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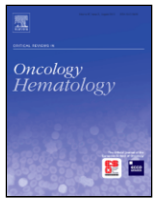

Combination of EGFR-TKIs and chemotherapy in advanced EGFR mutated NSCLC: Review of the literature and future perspectives

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ABSTRACT

Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) improved clinical outcome compared to chemotherapy in EGFR-mutated advanced non-small cell lung cancer (NSCLC) patients. Nonetheless, acquired resistance develops within 10–14 months and 20–30% of EGFR-mutated patients do not respond to EGFR-TKI. In order to delay or overcome acquired resistance to EGFR-TKIs, combination therapies of EGFR-TKIs with chemotherapy have been investigated with conflicting results. Early studies failed to show a survival benefit because of a lack of patient selection, but more recently clinical studies in EGFR-mutated patients have shown promising results. This review summarizes preclinical and clinical studies on the combination of EGFR-TKIs, including the third-generation EGFR-TKI osimertinib, with chemotherapy in first- and second-line settings, using concurrent or intercalated treatment strategies. In the new era of third-generation EGFR-TKIs, new studies of this combination strategy are warranted.

1. Introduction

The advances in molecular knowledge of non-small cell lung cancer (NSCLC) have led to a new era of targeted therapy for metastatic disease. The choice of first-line therapy in advanced NSCLC is currently based on the presence or not of driver gene alterations, such as epidermal growth factor receptor (*EGFR*) activating mutations, anaplastic lymphoma kinase (*ALK*) and proto-oncogene tyrosine-protein kinase reactive oxygen species (*ROS-1*) translocations and B-Raf proto-oncogene (*BRAF*) mutations (Planchard et al., 2018). *EGFR* mutations are the most frequent (~50%) driver gene mutations in Asian patients with non-squamous NSCLC and the second most frequent (~10-15%) in Western patients after Kirsten rat sarcoma viral oncogene homolog (*K-RAS*) mutation (Cooper et al., 2013). The first-line therapy for NSCLC patients harbouring activating *EGFR* mutations is the treatment with EGFR-tyrosine kinase inhibitors (EGFR-TKIs), which showed to significantly improve progression-free survival (PFS), objective responses rate (ORR) and quality of life compared to chemotherapy (Planchard et al., 2018).

Despite the PFS improvement, acquired resistance to first- or second-generation EGFR-TKIs inevitably develops within 10–14 months, and it is mediated by various mechanisms including the acquisition of the T790M secondary mutation of *EGFR* (50–60%), the activation of downstream or parallel pathways to *EGFR* signalling (*HER2* amplification, *MET* amplification, etc), the transformation to small-cell lung cancer (SCLC) or the epithelial-to-mesenchymal transition (EMT) (Westover et al., 2018). Moreover, 20–30% of patients, although harbouring *EGFR* mutations, do not respond to EGFR-TKI and are defined as intrinsic or primary resistant (Westover et al., 2018). For many years, chemotherapy has been the only treatment option for patients developing resistance to EGFR-TKIs.

In order to improve the outcome with EGFR-TKI treatment, the combination therapy of EGFR-TKIs with other anticancer drugs, especially chemotherapy, as concurrent, intercalated or sequential combination, has been under investigation with conflicting results either in preclinical or in clinical studies (Zhang et al., 2016; Leung et al., 2012). Early studies of EGFR-TKIs and chemotherapy combination failed to show a positive impact because of a lack of patient selection, while more recent studies with *EGFR* mutation status selection have shown promising results (Iwama et al., 2018). However, to date, this combination therapy has no a definitive role in clinical practice.

In recent years, the efficacy of osimertinib, a third-generation EGFR-TKI, has been validated in T790M-positive advanced non-squamous NSCLC progressed to first- and second-generation EGFR-TKIs (Mok

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et al., 2017a). For its superior efficacy in terms of PFS compared with gefitinib or erlotinib, it was also approved as first-line treatment (Soria et al., 2018). Osimertinib is, therefore, the treatment of choice as first-line treatment in EGFR-mutated advanced NSCLC patients and as second-line treatment of acquired T790 M-positive advanced NSCLC patients.

However, acquired resistance occurs also in patients treated with osimertinib and the mechanisms of acquired resistance to third-generation EGFR-TKIs are extremely heterogeneous and more complex than those developed to first- and second-generation EGFR-TKIs (Minari et al., 2016). Moreover, after progressive disease to osimertinib, no other approved targeted therapies are available in clinical practice to overcome acquired resistance (Suda et al., 2017). Chemotherapy remains the only therapeutic option for patients who progressed to third-generation or first- and second-generation EGFR-TKIs without the T790 M mutation.

The optimal management of patients who develop resistance to EGFR-TKIs is still a challenging open question. Given the wide variety of EGFR-TKI resistance mechanisms identified so far, it could be difficult to implement a clinical strategy based on the sequential introduction of the specific treatment for the EGFR-TKI resistance individually and accurately identified after TKIs failure. An alternative strategy may be to identify a combined treatment approach that prevents or delay the occurrence of more than one resistance mechanism.

Therefore, we performed this review focusing on the combination therapy of EGFR-TKIs and chemotherapy as a potential mean to delay or overcome resistance to EGFR-TKIs, both first-/second-generation and third-generation EGFR-TKIs.

2. Preclinical evidence

Many preclinical studies have explored the effects of combining first-generation EGFR-TKIs with chemotherapeutic agents.

