* mutations were detected in 70/800 evaluable tumours. No significant differences were found in outcome between treatment groups in these patients. Prognosis was worse in each treatment arm for patients with *BRAF* tumour mutations than those with *BRAF* wild-type tumours. A *BRAF* mutation does not appear to be a predictive biomarker in this setting, but is a marker of poor prognosis. In 2006, the FDA approved P-mab for EGFR-expressing mCRC patients who had progressed on fluoropyrimidine, OX, and IRI containing regimens. The benefit of P-mab is also concentrated in the *K-ras* wild-type population. A Phase III randomized trial of FOLFOX with or without P-mab in previously untreated patients (PRIME) was performed [54]. The PFS time was longer in the investigational arm (9.6 months versus 8.0 months). The median OS time had not yet been reached in the investigational arm at the interim analysis. In combination with FOLFIRI for second-line treatment [55], the primary endpoint of a PFS difference (5.9 months versus 3.9 months; p=0.004) was fulfilled with the addition of P-mab but the OS endpoint was not met. *K-ras* mutations have been shown to predict the response to C-mab and P-mab for mCRC. On the other hand, the *K-ras* mutational status has been assessed in NSCLC patients receiving cetuximab [47]. Interestingly, correlative analyses accompanying FLEX suggest no differences in clinical outcomes on the basis of the *K-ras* status [56]. In addition to laboratory biomarkers, much interest surrounds the use of a rash as a predictor of C-mab efficacy.

EGFR tyrosine kinase inhibitors (TKI): Gefitinib and erlotinib are orally bioavailable synthetic anilinoquinazolines that selectively and reversibly prevent ATP binding and autophosphorylation of the EGFR tyrosine kinase. Clinical trials of gefitinib and erlotinib have shown the therapeutic viability of targeted agents in NSCLC (Table 4). Gefitinib was the first anti-EGFR agent shown to have clinical activity. In two phase II trials gefitinib was evaluated in patients with advanced NSCLC, stage III or IV, who were treated with one or more regimens containing cisplatin or carboplatin and docetaxel and had progressed. The IDEAL-1 study [57] was carried out primarily in Europe and Japan in 2002. RR was 18.4% in the 250 mg/day group and 19.0% in the 500 mg/day group. Also, in the IDEAL-2 study [58], carried out in the USA, RR was almost the same between the 250 mg/day group (11.8%) and the 500 mg/day group (8.8%), and there was no difference in the survival period between these two dose groups. In a subgroup analysis, RR was significantly higher in females, patients with adenocarcinoma, and Japanese patients. On the basis of these results, the Japanese Regulatory Authority approved the use of gefitinib in 2002, earlier than in other countries around the world. ISEL [59] was a phase III clinical study in which previously treated patients with NSCLC were randomly allocated to gefitinib and control. Results revealed that RR was significantly higher in the gefitinib group than that in the control group (8% *vs.* 1%, p<0.0001). As concerns the MST which is the primary endpoint, there was no significant difference between the two groups (p=0.087). However, in subgroup analysis, gefitinib was shown to extend survival in non-smokers (MST: 8.9 months *vs.* 6.1 months, p=0.012) and Asian patients (MST: 9.5 months *vs.* 5.5 months, p=0.01). In 2008, a paradigm shift towards molecular profiling in our treatment choice resulted from the IPASS study [60] which enrolled East Asian patients and compared first line carboplatin / paclitaxel with gefitinib in patients with adenocarcinoma who were light or never smokers. In this selected population, PFS was superior with gefitinib therapy. In subset analyses, patients with an *EGFR* mutation had superior PFS with gefitinib, whereas patients with a wild-type *EGFR* had superior PFS with chemotherapy. This was the first study to definitively identify the mutation status as an important predictive marker for EGFR-TKI therapy.

Study name	*K-ras* status	Treatment	RR (CR+PR) (%)	mPFS (months)	mOS (months)
CRYSTAL	Wild type (n=348)	FOLFIRI+C-mab FOLFIRI	59.3 (p=0.003) 43.2	9.9 (p<0.017) 8.7	24.9 21.0
	Mutant (n=192)	FOLFIRI+C-mab FOLFIRI	36.2 (p=0.46) 40.2	8.1 7.6	17.5 17.7
OPUS	Wild type (n=134)	FOLFOX+C-mab FOLFOX	60.7 (p<0.011) 37	7.7 (p<0.016) 7.2	N.R. N.R.
	Mutant (n=99)	FOLFOX+C-mab FOLFOX	32.7 49	8.6 (p<0.02) 5.5	N.R. N.R.

RR: response rate, CR: complete response, PR: partial response, mPFS: median progression free survival, mOS: median overall survival, N.R.: not reported. C-mab: cetuximab, FOLFIRI: irinotecan/5-FU/leucovorin, FOLFOX: oxaliplatine/5-FU/leucovorin,

Table 3: The CRYSTAL and OPUS trials: Overall efficacy based on *K-ras* status.

