Moving from histological subtyping to molecular characterization: new treatment opportunities in advanced non-small-cell lung cancer

Moving from histological subtyping to molecular characterization: new treatment opportunities in advanced non-small cell lung cancer

Journal:	*Expert Review of Anticancer Therapy*
Manuscript ID:	ERT-2014-0065.R1
Manuscript Type:	Reviews
Keywords:	Non-small cell lung cancer, tyrosine kinase inhibitors, immunotherapy, vaccines, monoclonal antibodies

TABLE A. Generations of EGFR Kinase Inhibitors for the treatment of NSCLC

Generations	Name or acronym	Stage of development	Therapeutic target
First Generations			
	Gefitinib	**Approved**	**EGFR**
	Erlotinib	**Approved**	**EGFR**
	Lapatinib	**Not approved**	**EGFR,HER2**
	Canertinib	**Not approved**	
Second Generations			
	XL647	**Phase II data**	**EGFR,HER2,VEGFR2,EphB4**
	BMS-690514	**Phase II data**	**EGFR,HER2,HER4,VEGFR1-4**
	Afatinib	**Approved**	**EGFR,HER2,HER3,HER4**
	Icotinib	**Approved (China only)**	**EGFR**
	Neratinib	**Not approved**	**EGFR,HER2,HER3,HER4**
	Dacomitinib	**Phase III data**	
Third Generations			
	WZ4002	**Preclinical data**	**EGFR mutant-selective**
	CO-1686	**Phase I/II data**	**EGFR mutant-selective**
	AP26113	**Phase I data**	**EGFR mutant-selective, ALK inhibitor**
	TAS-2913	**Preclinical data**	**EGFR mutant-selective**
	AZD9291	**Phase I/II data**	**EGFR mutant-selective**
	Z650	**Preclinical data**	**EGFR mutant-selective**

EGFR: *epidermal growth factor receptor;* ***ALK:*** *anaplastic lymphoma kinase;* ***HER2:*** *human epidermal growth factor receptor 2;* ***HER3:*** *human epidermal growth factor receptor 3;* ***HER4:*** *human epidermal growth factor receptor 4;* ***VEGFR2:*** *vascular endothelial growth factor receptor 2; VEGFR1-4: vascular endothelial growth factor receptor 1 to 4*

TABLE B. ALK inhibitors in development for NSCLC

Name or acronym	Therapeutic target	Other ALK mutations target	Stage of development
Crizotinib	ALK, ROS1, MET		Approved
Ceritinib (LDK378)	ALK, ROS1		Approved (only in USA)
Alectinib (CH5424802)	ALK, GAK, LTK	L1196M, F1174C, R1275Q,C1156Y	Phase III
AP26113	ALK, EGFR	L1196M, F1174C, I1171T, F1245C, E1210K, S1206R, G1269S	Phase II
X-376	ALK, MET		Preclinical
X-396	ALK, MET	L1196M, C1156Y	Phase I
X- 276	ALK		Preclinical
ASP3026	ALK, ROS1, MET	L1196M	Phase I
CEP-37440	ALK		Phase I
NMS-E628	ALK, IGF-1R, AURORA B	L1196M, C1156Y	Preclinical
TSR-011	ALK, TRKA, TRKB, TRKC		Phase I/IIa
PF-06463922	ALK, ROS1		Phase I/II

ALK: anaplastic lymphoma kinase; EGFR: epidermal growth factor receptor; ROS1: c-ros oncogene 1 GAK: cyclin G-associated kinase; MET: hepatocyte growth factor receptor; IGF-1R: insulin-like growth factor 1 receptor; AURK B: Aurora B kinase; TRK: tropomyosin-related kinases.

TABLE C. Potential treatment related adverse events with immune checkpoint inhibitors [~~137,138, 141,144~~ 160,161,162,163,164,166,167]

TYPES	FREQUENCY	ADVERSE EVENTS
All types and grades	40-85%	
Grade 3 or 4	3-20%	
Gastrointestinal events	9-32%	Diarrhea, nausea, abdominal pain, colitis
Infusion-related reactions	3-10%	
Endocrine disorders	2-8%	Hypothyroidism, hyperthyroidism, adrenal insufficiency, hypophysitis
Pulmonary	3-6%	Pneumonitis, pulmonary edema
Hepatic/Pancreatic		Hepatitis, hyperglycemia, increased liver function enzymes and lipase levels
Skin disorders	6%	Dermatitis acneiform
Ocular		Uveitis, pruritus
Adverse event-related deaths	0-1,5%	

Moving from histological subtyping to molecular characterization: new treatment opportunities in advanced non-small cell lung cancer

Simona Carnio, Silvia Novello, Paolo Bironzo, Giorgio Vittorio Scagliotti
University of Torino, Department of Oncology, Torino (Italy)

Address for correspondence:

Scagliotti Giorgio Vittorio

University of Torino, Department of Oncology

S. Luigi Hospital

Regione Gonzole 10, 10043 Orbassano (Torino), Italy

Tel. +390119026414

Fax +390119015184

e-mail : giorgio.scagliotti@unito.it

Summary

In last ten years the systemic treatment of advanced non-small cell lung cancer (NSCLC) progressively moved away from the "one fits all" approach to histological subtyping and currently there is a progressive implementation of targeted therapies based on specific molecular characteristics such as the epidermal growth factor receptor (EGFR) sensitizing mutations and the anaplastic lymphoma kinase (ALK) rearrangements. Despite the availability of effective agents against these abnormalities, acquired resistance is still a major issue. A new generation of tyrosine kinase inhibitors (TKIs) for EGFR and ALK targeting acquired resistance mechanisms have been recently investigated. Several promising TKIs that hit other targets are also in clinical development including RAS/MEK, BRAF1, PIK3A, c-MET, ROS1, RET, HER2, FGFR, VEGFR, PDGFR, DDR2. Furthermore, new advances in immunology have been achieved through the discovery of vaccines and immune checkpoint pathways such as the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1) and its ligands.

Keywords: Non-small cell lung cancer, tyrosine kinase inhibitors, immunotherapy, vaccines, monoclonal antibodies

Introduction

In the last 10 years the systemic treatment of Non-Small Cell Lung Cancer (NSCLC) has undergone relevant changes leading to treatment customization based on histological findings and specific genomic abnormalities, some of them have been fully integrated in the therapeutic guidelines such as the Epidermal Growth Factor Receptor (EGFR) sensitizing mutations and anaplastic lymphoma kinase (ALK) rearrangements. A whole spectrum of genomic alterations (point mutations, translocations, gene amplifications) have been detected in adenocarcinoma [1,2,3,4,5], and more recently, in other types of lung cancer. This extensive amount of work led to the development of platforms for molecular genetics [6,7] and to initiate studies aimed to test, in the clinical setting, the role of specific targeted therapies. Similarly to that already reported for other solid tumors, lung cancer cells contain multiple genetic and epigenetic abnormalities but despite this complexity, their growth and survival can often be impaired by genetic changes of a single oncogene. This phenomenon, called "oncogene addiction," provides a rationale for molecular targeted therapy [8]. One of the common characteristics of these oncogene "drivers" is the their low frequency that transforms an extremely common disease such as NSCLC in a wide array of distinct, rare entities. Because of such limitations broad tumor profiling would enable centers to participate in multiple nested clinical trials and facilitate patient enrollment in such trials. However, it should be stressed that the detected genomic changes beyond EGFR mutations and ALK rearrangements may be considered clinically experimental at the present time. Secondly, although genomic testing is widely endorsed, the clinical utility of routine multiple genetic testing remains unclear [9].

NSCLC with EGFR sensitizing mutations are highly responsive to therapy with EGFR tyrosine kinase inhibitors (TKIs) [10,11,12] and those with ALK rearrangements to treatment with the ALK inhibitors [13]. Unfortunately, among Caucasians no more than 10-15% of patients with NSCLC, mainly with adenocarcinoma, are candidates for treatment with specific targeted agents. In

addition, not all patients with a specific genotype are responsive to specific targeted therapy, being the response rate in the range of 60-70%, and for those that do benefit, acquired resistance is expected approximately within one year. A new generation of EGFR and ALK kinase inhibitors that is currently under investigation aims to overcome acquired resistance to existing agents.

Recent advances in the understanding of immunology and antitumor immune responses have led to the development of new drugs that are now being tested also in lung cancer. These new strategies include vaccines and monoclonal antibodies that inhibit immune checkpoint pathways and associated with unconventional toxicity and response.

Epidermal Growth Factor Receptor Tyrosine Kinase inhibitors

Activating mutations in the EGFR are present from 10% to 15% of patients of European descent and approximately 30% of patients of East Asian descent [14]. Currently there are clinical data available for three different generations of EGFR-TKIs (Table A)

Gefitinib and **erlotinib,** two first-generation reversible EGFR TKIs inhibitors, in first-line treatment of EGFR mutated NSCLC have demonstrated superior outcomes when compared to standard chemotherapy, in terms of higher response rates (RR), longer median progression-free survival (PFS), less toxicity and better quality of life [2,3,15,16,17,18]. Overall survival (OS) ranges from 21.6 to 30.5 months and is highly influenced by massive crossover rates, suggesting that these agents are effective independently from the line of therapy. Unfortunately, all patients treated with EGFR-TKI develop acquired resistance [19,20]. The commonest mechanism associated with acquired resistance is the threonine-790–methionine (T790M) gatekeeper point mutation detected in approximately in 50% of the cases at the time of the clinical progression. The incidence of T790 mutation at baseline is reported to be in the range of 5% but this is still a matter of extensive investigation and one study demonstrated that positive patients had a PFS of approximately 3

months [21]. Other mechanisms include c-MET amplification (15%–20% of cases), in-frame duplications and/or insertion in exon 20 (5% of the cases) of the EGFR gene, PIK3CA mutations (5%), HER-2 amplification and unknown mechanisms (25%–30% of the cases) [22,23,24]. Occasionally (3%) histological switch to small cell lung cancer has been demonstrated [12].

Despite early encouraging phase I data, **canertinib** (CI-1033) and **neratinib** (HKI-272), two pan-HER inhibitors, did not show significant clinical activity in NSCLC [25,26].

In a Chinese study **icotinib,** another reversible EGFR-TKI, was compared to gefitinib in the second/third-line setting and no significant difference in terms of clinical outcome was observed [27].

Afatinib (BIBW2992) is an irreversible EGFR inhibitor, targeting also HER2 and HER4, with potent activity against NSCLC harboring activating EGFR mutations and/or the gatekeeper mutation T790M. The phase III trial LUX-Lung 3 established the superiority of afatinib *versus* cisplatin plus pemetrexed as first-line treatment for patients with advanced adenocarcinoma harboring EGFR-activating mutations in terms of median PFS (11.1 *versus* 6.9 months, hazard ratio [HR] 0.58; p=0.0004) and overall response rate (ORR, 56% *versus* 23%, p=0.001)[28]. Afatinib showed also activity in patients previously treated with erlotinib or gefitinib (LUX-Lung 1 and LUX-Lung 4 trials) [29,30]. A phase II trial of afatinib *versus* gefitinib as first-line treatment (LUX-lung 7, NCT01466660) is ongoing in patients with EGFR activating mutations. In an exploratory combined analysis of LUX-Lung 3 and LUX-Lung a benefit of 10 months in OS favoring afatinib was documented in EGFR Del19 patients while no significant difference was observed in patients with L858R mutations, individually or in exploratory combined analysis [31].

In a randomized phase II study **dacomitinib** (PF-00299804), another irreversible EGFR-TKI, was compared with erlotinib and PFS was improved (2.86 *versus* 1.91 months) but not in the EGFR-mutant subset (7.44 months in both arms) [32] without reporting significant differences in the

toxicity profile. A phase III study comparing the efficacy and safety of dacomitinib to gefitinib is currently enrolling patients with EGFR-activating mutations NSCLC having as primary end point PFS; results are awaited in 2015. Two negative phase III studies investigating the role of this agent have been recently presented [33,34]. The ARCHER 1009 trial included patients previously treated with chemotherapy (second/third line) and it did not meet its primary end point of demonstrating statistically significant improvement in PFS when compared with erlotinib. In the second study dacomitinib was compared to placebo (NCIC CTG BR.26) in patients with advanced NSCLC previously treated with both chemotherapy and an EGFR TKI and it failed to improve overall survival (OS).

CO-1686 (AVL-301, CNX-419) and **AZD9291** are oral covalent, third-generation EGFR-TKIs that target both the activating EGFR mutations and the T790M resistance mutation, while sparing the wild-type EGFR. These agents have the potential either as first line agents in patients with both sensitizing mutations and T790 mutation at baseline or as second line/third line agents in patients with acquired resistance to first- and second generation EGFR TKI.

An ongoing phase I/II trial of CO-1686 in previously EGFR mutant NSCLC patients with documented progression following a first-line EGFR inhibitor, such as erlotinib, gefitinib, neratinib, afatinib, or dacomitinib, and the expansion cohort indicated the recommended phase II dose at 750 mg twice daily. Available data indicate an ORR of 64% in patients with T790M mutation and it remains above 50% even when patients with T790M-unknown status are included. Median PFS in T790M+ patients is exceeding 6 months [35]. Main adverse events (AEs) of all grades include nausea (25%), fatigue (21%), impaired glucose tolerance/hyperglycemia (21%). Dose-related WT-driven diarrhea and rash has not been observed. An extensive phase 2/3 program called TIGER (Third-Generation Inhibitor of mutant EGFR in lung cancer) has been planned. The phase II/III study TIGER I will compare CO-1686 *versus* erlotinib in first-line mutant EGFR patients not screened for T790M

status. TIGER 2 is a phase II single arm study that will evaluate CO-1686 as a second line treatment in mutant EGFR NSCLC, T790M+ after progression from first-line with other EGFR-TKIs and TIGER 3 will randomized patients with EGFR mutant NSCLC to receive CO-1686 or chemotherapy in T790M+, after progression from first-line with EGFR-TKi [36].