A synergistic cytotoxicity was documented with the concomitant exposure of erlotinib and the antimetabolite agent pemetrexed (Li et al., 2007; Giovannetti et al., 2008; Feng et al., 2017) in different NSCLC cell lines mainly in erlotinib-sensitive cells, while an antagonistic effect was observed when erlotinib was administered before pemetrexed. A possible explanation is the G1-phase arrest of tumor cells induced by the EGFR-TKI, which conferred a protection against the cytotoxic activity of pemetrexed (Li et al., 2007; Giovannetti et al., 2008; Feng et al., 2017). Interestingly, it has been reported that removing erlotinib for at least 8 h before exposing cells to pemetrexed was able to avoid the antagonistic effect of the sequential treatment at least *in vitro* (Li et al., 2007). Accordingly, our results from PC9-xenograft models indicated that combination of gefitinib with pemetrexed intercalated with gefitinib alone, and pemetrexed administered first with intercalated gefitinib resulted in tumor regression and delayed the development of gefitinib resistance mediated by T790 M mutation or EMT. By contrast, the application of an intermittent treatment with gefitinib administered first with intercalated pemetrexed was ineffective (La Monica et al., 2016).

Controversial results regarding the combination of pemetrexed and EGFR-TKI in TKI-resistant models have been reported: a significant anti-proliferative effect in TKI-acquired resistant PC9/GR cells was described by Wu M et al. (Wu et al., 2014), while combination therapy did not exhibit enhanced anti-tumor effects in H1975 (L858R/T790M-positive) xenografts (Takezawa et al., 2010).

As reported for pemetrexed, also the sequence of the anti-microtubule agent paclitaxel followed by gefitinib exerted stronger activity than concurrent or inverse sequence in NSCLC cell lines with wild-type or mutated *EGFR* gene (Cheng et al., 2011a, b). In these cell models, paclitaxel induced the activation of EGFR phosphorylation depending on TGFα release enhancing the efficacy of the subsequent exposure to EGFR-TKI.

H1975 NSCLC cell line and the *EGFR* wild-type cell line A549 showed more sensitivity to the sequential schedule of vinorelbine followed by gefitinib compared with single treatments or the reverse schedule (Dal Bello et al., 2015). In this study, the concomitant administration was not tested. Tsai et al., evaluating the effect of the concomitant combination of gefitinib with paclitaxel, docetaxel or vinorelbine in several NSCLC cell lines, demonstrated that the cells harbouring sensitizing *EGFR* mutations showed a tendency toward consistent antagonism to the tested doublets (Tsai et al., 2012). The same authors tested also six different gefitinib/chemotherapeutic doublets (cisplatin, gemcitabine, pemetrexed, paclitaxel, docetaxel, or vinorelbine) in NSCLC cell lines with or without activating mutations indicating that EGFR-TKI-sensitive cells treated concomitantly are mostly chemo-refractory. In particular, the concomitant gefitinib/cisplatin combination showed antagonism due to the interference of gefitinib in the cisplatin influx into cells but gefitinib/pemetrexed combination showed antithetical results in the three mutant-EGFR lung cancer cell lines tested: synergism in H3255, additivism in HCC827 and antagonism in PC9 cell line (Tsai et al., 2013; Tsai et al., , 2011). Differently from these results, we have previously reported an additive effect when PC9 and HCC827 cells were simultaneously treated with gefitinib and pemetrexed (La Monica et al., 2016). Very recently, a study published by our group (La Monica et al., 2019) investigated the effect of combining the third-generation EGFR-TKI osimertinib with chemotherapy. To the best of our knowledge, this is the first preclinical study evaluating the efficacy of such combination. Different schedules of administration between osimertinib and pemetrexed or cisplatin were tested both *in vitro* and *in vivo* in *EGFR*-mutated NSCLC models. In all mutant-EGFR cell lines tested, the combination treatment significantly suppressed cell growth and enhanced apoptosis signaling. In xenograft models, the treatment with osimertinib alone induced acquired resistance in 50% of mice. In contrast, a strong anti-tumor effect was observed when osimertinib was combined with pemetrexed or cisplatin intercalated, every week, with osimertinib alone and the combination treatment enhanced the percentage of fibrotic tissue within the xenograft tumors. In addition, the small tumors did not regrow after stopping therapy, indicating that the addition of chemotherapy may potentiate the efficacy of osimertinib in eradicating parenchymal tumor cells. Moreover, our results indicate that osimertinib given before pemetrexed or cisplatin was the worst therapy to suppress tumor growth and delay resistance.

Preclinical data indicate that the combination of EGFR-TKIs with cytotoxic agents has shown more efficacy than monotherapy depending on the drug and on the schedule of administration. Based on preclinical results, a concomitant or intercalated approach of pemetrexed and osimertinib in first-line setting may currently represent a potential treatment strategy either in term of efficacy and prevention of acquired resistance to study in *EGFR* mutated NSCLC patients.

The chemotherapy in association with EGFR-TKI, by enhancing cell death, may affect the emergence of resistance, overcoming the problem of tumor genetic heterogeneity. However, chemotherapy itself exerts a selective pressure influencing tumor clonal evolution. Therefore, the combined approach, even if not completely able to prevent, might at least postpone the appearance of tumor resistance.