Study name	Setting	Treatment	Pts (n)	RR (CR+PR) (%)	mPFS (months)	mOS (months)
IDEAL-1 (phase II)	2nd and 3rd-line	gefitinib (250 mg) (500 mg)	103 106	18.4 19.0	2.7 2.8	7.6 8.0
IDEAL-2 (phase II)	3rd-line	gefitinib (250 mg) (500 mg)	102 114	12 9	N.R. N.R.	7.0 6.0
ISEL (phase III)	2nd and 3rd-line	gefitinib (250 mg) placebo	1129 563	8 (p<0.0001) 1	N.R N.R.	5.6 5.1
BR.21 (phase III)	2nd and 3rd-line	erlotinib (150 mg) placebo	448 243	9 (p<0.0001) <1	2.2 (p<0.001) 1.8	6.7 (p=0.001) 4.7
IPASS (phase III)	1st-line *EGFR* Mutation positive patients	gefitinib (250 mg) chemotherapy	609 608	43 (p<0.001) 32.2	5.7 5.8	18.6 17.3
		gefitinib (250 mg) chemotherapy	132 129	71.2 (p<0.001) 47.3	9.5 (p<0.001) 6.3	N.R N.R.

RR: response rate, CR: complete response, PR: partial response, mPFS: median progression free survival, mOS: median overall survival, N.R.: not reported

Table 4. Clinical trials of EGFR-TKIs for advanced NSCLC.

A PhaseIII study (BR.21) [61] was carried out by the National Cancer Institute of Canada Clinical Trial Group (NCIC), where previously treated patients with NSCLC were allocated randomly to an erlotinib group and a placebo group at a ratio of 2:1. In analysis of primary endpoints, erlotinib was significantly superior in terms of both OS and PFS. On the basis of the results of this study, erlotinib was approved in 2004 in the USA and in 2007 in Japan. Significant differences in response were recorded for gefitinib and erlotinib than that with placebo in both the ISEL and BR21 trials, respectively. Regarding this discrepancy, the influence of pharmacological differences has been pointed out, such as differences in the dose level (erlotinib dose level equal to MTD and gefitinib dose level equivalent to about 1/3 of MTD) and the differences in the affinity for EGFR [62].

Predictive marker of EGFR-TKI: A number of somatic mutations have been identified in the *EGFR* gene in NSCLC. In general, these mutations can be classified into three major types: in-frame deletion, insertion, and missense mutation. Most mutations are located in the tyrosine kinase coding domain (exons 18-21) of the *EGFR* gene. The amino acids 746~753, encoded by exon 19, and amino acid 858, encoded by exon 21, are two mutation hotspots, that account for over 80% of all detected mutations [63]. Deletion of 5 amino acids in exon 19 and the L858R point mutation of exon 21 have been reported to account for more than 80% of all mutations of the *EGFR* gene [64,65]. Research is currently focusing on two main resistance mechanisms including the secondary T790M [66] mutation which has been reported to occur in approximately 50% at time of tumour growth and the amplification of the *MET* proto-oncogene, which drives the ErbB3 (HER3)-dependent activation of PI3K and is detected in about 20–30% of cases [67]. Laboratory-based efforts have focused on developing agents to target this mutation. These compounds that showed preclinical inhibition of T790M include afatinib, a dual inhibitor of EGFR and HER2 and neratinib, a pan ErbB inhibitor that inhibits ErbB-1, -2, and -4.

Other molecular-targeted drugs: *ALK* (anaplastic lymphoma kinase) encodes a tyrosine kinase normally expressed only in certain neuronal cells. The *ALK* gene was originally identified through cloning of the t(2;5)(p23;q35) translocation found in a subset of anaplastic large cell lymphomas [68]. In a rare subset of NSCLCs, interstitial deletion and inversion within chromosome 2p results in fusion of the N-terminal portion of the protein encoded by the echinoderm microtubule-associated protein-like4 (*EML4*) gene with the intracellular signaling portion of the ALK receptor tyrosine kinase. In 2007, rearrangements of the *ALK* gene in NSCLC were reported [69]. Clinicopathological features of *EML4-ALK* NSCLC, which represents ~5% of all NSCLCs harboring tend to be younger and have little to no smoking history. Almost all cases have been adenocarcinomas, predominantly the signet-ring cell type with abundant intracellular mucin. While this histologic pattern is well recognized in gastrointestinal and breast adenocarcinomas, it is rarely observed in lung cancer. *EML4-ALK* rearrangements appear to be mutually exclusive to *EGFR* and *KRAS* mutations [70]. This finding led to the rapid development of the oral ALK inhibitor crizotinib, which has demonstrated efficacy and tolerability in patients with advanced NSCLC. To assess whether crizotinib affects overall survival in these patients, Alice et al. [71] did a retrospective study comparing survival outcomes in crizotinib-treated patients in the trial and crizotinib-naive controls screened during the same time period. They examined OS in patients with advanced, ALK-positive NSCLC who enrolled in the phase 1 clinical trial of crizotinib, focusing on the cohort of 82 patients. Controls were 36 ALK-positive patients from trial sites who were not given crizotinib (ALK-positive controls), 67 patients without the *ALK* rearrangement but who were positive for the *EGFR* mutation, and 253 wild-type patients lacking either the *ALK* rearrangement or *EGFR* mutation. Among 82 ALK-positive patients who were given crizotinib, median overall survival from initiation of crizotinib has not been reached (95% CI 17 months to not reached); 1-year OS was 74%, and 2-year OS was 54%. OS did not differ based on age, sex, smoking history, or ethnic origin. Based on the positive results of phase I and II trials with ORRs over 50%, duration of response >40 weeks and strong OS trends, the US Food and Drug Administration (FDA) granted accelerated approval in August 2011 for the use of crizotinib to treat advanced NSCLC patients with ALK-positive disease [72,73] (Figure 6).