AZ9291 is another third generation EGFR-TKI with activity on the same targets of CO-1686 and a large phase I open label study is ongoing. Preliminary data indicate clinical activity at all doses studied so far and a good tolerability [37,38]. The overall disease control rate in T790M+ patients is 96% (85/89) including responses in patients with brain metastases while an inferior activity was noted in T790 - tumors. To date the longest duration of response is >8 months and no dose limiting toxicities were observed. Grade 3/4 AEs occurred in 16% of patients. [39,40].

Data support the existence of a cross-link between Vascular Endothelial Growth Factor Receptor (VEGFR) and EGFR pathway. VEGF signaling is up-regulated by EGFR expression [41,42,43] and, conversely, VEGF up-regulation independent of EGFR signaling seems to contribute to resistance to EGFR inhibition [44]. Therefore, inhibition of both pathways could improve antitumor efficacy and overcome resistance to EGFR inhibition. **XL647** is an oral multiple receptor tyrosine kinase inhibitor, including EGFR, VEGFR2, HER2 and Ephrin type-B receptor 4 (EphB4) tested in an phase II study in patients with adenocarcinoma and EGFR sensitizing mutations or patients of Asian ethnicity and/or female, and/or minimal or no smoking history. The ORR was of 19,6% and in 14 EGFR mutated patients the PFS was 9.3 months [45]. In a second study in patients with acquired resistance to EGFR TKI, the ORR was only 3% and none of the 12 patients with a known T790M mutation responded [46]. Tissue analysis was performed, at the time of progression, in patients with EGFR-mutant adenocarcinoma treated first-line with XL647 and molecular analyses indicate that this agent does not necessarily select for T790M resistant clones and this finding allows the sequential use of EGFR TKIs such as erlotinib [47].

BMS-690514 is a potent, reversible oral inhibitor of EGFR/HER-1, HER-2 and -4, and VEGFRs-1 to -3. In a phase II study in advanced NSCLC, erlotinib-naïve and resistant patients were treated. Disease control (for at last 4 months) and ORR were 43.3% and 3.3% in erlotinib-naive and 22.6% and 3.2% in erlotinib-resistant patients, respectively. Six of 21 (29%) NSCLC patients with wild-type EGFR achieved disease control *versus* seven of 10 (70%) patients with EGFR mutations (including T790M positive tumors) [48]. An international randomized phase II trial comparing BMS-690514 with erlotinib in patients with advanced NSCLC that have progressed on platinum therapy has been also completed (NCT00743938), but results are not yet available.

AP26113 is a reversible, dual EGFR/ALK inhibitor with preferential activity against mutant EGFR *versus* wild type. In a phase I/II study AP26113 showed activity in EGFR mutated patients progressed to prior EGFR-TKI [49] and the phase II study is ongoing and includes a cohort of patients with T790M (NCT01449461).

Anaplastic Lymphoma Kinase (ALK) inhibitors

The EML4-ALK fusion gene is detected in 4%–6% of the adenocarcinomas of the lung [50],is oncogenic, and can be suppressed by specific ALK-inhibitors. EML4-ALK positive NSCLC patients share many of the clinical features of patients harboring EGFR mutations but, almost invariably, EML4-ALK and EGFR mutations are mutually exclusive [51]

Crizotinib is a MET inhibitor that also has activity on ALK and c-ROS1 oncogene (ROS1) pathways. In a phase I/II study (PROFILE 1001) in ALK-translocated patients crizotinib induced tumor shrinkage in 61% of patients and the median PFS was 10 months [52]. These data were confirmed by a large multicenter second-line study (PROFILE 1005) in the same patient population with ORR of 51% [53]. In both trials, the majority of responses were observed during the first 8 weeks of treatment and median duration of responses was 48.1 and 41.9 weeks, respectively. In a phase III

second line study, crizotinib confirmed superior activity *versus* pemetrexed or docetaxel with a statistically significant increase of PFS (7.7 *versus* 3.0 months, respectively; HR 0.49, p=0.0001) and higher ORR (65% *versus* 20%, p=0.0001). No difference in OS was observed between the treatment arms [54]. The PROFILE 1014 trial investigated crizotinib *versus* cisplatin or carboplatin plus pemetrexed as first-line treatment in untreated ALK-positive non-squamous NSCLC patients [55]. Crizotinib significantly prolonged PFS, the primary end point of the study, (median 10.9 *versus* 7.0 months; HR: 0.454, p<0.0001) and also ORR favored crizotinib (74% *versus* 45%; p<0.0001) with an acceptable safety profile [56].

Similarly to EGFR-TKI, mechanisms of acquired resistance to crizotinib are multiple, including ALK mutations or gene amplification documented in approximately one-third of patients at progression. L1196M (gatekeeper mutation) and C1156Y are two non-overlapping mutations in the ALK tyrosine kinase domain and each of them independently confers *in vitro* resistance to crizotinib. Other resistance mutations in the ALK kinase domain are G1269A, 1151Tins, L1152R, G1202R, and S1206Y [57]. Additional resistance mechanisms involves activation of alternative signaling pathways that can bypass ALK as, for instance, up-regulation of EGFR, cKIT gene amplification, activating mutations in EGFR and KRAS [58,59].

Following treatment with crizotinib, relapses are most commonly reported in central nervous system (CNS). A study assessed the sites progression in EGFR mutant or ALK translocated tumors and CNS was the first site of disease progression in 13 of 28 ALK-positive patients (46%) treated with crizotinib [60]. The low level of crizotinib in the cerebrospinal fluid suggests poor drug penetration into the CNS, probably related to pharmacokinetic issues [61]. The development of the second-generation ALK inhibitors could overcome this problem as these drugs already demonstrated activity against CNS metastases.

Ceritinib (LDK378), a highly potent and selective, second generation ALK inhibitor, in a phase I

study (ASCEND-1 trial) in ALK-positive patients induced objective responses in 58% of the cases (57% in crizotinib-resistant, 60% in crizotinib naive patients) with median PFS and duration of response (DOR) of 8.6 and 8.2 months, respectively [62]. Treatment was reasonably tolerated and the most common adverse events included diarrhea (84%), nausea (77%), vomiting (57%), fatigue (36%), and ALT increased (36%). [63]. Phase II and III studies are currently ongoing either in crizotinib-resistant and in crizotinib-naive patients. Recently based on phase I data reported above ceritinib granted approved by FDA for crizotinib resistant patients.

Alectinib (CH5424802) is another potent selective orally available ALK inhibitor with activity against L1196M gatekeeper mutation and other mutations such as F1174L and R1275Q [64]. In a phase I/II study, alectinib was investigated in ALK-positive, crizotinib-pretreated patients. ORR was 93% and tolerability was good [65]. Another phase I trial enrolled 31 evaluable patients reporting an ORR of 48% and disease stabilization in 34% of the patients. [66]. At baseline sixteen patients had CNS disease, either parenchymal or leptomeningeal disease, and responses were reported after 3-6 weeks of treatment [67]. A phase II study is currently ongoing. A randomized phase III trial will compare alectinib *versus* crizotinib in treatment-naive ALK-positive advanced NSCLC patients.

AP26113 is active against L1196M "gatekeeper" mutation and other resistance mutations that have been observed clinically in patients who initially responded to crizotinib and then relapsed. This agent is also active against ROS1 and, to a lesser extent, mutant EGFR with T790M mutation. An ongoing phase I/II study showed an ORR of 63% (75% in crizotinib-resistant patients) with significant clinical activity against brain metastases [68]. The most common adverse events were nausea (38%), diarrhea (31%), fatigue (31%), cough (23%), and headache (20%), which were generally grade 1/2 in severity. Early onset of signs of potential pulmonary toxicity were in 13% of patients treated at 180 mg QD but not reported at lower doses [69].

X-376 and **X-396** are two potent ALK and, to a lesser extent, MET inhibitors. These agents have *in vivo* potent antitumor activity with favorable toxicity profiles [70]. A phase I trial with X-396 is ongoing and the drug is generally well tolerated at doses up to 250 mg daily. The most common adverse events observed are rash (36%, G1-G3), fatigue (30%, G1-G2), nausea (27%, G1), vomiting (27%, G1) and edema (20%, G1-G2). Grade3/4 treatment related AEs were rash and edema. Efficacy data are still preliminary but activity in ALK positive tumors was seen in crizotinib naïve and pretreated patients including responses in brain metastases [71].

ASP3026 is a dual ALK/MET inhibitor acting through an ATP-competitive mechanism with significant activity against resistant cells to crizotinib with L1196M mutation. In a mouse model, ASP3026 enhanced the antitumor activities of paclitaxel and pemetrexed producing tumor shrinkage and prolonging survival [72]. A phase I study (NCT01401504) is ongoing in patients with relapsed/refractory solid tumor including NSCLC and in patients with clinical progression after prior exposure to crizotinib, the ORR rate was 44% with a toxicity profile similar to other agents of the same class [73]. A summary of ALK inhibitors currently in development for NSCLC is provided in Table B.

Heat-Shock Protein 90 (HSP90) inhibitors

HSP90 inhibitors induce growth inhibition and tumor regression in NSCLC cell lines and xenograft models, both as monotherapy and in combination with other agents [74,75] and showed efficacy in EGFR-mutated and ALK-rearranged NSCLC [76]. Collectively, phase II studies with **IPI-504** [77], **AUY922** [78] and **ganetespib** [79] indicated a clinically meaningful level of activity in ALK rearranged tumors, including patients with acquired resistance to crizotinib [80].

Several combination studies are currently ongoing and investigate crizotinib with ganetespib , crizotinib with **AT13387**, and LDK378 in combination with **AUY922**.

RAS (rat sarcoma gene)/MAPK (mitogen-activated protein kinase) pathway

Activating mutations in the KRAS gene, usually missense mutations, with an aminoacid substitution at position 12, 13 or 61, are documented in about 25-30% of lung adenocarcinoma and lead to the constitutive activation of downstream kinases including PI3K/AKT/mTOR and RAS/RAF/MEK/MAPK [81]. To date, despite several therapeutic attempts there are no selective drug targeting KRAS. New treatment possibilities arise from the promising activity of multi-kinase inhibitors that target downstream RAS effectors in both the MAPK and PI3K pathways. **Sorafenib** targets RAF, VEGFR1-3, PDGFR-beta, cKIT, FLT-3 and RET; in NSCLC this agent already failed as monotherapy [82] and in combination with platinum-based chemotherapy [83,84] but additional findings reported from the BATTLE-1 trial suggested that patients with EGFR-wild-type and KRAS mutation or KRAS/BRAF mutation may derive benefit from sorafenib [85].

MEK mutations are documented around 1% of all NSCLC and MEK1 and MEK2 mutations have been identified in chemotherapy-resistant tumors. **Selumetinib** (AZD6244), an oral MEK inhibitor, has shown synergistic activity with docetaxel in preclinical models. Recently a randomized phase II trial investigated docetaxel with or without selumetinib in KRAS-mutant NSCLC patients. The combination arm resulted in a statistically non-significant prolongation of OS with an impressive ORR of 37% (*versus* 0% in the docetaxel alone group, $p < 0.0001$) and a significant increase of PFS (median 5.3 *versus* 2.1 months, $p=0.014$). Adverse events of grade 3 or higher were increased in the combination arm in terms of neutropenia (67 *versus* 55%), neutropenic fever (18% *versus* none) and asthenia (9% *versus* none) [86]. However, it should be noted that the median survival time in docetaxel arm was inferior compared with other studies that tested the same agent in unselected patient populations. Secondly, the experimental arm had an unbalance in terms of positive prognostic factors. The ongoing phase III trial will prove whether selumetinib added to

docetaxel improves survival in patients with advanced KRAS-mutant NSCLC. Currently, a phase I study is evaluating selumetinib in combination with standard first-line platinum-containing doublet chemotherapy.

Trametinib is another MEK inhibitor that has been also compared with docetaxel in KRAS-mutant NSCLC. No significant difference in median ORR and PFS were observed [87]. Two studies investigated trametinib in combination with chemotherapy, one in combination with docetaxel (NCT01192165) and another with pemetrexed [88]. In the latter the ORR was 17% for the combination, with 48% of patients achieving stable disease (SD). The ORR was much higher in patients with the G12C KRAS mutation compared to all other mutations (40 *versus* 20%).

BRAF (v-raf murine sarcoma viral oncogene homolog B1) inhibitors

Somatic mutations in BRAF are detected in 1-2% of NSCLC, particularly in adenocarcinoma and former/current smokers. The most common is the point mutation V600E but, in lung cancer, BRAF mutations within the kinase domain also occur at different positions with potential therapeutic differences between lung cancer and melanoma in terms of response to selective BRAF inhibitors.