3. First-/second-generation EGFR-TKIs plus chemotherapy in unselected NSCLC patients

3.1. Concurrent combinations

The initial assessment on the first-line combination EGFR-TKIs plus chemotherapy in advanced NSCLC patients included several randomized phase 3 trials (TRIBUTE, TALENT, INTACT-1, INTACT-

2) (Herbst et al., 2005; Gatzemeier et al., 2007; Giaccone et al., 2004; Herbst et al., 2004), which evaluated the concurrent combination of platinum-doublet chemotherapy with erlotinib or gefitinib vs. chemotherapy alone in unselected population. All these trials reported negative results. Nevertheless, subgroup analyses showed improvement of PFS and/or overall survival (OS) for the combination over chemotherapy alone in never smoker or adenocarcinoma histology patients, features highly associated with activating *EGFR* mutations (Herbst et al., 2005; Gatzemeier et al., 2007; Herbst et al., 2004). Subsequently, these subgroup results were unconfirmed by the CALGB 30406 randomized phase II trial (Janne et al., 2012), which showed no survival differences between concurrent combination of chemotherapy and erlotinib vs. erlotinib alone in patients clinically selected (lung adenocarcinoma and never or light former smokers). The subgroup analysis on *EGFR*-mutated patients reported longer survival in both treatment arms compared to *EGFR* wild-type patients, showing, therefore, the lack of additional benefit of the combination treatment in this subgroup of patients (Janne et al., 2012).

These studies failed to demonstrate a survival benefit of the concurrent combination of EGFR-TKIs plus chemotherapy over chemotherapy alone, findings also confirmed by several meta-analyses (Zhang et al., 2016; Feld et al., 2006). Two hypotheses have been proposed for these negative results: one regarding the inadequate selection of patients and one regarding the cell-cycle timing interference between TKI and chemotherapy (La Salvia et al., 2017; Takahashi and Saito, 2016). The main issue is that NSCLC patients were not selected for *EGFR* mutational status and so *EGFR* wild-type patients, who are not likely to respond to EGFR-TKIs, were included in these studies. Moreover, as indicated above, preclinical data showed a possible antagonist effect between EGFR-TKIs and concurrent or subsequent chemotherapy (Yang et al., 2018a). For this reason, alternative strategies for combining chemotherapy with EGFR-TKIs have been investigated, such as intercalated or sequential treatment to achieve pharmacodynamic separation of the two drugs (Zhang et al., 2016).

3.2. Intercalated combinations

Intercalated combinations of EGFR-TKIs and chemotherapy were explored mainly in two FASTACT randomized trials (FASTACT-1 and FASTACT-2) in unselected patients with untreated advanced NSCLC (Mok et al., 2012; Wu et al., 2013). In these studies, EGFR-TKIs were administered as an intercalated regimen with chemotherapy (combination phase) and subsequently as monotherapy (maintenance therapy) after the end of chemotherapy.

The phase II FASTACT-1 evaluated erlotinib 150 mg/die or placebo on days 15–28 of a 4-week cycle of chemotherapy (gemcitabine plus cisplatin or carboplatin) followed by a maintenance phase of erlotinib/placebo (Mok et al., 2012). This study reported a statistically significant PFS improvement with the combination therapy, but no significant difference in OS (Mok et al., 2012).

The phase III FASTACT-2 study (Wu et al., 2013) included the same treatments and showed a survival benefit both in terms of PFS and OS of the intercalated regimen over chemotherapy alone for the entire cohort. The subgroup analysis showed a significant survival benefit only in *EGFR*-mutated patients, while no difference was observed in *EGFR* wild-type ones (Wu et al., 2013).

Several other studies and meta-analyses assessed the use of intercalated regimens of chemotherapy plus EGFR-TKIs in unselected, or clinically selected, NSCLC patients both in first- and second-line (Aerts et al., 2013; Auliac et al., 2014; Lee et al., 2013; Yu et al., 2014; Hirsch et al., 2011), suggesting a potential benefit of the intercalated therapy limited to *EGFR*-mutated patients (Zhang et al., 2016; La Salvia et al., 2017).

4. First-/second-generation EGFR-TKIs plus chemotherapy in EGFR-mutated NSCLC patients

The discovery of the predictive role of *EGFR* mutational status and the survival advantage of first-line EGFR-TKIs over chemotherapy in *EGFR*-mutated NSCLC patients provided a new viewpoint to combination therapy for advanced NSCLC patients (Iwama et al., 2018). After the negative results of the unselective studies and the interesting results of subgroup analyses of the earlier studies, recently many phase II-III trials have investigated the EGFR-TKIs plus chemotherapy combination in selected NSCLC patients harbouring *EGFR* activating mutations (Table 1).

4.1. First-line EGFR-TKIs plus chemotherapy

4.1.1. Concurrent combinations

Several studies evaluated the combinations of single-agent or platinum-based doublet chemotherapy combined with EGFR-TKIs as first-line treatment in *EGFR* mutated NSCLC patients (Table 1).

In 2015 Tamiya et al. published the results of a single-arm phase II trial on a triplet combination therapy of gefitinib, carboplatin and S-1 as first-line treatment of *EGFR*-mutated advanced NSCLC patients (Tamiya et al., 2015). Patients received four courses of triplet therapy in 3–4 weeks cycles with the possibility of continuing with S-1 and gefitinib as maintenance therapy until progressive disease. The mPFS and ORR were similar to those reported by the combination arms of the randomized NEJ005, NEJ009 and JMIT trials, reported below, while the mOS was not reached (NR) (Tamiya et al., 2015).

The phase 2 NEJ005/TCOG0902 was the first randomized study to investigate the efficacy of EGFR-TKI and chemotherapy combination in 80 EGFR-mutated patients (Sugawara et al., 2015). This study evaluated the efficacy of concurrent or sequential alternating combination of platinum-based chemotherapy and gefitinib in untreated advanced non-squamous NSCLC patients with sensitive *EGFR* mutations. The concurrent group consisted in concurrent carboplatin-pemetrexed in a 3-week cycle and gefitinib for up to 6 cycles, followed by concurrent gefitinib and pemetrexed maintenance, while in the sequential/alternating one, the patients received 8 weeks of gefitinib followed by 2 cycles of carboplatin-pemetrexed, repeated 3 times, followed by alternating gefitinib for 8 weeks and two cycles of pemetrexed. The updated survival data of 2018 (Oizumi et al., 2018), at the median follow-up time of 35.6 months, showed significantly longer OS, numerically better PFS and similar ORR with the concurrent regimen compared with the sequential/alternating one. These results confirmed preclinical data on the antagonist effect of the sequential TKI and chemotherapy regimen (Li et al., 2007; Feng et al., 2017; La Monica et al., 2016).

