

# Expanded molecular interrogation for potential actionable targets in non-squamous non-small cell lung cancer

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**Abstract:** The advent of targeted therapies has established new standards of care for defined molecular subsets of non-small cell lung cancer (NSCLC). Not only has this led to significant changes in the routine clinical management of lung cancer e.g., multiplexed genomic testing, but it has provided important principles and benchmarks for determining “actionability”. At present, the clinical paradigms are most evolved for EGFR mutations and ALK rearrangements, where multiple randomized phase III trials have determined optimal treatment strategies in both treatment naïve and resistant settings. However, this may not always be feasible with low prevalence alterations e.g., ROS1 and BRAF mutations. Another emerging observation is that not all targets are equally “actionable”, necessitating a rigorous preclinical, clinical and translational framework to prosecute new targets and drug candidates. In this review, we will cover the role of targeted therapies for NSCLC harbouring BRAF, MET, HER2 and RET alterations, all of which have shown promise in non-squamous non-small cell lung cancer (ns-NSCLC). We further review some early epigenetic targets in NSCLC, an area of emerging interest. With increased molecular segmentation of lung cancer, we discuss the upcoming challenges in drug development and implementation of precision oncology approaches, especially in light of the complex and rapidly evolving therapeutic landscape.

**Keywords:** Molecular profiling; novel targets; epigenetic; actionable alterations

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## Introduction

The adoption of potent and specific kinase inhibitors against epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangement has necessitated significant change in the diagnostic and clinical algorithms in the management of non-small cell lung cancer (NSCLC) (1). It is now routine to mandate a good quality tissue biopsy for thorough histological review (rather than cytology specimens), routine genomic testing for non-squamous non-small cell lung cancer (ns-NSCLC), prior

to making treatment recommendations (2). The burden of proof supporting this approach is most substantial for EGFR and ALK, where phase III trials in biomarker-selected patients, have incontrovertibly confirmed higher efficacy of targeted agents against platinum-based chemotherapy (1).

The striking success with targeted therapies has led to significant enthusiasm for the identification of additional molecularly-defined subsets. To this end, comprehensive large-scale sequencing studies have provided an unabridged list of frequently recurring genomic events that may

represent potential targets (3), although there are several limitations. First, with each tumor harboring multiple non-synonymous events, not all genetic alterations may be equally functional and as a consequence druggable. Second, the genomic context of these alterations has become increasingly relevant, where pan-cancer analysis has suggested that alterations such as PIK3CA often occur as subclonal events, consistent with the variable experience observed in the clinic to date in PI3K targeting (4,5). Similarly, some alterations may be involved in tumor initiation and have a lesser contribution to the on-going progression malignant phenotype. Third, depending on the relevance of the target to human physiology, and the specificity of the drug, there may not be a sufficient therapeutic index to effect anti-tumor activity (6). Lastly, not all NSCLC cases are accounted for by known recurrent somatic events, reflecting the need to examine other non-genomic mechanisms such as epigenetic and transcriptomic changes to delineate the entire spectrum of tumor vulnerabilities.

Nevertheless, the establishment of EGFR, ALK and ROS1 as bona fide oncogene-addicted NSCLC have set the benchmark for future credentialing of actionable targets (2). Additional targets such as BRAF, MET, RET and HER2 have shown early promise (7), with several currently undergoing clinical evaluation in early phase trials. In this review, we will discuss the emerging data on the genomic alterations in non-squamous NSCLC, some of the novel epigenetic targets in development, and the anticipated challenges in the expanding scope for molecular interrogation and defining new standards of care for rare molecular subsets.