Vemurafenib and **dabrafenib** are two targeted agents currently being investigated in BRAF-mutant lung adenocarcinomas. A phase II trial of dabrafenib in pre-treated NSCLC with a V600E BRAF mutation is ongoing (NCT01336634) and preliminary results indicate an ORR of 54% with the longest duration of response of 49 weeks. [89]. Molecular analyses indicated, in addition to the known mutations, three new mutations including an activating mutation in KRAS, upstream of BRAF. Therefore, acquired KRAS activation may be a mechanism of resistance to BRAF inhibitors [90]. Preclinical data indicate MEK inhibition as a viable strategy in BRAF-mutated NSCLC [91]. Trials with several MEK inhibitors are ongoing in BRAF mutated tumors. A pooled shRNA-drug screen strategy identified genes that, when inhibited, cooperate with MEK inhibitors to effectively

inhibit KRAS mutant cancer cells. The anti-apoptotic BH3 family gene BCL-XL emerged as a top hit. ABT-263 (navitoclax), a inhibitor that blocks the ability of BCL-XL to bind and inhibit pro-apoptotic proteins, in combination with a MEK inhibitor led to dramatic apoptosis in many KRAS mutant cell lines. This combination also caused marked *in vivo* tumor regressions in KRAS mutant xenografts and in a genetically engineered KRAS-driven lung cancer mouse model, supporting the potential of this combination as a new therapeutic approach for KRAS mutant cancers [92].

PIK3CA (phosphatidylinositol-4,5-bisphosphate3-kinase, catalytic subunit alpha) inhibitors

PIK3CA mutations are more frequently detected in squamous NSCLC, as well as AKT and PTEN mutations, [93] and they have been also associated with acquired EGFR TKI resistance. AKT1 is the signaling partner of PI3K and PTEN is its negative regulator. The first PI3K inhibitor rapamycin did not show activity in NSCLC. Currently a new generation of PI3K/mTOR inhibitors is being investigated with promising data in KRAS-mutant human NSCLC xenografts [94].

Buparlisib (BKM-120) reduces AKT activation without inducing mTOR inhibition. Trials combining buparlisib with chemotherapy in squamous NSCLC and a phase II study in patients with NSCLC that harbor an activating PI3K mutation are ongoing.

Pictilisib (GDC-0941) is another the PI3K inhibitor and preliminary data from an ongoing study in combination with carboplatin, paclitaxel and optional bevacizumab showed an ORR of 44% [95]. Other PI3K inhibitors have been evaluated in phase I studies (**XL765, PX-866**) [96].

c-MET (c-mesenchymal-epithelial transition) inhibitors

Physiologic MET activation facilitates cell processes for embryonic development, wound healing, and tissue regeneration. A gene located on chromosome 7 encodes for the MET tyrosine kinase receptor that binds the hepatocyte growth factor (HGF or scatter factor). Both tumor and stromal

cells have been identified as potential sources of HGF. In vitro studies have demonstrated that fibroblast-dependent carcinoma cell growth and invasion is inhibited by anti-HGF antibodies, highlighting the relevance of stroma-derived HGF in tumor maintenance and progression. HGF can act either as a paracrine factor, causing positive feedback leading to c-MET transcription or by an autocrine mechanism [97].

MET protein is overexpressed in approximately 25% to 75% of early stage NSCLC and gene amplification and overexpression have been associated with poor prognosis [98,99,100]. *MET* gene amplification occurs in approximately 4% to 7% of untreated NSCLC [101,102], therefore rarely underlying primary resistance to EGFR tyrosine kinase inhibitors while it also occurs more frequently in approximately in 20% of patients previously treated with EGFR TKIs [101]. Elevated serum level of HGF has been associated with more aggressive biology, poor prognosis in both NSCLC and small-cell lung cancer [103,104] and with intrinsic and acquired resistance to EGFR TKI [105,106]. Three strategies have been developed to antagonize MET including anti-HGF monoclonal antibodies (mAbs) such as ficlatuzumab, rilotumumab and TAK701, anti-MET mAb such as onartuzumab and selective and non-selective TKIs.

Ficlatuzumab is a humanized IgG1 antibody that binds HGF with high affinity and specificity and as single agent decreased tumor growth in a dose-dependent manner and led to significant reductions in phospho-c-MET and phospho-AKT levels, but produced a concomitant increase in phospho-EGFR levels. In combination with erlotinib or cetuximab, ficlatuzumab showed increased antitumor activity when compared to the single agent activity [107]. In phase I studies, as a single agent or in combination with gefitinib, showed a good tolerability [108,109]. In a randomized phase II trial gefitinib was compared with the combination of gefitinib and ficlatuzumab. The study enrolled 188 Asian treatment naïve patients with lung adenocarcinoma not selected for EGFR mutation status, even if the study population had clinical characteristics frequently associated with

the presence of EGFR mutations. The study did not reach its primary endpoint of improving the ORR but interesting observations may guide further development of this agent. Subgroup analyses indicated that the combination was more effective in patients with low MET expression. In particular, patients with EGFR activating mutations and low c-MET levels benefited more from the combination in terms of PFS, indicating that c-MET/HGF inhibition may delay EGFR TKI resistance [110].

Several studies indicate that **rilotumumab** (AMG102), an anti-HGF IgG2 monoclonal antibody, decreases c-MET phosphorylation and can delay the progression of some solid tumors [111]. To date, a phase I/II trial of AMG 102 at the dose of 15 mg and erlotinib 150 mg in previously treated patients with advanced NSCLC is ongoing.

Ornatuzumab (MetMAb), an anti-MET monovalent monoclonal antibody inhibits HGF-mediated activation, in a phase II trial in combination with erlotinib *versus* erlotinib alone was investigated as second- or third-line treatment of NSCLC without previous exposure to EGFR-TKIs. Time to progression (3 *versus* 1.5 months; p= 0.01) and in OS (12.6 *versus* 4.6 months; p = 0.002) were improved in the MET-positive tumors (+2/+3 at immunohistochemistry) [112]. A randomized phase III study (METLung) in MET-positive NSCLC patients has been then performed but in early March 2014 the Independent Data Monitoring Committee (IDMC) has recommended the study closure due to a lack of any clinically meaningful efficacy[113].

Tivantinib (ARQ-197) is a non-ATP competitive MET inhibitor that in a randomized phase 2 study in combination with erlotinib showed trends toward improved PFS and OS when compared to erlotinib alone, with enhanced outcomes in the non-squamous subpopulation [114].

A multicenter, open-label, single arm Phase II combining erlotinib and tivantinib was performed in Japan enrolling 45 EGFR-mutated NSCLC patients with acquired EGFR-TKI resistance and it showed erlotinib plus tivantinib may be beneficial in tumors with high c-Met expression or those without

resistance mutations in EGFR gene (i.e. T790M or Exon 20 ins) [115].

The Phase III MARQUEE trial was designed to further test this combination, but is been halted based on IDMC recommendation following a futility analysis [116]. Tivantinib plus erlotinib did improve OS in the subgroup of tumors with high MET expression suggesting the potential for efficacy in a biomarker-selected population.

Erlotinib plus tivantinib versus erlotinib plus placebo were compared for OS in a phase III trial (ATTENTION) in Asian patients with pretreated NSCLC with WT-EGFR. Enrollment was stopped when 307 patients had been randomized, following the Safety Review Committee's recommendation because of an imbalance in the incidence of interstitial lung disease between the groups. OS was numerically prolonged but this did not reach statistical significance and hopefully additional exploratory biomarker analysis will be able to identify subsets that benefit from this combination [117].

Cabozantinib (XL-184) is an oral, potent inhibitor of MET, RET, VEGFR2 and AXL. In a phase II trial, the ORR was 10% with overall disease control rate of 40% [118]. Several studies are exploring the combination of cabozantinib plus erlotinib and in monotherapy in patients with KIF5B/RET mutations. In patients with advanced EGFR-mutant NSCLC at progression after EGFR-TKis the combination of cabozantinib and erlotinib delayed tumor growth in 87% of the cases as documented through the assessment of the tumor doubling time. Ongoing correlative studies are assessing the correlation between tumor response and MET amplification and/or T790M mutation [119].

Crizotinib is also a MET inhibitor and therefore might have a role either in tumors with *de novo* MET amplification or in acquired resistance following EGFR-TKIs and MET gene amplification. The combination of crizotinib and erlotinib is currently investigated as a potential strategy to delay resistance to EGFR-targeted therapy. Furthermore, in a phase I study a cohort of patients with MET

amplified tumors are treated with crizotinib. Preliminary data indicate that tumors with an increase in the ratio of MET gene copy number gain relative to centromere 7 (CEP7) ≥ 5 had the higher benefit from crizotinib. However, the exploration of the optimal MET/CEP7 ratio associated with clinical benefit is ongoing [120].

ROS1 (c-ros oncogene 1) inhibitors

ROS1 rearrangement is detectable in about 1% of lung adenocarcinoma [121] and it represents a unique molecular subset of NSCLC with no overlap with other oncogene drivers. Preliminary results demonstrated good activity of crizotinib in ROS1-rearranged NSCLC with an ORR of 57% and a disease control rate of 80% [122]. Currently, trials with the second-generation ALK inhibitors (AP26113, ASP3026) are ongoing in ROS1 fusion positive tumors.

RET (REarranged during Transfection) inhibitors

The RET fusion gene has been recently reported in 1.7% of lung adenocarcinomas and related patients have identifiable clinical-pathologic characteristics [123]. Currently RET-selective inhibitors are not yet available, but in several trials the multikinase inhibitors such as vandetanib and cabozantinib showed activity in RET positive tumors [124,125] and ongoing trials are further assessing the activity of these agents. Preliminary data from a prospective phase II trial indicate that in the first three RET fusion-positive NSCLC patients treated with cabozantinib a partial response was observed in two patients, including one harboring a novel TRIM33-RET fusion. The third patient with a KIF5B-RET fusion has had prolonged stable disease approaching 8 months [126].

Several exploratory studies are currently exploring the role of other multitargeted inhibitors such as lenvatinib, ponatinib (AP24534), dovitinib and sunitinib.

HER2 (human epidermal growth factor receptor 2)

Insertions or point mutations in exon 20 that lead to constitutive activation of HER2 are documented in approximately 1-2% of NSCLC, representing 6% of EGFR/KRAS/ALK-negative specimens [127]. Preclinical data suggest that HER2 mutation may be associated with primary resistance to first generation EGFR-TKI [128] and a potential role of afatinib in patients with mutation of HER2 was suggested but in a phase II study no objective responses were observed in seven patients with HER2 mutations, although five patients maintained stable disease [129]. In a single patient with HER2 amplified NSCLC the combination of dacomitinib and trastuzumab induced tumor shrinkage [130] and a phase II study of single agent dacomitinib (NCT00818441) included a cohort of patients with HER2 mutation or amplification. The ORR was 13% (3/26) in patients with *HER2* exon 20 insertions (2/5 patients with 9 base pair insertions). Partial responses lasted 13, 14, and 4+ months. No responses were documented in 4 patients with *HER2* amplification.[131]. Further studies are warranted to explore dacomitinib in a larger cohort of patients with 9 base pair insertions, patients with *HER2* amplification and point mutations and patients with extracellular domain mutations.

FGFR (fibroblast growth factor receptor) inhibitors

FGFR1 is a member of FGFR family of 4 highly conserved RTKs whose activation leads to downstream signaling through PI3K/AKT and RAS/RAF/MEK/MAPK pathways [132]. Copy number changes can be detected by different techniques, including fluorescence in situ hybridization (FISH) analysis; at the present time the cut-off level for true amplification remains to be determined. Data suggest that the FGFR pathway activation can mediate resistance to other targeted therapies such as EGFR, BRAF, HER, MET, and angiogenesis inhibitors [133,134,135]. FGFR1 amplification has been reported in 20% of squamous cell carcinomas mainly in former/current smokers and 5% of

small cell carcinomas [136,137,138].

Dovitinib (TKI258), a multiple TKI against PDGFR-beta, VEGFR1-3, c-kit, FLT3, CSFR1, Trk, RET, FGFR1-3 is currently investigated in a phase II in patients with solid tumor harboring mutations, amplifications or translocations of these targets. In a second line study single agent **nintedanib** (BIBF 1120), a FGFR1/2/3, VEGFR1/2/3 and PDGFRa/b inhibitor, showed modest single activity in unselected NSCLC patients. In platinum-pre-treated patients a phase III trial of nintedanib plus pemetrexed versus pemetrexed plus placebo demonstrated a minimal PFS improvement (4.4 *versus* 3.6 months) [139]. Conversely a statistically significant improvement in PFS was observed in another phase III study where nintedanib was associated to docetaxel and compared to docetaxel plus placebo (median 3·4 *versus* 2·7 months; hazard ratio [HR] 0.79; p=0.0019). In the combination arm OS was significantly improved for patients with adenocarcinoma who progressed within 9 months from the start of first-line treatment (median 10.9 months *versus* 7.9 months HR 0.75, p=0.0073) [140]. Currently, nintedanib is explored in a phase II trial in patients with NSCLC mutation-positive FGFR1.

Several phase I-II trials with FGFR1-3 kinase inhibitors alone or in combination with cytotoxics are ongoing and exploring the activity of **lucitanib** (E-3810), **AZD4547** and **BGJ398**.

XL999 is a new chemical entity that inhibits a spectrum of receptor tyrosine kinases (RTKs) with growth promoting and angiogenetic properties, including FGFR 1/3, PDGFRa/b, VEGFR2/KDR, KIT, and FLT3. XL999 also inhibits FLT4 and SRC [141].

Recently, V555M a secondary gatekeeper mutation has been identified in FGFR3 gene, which may represent a potential mechanism of acquired resistance to FGFR inhibitors [142].

PDGFR (Platelet derived grow factor receptor) inhibitors

Platelet-derived growth factor receptor α (PDGFRα) is a receptor tyrosine kinase that promotes cell

survival and is expressed in both the tumor and the stromal components of human cancers. The expression of activated PDGFR promotes oncogenic transformation. In preclinical studies, amplification at chromosomal segment 4q12 is associated with a potential oncogenic role of PDGFRα and KIT. This amplification has been identified in 3-7% of lung adenocarcinomas and 8-10% of squamous NSCLC [143]. Multikinase inhibitors, such as imatinib and sunitinib, that include KIT and PDGFRα among the targets, might have a potential therapeutic role in tumors harboring these aberrations. Single agent sunitinib induced objective responses in 2-11% of patients with previously treated, molecularly unselected advanced NSCLC [144,145]. Currently a phase II trial is investigating the role of sunitinib in patients with KIT or PDGFRα mutations while in another exploratory study dovitinib is administered to patients with molecularly defined tumors including those harboring PDGFR mutations.