On the basis of the results of the NEJ005 trial, a randomized phase 3 study (NEJ009) was conducted to assess the concurrent combination of gefitinib with carboplatin-pemetrexed followed by gefitinib plus pemetrexed compared to gefitinib alone (Seike et al., 2018). In the gefitinib alone arm, the recommended second-line treatment consisted of carboplatin-pemetrexed. The results of the NEJ009 study (Seike et al., 2018; Nakamura et al., 2018) showed longer PFS (20.9 vs 11.2 months, HR 0.493, $p < 0.001$) and OS (52.2 vs 38.8 months, HR 0.695, $p = 0.013$) and superior ORR (84% vs 67.4%) of the combination compared to gefitinib alone.

In 2016 another randomized phase 2 study, the JMIT trial, evaluated gefitinib with and without concurrent pemetrexed as first-line therapy in advanced *EGFR*-mutated NSCLC patients, showing longer PFS and similar ORR (80 vs 74%) of the combination therapy compared to gefitinib alone (Cheng et al., 2016). The updated analysis confirmed a significantly longer PFS but reported a not significantly longer OS (43.4 vs 36.8 months, HR 0.77, $p = 0.105$) with the

Table 1
First-line treatment combination of EGFR-TKI and chemotherapy in advanced NSCLC patients with activating EGFR mutations.

Author(s) (Year)	Phase	Treatment regimens	N	ORR	p value	mPFS (mo)	HR (95% CI)	p value	mOS (mo)	HR (95% CI)	p value
Concurrent											
Tamiya et al. (2015)	II	G + Ca + S1	35	85.7%	–	17.6	–	–	NR	(27.9 – NR)	–
Oizumi et al. (2018) (NEJ005 update)	II	G + Ca + P cuncurrent	80	90.2%	0.34	17.5	0.68 (0.42-1.12)	0.13	41.9	0.58 (0.34- 0.97)	0.036
		vs sequentially alternating		82.1%		15.3			30.7		
Seike et al., 2018; Nakamura et al., 2018 (NEJ009)	III	G + Ca + P	345	84%	–	20.9	0.493 (0.39-0.62)	< 0.001	52.2	0.695 (0.52-0.927)	0.013
		G		67.4%		11.2			38.8		
Yang et al. (2018b) (JMIT-update)	II	G + P	191	–	–	16.2	0.67 (0.5-0.9)	0.009	43.4	0.77	0.105
		G				11.07			36.8		
Noronha et al. (2019)	III	G + P/Ca	350	75.3%	0.01	16	0.51 (0.39-0.66)	< 0.001	NR	0.45 (0.31- 0.65)	< 0.001
		G		62.5%		8			17		
Intercalated											
Yoshimura et al. (2015)	II	G + P	26	84.6%	–	18	–	–	32	–	–
An et al. (2016)	II	G + P	90	80%	< 0.05	18	–	< 0.05	34	–	> 0.05
		G + Pl		73%		14			32		
Kanda et al. (2015)	II	G → Cis + D → G (insertion)	33	–	–	19.5	–	–	48	–	–
Han et al. (2017)	II	G + Ca + P	121	82.5%	–	17	0.48* (0.29–0.78)	0.003	32.6	0.36* (0.20–0.67)	0.001
		G		65.9%		11.9	0.16** (0.09–0.29)	< 0.001	25.8	0.46**(0.24–0.87)	0.016
		Ca + P		32.5%		5.7			24.3		
Wen et al. (2018)	Retrospective	G/E + Cis + D	92	–	–	20.5	1.76*	0.036	36	1.52*	0.19
		G/E				16	2.78**	< 0.0001	29	2.86**	0.001
		Cis + D				12			18	**	
Yan et al. (2019)	Retrospective	G/E/I + Cis/Ca + P/D/Pa/Ge	76	55.9%	0.181	7.9	–	0.015	25.8	–	0.047
		G/E/I		40.5%		5.9			19.8		

N number of patients, *ORR* overall response rate, *mPFS* median progression-free survival, *mo* months, *HR* hazard ratio, *CI* confidence interval, *mOS* median overall survival, *NR* not reached, *Ca* carboplatin, *Cis* cisplatin, *P* pemetrexed, *G* gefitinib, *E* erlotinib, *D* docetaxel, *I* icotinib, *Pa* paclitaxel, *Ge* gemcitabine.
* HR of combination therapy vs EGFR-TKI alone.
** HR of combination therapy vs chemotherapy alone.

combination of pemetrexed-gefitinib compared to gefitinib alone (Yang et al., 2018b).

At the annual ASCO meeting of 2019, Noronha et al. presented the results of a phase III trial on the combination of gefitinib and pemetrexed-carboplatin compared to gefitinib alone in untreated *EGFR*-positive NSCLC patients (Noronha et al., 2019). They reported the most interesting results on the concurrent combination of EGFR-TKI and chemotherapy with statistical significance reached in all end-points. Significantly higher ORR (75% vs 63%), doubled PFS (16 vs 8 months; HR 0.51, 95% CI 0.39 – 0.66) and improved OS (NR vs 17 months, HR 0.45, 95% CI 0.31 – 0.65) were showed with the combination therapy compared with gefitinib alone.