However, despite these remarkable initial responses, cancers eventually develop resistance to crizotinib, usually within 1y, thereby limiting the potential clinical benefit. Choi et al. [74] reported the discovery of two secondary mutations (C1156Y and L1196M) within the kinase domain of EML4-ALK in tumor cells isolated from a

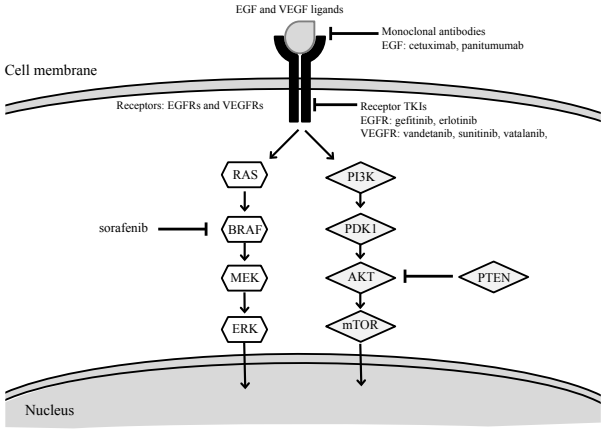

Figure 5: Schematic representation of therapy targeting the EGFR signaling pathway. mTOR: mammalian target of rapamycin, PDK1: 3-phospoinositide-dependent protein kinase-1, PI3K: phosphatidylinositol 3-kinase, PTEN: phosphatase and tensin, RAS: rat sarcoma, TKIs, tyrosine kinase inhibitors.

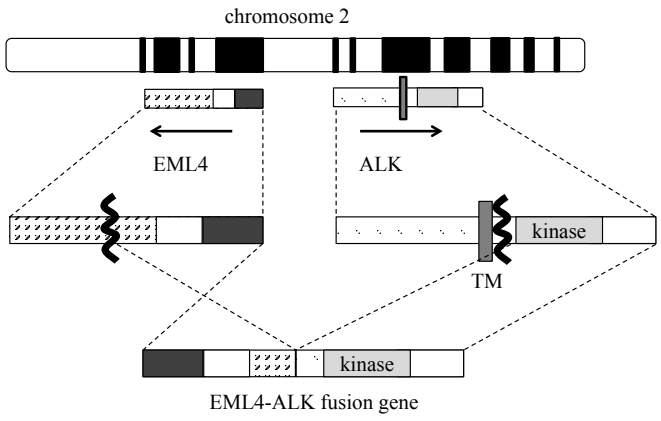

Figure 6: Fusion of the N-terminal portion of EML4 to the intracellular region of ALK. The inversion within the short arm of chromosome 2 was found to result in the ligation of *EML4* and *ALK*, leading to the production of a fusion protein consisting of the EML4 and the intracellular region of the protein tyrosine kinase ALK. This portion of EML4 mediates the constitutive dimerization and activation of EML4-ALK. TM: transmembrane domain.

patient during the relapse phase of treatment with an ALK inhibitor. It is predicted that L1196M is a "gatekeeper" mutation, which confers resistance to tyrosine kinase inhibitors via altered ATP binding and steric hindrance of drug binding. The mechanism of resistance of C1156, an activating mutation on the N-terminal side of ALK, is less clear. Sakamoto et al. [75] identified a second generation ALK inhibitor, CH5424802 that blocked EML4-ALK L1196M-driven cell growth.

Conclusion

The clinical application of molecular diagnostic techniques has allowed a more precise and rapid assessment of advanced cancers and will help to triage the patient to "personalized" therapies that will have the highest success rates for eradicating the tumor. As a result of such new drug developments, the median survival of mCRC increased from 5 months to 2 years between 1993 and 2009. Regarding advanced NSCLC, modern chemotherapy doublets have increased MST to 8-11 months and the use of biologic agents leads MST in some cases exceed 12 months. However, questions remain regarding the molecular targeted treatment of metastatic disease. In particular, biomarker correlates will likely prove the key in identifying patients most likely to benefit from newer targeted agents.

Such personalized medicine will now be forced to discuss the issues surrounding economical point of view. If a drug supports only a small fraction of that population, pharmaceutical companies cannot afford to bring the drug to market. Our current healthcare systems pay for volume rather than for value; therefore, we cannot measure the quality of delivered healthcare and this is going to slow down personalized medicine adoption if we don't solve this problem.

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