DDR2 (Discoidin death receptor 2) inhibitors

DDR2 gene mutations have been identified approximately in 2 % of NSCLC, mainly in squamous histology. A DDR2 S768R mutation was found in a case of squamous cell carcinoma of the lung EGFR wild type responsive to the combination of dasatinib and erlotinib. [146]. In a case report a patient with chronic myelogenous leukemia and squamous cell carcinoma of the lung harboring a DDR2 S768R mutation responded to dasatinib [147]. Two distinct mechanisms of acquired resistance to dasatinib have been observed including NF1 loss and a second-site mutation in DDR2 [148]. A prospective phase II study of dasatinib in NSCLC with a DDR2 mutation or inactivating B-RAF mutation is ongoing.

THE CHECKMATE OF IMMUNOTHERAPY IN ADVANCED NSCLC

Lung tumors are recognized by the immune system and a stronger antitumor immune response as

documented, in the tumor stroma, by a higher percentage of tumor-infiltrating CD4+ T-cells, CD8+ T-cells, dendritic cells (DCs) and or natural killer (NK) cells may improve survival [149]. In the context of immunotherapy the primary aim is to stimulate and increase the host immune response against tumor cells and the therapeutic effect is associated with a unique timing to tumor response and also holds different associated toxicities compared to chemotherapy or targeted therapy. Most of the available data have been reported with vaccines and, more recently, immune-checkpoint inhibitors targeting CTLA-4 and the PD-1 pathway, which have shown antitumor activity and manageable toxicities. Immunotherapy-related toxicities are listed in Table C.

New immunotherapy agents showed also activity in squamous lung cancers, a subgroup of patients with limited therapeutic opportunities. To date, immunotherapy has been mainly developed in the context of second-line setting for metastatic NSCLC; however, its role in the context of the control of minimal residual disease in different disease settings is expected.

Talactoferrin

Talactoferrin is an orally bioavailable recombinant of human lactoferrin produced in the fungus Aspergillus niger with potential antineoplastic and immunomodulating activities. Following oral administration, talactoferrin is transported into small intestinal Peyer's patches of the gut-associated lymphoreticular tissues (GALT), where it recruits circulating immature dendritic cells bearing tumor antigens and induces their maturation. In the GALT, DC maturation in the presence of tumor antigens and lymphoid effector cells may induce systemic innate and adaptive immune responses mediated by anti-tumor natural killer (NK) cells, cytotoxic T lymphocytes, and natural killer T (NKT) cells; activation of tumor-draining lymph nodes, cellular infiltration of distant tumors, and tumor-cell death may follow. In a phase II study patients with locally advanced or metastatic NSCLC received either talactoferrin or placebo in combination with paclitaxel/carboplatin. The ORR

in the talactoferrin arm was 47% compared to 29% in the placebo arm (p=0.05) without difference in OS [150]. In a phase III study talactoferrin in pretreated patients with advanced NSCLC was compared with BSC and no statistically significant difference in OS, PFS and disease control rate (DCR) was observed between the two arms [151]. Another phase III trial is evaluating talactoferrin in combination with carboplatin and paclitaxel *versus* placebo plus chemotherapy as first-line in treatment-naïve patients.

Belagenpumatucel-L is prepared by transfecting allogeneic non-small cell lung cancer (NSCLC) cells with a plasmid containing a TGF-beta2 antisense transgene, expanding the cells, and then irradiating and freezing them. Upon administration, this agent may elicit a cytotoxic T lymphocyte (CTL) response against host NSCLC cells, resulting in decreased tumor cell proliferation. In a phase II trial in patients with NSCLC stage II–IV three different doses of belagenpumatucel-L were evaluated. The estimated median survival time for patients treated with the two higher doses was significantly higher than for patients receiving the lowest dose (581 days *versus* 252 days; P=0.0186) [152]. A phase III trial of belagenpumatucel-L in advanced NSCLC is currently underway .

MUC1 (Mucinous glicoprotein-1)

Tecemotide (L-BLP25) is an investigational MUC1 antigen-specific cancer immunotherapy that is designed to stimulate the body's immune system to identify and target cells expressing the cell-surface glycoprotein MUC1. MUC1 is expressed in many cancers, including NSCLC, and has multiple roles in tumor growth and survival. In a randomized phase IIB trial, tecemotide was administered as maintenance therapy *versus* best supportive car in patients with stage IIIB/IV NSCLC in response or stable disease after first-line chemotherapy/chemoradiation. Median OS benefit was 4.4 months in favor of tecemotide. The survival advantage was statistically significant only in patients with stage IIIB disease, in which there was a strong positive trend in 3-year survival [153]. In a phase III

study (START) patients with stage III NSCLC were randomized to receive either IV cyclophosphamide followed by weekly maintenance tecemotide or placebo. In the intent to treat population no OS difference was observed between the two arms (median OS of 25.6 *versus* 22.3 months, respectively; p=0.123). In a preplanned subgroup analysis a statistically significant improvement in OS was documented in patients who received concurrent chemo-radiation followed by vaccination compared to those receiving placebo (30.8 *versus* 20.6 months, respectively; p=0.016) [154]. Two additional trials are ongoing; a phase III trial of tecemotide *versus* placebo in Asian patients (INSPIRE) and a second of phase II study of tecemotide with bevacizumab following chemoradiation for stage IIIA-IIIB NSCLC.

TG4010 contains a genetically modified virus that expresses both MUC1 and interleukin-2, resulting in activation of T-cells and NK cells. In a phase IIb study TG4010 was evaluated in patients with stage IIIB or IV MUC1-positive NSCLC in association with chemotherapy (cisplatin plus gemcitabine). Estimated 6-month PFS was 43.2 % in the TG4010 treated group, *versus* 35.1 % in the chemotherapy only arm (p=0.307) while median OS was similar for the two groups[155]. A phase IIb/III trial of TG4010 *versus* placebo in association with first-line chemotherapy in patients with stage IV NSCLC is ongoing.

Anti –EGF vaccine

Recombinant EGF was bound to a carrier protein and consequently an anti-EGF antibody that inhibits the interaction between endogenous EGF and the EGFR was generated leading to inhibition of growth and proliferation in cancer cells. In a randomized phase II study patients with stage IIIB/IV NSCLC at the end of first-line chemotherapy were randomly assigned to receive the EGF vaccine or best supportive care. The OS advantage for vaccinated patients was statistically significant only in the subgroup of younger patients (< 60 years, median survival 11.6 months

versus 5.3 months, p=0.0124). In patients with an antibody response a better OS was observed (11.7 *versus* 3.6 months, respectively) compared with those with a poor antibody response [156]. A randomized phase III trial of the EGF vaccine *versus* BSC in patients with stage IIIB/IV disease is ongoing.

Monoclonal antibodies against checkpoint inhibitors

Immune checkpoints refer to a plethora of inhibitory pathways hardwired into the immune system that are crucial for maintaining self-tolerance and modulating the duration and amplitude of physiological immune responses in peripheral tissues in order to minimize collateral tissue damage. It is now clear that tumors co-opt certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T cells that are specific for tumor antigens. Because many of the immune checkpoints are initiated by ligand-receptor interactions, they can be readily blocked by antibodies or modulated by recombinant forms of ligands or receptors. Recently antibodies that bind to and inactivate these inhibitory stimuli have been evaluated in several solid tumors including NSCLC.

Ipilimumab

Ipilimumab is a fully human IgG1 monoclonal antibody that binds to CTLA-4 and prevents cytotoxic T-cell down-regulation at early stages of T-cell activation. In a pivotal study phase III study in patients with previously treated metastatic melanoma, ipilimumab administered with or without a glycoprotein100 (gp100) peptide vaccine was compared with gp100 alone. The median overall survival was 10.0 months among patients receiving ipilimumab plus gp100, as compared with 6.4 months among patients receiving gp100 alone (HR, 0.68; P<0.001) and relevantly 2-year survival rate (24%) was significantly higher with ipilumumab [157].

In a multicenter phase II study, ipilimumab in combination with paclitaxel and carboplatin was

evaluated in patients with chemotherapy-naive stage IIIb/IV NSCLC. Patients were randomized to a concurrent ipilimumab plus paclitaxel/carboplatin followed by two doses of placebo plus paclitaxel and carboplatin, a phased ipilimumab (two cycles of placebo plus paclitaxel and carboplatin followed by four doses of ipilimumab plus paclitaxel and carboplatin), or a control regimen (placebo plus paclitaxel and carboplatin) for a maximum of 18 weeks. Patients with response/stable disease received maintenance treatment with ipilimumab or placebo until disease progression or intolerance. Patients receiving phased ipilimumab experienced an improved immune-related PFS (irPFS) as compared to those receiving PC alone (irPFS 5.7 *versus* 4.6 months, respectively, p=0.05). Phased ipilimumab improved the activity of chemotherapy mainly in patients with squamous histology [158]. The toxicity profile was similar to that observed in melanoma patients, despite a higher dose of ipilimumab (10 mg/kg *versus* 3 mg/kg). A Phase III study of phased ipilimumab with chemotherapy in patients with stage IV/recurrent squamous NSCLC is ongoing.

Monoclonal antibodies anti PD-1

The inhibitory receptor PD-1 is expressed on activated T cells and modulates T cell function mainly during the effector phase in peripheral tissues including tumors (as opposed to the up-regulation of CTLA-4 during the early activation phase in the lymph node). The PD-1 ligand, PD-L1, is expressed on epithelial and endothelial cells in addition to different types of immune cells such as antigen-presenting cells (dendritic cells, macrophages, B cells). PD-L1 can be both constitutively expressed and up-regulated by interferon in inflammatory conditions such as a chronic tissue infection. It is thought that tumors “exploit” this regulatory function to evade a tumor-directed T cell response. PD1 up regulation on the surface of tumor infiltrating CD8+ lymphocytes in patients with NSCLC has been associated with a diminished anti-tumor cellular immune response [159].

Nivolumab (BMS-936558, MDX-1106) is a fully - human IgG4 PD-1 receptor blocking antibody with

no antibody-dependent cell death activity (consistent with its IgG4 Fc-domain) that in a phase I trial in several tumor types induced objective responses in NSCLC in approximately 20% of the patients, most of them heavily pretreated [160,161]. Updated results from the expansion cohort of the phase I trial included 129 patients. For those treated at 3 mg/kg of nivolumab the ORR was 24.3 % and median survival was 14.9 months (9.5 for squamous NSCLC; 18.2 for non-squamous NSCLC). At a median 20.3-month follow-up, OS rates were 42% at 1 year and 24% at 2 years across all cohorts of NSCLC, regardless of the dose received [162]. Recently, the association between PD-L1 expression by immunohistochemistry (IHC) analysis and response to nivolumab was investigated. ORR was 16.1% (5/31) in PDL-1 positive and 12,5% (4/32) in PD-L1 negative tumors respectively. No apparent association between PD-L1 protein expression and NSCLC histology was observed [163]. Additional evaluation of PD-L1 as a molecular marker of nivolumab efficacy is needed.

Phase III studies of second-line nivolumab compared to docetaxel for advanced/metastatic non-squamous and squamous NSCLC are currently active. Recently, data about a multi-arm phase I trial in front-line of nivolumab plus different platinum-based doublets, including gemcitabine/cisplatin (Arm A), pemetrexed/cisplatin (Arm B), carboplatin/paclitaxel (Arm C) were presented. Forty-three patients were treated with nivolumab plus platinum-based doublets. No dose-limiting toxicities (DLTs) were seen with 10 mg/kg nivolumab. Grade 3-4 treatment-related adverse events were 49% across arms, and 25%, 47%, and 69% of patients in Arms A, B, and C, respectively, had relevant toxicity. Three grade 3 pneumonitis have been observed and one patient died of disease progression with unresolved pneumonitis. Confirmed ORRs were 33%, 33%, and 31% in Arms A, B, and C, respectively [164].

A phase III trial in front-line is currently randomizing patients with stage IV or recurrent NSCLC to nivolumab at a dose 3 mg/kg IV every two weeks versus investigator's choice chemotherapy using

PD-L1 expression level and histology as stratification factors. The primary end point of the study is PFS and optional cross-over to nivolumab is allowed [165].

Pembrolizumab (MK-3475)

MK-3475 is a humanized IgG4 monoclonal antibody that binds PD-1 already evaluated at the dose of 10 mg/kg every 2 weeks or 3 weeks in squamous and non-squamous NSCLC who had received two prior systemic regimens. Assessment of drug activity was carried out every 9 weeks using immune-related response criteria (irRC). ORR (confirmed & unconfirmed by irRC/RECIST) in all patients was 15%/21% (16%/24% in PD-L1 positive *versus* 10%/8% in PD-L1 negative tumors). Most of the toxicities reported were grade 1-2 in severity, most commonly fatigue (13%), decreased appetite (6.5%), arthralgia (6.1%), pruritus (5.4%), rash (4.7%), and pyrexia (3.6%). The incidence of grade 3/4 drug-related AEs was 6%. There were 3 cases of drug-related grade 3/4 pneumonitis. [166]. A phase II/III study is currently recruiting to compare MK-3475 and docetaxel in patients with NSCLC previously treated with platinum-containing chemotherapy (KEYNOTE -010).