Differently from preclinical data giving contradictory results on the simultaneous combination (La Monica et al., 2016; Tsai et al., 2013, 2011), all the above-mentioned studies showed that the first-line combination therapy of an EGFR-TKI plus chemotherapy offers promising efficacy and survival improvement in *EGFR*-mutated NSCLC patients. Severe adverse events (AEs) were more common with the combination therapy arms, especially the hematological AEs, but were predictable and clinically manageable (Nakamura et al., 2018; Cheng et al., 2016; Noronha et al., 2019) with similar discontinuation rate (Yang et al., 2018a; Nakamura et al., 2018).

4.1.2. Intercalated combinations

The intercalated combination of EGFR-TKI and chemotherapy has been investigated also in advanced NSCLC harbouring activating *EGFR* mutations (Table 1).

In 2015 Yoshimura et al. (2015) reported the results of the first single-arm phase II evaluating the efficacy of the intercalated combination of pemetrexed and gefitinib as first-line therapy in *EGFR*-mutated advanced NSCLC patients. Patients received pemetrexed (on day 1) plus gefitinib (on days 2–16), every 3 weeks until disease progression. The combination regimen showed high ORR (85%), long PFS (18 months) and OS (32 months) with acceptable toxicity.

Similar results were observed in a randomized phase II study conducted by An et al. on the efficacy of the same intercalated combination of gefitinib plus pemetrexed vs gefitinib plus placebo (An et al., 2016). The results obtained with the intercalated combination

regimen were similar to those reported by Yoshimura et al. with statistically significant higher ORR (80% vs 73%, $p < 0.05$) and PFS (18 vs 14 months, $p < 0.05$) compared to gefitinib alone (An et al., 2016). The median OS was 34 vs 32 months respectively, but the difference was not statistically significant (An et al., 2016).

Another single-arm phase II trial on intercalated combination of EGFR-TKI and chemotherapy was conducted in 2015 (Kanda et al., 2015). Kanda et al. carried out a phase II study assessing the efficacy of gefitinib with three inserted cycles of chemotherapy: patients received gefitinib on days 1–56 (250 mg daily) and then, after a two-week drug-free period, three cycles of cisplatin (80 mg/m^2) and docetaxel (60 mg/m^2) on days 71, 92, and 113 (Kanda et al., 2015). Thereafter, gefitinib was re-started on day 134 and continued until disease progression. The median PFS (mPFS) and OS (mOS) were 19.5 and 48.0 months, respectively. To confirm these findings, a phase III clinical trial (AGAIN study, JCOG1404/WJOG8214L) comparing gefitinib alone and gefitinib combined with inserted cisplatin plus pemetrexed was planned and is currently ongoing (Kanda et al., 2018).

In 2017 Han et al conducted the only phase II trial on three treatment arms comparing the combination of intercalated gefitinib (on days 5–21) plus pemetrexed-carboplatin every four weeks, carboplatin plus pemetrexed and gefitinib alone (Han et al., 2017). The ORR was higher in the combination therapy compared with both gefitinib and chemotherapy groups (83%, 66% vs 33% respectively). The mPFS and mOS were significantly longer in the combination group compared with both gefitinib (PFS HR 0.48, $p = 0.003$ and OS HR 0.36, $p = 0.001$) and chemotherapy (PFS HR 0.16, $p < 0.001$; OS HR 0.46, $p = 0.016$).

In 2018 and 2019 two retrospective analyses evaluated the efficacy of the intercalated combination therapy compared to chemotherapy and/or EGFR-TKI monotherapy (Wen et al., 2018; Yan et al., 2019). Wen et al. retrospectively assessed the efficacy of intercalated EGFR-TKI (gefitinib or erlotinib) with chemotherapy (docetaxel plus cisplatin) compared to chemotherapy alone and EGFR-TKI alone (Wen et al., 2018). Similarly to the previous phase II trial, the intercalated combination significantly improved PFS compared with both EGFR-TKI and chemotherapy groups. In terms of OS, no statistically significant difference was instead observed between combination therapy and EGFR-TKI therapy, while mOS was significantly longer in the combination therapy compared to chemotherapy alone.

Yan et al. retrospectively analysed advanced NSCLC patients harbouring low-abundance *EGFR* mutations receiving combination of EGFR-TKI therapy (gefitinib/erlotinib/icotinib on day 8 of each chemotherapy cycle to day 1 of the next chemotherapy cycle) plus a platinum-based regimen (cisplatin/carboplatin plus paclitaxel, docetaxel, pemetrexed or gemcitabine) or EGFR-TKI monotherapy (Yan et al., 2019). The study reported no significant difference in ORR between the two treatment groups, while mPFS and mOS were significantly longer in the combination group than in the monotherapy one.

The studies on first-line intercalated combination of an EGFR-TKI plus chemotherapy reported promising efficacy and survival improvement compared to EGFR-TKI and chemotherapy alone in *EGFR*-mutated NSCLC patients. They concluded that the intercalated combination treatment induced generally an AEs increase compared with the monotherapy arms but, in any case, expected and clinically manageable (An et al., 2016; Han et al., 2017; Wen et al., 2018; Yan et al., 2019).

Considering all these studies reported, at the moment, it is not possible to conclude what is the better strategy of combination (concomitant or intercalated); even if both showed a superior efficacy than EGFR-TKI alone, stronger evidence (2 phase III trials, (Nakamura et al., 2018; Noronha et al., 2019) with more 700 patient randomised) is available for the strategy of concomitant combination.