### **Determinants of cancer traits and scope of actionable targets**

Anticancer therapies aim to subvert mission-critical mechanisms of tumorigenesis and cell survival. Within a tumor ecosystem, this is dictated by multiple layers of regulation, starting with intercellular differences in genomic alterations, epigenetic and transcriptomic changes, as well as clonal diversity, tumor microenvironment and the immune landscape. The treatment for NSCLC has been revolutionized due to the biomarker-driven treatment paradigm, exemplified by EGFR mutations and ALK rearrangements. Having elucidated the genomic landscape of NSCLC through large-scale sequencing studies (8-10), we now have a slew of uncommon yet equally actionable

targets e.g., MET exon 14 skipping mutations, BRAF alterations (7). With new drug discovery technologies, such as fragment-based design and ultra-high throughput screens, coupled with enhanced molecular profiling of patients, the number of patients who could potentially benefit from new highly specific and optimized investigational drugs are set to increase. Similarly, tumor mutational burden and signatures can provide complementary information, such as those relating to biomarkers for immune checkpoint inhibitors and potential etiologies to cancer (11,12).

While genomics has been widely adopted and remains the cornerstone of our efforts in precision oncology, it is important to explore a broader repertoire of potential actionable targets—one area of high interest is epigenetic targets. Epigenetics is defined as heritable changes in gene expression without alteration of the DNA sequence, broadly encompassing DNA methylation, histone modification and non-coding RNA (13). Chromatin structure has been shown to influence the mutation rates in cancer cells (14-16). The associated changes in chromatin accessibility and modifications are thought to be a major feature in shaping the mutational landscape between different cell of origins (16). At present, majority of drug development into epigenetic targets has been focused on DNA methylation and histone modifications. Of note, signalling pathways can also be influenced by chromatin structure and dynamics (17), highlighting the complex interplay between oncogenic signalling and the cancer epigenome. Unlike genetic mutations, epigenetic aberrations are potentially reversible, providing an attractive approach to modulate the effects of multiple signalling pathways (18).

The next sections will describe a range of promising genomic and epigenetic targets in development and the importance of broad comprehensive molecular profiling.

### **Emerging actionable genomic targets in NSCLC**

#### ***BRAF mutations***

BRAF acts as a kinase that connects the RAS guanosine triphosphate to the proteins found within the mitogen-activated protein kinase (MAPK) pathway (19). A BRAF mutation promotes tumorigenesis by activating the proliferative pathway and MAPK2 and MAPK3 (20). Although this proto-oncogene is most commonly found in melanoma patients, BRAF mutations are still found amongst 1–3% of lung adenocarcinoma patients, about half of which harbor the V600E mutation, where valine is substituted for

glutamic acid in exon 15 (21). BRAF mutations have been reported in smokers and never smokers, suggesting that enrichment molecular screening strategies are probably inadequate (21,22). Past studies have shown these patients are less responsive to platinum-based chemotherapy and have poor clinical outcomes (22), underscoring the importance of identifying such patients early on in the disease course. The latest clinical trials have shown promise and summarised below (*Table 1*).

Single agent basket trials have generally seen overall response rates of 42–43% (23,24). An ACSE Phase II trial of vemurafenib (NCT02304809) in advanced NSCLC and other BRAF<sup>V600E</sup> cancer patients demonstrated a 43% overall response rate (ORR) for NSCLC patients (23). Another similar study (NCT01524978) explored the tumor agnostic concept, where BRAF<sup>V600E</sup> mutant tumors regardless of tumor type were treated with vemurafenib. Notably, response rates were 42% in the NSCLC cohort and progression-free survival (PFS) 7.43 months (24). Subsequently, two phase II trials involving dabrafenib monotherapy and dabrafenib-trametinib combination were conducted in parallel (NCT01336634), examining the concept of vertical blockade. The combination of dabrafenib and trametinib achieved an overall response rate of 63.2% (26) compared to a 33.3% ORR for patients that only received dabrafenib (25). Of note, reported adverse events for either the monotherapy or the combinational therapy included skin-related toxicities and most strikingly the development of cutaneous squamous cell carcinoma and basal cell carcinoma. Notably, combinational therapy reduced the incidence of cutaneous squamous cell carcinoma to only 2 of 57 patients (4%) instead of 10 of 84 patients (12%) affected and none of the patients developed basal cell carcinoma in the combination group (25,26). The encouraging clinical results and decreased side effects led to granting of accelerated approval by the Food and Drug Administration (FDA), where the combination approach is the current accepted standard for advanced staged BRAF<sup>V600E</sup> NSCLC patients (27).