Early safety and clinical data of MK-3475 as initial therapy in patients with advanced NSCLC have been already presented. EGFR and ALK negative, PD-L1 positive ($\geqslant$ 1% positive cells) NSCLC were randomized to receive MK-3475 10 mg/kg every 3 or 3 weeks until progressive disease. Forty-five patients were eligible for treatment with evaluable imaging at baseline by irRC and 42 by RECIST criteria. Reported ORR was 26% by RECIST and 47% by irRC. Most commonly reported adverse events were fatigue (14%), pruritus (8%), dermatitis acneiform (6%), diarrhea (6%) and dyspnea (6%) [167]. A phase III of single agent MK-3475 versus chemotherapy in PD-L1 positive metastatic NSCLC will start recruitment in September 2014.

Anti PD-L1 and 2 monoclonal antibodies

PD-1 binds to its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), which are expressed on antigen

presenting cells but also on cancer cells to escape to the immune system overexpressing PD-1 ligands on their surface and inhibition of PD-L1 on the tumor cell surface may represent another therapeutic strategy. The downside of this inhibition includes the potential ability of tumors to aberrantly up-regulate expression of other inhibitory ligands for PD-1, such as B7-DC and the development of drug-resistant clones [168]. PD-L1 expression has been reported approximately in 50% of NSCLC [169].

BMS-936559 is an IgG4 monoclonal antibody targeting PD-L1 that in a phase I study in several tumor types induced objective responses in 5 out of 49 evaluable patients with NSCLC. Response duration ranged between 2.3 and 16.6 months with PFS rate at 24 week of 31% [170].

At the interim analysis of 40 NSCLC patients treated in a phase I trial with **MPDL-3280A**, an IgG1 monoclonal antibody directed against PD-L1, ORR was 23% and PFS rate at 24 weeks 46% with only 11% drug-related adverse events. Smokers or former smokers, had a better ORR (26%) compared to never-smokers (10%) [171]. Furthermore, a retrospective assessment of tumor PD-L1 expression suggests that PD-L1 may eventually be a predictive biomarker; a correlation has been observed between strong IHC (3+) baseline PD-L1 expression and responses to MPDL3280A [172]. Two-phase II trials in NSCLC in patients with PD-L1+ by IHC are recruiting patients. Furthermore an association between a T-cell gene signature (including CD8, IFNg and Granzyme-A) and treatment response was identified. Conversely, non-responders showed minimal tumor CD8+ T-cell infiltration and the absence of T-cell activation (measured by Granzyme-A and Perforin expression). Additionally, a subpopulation of patients exhibited changes in circulating cytokines and activated T-cell subsets [133]. An evaluation of 60 NSCLC tumor samples indicated that genes associated with tumor progression and signaling pathways were over-expressed in PD-L1+ tumors. PD-L1 positivity was identified amongst KRAS mutation positive (8/10) and wild-type tumors (15/43). PD-L1 positivity was not associated with PTEN, EGFR protein expression and histology. A

single arm phase II trial in patients with PD-L1-positive in advanced/metastatic NSCLC is in progress. A phase III open-label, multicenter randomized study is comparing MPDL3280A with docetaxel in patients with NSCLC after failure with platinum containing chemotherapy.

MEDI4736 is another IgG1-kappa PD-L1 inhibitor that in a phase I trial have shown promising activity and low toxicity in several solid cancers including NSCLC [173]. In addition a phase Ib study of MEDI4736 in combination with tremelimumab is recruiting patients while a phase II study is testing the drug activity in patients with stage IIIB/IV NSCLC already treated with at least two prior systemic treatments. A phase I, open-label, multicenter study is evaluating MEDI4736 in association with gefitinib.

AMP-224 is a recombinant fusion protein comprised of the extracellular domain of PD-L2 and the Fc region of human IgG. Promising preclinical studies [159] led to a phase I trial in advanced cancer not responsive to standard therapy. The dose escalation study is concluded and currently patients are enrolled in an expansion cohort in order to assess the pharmacodynamic activity and therapeutic potential [174].

KIR Inhibition and other Checkpoint Proteins

Lirilumab (IPH2102/BMS-986015) is a fully human monoclonal antibody blocking the interaction between killer-cell immunoglobulin-like receptors (KIR) on natural killer (NK) cells with their ligands. Inhibitory KIRs seem to be selectively expressed in the peritumoral NK cell infiltrate and thus be involved in the checkpoint pathway co-opted by tumors [175]. In a preclinical xenograft tumor model lirilumab showed anti-tumor efficacy [176] and it is currently tested in a phase I trial in combination with nivolumab in patients with advanced solid tumors including NSCLC and also in combination with ipilimumab.

Phosphatidylserine (PS) is a cell membrane phospholipid physiologically expressed by normal cells

that during apoptosis moves to the outer membrane surface, suppressing the excessive immune activation [177] and it was found overexpressed in some tumor cells. **Bavituximab** is a chimeric IgG3 antibody against PS that in a phase II study was combined with carboplatin and paclitaxel reporting a 52% overall response rate in first line NSCLC [178].

CONCLUSION

Worldwide, NSCLC is the most common cause of cancer-related death and, unfortunately, the overall 5-year survival rate remained unchanged in the last 10 years. However, the advent of systematic genomic sequencing and the discovery of a clinically relevant activity of molecular targeted therapies in specific subgroups of patients as well as the identification of a potential role for immunotherapy open new treatment avenues in every histological subtype of lung cancer, including small cell lung cancer.

Cancer is a genetic somatic disease (5% inherited), it is usually caused by genetic alterations of a handful of genes (the so called “oncogenes or driver genes’) and today is often and increasingly possible to identify these genetic lesions by molecular diagnosis. There is already established paradigms indicating the targeted therapies are only effective when aimed at the alteration of the drive gene(s) such in the case of EGFR-TKI and EGFR sensitizing mutations or ALK translocation and ALK-inhibiting agents which have already proven the their superior efficacy when compared to the standard of care.

In the next five years the role of biomarkers will become even more relevant. With targeted therapies, biomarkers have the potential for providing added value through an integrated approach to prediction using the genetic makeup of the tumor and the genotype of the patient for treatment selection and patient management. Specifically, biomarkers can aid in patient stratification (risk assessment), treatment response identification (surrogate markers), or

differential diagnosis (identifying individuals who are likely to respond to specific drugs).

EXPERT COMMENTARY

Currently EGFR sensitizing mutations and ALK translocations are established molecular targets mainly in patients of Asian ethnicity, never smokers with adenocarcinoma of the lung. Even if these clinical factors are enrichment criteria for the specific molecular abnormalities only the appropriate molecular tests are associated with the best sensitivity and specificity. Worldwide erlotinib, gefitinib and afatinib got already approval for front-line treatments in EGFR mutation-positive patients while crizotinib is registered, with different indications in US and Europe, for the treatment of NSCLC patients with ALK translocation.

While these molecular agents are standard of care in the setting of metastatic NSCLC, their role in the early and locally advanced disease setting remains to be established and appropriate and well-designed clinical trials are largely awaited.

The crucial understanding of mechanisms of acquired resistance to molecular targeted therapies, leading clinically to disease progression, is today one of the biggest therapeutic challenges. Because of the urgent need of a better understanding of the resistance mechanisms in addition to the diagnostic relevance of initial tissue acquisition leading to biomarker testing, the opportunity of the re-biopsy at the time of disease progression, although it is not a standardized requirement according to current diagnostic guidelines, should be strongly advocated at least in the context of appropriated designed clinical trials because provides information about changes in genomic asset and histological findings , that could theoretically guide new therapies. At this stage the optimal clinical management of the NSCLC at the time of radiological progression on specific targeted therapies remains to be determined. There is evidence from retrospective studies that in the case of oligo-metastatic progression patients can benefit from a loco-regional treatment modality and

may continue the same drug. In case of diffuse progression therapeutic alternatives include the addition of chemotherapy to the targeted therapy, the administration of a different targeted therapy based on specific resistance mechanism, or the switch to systemic chemotherapy alone. None of these approaches have been validated in the context of prospective clinical trials.

The current state of knowledge of the genomic alterations in small cell and squamous cell lung cancer lags behind compared to what is known in adenocarcinoma, but as more attention is focused on this topic, we expect that new targeted therapies for this histological subtype will be quickly developed.

Although immunotherapy studies in NSCLC had invariably failed to improve survival, new therapeutic targets have emerged particularly the immune checkpoint pathways and, based on data generated so far, specific inhibitory monoclonal antibodies, alone or in combination, may hold the key to making immunotherapy a reality in the treatment of NSCLC.

FIVE-YEAR REVIEW

In the next five years it will become increasingly evident that there are molecularly distinct subtypes of lung cancers, with different therapeutic approaches required for each subtype. The extensive understanding of cancer pathogenesis will pave the way to new treatment options, including targeted agents and cancer immunotherapy. Targeted approaches aim to inhibit molecular pathways that are crucial for tumor growth and maintenance whereas, immunotherapy endeavors to stimulate a host immune response that effectuates long-lived tumor destruction. Targeted therapies and cytotoxic agents also modulate immune responses, which raises the possibility that these treatment strategies might be effectively combined with immunotherapy to improve clinical outcomes. Immunotherapy itself is moving in the direction of personalized medicine searching for a single or combinations of biomarkers to identify the subgroup of patients

deriving the highest benefit from this approach.

The establishment of molecular platforms accessible to all lung patients will be a critical requirement, with a rapid turnaround time to direct patients to the appropriate therapy. This epidemiological enterprise needs to be tightly linked to clinical trials, even in the context of the current limited molecular testing accessibility that hopefully will become less relevant when next generation sequencing facilities will become widely available.

The International Cancer Genome Consortium (ICGC) and the Cancer Genome Atlas (TCGA) are examples of large-scale integrative programs whose goal is to harness the full potential of the cancer genome to deliver personalized cancer medicine through multidisciplinary programs. The value of these prospective data collections will benefit our knowledge about molecular epidemiology of lung cancer but provides also values for health economical assessments.

The fact that most targeted cancer therapies have highly favorable toxicity profiles relative to cytotoxic chemotherapy is cause for optimism. However, the current focus on single agent therapy could complicate the investigation of tolerable combinations because dose and schedule are selected exclusively based on tolerability of single agent studies. Combination studies of targeted therapies are largely awaited by evaluating tolerability of each single agent given simultaneously. If additive toxicities are observed (as might be expected), the combination might be abandoned prematurely. If early development decisions will be, instead, driven by strategies that plan for combination therapy rather than single agent therapy, clinical safety and efficacy could be assessed more quickly.

It should be acknowledged that for the next five years the role of histology will be still critical in defining the treatment approach for a considerable proportion of patients with advanced lung cancer. Furthermore, after failure of one or more targeted therapies chemotherapy still remains a therapeutic opportunity sometime during the clinical course of the disease. Finally, the

identification of the best combinations to be used (e.g. chemotherapy and immunotherapy, molecular targeted therapy and immunotherapy, radiotherapy and immunotherapy and others) remains to be explored. For instance, it is well known that some chemotherapeutic agents may effect the immune system through different mechanisms including up-regulation of major histocompatibility complex–1 expression, dendritic cells and T-cell activation. Erlotinib can increase class I and class II major histocompatibility complex expression and can induce inhibition/activation on the T-cell proliferation through down-regulation of the c-Raf/ERK cascade and Akt signaling pathway. Furthermore, similar types of interaction may be observed with radiotherapy in a phenomenon called “abscopal effect”. This event is based on the fact that radiation locally can trigger systemic immune activation and lead to spontaneous regression of tumors or metastases that are outside the radiation field. Radiation therapy has the potential to partner with immune-checkpoint blockade, because high-dose radiation appears to be associated with increased antigen expression and induction of immunogenic cell death.

In conclusion, the efficacy of various targeted therapies in such dismal disease such has been lung cancer for a long period of time, suggests that we are entering an era in which treatment decisions will be based on tumor molecular abnormality profile or “signature,” rather than tumor tissue type or anatomical site of origin, improving patient prognosis and quality of life.

KEY ISSUES

- Molecular profiling of lung cancer is an absolute priority and large molecular screening consortium should be largely encouraged.
- All patients with EGFR mutations and ALK gene rearrangements should get therapy with appropriate agents.

- New generations of EGFR-TKIs active also against T-790 mutation and a second generation of ALK-inhibiting agents active also against brain metastases are currently in clinical development
- Acquired resistance to specific targeted therapies arises within approximately one year and it is characterized by multiple mechanisms.
- In adenocarcinoma multiple new targets have been identified and for some of them specific inhibitors are already in clinical trials (RAS,MEK, BRAF1, PIK3A, c-MET, ROS1, RET, HER2).
- There is a growing interest to further explore the role of immunotherapy because of early significant results with immune checkpoint pathways inhibitors such as the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1) and its ligands PD1-L1.