4.2. EGFR-TKIs plus chemotherapy after EGFR-TKI-progression

Another treatment strategy assessed to overcome acquired resistance to first-line EGFR-TKIs is to continue TKI beyond progression with the addition of other treatments, including chemotherapy. This strategy has been proposed considering the possibility of disease flare at the EGFR-TKI treatment discontinuation, suggesting that activated EGFR may have still a role even after the acquisition of EGFR-TKI resistance. Several studies assessed the concurrent or intercalated combination of EGFR-TKI and chemotherapy after the TKI progression (Table 2).

Two single-arm phase II studies assessed the continuation of EGFR-TKI (gefitinib and/or erlotinib) beyond progression plus pemetrexed as intercalated (Yoshimura et al., 2013) or as concurrent (Uchibori et al., 2018) combination in *EGFR*-positive patients. They reported similar ORR (≈25%) and mPFS (7 months) but different mOS (11.4 and 24.3 months, respectively). Uchibori et al. reported also no significant difference according to T790M mutational status (PFS of 5.9 vs. 7.0 months for T790M positive vs. negative patients, respectively; $p = 0.48$) (Uchibori et al., 2018).

A randomized phase II trial compared single-agent chemotherapy (pemetrexed or docetaxel) and intercalated combination of chemotherapy plus erlotinib in patients progressed to first-line erlotinib. Patients were treated with the EGFR-TKI regardless *EGFR* mutational status (Halmos et al., 2015). The combination was associated with higher ORR but with shorter survival, also in *EGFR*-mutated patients (67%) (Halmos et al., 2015). The study was stopped prematurely because of poor enrolment and was therefore likely underpowered. To date, no evidence supports a survival benefit of intercalated EGFR-TKI combined with chemotherapy over chemotherapy alone after progression to front-line EGFR-TKIs.

Two retrospective studies assessed patients who progressed to first-line EGFR-TKI comparing continuing EGFR-TKI with the addition of chemotherapy (platinum-based combinations and single agent therapies) vs. switching therapy to chemotherapy alone (Goldberg et al., 2013; Ding et al., 2017). Goldberg et al. reported that ORR of the combination therapy was more than two-fold of chemotherapy alone but no survival improvement was observed between the two treatment groups (Goldberg et al., 2013). Ding et al. showed higher ORR and longer PFS with the combination therapy, especially in T790M-negative patients and in particular in pemetrexed-based chemotherapy (Ding et al., 2017). This study, therefore, suggested that T790M-negative patients may derive clinical benefit from continuing gefitinib beyond progression. Our previous preclinical results confirmed that in HCC827 GR5 gefitinib resistant cell lines T790M negative and with *MET* amplification the maintenance of gefitinib exerted positive effects by inhibiting migration, invasion and EMT (La Monica et al., 2013).

The IMPRESS trial was the only randomized placebo-controlled phase III trial which evaluated whether advanced *EGFR*-mutated NSCLC patients who progressed to first-line gefitinib might benefit from the continuation of gefitinib in concomitant combination with platinum-doublet chemotherapy (cisplatin/pemetrexed) (Soria et al., 2015). No benefit was observed in PFS with the combination vs. chemotherapy alone (Soria et al., 2015). The updated analysis of 2017 reported the mature OS data and the results of the pre-planned exploratory biomarker analyses on T790M mutational status assessed using cell-free plasma tumor DNA at time of progression to first-line gefitinib (Mok et al., 2017b). The continuation of gefitinib plus cisplatin/pemetrexed was detrimental in OS compared with chemotherapy alone in overall population (13.4 vs 19.5 months, HR 1.44; $p = 0.016$) and in particular in T790M positive patients (HR 1.49; $p = 0.0432$), but not in T790M negative ones (HR 1.15; $p = 0.6093$) (Mok et al., 2017b).

Table 2
Combination of EGFR-TKI and chemotherapy after first-line EGFR-TKI therapy in advanced EGFR-positive NSCLC patients.

Author(s) (Year)	Phase	Treatment strategy	Treatment regimens	N	ORR	p value	mPFS (mo)	HR (95% CI)	p value	mOS (mo)	HR (95% CI)	p value
Yoshimura et al. (2013)	II	Intercalated	E/G + P	27	25.9	–	7.0	–	–	11.4	–	–
Uchibori et al. (2018)	II	Concurrent	G + P	36	22.9%	–	6.7	–	–	24.3	–	–
Halmos et al. (2015)	II	Intercalated	E + D/P	46	17%	0.37	4.4	–	0.699	14.2	–	0.369
			D/P		13%		5.5			16.4		
				EGFR + (67%)	–	–	–	–	0.332	–	–	0.346
Goldberg et al. (2013)	Retrospective	Concurrent	E + CT	78	41%	0.02	44	0.79 (0.48–1.29)	0.34	14.2	0.75 (0.41–1.39)	0.37
			CT		18%		4.2			15		
Ding et al. (2017)	Retrospective	Concurrent	G + CTCT	170	30.4%	0.110	5	0.72(0.50–1.03)	0.071	–	–	–
					19.8%		4					
				T790 M +	25.0%	0.863	5.0	(0.40–1.61)0.80	0.52	–	–	–
					22.7%		5.5					
				T790 M -	35.1%	0.12	6.6	0.50 (0.29–0.88)	0.011	–	–	–
					19.5%		3.5					
Mok et al. (IMPRESS trial - update) (2017))	III	Concurrent	G + Cis + PCis + P + Pl	265	32%	0·76	5.4	0·86(0.65–1.13)	0.27	13.4	1.44(1.07-1.94)	0.016
					34%		5.4			19.5		
				T790 M +	28.4%	–	4.6	(0.67-1.42)0.97	0.8829	10.8	(1.02-2.21)1.49	0.0432
					39.3%		5.3			14.1		
				T790 M -	36.8%	–	6.7	0.67 (0.43 - 1.03)	0.0745	21.4	1.15 (0.68 - 1.94)	0.6093
					32.3%		5.4			22.5		

N number of patients, *ORR* overall response rate, *mPFS* median progression-free survival, *mo* months, *HR* hazard ratio, *CI* confidence interval, *mOS* median overall survival, *NR* not reached, *G* gefitinib, *E* erlotinib, *CT* chemotherapy, *Cis* cisplatin, *P* pemetrexed, *Pl* placebo, *D* docetaxel.

No difference in mPFS was observed in T790 M positive patients between the two treatment groups, similarly to the entire cohort, while T790 M negative ones had a trend towards benefit with combined treatment (mPFS: 6.7 vs 5.4 months, HR 0.67; p = 0.0745) with higher ORR (Mok et al., 2017b).

According to the reported studies, the combination of EGFR-TKI plus chemotherapy after progression to first-line TKI was not associated with survival benefit compared with the results observed in the first-line setting and, therefore, this strategy with first- or second-generation-EGFR-TKI probably does not require further evaluations.

5. Third-generation EGFR-TKI plus chemotherapy

Despite the promising clinical results with osimertinib, advanced NSCLC patients inevitably develop acquired resistance to osimertinib when administered both front-line and after failure of previous EGFR-TKI (Minari et al., 2016). Acquired resistance mechanisms to osimertinib are grouped into EGFR-dependent or EGFR-independent mechanisms (Minari et al., 2016). EGFR-dependent mechanisms include the development of tertiary mutations (*EGFR* C797S, L792X, L718Q and L844V) or amplifications within the *EGFR* gene. EGFR-independent mechanisms of resistance regard activation of alternative bypass pathways, aberrant downstream signalling or histological transformation. They include loss of T790M, *c-MET* gene amplification, *HER2* amplification, RAS-MAPK pathway aberrations, *PIK3CA* mutation/amplification, *PTEN* deletion and oncogenic fusions. Histological transformation includes the transition to SCLC and EMT. All these mechanisms may coexist as reflection of intratumor heterogeneity with important research and clinical implications (Gao et al., 2019).

5.1. Clinical evidence in the literature

To date, little clinical evidence (only case reports) regarding the combination of osimertinib plus chemotherapy is available (Wang et al., 2018; Metro et al., 2018; Hirakawa et al., 2018; Yoshida et al., 2018). Moreover, several clinical trials are ongoing on this combination as a therapeutic strategy to overcome acquired resistance to osimertinib (Okada et al., 2018; Piotrowska et al., 2018).

There are few case reports in the literature on the efficacy of the combination of osimertinib and chemotherapy in pre-treated *EGFR*-mutated patients. Two case reports showed a successful brain and leptomeningeal metastases response (Wang et al., 2018; Yoshida et al., 2018) : Wang et al. successfully treated their patient with the combination of osimertinib, temozolomide (250 mg/die for 5 days), intrathecal injections of cytarabine and whole-brain radiation therapy (50 Gy in 25fractions for 3 weeks) (Wang et al., 2018); Yoshida et al. reported the combination of osimertinib and chemotherapy with carboplatin, pemetrexed and bevacizumab (Yoshida et al., 2018).

Another case report showed the efficacy of osimertinib re-challenge after intervening chemotherapy (cisplatin 75 mg/m^2 plus pemetrexed 500 mg^2), as a treatment strategy to eradicate EGFR-TKI-resistant

cancer cell (Metro et al., 2018). Finally, two papers reported the copresence of two tumor sites with different mutational status, which grew differently depending on the treatment administered (Hirakawa et al., 2018; Yoshida et al., 2018). Hirakawa et al. alternated osimertinib and carboplatin plus irinotecan in order to control the two different tumor types (right pleural dissemination and pleural tumor near the right diaphragm) (Hirakawa et al., 2018). In the aforementioned case of Yoshida et al. the combination of osimertinib and chemotherapy was used to control respectively the cranial and the extracranial disease (Yoshida et al., 2018). In these cases, the concurrent or alternating combination of osimertinib and chemotherapy led to the global control of the disease (Hirakawa et al., 2018; Yoshida et al., 2018).

At ASCO 2018 Okada et al. and Piotrowska et al. reported the safety analysis of the combination of osimertinib and chemotherapy (Okada et al., 2018; Piotrowska et al., 2018). Okada et al. conducted an open-label randomized phase 2 trial of osimertinib alone vs osimertinib plus carboplatin-pemetrexed in 24 T790 M positive NSCLC patients who progressed to EGFR-TKI (Okada et al., 2018). The planned safety review of the first treatment course showed a good safety profile for the combination treatment: adverse events frequency of the combination therapy was similar to that shown in previous studies on carboplatin-pemetrexed and no exaggeration of osimertinib-related adverse events were observed with the combination therapy (Okada et al., 2018).

The retrospective analysis of Piotrowska et al. on 18 advanced *EGFR*-mutated NSCLC patients treated with concurrent combination of osimertinib plus different chemotherapy regimens, reported that osimertinib does not add significant toxicity to chemotherapy regimens (Piotrowska et al., 2018).