References

Papers of special note have been highlighted as:

● of interest

●● of considerable interest

[1] Ruppert AM, Antoine M, Beau-Faller M, Cadranel J, Wislez M. Which platform to support a personalized lung cancer treatment? Bull Cancer. 99(5),529-34 (2012)

[2] Imielinski M, Berger AH, Hammerman PS et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell. 14;150(6):1107-20 (2012)

[3] Nan Y, Jin F, Yang S et al. Discovery of a set of biomarkers of human lung adenocarcinoma through cell-map proteomics and bioinformatics Med Oncol. 27(4),1398-406 (2010)

[4] Weir BA, Woo MS, Getz G et al. Characterizing the cancer genome in lung adenocarcinoma. Nature. 450(7171), 893-8 (2007)

[5] Kris MG, Johnson BE, Kwiatkowski DJ et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: the NCI's Lung Cancer Mutation Consortium (LCMC). J Clin Oncol 29:abstract CRA7506 (2011)

[6] Kim Y, Hammerman PS, Kim J et al. Integrative and comparative genomic analysis of lung squamous cell carcinomas in East Asian patients. J Clin Oncol. 32(2),121-8 (2014)

[7] Kao S, Shiau CK, Gu DL et al. IGDB. NSCLC:integrated genomic database of non-small cell lun cancer. Nucleic Acids Res. 40(Database issue),D972-7 (2012)

[8] Weinstein IB, Joe A. Oncogene addiction. Cancer Res. 68(9):3077-80 (2008)

[9] Hall MJ. Conflicted confidence: Academic oncologists' views on multiplex pharmacogenomic testing. J Clin Oncol. 32(2014)

[10] Rosell R, Carcereny E, Gervais R et al. Erlotinib *versus* standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomize phase 3 trial. Lancet Oncol. 13:239-46 (2012)

[11] Inoue A, Kobayashi K, Maemondo M et al. Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin paclitaxel for chemo-naive non-small cell lung cancer with sensitive EGFR gene mutations (NEJ002). Ann Oncol. 24:54-9 (2013)

[12] Sequist LV, Yang JC, Yamamoto N et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol. 31:3327-34 (2013)

[13] Shaw AT, Kim DW, Nakagawa K et al. Crizotinib *versus* chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 368:2385-94 (2013)

[14] Rosell R , Moran T , Queralt C et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med. 361 : 958 – 67 (2009)

[15] Thongprasert S, Duffield E, Saijo N et al. Health-related quality-of-life in a randomized phase III first-line study of gefitinib *versus* carboplatin/paclitaxel in clinically selected patients from Asia with advanced NSCLC (IPASS). J Thorac Oncol. 6(11),1872–80 (2011)

[16] Fukuoka M, Wu YL, Thongprasert S et al. Biomarker analyses and final overall survival results from a phase III randomised, open-label, first-line study of gefitinib *versus* carboplatin/paclitaxel in clinically selected patients with advanced non-small cell lung cancer in Asia (IPASS). J Clin Oncol. 29: 2866–74 (2011)

[17] Zhou C, Wu YL, Chen G et al. Erlotinib versus chemotherapy as first line treatment for patients with advanced EGFR mutation positive non-small cell lung cancer (OPTIMAL, CTONG-802): a multicentre, open label, randomised phase 3 study. Lancet Oncol. 12:735–42 (2011)

[18] Maemondo M, Inoue A, Kobayashi K et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med. 362:2380–8 (2010)

[19] Sequist LV, Waltman BA, Dias-Santagata D et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 3(75),75ra26. (2011)

[20] Doebele RC, Pilling AB, Aisner DL et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res. 18(5),1472-82 (2013)

[21] Riely GJ, Yu HA, Arcila ME et al. Response to erlotinib and prognosis for patients with *de novo* epidermal growth factor receptor (EGFR) T790M mutations. J Clin Oncol. 31:abstract 8018 (2013)

[22] Oxnard GR, Arcila ME, Sima CS et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. Clin Cancer Res. 17:1616–22 (2011)

[23] Engelman JA, Zejnullahu K, Mitsudomi T et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 316:1039-43(2007)

[24] Kobayashi S, Ji H, Yuza Y et al. An alternative inhibitor overcomes resistance caused by a mutation of the epidermal growth factor receptor. Cancer Res. 65:7096–101(2005)

[25] Janne PA, von Pawel J, Cohen RB et al. Multicenter, randomized, phase II trial of CI-1033, an irreversible pan-ERBB inhibitor, for previously treated advanced non small-cell lung cancer. J Clin Oncol. 25:3936-44 (2007)

[26] Sequist LV, Besse B, Lynch TJ et al. Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. J Clin Oncol. 28:3076-83 (2010)

[27] Shi YS, Zhang L, Liu X et al. A randomized, double-blind phase III study of icotinib *versus* gefitinib in patients with advanced non-small cell lung cancer (NSCLC) previously treated with chemotherapy (ICOGEN). J Clin Oncol 29:abstr 7522 (2011)

[28] Sequist LV, Yang JC, Yamamoto N et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol. 31:3327-34 (2013)

[29] Miller VA, Hirsh V, Cadranel J et al. Afatinib *versus* placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. Lancet Oncol. 13:528-38 (2012)

[30] Katakami N, Atagi S, Goto K et al. LUX-Lung 4: a phase II trial of afatinib in patients with advanced non-small-cell lung cancer who progressed during prior treatment with erlotinib, gefitinib, or both. J Clin Oncol. 31:3335-41(2013)

[31] Yang JCH, Sequist LV, Schuler MH et al. Overall survival (OS) in patients (pts) with advanced non-small cell lung cancer (NSCLC) harboring common (Del19/L858R) epidermal growth factor receptor mutations (EGFR mut): Pooled analysis of two large open-label phase III studies (LUX-Lung 3 [LL3] and LUX-Lung 6 [LL6]) comparing afatinib with chemotherapy (CT). J Clin Oncol 32:5s, abstract 8004 (2014)

[32] Ramalingam SS, Blackhall F, Krzakowski M et al. Randomized phase II study of dacomitinib (PF00299804), an irreversible pan-human epidermal growth factor receptor inhibitor, *versus* erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol. 30:3337-44 (2012)

[33] Ramalingam SS, Janne PA, Mok T et al. Randomized, double-blinded study of dacomitinib, an irreversible pan-human epidermal growth factor receptor (HER) inhibitor, versus erlotinib for second-

line/third-line therapy of locally advanced/metastatic non-small cell lung cancer (ARCHER 1009). J Clin Oncol 32:5s, abstract 8018 (2014)

[34] Ellis PM, Liu G, Millward M et al. NCIC CTG BR.26: A phase III randomized, double blind, placebo controlled trial of dacomitinib versus placebo in patients with advanced/metastatic non-small cell lung cancer (NSCLC) who received prior chemotherapy and an EGFR TKI. J Clin Oncol 32:5s abstract 8036 (2014)

[35] Wakelee H, Soria J, Camidge R et al. First-in-human evaluation of C0-1686, an irreversible, highly selective tyrosine kinase inhibitor of mutations of EGFR (activating and T790M) Abstract presented at 4th European Lung Cancer Conference, March 26–29, Geneva, Switzerland (2014)

[36] Sequist LV, Soria JC, Gadgeel SM et al. First-in-human evaluation of CO-1686, an irreversible, highly selective tyrosine kinase inhibitor of mutations of EGFR (activating and T790M). J Clin Oncol. 32:5s abstract 8010 (2014) **●● potential role for improved activity and tolerability of EGFR TKIs in NSCLC in particular with T790M+ disease**

[37] Ranson M, Pao W, Kim DW. AZD9291: an irreversible, potent and selective tyrosine kinase inhibitor (TKI) of activating (EGFR+) and resistance (T790M) mutations in advanced NSCLC The AURA study. J Thorac Oncol. 11–034. suppl; abstr P1.11. (2013)

[38] Ranson M, Pao W, Kim DW et al. AZD9291: an irreversible, potent and selective tyrosine kinase inhibitor (TKI) of activating (EGFRM+) and resistance (T790M) mutations in advanced NSCLC, Abstract MO21.12 presented at the 15th International Association for the Study of Lung Cancer World Conference on Lung Cancer, October 27–30, Sydney, Australia. (2013)

[39] Janne PA, Ramalingam SS, Yang JCH et al. Clinical activity of the mutant-selective EGFR inhibitor AZD9291 in patients (pts) with EGFR inhibitor–resistant non-small cell lung cancer (NSCLC). J Clin Oncol 32:5s, abstract 8009 (2014)

[40] Dearden SP, Brown H, Cole R, Jenkins S. EGFR T790M mutation testing within a phase I study with AZD9291.J Clin Oncol 32, abstract e19051 (2014)

[41] Viloria-Petit A, Crombet T, Jothy S et al. Acquired resistance to the antitumor effect of epidermal

growth factor receptor-blocking antibodies in vivo: a role for altered tumor angiogenesis. Cancer Res. 61:5090–101 (2001)

[42] Heymach JV, Paz-Ares L, De Braud F et al. Randomized phase II study of vandetanib alone or with paclitaxel and carboplatin as first-line treatment for advanced non-small-cell lung cancer. J Clin Oncol. 26:5407–15 (2008)

[43] Pakkala S, Ramalingam SS. Combined inhibition of vascular endothelial growth factor and epidermal growth factor signaling in non-small-cell lung cancer therapy.Clin Lung Cancer. 1:S17-23 (2009)

[44] Naumov GN, Nilsson MB, Cascone T et al. Combined vascular endothelial growth factor receptor and epidermal growth factor receptor (EGFR) blockade inhibits tumor growth in xenograft models of EGFR inhibitor resistance. Clin Cancer Res. 15:3484–3494 (2009)

[45] Pietanza MC, Gadgeel SM, Dowlati A et al. A Phase II study of the multitargeted tyrosine kinase inhibitor XL647 in patients with non-small-cell lung cancer. J Thorac Oncol. 7(5),856-65 (2012)

[46] Pietanza MC, Lynch TJ Jr, Lara PN Jr et al. XL647 a multitargeted tyrosine kinase inhibitor: results of a phase II study in subjects with non-small cell lung cancer who have progressed after responding to treatment with either gefitinib or erlotinib. J Thorac Oncol. 7:219-26 (2012)

[47] Chmielecki J, Pietanza MC, Aftab D et al. EGFR mutant lung adenocarcinomas treated first-line with the novel EGFR inhibitor, XL647, can subsequently retain moderate sensitivity to erlotinib. J Thorac Oncol. 7(2),434–442 (2012)

[48] Soria JC, Baselga J, Hanna N et al. Phase I-IIa study of BMS-690514, an EGFR, HER-2 and -4 and VEGFR-1 to -3 oral tyrosine kinase inhibitor, in patients with advanced or metastatic solid tumours. Eur J Cancer49:1815-24 (2013)

[49] Camidge DR, Bazhenova L, Salgia R et al. First-in-human dose-finding study of the ALK/EGFR inhibitor AP26113 in patients with advanced malignancies: updated results. J Clin Oncol. 31:abstract 8031 (2013)

[50] Koivunen JP, Mermel C, Zejnullahu K et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin. Cancer Res. 14:4275–83 (2008)

[51] Wong DW, Leung EL, So KK et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from non smokers with wild-type EGFR and KRAS. Cancer. 115:1723–33(2009)

[52] Camidge DR, Bang Y, Kwak EL et al. Progression-free survival (PFS) from a phase I study of crizotinib (PF 02341066) in patients with ALK-positive non-small-cell lung cancer (NSCLC). J Clin Oncol. 29(suppl): abstract 2501 (2011)

[53] Crino` L, Kim D, Riely G et al. Initial phase II results with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC): PROFILE 1005. J Clin Oncol. 29(suppl): abstract 7514 (2011)

[54] Shaw AT, Kim D-W, Nakagawa K et al. Crizotinib *versus* chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 368:2385–94 (2013)

[55] Pilotto S, Peretti U, Novello S et al. PROFILing non-small-cell lung cancer patients for treatment with crizotinib according to anaplastic lymphoma kinase abnormalities: translating science into medicine. Expert Opin Pharmacother. 14(5),597-608 (2013)

[56] Mok T, Kim DW, Wu YL et al. First-line crizotinib versus pemetrexed–cisplatin or pemetrexed–carboplatin in patients (pts) with advancedALK-positive non-squamous non-small cell lung cancer (NSCLC): results of a phase III study (PROFILE 1014). J Clin Oncol 32:5s abstract 8002 (2014)

[57] Kim S, Kim TM, Kim DW et al. Heterogeneity of genetic changes associated with acquired crizotinib resistance in ALK-rearranged lung cancer. *J. Thorac. Oncol*. 8, 415–422 (2013). ● **interesting genetic changes in patients who respond to crizotinib and subsequently develop resistance.**

[58] Choi YL, Soda M, Yamashita Y et al. ALK Lung Cancer Study Group. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N. Engl. J. Med*. 363, 1734–1739 (2010)

[59] Huang D, Kim DW, Kotsakis A et al. Multiplexed deep sequencing analysis of ALK kinase domain identifies resistance mutations in relapsed patients following crizotinib treatment. *Genomics* 102, 157–162 (2013).

[60] Weickhardt AJ, Scheier B, Burke JM et al. Continuation of EGFR/ALK inhibition after local therapy of oligoprogressive disease in EGFR mutant (Mt) and ALK+ non-small cell lung cancer (NSCLC). J. Clin. Oncol. 30 (suppl): abstr. 7526 (2012).

[61] Costa DB, Kobayashi S, Pandya SS et al. CSF concentration of the anaplastic lymphoma kinase inhibitor crizotinib. J Clin Oncol. 20;29(15), e443-5 (2011)

[62] Shaw AT, Kim DW, Mehra R et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. N Engl J Med. 370(13):1189-97 (2014) ● **A new option for patients in relapse after crizotinib**

[63] Kim DW, Mehra R, Tan DSW et al. Ceritinib in advanced anaplastic lymphoma kinase (ALK)-rearranged (ALK+) non-small cell lung cancer (NSCLC): Results of the ASCEND-1 trial. J Clin Oncol 32:5s, abstract 8003 (2014)

[64] Kinoshita K, Asoh K, Furuichi N et al. Design and synthesis of a highly selective, orally active and potent anaplastic lymphoma kinase inhibitor (CH5424802). Bioorg. Med. Chem. 20, 1271–1280 (2012).