5.2. Ongoing clinical trials

Studies on the combination of osimertinib with chemotherapy in *EGFR* positive NSCLC are currently ongoing. The TAKUMI trial (LOGIK1604/NEJ032A) (Tanaka et al., 2017) is a multicenter randomized phase II study evaluating osimertinib alone vs osimertinib plus carboplatin and pemetrexed followed by maintenance therapy with osimertinib plus pemetrexed in advanced T790 M positive NSCLC patients whose disease had progressed to previous EGFR-TKI. A phase I trial is also assessing the combination of osimertinib with cisplatin/carboplatin and etoposide (NCT03567642) in metastatic *EGFR* positive lung cancers with concurrent retinoblastoma 1 gene (*RB1*) and *TP53* alterations.

In July 2019 the FLUARA2 started recruiting and it is a phase III, open-label, randomized study of osimertinib with or without platinum-pemetrexed chemotherapy as first-line treatment in *EGFR* mutated advanced NSCLC patients (ClinicalTrials.gov Identifier: NCT04035486).

6. Concluding remarks and future directions

The combination therapy of EGFR-TKI and chemotherapy has long been evaluated as a therapeutic strategy to overcome resistance to EGFR-TKIs in advanced NSCLC since the early 2000s (Yang et al., 2018a). First clinical trials failed to demonstrate a survival benefit mainly due to the lack of patient selection according to *EGFR*-mutational status (Iwama et al., 2018). The discovery of the predictive role of activating *EGFR* mutations gave a new viewpoint to this treatment strategy as a new mean to better select patients who may most benefit from the combination therapy. In fact, the following clinical trials on *EGFR*-mutated patients have shown promising results. Nevertheless, first-line therapy with EGFR-TKI plus chemotherapy has not been widely used in clinical practice due to a lack of strong long-term survival advantage and controversial results between clinical trials, meta-analyses and systematic reviews. Moreover, concerns about potential toxicity increase contributed to the limited use of EGFR-TKI plus chemotherapy combination, despite the majority of studies reported little additive toxicity, which resulted predictable and clinically manageable.

On the basis of the results of the FLAURA and AURA studies (Mok et al., 2017a; Soria et al., 2018), osimertinib has become the new standard treatment for advanced *EGFR*-positive NSCLC patients in the first-line setting and after failure to first/second-generation EGFR-TKIs with T790 M mutation. Chemotherapy is currently the treatment of choice in patients who progressed to osimertinib and to first-line first/second-generation EGFR-TKIs without T790 M mutation, as the optimal targeted treatment strategy for these patients has not been established.

In the era of third-generation EGFR-TKIs, according to the complexity of EGFR resistance mechanisms, preclinical and early clinical studies are focused on the characterization of resistance clones both at time of progression and through therapy and on the new TKIs sensitivity and resistance profile (Leonetti et al., 2019; Tomasello et al., 2018).

Due to tumor heterogeneity, multiple resistance mechanisms may be present simultaneously or sequentially in an individual patient, so it is fundamental to perform real-time genetic examination in order to combine tailored treatments and propose a more individualized therapy (Gao et al., 2019; Hirakawa et al., 2018). In this context, recent evidences highlighted the correlation between high tumor mutational burden (TMB) and poorer outcomes in *EGFR*-positive patients treated with TKI therapy, probably due to the great heterogeneity in pre-existing subclones or at time of recurrence for a higher propensity of mutagenesis. *EGFR*-mutated NSCLC patients with high TMB might benefit from intensified treatment with TKI combination strategies (Cheng and Oxnard, 2019).

More comprehensive and sensitive molecular diagnostics, including next-generation sequencing or single-cell profiling technique, and dynamic monitoring technology using serial liquid biopsies (circulating tumor DNA and other biomarkers) are under evaluation, in order to define spatial and temporal heterogeneity, clonal selection and evolution of resistant cancer cells (Nakamura et al., 2018; Murtuza et al., 2019).

Intertumoral and intratumoral heterogeneity make biopsy samples potentially biased and misleading, while the liquid biopsy could reveal the complex genetic landscape of the tumor, making plasma-based genomic strategies the most interesting research field under evaluation to provide surveillance of the tumor's mutations.

Despite the occurrence of new acquired resistance molecular changes, the original *EGFR* mutation may remain detectable at time of resistance, so continuing treatment beyond progression may be still the best treatment option with the addition of other therapeutic agents. Studies evaluating the safety and efficacy of the addition of other agents to osimertinib are underway and include combinations with targeted agents of different pathways (e.g. MET, MAPK, BCL-2 and JAK activation), immune checkpoint inhibitors and chemotherapeutic agents (Nakamura et al., 2018). In this scenario, intervening chemotherapy may also be used to eradicate cancer cells clones which are responsible for clinical resistance to EGFR-TKI in order to allow regrowth of EGFR-TKI-sensitive cancer cells and so treatment rechallenge of a previous EGFR-TKIs (Okada et al., 2018).

In conclusion, osimertinib is only used as monotherapy in clinical practice and the EGFR-TKI plus chemotherapy combination is not currently standard of care, but clinical trials on this combination are still underway. However, the therapeutic strategies, the selection biases and the conflicting results of the past should be the basis for new therapeutic strategies and patients' selection in the new era of third-generation EGFR-TKIs. Our preclinical results (La Monica et al., 2019) strongly provide a rationale for randomized studies comparing osimertinib vs osimertinib combined with platinum-pemetrexed. Moreover, the good profile of toxicity of osimertinib, better than previous generation EGFR-

TKI (Soria et al., 2018; Li and Gu, 2019) could allow a safe combination with chemotherapy.

Thus, pharmacokinetic/pharmacodynamic testing and well-designed randomized clinical trials are warranted to assess the efficacy of sequential or combination therapies as a strategy to delay or overcome the onset of EGFR-TKI resistance mechanisms in advanced NSCLC patients.

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Declaration of Competing Interest

No potential conflicts of interest were disclosed.

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