[65] Nakagawa K, Kiura K, Nishio M et al. A phase I/II study with a highly selective ALK inhibitor CH5424802 in ALK-positive non-small cell lung cancer (NSCLC) patients: updated safety and efficacy results from AF-001JP. J Clin Oncol. 31(suppl): abstr 8033 (2013)

[66] Gadgeel S, Ou SI, Chiappori AA et al. A phaseI dose escalation study of a new ALK inhibitor, CH542480202, in ALK+ non-small cell lung cancer patients who have failed crizotinib. Abstract O16.06. presented at the 15th International Association for the Study of Lung Cancer World Conference on Lung Cancer, October 27–30, Sydney, Australia (2013)

[67] Ou SI, Gadgeel S, Chiappori AA et al. Consis-tent therapeutic efficacy of CH5424802/RO5424802 in brain metastases amongcrizotinib-refractory ALK-positive non-small cell lung cancer patients in anongoing phase I/II study. Abstract O16.07. presented at the 15th International Association for the Study of Lung Cancer World Conference on Lung Cancer, October 27–30, Sydney, Australia (2013)

[68] Camidge DR, Bazhenova L, Salgia R et al. First-in-human dose-finding study of the ALK/EGFR inhibitor AP26113 in patients with advanced malignancies: Updated results. J. Clin. Oncol. 31 (suppl.; abstr. 8031) (2013).

[69] Gettinger SN, Bazhenova L, Salgia R et al. Updated efficacy and safety of the ALK inhibitor AP26113 in patients (pts) with advanced malignancies, including ALK+ non-small cell lung cancer (NSCLC).J Clin Oncol 32:5s abstract 8047 (2014)

[70] Lovly CM, Heuckmann JM, de Stanchina E et al. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinase inhibitors. Cancer Res. 15;71(14),4920-31 (2011)

[71] Horn LL, Jeffrey R. Infante JR, Blumenschein GR. Et al A phase I trial of X-396, a novel ALK inhibitor, in patients with advanced solid tumors. J Clin Oncol 32:5s abstract 8030 (2014)

[72] Mori M, Ueno Y, Konagai S et al The Selective Anaplastic Lymphoma Receptor Tyrosine Kinase Inhibitor ASP3026 Induces Tumor Regression and Prolongs Survival in Non-Small Cell Lung Cancer Model Mice.Mol Cancer Ther. 13(2),329-40 (2014)

[73] Maitland ML, Ou SHI, Tolcher AW et al. Safety, activity, and pharmacokinetics of an oral anaplastic lymphoma kinase (ALK) inhibitor, ASP3026, observed in a "fast follower" phase 1 trial design. J Clin Oncol 32:5s abstract 2624 (2014)

[74] Socinski MA, Goldman J, El-Hariry I et al. A multicenter phase II study of Genetespib monotherapy in patients with geno4.typically defined advanced non-samll cell lung cancer. Clin. Cancer Res.19:3068–77 (2013)

[75] Ramalingam SS, Goss GD, Andric ZG et al. A randomized study of ganetespib, a heat shock protein 90 inhibitor, in combination with docetaxel *versus* docetaxel alone for second line adenocarcinoma (Galaxy-1). J Clin Oncol.31(suppl): abstract LBA 8007(2013)

[76] Pillai RN, Ramalingam SS. Heat shock protein 90 inhibitors in non-small-cell lung cancer.Curr Opin Oncol. 26(2),159-64 (2014)

[77] Sequist LV, Gettinger S, Senzer NN et al. Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. J. Clin. Oncol. 28, 4953–4960 (2010).

[78] Felip E, Carcereny E, Barlesi F et al. Phase II activity of the HSP90 inhibitor AUY922 in patients with ALK-rearranged (ALK+) or EGFR-mutated advanced non-small cell lung cancer. Ann Oncol. 23, ix152; abstract 4380 (2012).

[79] Socinski MA, Goldman J, El-Hariry I et al. A multicenter phase II study of ganetespib monotherapy in patients with genotypically defined advanced non small cell lung cancer. Clin. Cancer Res. 19, 3068–3077 (2013) ● **Well-designed clinical trial on the basis of mutational patterns, promising role of Ganetespib in tumors harboring ALK gene rearrangement**

[80] Sang J, Acquaviva J, Friedland JC et al. Targeted inhibition of the molecular chaperone Hsp90 overcomes ALK inhibitor resistance in non-small cell lung cancer. Cancer Discov. 3, 430–443 (2013).

[81] Brose MS, Volpe P, Feldman M et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res. 62(23),6997-7000 (2002)

[82] Paz-Ares L, Hirsh V, Zhang L et al. Monotherapy administration of sorafenib in patients with non-small cell lung cancer: phase III, randomized, double blind, placebo controlled MISSION study. Ann Oncol. 23(suppl 9):LBA 33 PR (2012)

[83] Scagliotti G, Novello S, von Pawel J et al. Phase III study of carboplatin and paclitaxel alone or with sorafenib in advanced non small-cell lung cancer. J Clin Oncol. 28: 1835–42 (2010)

[84] Gatzemeier U, Eisen T, Santoro A et al. Sorafenib (S) þ gemcitabine/cisplatin (GC) *vs*. GC alone in the first-line treatment of advanced non-small cell lung cancer (NSCLC): Phase III NSCLC research experience utilizing sorafenib (NEXUS) trial. Ann Oncol. 21:viii (2010)

[85] Kim ES, Herbst RS, Wistuba II et al. The BATTLE trial: personalizing therapy for lung cancer. Cancer Discov. 1(1),44-53 (2011)

[86] Janne PA, Shaw AT, Pereira JR et al. Selumetinib plus docetaxel for KRAS mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol . 14:38–47 (2013)

[87] Gandara DR, Hiret S, Blumenschein GR et al. Oral MEK1/ MEK2 inhibitor trametinib (GSK1120212) in combination with docetaxel in KRAS-mutant and wild-type (WT) advanced non-small cell lung cancer (NSCLC): a phase I/Ib trial. J Clin Oncol. 31:abstract 8028 (2013)

[88] Kelly K, Leighl NB, Barlesi F et al. Oral MEK1/MEK2 inhibitor trametinib (GSK1120212) in combination with pemetrexed in KRAS-mutant and wildtype (WT) advanced non-small cell lung cancer (NSCLC): a phase I/Ib trial. J Clin Oncol. 31:abstract 8027 (2013)

[89] Planchard D, Mazieres J, Riely GJ et al. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation-positive non-small cell lung cancer (NSCLC) patients. J Clin Oncol. 31:abstract 8009 (2013) ●● **important response in patients with BRAF mutation**

[90] Rudin CM, Hong K, Streit M. Molecular characterization of acquired resistance to the BRAF inhibitor dabrafenib in a patient with BRAF-mutant non-small-cell lung cancer. J Thorac Oncol. 8:e41-2 (2013)

[91] Trejo CL, Juan J, Vicent S et al. MEK1/2 inhibition elicits regression of autochthonous lung tumors induced by KRASG12D or BRAFV600E. Cancer Res. 72:3048-59 (2012)

[92] Corcoran RB, Cheng KA, Hata AN et al. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. Cancer Cell. 23(1),121-8 (2013)

[93] Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 489:519-25 (2012)

[94] Xu CX, Zhao L, Yue P et al. Augmentation of NVP-BEZ235's anticancer activity against human lung cancer cells by blockage of autophagy. Cancer Biol Ther. 12(6),549-55 (2011)

[95] Besse B, Soria J, Gomez-Roca C et al. A phase Ib study to evaluate the PI3-kinase inhibitor GDC-0941 with paclitaxel (P) and carboplatin (C), with and without bevacizumab (BEV), in patients with advanced non-small cell lung cancer. J Clin Oncol 29:abstract 3044 (2011)

[96] Von Hoff DD, P. LoRusso P, Tibes R et al. A first-in-human phase I study to evaluate the pan-PI3K inhibitor GDC-0941 administered QD or BID in patients with advanced solid tumors. J Clin Oncol. 28: abstract 2541 (2010)

[97] Navab R, Liu J, Seiden-Long I et al. Co-overexpression of Met and hepatocyte growth factor promotes systemic metastasis in NCI-H460 non-small cell lung carcinoma cells. Neoplasia11:1292–1300 (2009)

[98] Ichimura E, Maeshima A, Nakajima T, Nakamura T. Expression of c-met/HGF receptor in human

non-small cell lung carcinomas *in vitro* and *in vivo* and its prognostic significance. Jpn J Cancer Res. 87(10),1063-9 (1996)

[99] Ma PC, Jagadeeswaran R, Jagadeesh S et al. Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. Cancer Res. 65(4),1479-88 (2005)

[100] Benedettini E, Sholl LM, Peyton M et al. Met activation in non-small cell lung cancer is associated with de novo resistance to EGFR inhibitors and the development of brain metastasis. Am J Pathol. 2010 Jul;177(1):415-23

[101] Cappuzzo F, Janne PA, Skokan M et al. MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. Ann Oncol. 20:298-304 (2009)

[102] Bean J, Brennan C, Shih J-Y et al. *MET* amplification occurs with or without *T790M* mutations in *EGFR* mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A.104:20932–20937 (2007)

[103] Ujiie H,Tomida M, Akiyama H et al. Serum Hepatocyte Growth Factor and Interleukin-6 Are Effective Prognostic Markers for Non-small Cell Lung Cancer. Anticancer Res.32:3251-3258 (2012)

[104] Hosoda H, Izumi H, Tukada Y et al. Plasma hepatocyte growth factor elevation may be associated with early metastatic disease in primary lung cancer patients. Ann Thorac Cardiovasc Surg.18(1),1-7 (2012)

[105] Masago K, Togashi Y, Fujita S et al. Clinical significance of serum hepatocyte growth factor and epidermal growth factor gene somatic mutations in patients with non-squamous non-small cell lung cancer receiving gefitinib or erlotinib. Med Oncol. 29:1614–1621 (2012)

[106] Han JY, Kim JY, Lee SH, Yoo NJ, Choi BG. Association between plasma hepatocyte growth factor and gefitinib resistance in patients with advanced non-small cell lung cancer. Lung Cancer. 74:293–299 (2011).

[107] Meetze KA, Boudrow A, Connoly K et al. Anti-tumor activity of SCH 900105 (AV299), an anti-HGF antibody, in non-small cell lung cancer models. Mol Cancer Ther. 8(Suppl 12, abstract C173 (2009)

[108] Patnaik A, Weiss GJ, Papadopoulos K et al. Phase I study of SCH 900105 (SC), an anti-hepatocyte growth factor (HGF) monoclonal antibody (MAb), as a single agent and in combination with erlotinib (E) in

patients (pts) with advanced solid tumors. J Clin Oncol. Abstract 2525 (2010)

[109] Tan E, Park K, Lim WT et al. Phase Ib study of ficlatuzumab (formerly AV-299), an anti-hepatocyte growth factor (HGF) monoclonal antibody (MAb) in combination with gefitinib (G) in Asian patients (pts) with NSCLC. J Clin Oncol. Abstract 7571 (2011)

[110] Mok TSK, Park K, Geater SL et al. A randomized phase (Ph) 2 study with exploratory biomarker analysis of ficlatuzumab (F) a humanized hepatocyte growth factor (HGF) inhibitory MAB in combination with gefitinib (G) versus G in Asian patients (pts) with lung adenocarcinoma (LA) Ann Oncol. 23(Suppl 9):ix391. Abstract 1198P (2012)

[111] Giordano S. Rilotumumab, a mAb against human hepatocyte growth factor for the treatment of cancer. Curr Opin Mol Ther 11: 448–455 (2009)

[112] Spigel DR, Ervin TJ, Ramlau RA et al.Randomized phase II trial of Onartuzumab in combination with erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol. 31(32):4105-14 (2013)

[113] Spigel DR, Edelman MJ, O'Byrne K et al. Onartuzumab plus erlotinib versus erlotinib in previously treated stage IIIb or IV NSCLC: Results from the pivotal phase III randomized, multicenter, placebo-controlled METLung (OAM4971g) global trial.Citation: J Clin Oncol 32:5s abstract 8000 (2014)

[114] Sequist LV, von Pawel J, Garmey EG et al. Randomized phase II study of erlotinib plus tivantinib *versus* erlotinib plus placebo in previously treated non-small-cell lung cancer. J Clin Oncol. 29:3307-15 (2011)

[115] Hirashima T, Azuma K, Yamamoto N et al. Phase II study of erlotinib plus tivantinib in patients with EGFR-mutation–positive NSCLC who failed in immediately previous EGFR-TKI therapy. J Clin Oncol 32:5s abstract 8052 (2014)

[116] Scagliotti GV, Novello S, Schiller JH et al. Rationale and design of MARQUEE: a phase III, randomized, double-blind study of tivantinib plus erlotinib versus placebo plus erlotinib in previously treated patients with locally advanced or metastatic, nonsquamous, non-small-cell lung cancer. Clin Lung Cancer. 13:391-5 (2012)

[117] Azuma K, Yoshioka H, Yamamoto N et al. Tivantinib plus erlotinib versus placebo plus erlotinib in

Asian patients with previously treated nonsquamous NSCLC with wild-type EGFR: First report of a phase III ATTENTION trial. J Clin Oncol. 32:5s abstract 8044 (2014)

[118] Hellerstedt BA, Edelman G, Vogelzang NJ et al. Activity of cabozantinib (XL184) in metastatic NSCLC: results from a phase IIrandomized discontinuation trial (RDT). J Clin Oncol. 30:abstract 7514 (2012)

[119] Reckamp KL, Frankel PH, Mack PC et al. Phase II trial of XL184 (cabozantinib) plus erlotinib in patients (pts) with advanced EGFR-mutant non-small cell lung cancer (NSCLC) with progressive disease (PD) on epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) therapy: A California Cancer Consortium phase II trial (NCI 9303). J Clin Oncol 32:5s abstract 8014 (2014)

[120] Camidge DR, Ou SHI, Shapiro G et al. Efficacy and safety of crizotinib in patients with advanced *c-MET*-amplified non-small cell lung cancer (NSCLC).J Clin Oncol 32:5s abstract 8001(2014)

[121] Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 30:863-70 (2012)

[122] Shaw AT, Camidge DR, Engelman JA et al. Clinical activity of crizotinib in advanced non-small cell lung cancer (NSCLC) harboring ROS1 gene rearrangement. J Clin Oncol. 30(suppl) abstr 7508 (2012)

[123] Wang R, Hu H, Pan Y et al. RET fusions define a unique molecular and clinic pathologic subtype of non-small-cell lung cancer. J Clin Oncol. 30:4352-9 (2012)

[124] Gautschi O, Zander T, Keller FA et al. A patient with lung adenocarcinoma and RET fusion treated with vandetanib. J Thorac Oncol. 8:e43–4 (2013)

[125] Drilon A, Wang L, Hasanovic A et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. Cancer Discov. 3:630–5 (2013)

[126] Drilon A, Wang L, Hasanovic A et al. Response to Cabozantinib in patients with RET fusion positive lung adenocarcinomas. Cancer Discov. 3(6),630-5 (2013)

[127] Arcila ME, Chaft JE, Nafa K et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin Cancer Res. 18:4910-18 (2012) ● **HER2 mutations is more present in EGFR/KRAS/ALK-negative NSCLC (" triple negative")**

[128] Wang SE, Narasanna A, Perez-Torres M et al. HER2 kinase domain mutation results in constitutive

phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. Cancer Cell. 10(1):25-38 (2006)

[129] De Greve J, Moran T, Graas MP et al. Phase II study of afatinib, an irreversible ErbB family blocker, in demographically and genotypically defined non-small cell lung cancer (NSCLC) patients. J Clin Oncol. 31:abstract 8063 (2013)

[130] Kelly RJ, Carter CA, Giaccone G. HER2 mutations in non-small-cell lung cancer can be continually targeted. J Clin Oncol. 30:3318-19 (2012)

[131] Kris MG, David Ross Camidge DR, Giaccone G et al. Results with dacomitinib (PF-00299804), an irreversible pan-HER tyrosine kinase inhibitor, in a phase II cohort of patients with HER2-mutant or amplified lung cance. Abstract P1.11-032 presented at the 15th International Association for the Study of Lung Cancer World Conference on Lung Cancer, October 27–30, Sydney, Australia. (2013)

[132] Kim HR, Kim DJ, Kang DR et al. Fibroblast growth factor receptor 1 gene amplification is associated with poorsurvival and cigarette smoking dosage in patients with resected squamous cell lung cancer. J Clin Oncol. 31:731-7(2013)

[133] Ware KE, Hinz TK, Kleczko E et al. A mechanism of resistance to gefitinib mediated by cellular reprogramming and the acquisition of an FGF2-FGFR1 autocrine growth loop. Oncogenesis. 2:e39 (2013)

[134] Metzner T, Bedeir A, Held G et al. Fibroblast growth factor receptors as therapeutic targets in human melanoma: Synergism with BRAF inhibition. J Invest Dermatol. 131:2087–95 (2011)

[135] Li JL, Sainson RC, Oon CE et al. DLL4-Notch signaling mediates tumor resistance to anti-VEGF therapy *in vivo*. Cancer Res. 71:6073–83 (2011)

[136] Dutt A, Ramos AH, Hammerman PS et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer PLoS One. 6(6),e20351(2011)

[137] Turner NC, Seckl MJ. A therapeutic target for smoking-associated lung cancer. Sci Transl Med. 2(62):62ps56 (2010)

[138] Weiss J, Sos ML, Seidel D et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med. 2(62),62ra93 (2010)

[139] Hanna NH, Kaiser R, Sullivan RN et al. Lume-lung 2: a multicenter, randomized, double-blind, phase III study of nintedanib plus pemetrexed versus placebo plus pemetrexed in patients with advanced nonsquamous non-small cell lung cancer (NSCLC) after failure of first-line chemotherapy. J Clin Oncol. 31:abstract 8034 (2013)

[140] Reck M, Kaiser R, Mellemgaard A et al. Docetaxel plus nintedanib *versus* docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. Lancet Oncol. 15(2),143-55.(2014)

[141] Kono SA, Heasley LE, Doebele RC, Camidge DR. Adding to the mix: fibroblast growth factor and platelet-derived growth factor receptor pathways as targets in non-small cell lung cancer. Curr Cancer Drug Targets. 12(2):107-23 (2012)

[142] Chell V, Balmanno K, Little AS et al. Tumour cell responses to new fibroblast growth factor receptor tyrosine kinase inhibitors and identification of a gatekeeper mutation in FGFR3 as a mechanism of acquired resistance. Oncogene. 32:3059–70 (2013)

[143] Ramos AH, Dutt A, Mermel C et al. Amplification of chromosomal segment 4q12 in non-small cell lung cancer. Cancer Biol Ther. 8:2042–50 (2009)

[144] Socinski MA, Novello S, Brahmer JR et al. Multicenter, phase II trial of sunitinib in previously treated, advanced non–small-cell lung cancer. J Clin Oncol.26(4),650-6 (2008)

[145] Novello S, Scagliotti GV, Rosell R et al. Phase II study of continuous daily sunitinib dosing in patients with previously treated advanced non-small cell lung cancer. Br Cancer. 101:1543–8 (2009)

[146] Hammerman PS, Sos ML, Ramos AH et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. Cancer Discov. 1(1),78-89 (2011)

[147] Pitini V, Arrigo C, Di Mirto C et al. Response to dasatinib in a patient with SQCC of the lung harboring a discoid-receptor-2 and synchronous chronic myelogenous leukemia. Lung Cancer. 82:171-2 (2013)

[148] Beauchamp EM, Woods BA, Dulak AM et al Acquired Resistance to Dasatinib in Lung Cancer Cell Line Conferred by DDR2 Gatekeeper Mutation and NF1 Loss. Mol Cancer Ther. 13(2),475-82 (2014)

[149] Dasanu CA, Sethi N, Ahmed N. Immune alterations and emerging immunotherapeutic approaches in lung cancer. Expert Opin Biol Ther. 12(7),923–937 (2012)

[150] Digumarti R, Wang Y, Raman G et al. A randomized, double-blind, placebo controlled, phase II study of oral talactoferrin in combination with carboplatin and paclitaxel in previously untreated locally advanced or metastatic non-small cell lung cancer. J Thorac Oncol. 6:1098–1103 (2011)

[151] Ramalingam S, Crawford J, Chang A et al Talactoferrin alfa *versus* placebo in patients with refractory advanced non-small-cell lung cancer (FORTIS-M trial). Ann Oncol. 24(11),2875-80 (2013)

[152] Nemunaitis J, Dillman RO, Schwarzenberger PO et al. Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. *J Clin Oncol*. 24(29),4721–4730 (2006)

[153] Butts C, Maksymiuk A, Goss G et al. Updated survival analysis in patients with stage IIIB or IV non-small-cell lung cancer receiving BLP25 liposome vaccine (L-BLP25): phase IIB randomized, multicenter, open-label trial. J Cancer Res Clin Oncol. 137(9),1337-42 (2011)

[154] Butts C, A. Socinski, Mitchell P et al. START: a phase III study of L-BLP25cancer immunotherapy for unresectable stage IIInon-small cell lung cancer. J Clin Oncol. 31:Abstract 7500 (2013)

[155] Quoix E, Ramlau R, Westeel V, et al. Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small-cell lung cancer: a controlled phase 2B trial. Lancet Oncol. 12(12):1125–1133 (2011)

[156] Garcia B, Neninger E, de la Torre A et al. Effective inhibition of the epidermal growth factor/epidermal growth factor receptor binding by anti-epidermal growth factor antibodies is related to

better survival in advanced non-smallcell lung cancer patients treated with the epidermal growth factor cancer vaccine. Clin Cancer Res. 14(3),840–6 (2008)

[157] Hodi FS, O'Day SJ, McDermott DF et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med.363(8):711–723 (2010)

[158] Lynch TJ, Bondarenko I, Luft A et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol. 30(17),2046–2054(2012) ●● **Demonstration of the important action of ipilimumab plus chemotherapy in NSCLC as first-line treatment**

[159] Zhang Y, Huang S, Gong D, Qin Y, Shen Q. Programmed death-1 up regulation is correlated with dysfunction of tumor-infiltrating CD8+ T lymphocytes in human non-small cell lung cancer. Cell Mol Immunol. 7(5),389–95 (2010)

[160] Brahmer JR, Drake CG, Wollner I et al. Phase I study of single-agent anti programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 28(19),3167–75 (2010)

[161] Topalian SL, Hodi FS, Brahmer JR et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med.366(26),2443–54 (2012)

[162] Brahmer JR, Horn L, Antonia SJ et al. Nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients with non-small cell lung cancer (NSCLC): overall survival and long-term safety in a phase 1 trial. Abstract MO18.03 presented at the 15th International Association for the Study of Lung Cancer World Conference on Lung Cancer, October 27–30. Sydney, Australia. (2013) ●● **Evidence of clinical activity of PD-1 blockade with BMS-936558 in NSCLC**

[163] Antonia SJ, Grosso JF, Horak CE et al. Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients with non-small-cell-lung cancer (NSCLC) treated with nivolumab (ANTI-PD-1; BMS-936558; ONO-4538). Abstract P2.11-035 presented at the 15th International Association for the Study of Lung Cancer World Conference on Lung Cancer, October 27–30, Sydney, Australia (2013)

[164] Rizvi N, Antonia SJ, Chow LQM et al. A phase I study of nivolumab (anti-PD-1; BMS-936558,ONO-4538) plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naive non-small cell lung cancer (NSCLC) patients (pts). J Clin Oncol.31:abstr 8072 (2013)

[165] Carbone DP, Socinski MA, Chen AC et al. A phase III, randomized, open-label trial of nivolumab (anti-PD-1; BMS-936558, ONO-4538) versus investigator's choice chemotherapy (ICC) as first-line therapy for stage IV or recurrent PD-L1+ non-small cell lung cancer (NSCLC). J Clin Oncol 32:5s abstract TPS8128

[166] Garon EB, Leighl NB, Rizvi NA et al. Safety and clinical activity of MK-3475 in previously treated patients (pts) with non-small cell lung cancer (NSCLC). J Clin Oncol 32:5s, abstract 8020 (2014) **●● data stimulants, boths in response that in safety**

[167] Rizvi NA, Edward B. Garon EB et al. Safety and clinical activity of MK-3475 as initial therapy in patients with advanced non-small cell lung cancer (NSCLC). J Clin Oncol 32:5s, abstract 8007 (2014)

[168] Konishi J, Yamazaki K, Azuma M et al. B7-H1 expression on nonsmall cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res. 10(15),5094-5100 (2004)

[169] Chen YB, Mu CY, Huang JA. Clinical significance of programmed death-1 ligand-1 expression in patients with non-small cell lung cancer: a 5-year-follow-up study. *Tumori* . 98:751–755 (2012)

[170] Brahmer JR, Tykodi SS, Chow LQ et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 366(26),2455–2465 (2012)

[171] Horn L, Herbst RS, Spigel DR et al. An analysis of the relationship of clinical activity to baseline EGFR status, PD-L1 expression and prior treatment history in patients with non-small cell lung cancer (NSCLC) following PD-L1 blockade with MPDL3280A (anti-PDL1). Abstract MO18.01 presented at the 15th International Association for the Study of Lung Cancer World Conference on Lung Cancer, October 27–30, Sydney, Australia. (2013)

[172] Powderly JD, Koeppen H, Hodi S et al. Biomarkers and associations with the clinical activity of PD-L1 blockade in a MPDL3280A study. J Clin Oncol. 31:abstr 3001 (2013).

[173] Khleif S. MEDI4736, an anti-PD-L1 antibody with modified Fc domain: preclinical evaluation and early clinical results from a phase 1 study in patients with advanced solid tumors. Abstract presented at the European Cancer Congress 2013; September 27 October. Amsterdam, The Netherlands (2013)

[174] Smothers F, Hoos A, Langermann S et al. AMP-224, a fusion protein that targets PD-1. Ann Oncol.24(suppl 1):i7.(2013)

[175] Carrega P, Morandi B, Costa R et al. Natural killer cells infiltrating human, nonsmall-cell lung cancer are enriched in CD56 bright CD16(−) cells and display an impaired capability to kill tumor cells. Cancer. 112(4),863-875 (2008)

[176] Sola C, Chanuc F, Thielens A et al. Anti-tumoral efficacy of therapeutic human anti-KIR antibody (Lirilumab/BMS-986015/IPH2102) in a preclinical xenograft tumor model. J Immunother Cancer. 1(Suppl 1):P40 (2013)

[177] Yin Y, Huang X, Lynn KD et al. Phosphatidylserine-targeting antibody induces M1 macrophage polarization and promotes myeloid-derived suppressor cell differentiation. *Cancer Immunol Res*. 1(4),256-268 (2013)

[178] Dragnev K, Attili S, Gagua R et al. A randomized, open-label, phase 2 trial of paclitaxel/carboplatin with or without bavituximab in patients with previously untreated locally advanced or metastatic non-squamous nonsmall-cell lung cancer. Paper presented at: Multidisciplinary Symposium in Thoracic Oncology; September 6,Chicago, IL (2012)