In contrast to BRAF<sup>V600E</sup>, non-V600 mutations, which account for the remaining half of BRAF mutations in NSCLC, are insensitive to currently approved BRAF inhibitors. Combinatorial therapy with dabrafenib (RAF inhibitor) and trametinib (MEK inhibitor) has demonstrated anti-tumor activity in NSCLC cell lines harbouring non-V600 BRAF mutations (28). The efficacy of trametinib in non-V600 mutated NSCLC is currently being addressed in the NCI-MATCH trial (29).

### *MET amplification and MET exon 14 skipping*

MET protein is located on the cell surface and is the tyrosine kinase receptor which binds to its ligand, hepatocyte growth factor (HGF). The binding leads to in MET dimerization, resulting in a cascade effect that ultimately leads to cell proliferation (30). While previous high profile phase III trial failures e.g. with the monoclonal antibody against MET, onartuzumab (31) were largely attributed to inadequate patient selection criteria, more robust biomarkers have since been developed. There are currently two types of MET abnormalities that show promise in the clinic—MET copy number gain and MET exon 14 skipping—with a 2–4% and 3–4% respective frequency within lung adenocarcinoma patients (32).

One key impetus in elucidating the therapeutic tractability of MET alterations in NSCLC, is the fact that crizotinib, approved for ALK and ROS1 alterations, has also demonstrated potent activity against c-MET (33,34). Thus, with the clinical availability of a potent MET inhibitor, there was a need to delineate the predictive value of MET alterations. Here, one of the key challenges is the definition of thresholds of MET copy number gain, and more recently MET exon 14 skipping mutations.

MET copy number gain was first established as a mechanism of resistance to EGFR TKI, where it has been described in up to 20% of patients (35,36). Its role in the treatment naïve setting has also been investigated using copy number cutoffs (37). Patients with high copy number are reported to be an indicator of a more aggressive disease (38). Complicating the interpretation of these cutoffs, is the fact that MET copy number gain, can occur in the context of focal amplification, polysomy (likely a reflection of ploidy) and even with other genomic alterations e.g., EGFR, KRAS in up to 56% of cases using a 5 copy threshold (37). Thus while MET fluorescent *in situ* hybridization (FISH) has been largely adopted to pre-select patients for trials, it is important to note that concurrent screening with broader next generation sequencing to exclude other drivers or immunohistochemistry (IHC) to ascertain protein expression may be complementary and allow further enrichment for potential c-MET addicted tumors (39).

The initial experience in targeting MET copy number gains was in the context of PROFILE 1001 (NCT00585185), where an extension cohort enrolled 14 patients with low, intermediate, or high copy number ratios. In this small cohort of patients, the ORR was 0%, 17% and 67% for the

**Table 1** Selected key trials targeting BRAF<sup>V600E</sup> mutations

Trial	Study type	Drug	Target	Molecular selection	Patient population	N =respective BRAF <sup>V600E</sup>	RR	PFS	Common adverse events
<b>Single agent studies</b>									
Blay <i>et al.</i> , 2016 (23), NCT02304809	Phase II	Vemurafenib	BRAF inhibitor	BRAF <sup>V600E</sup> mutated cancers screened using INCa molecular genetic platform	No longer suited for curative treatment upon failing standard treatment	78 (46 of which were NSCLC pt)	43% from cutoff date	Not reported	Grade 3 or above AE that are mainly related to skin and gastrointestinal toxicities
Hyman <i>et al.</i> , 2015 (24), NCT01524978	Phase II basket	Vemurafenib	BRAF inhibitor	All BRAF <sup>V600E</sup> mutants detected via Sanger [6], PCR [4], Sequenom [3], SNaPshot [4], NGS [3]	Never treated with MEK or BRAF inhibitor	122 (20 in NSCLC cohort)	8 (42%, 20–67)	7.43 months	19 (95%) of 20 pts reported AE, most common diarrhea
Planchard <i>et al.</i> , 2016 (25), NCT01336634	Phase II	Dabrafenib	BRAF inhibitor	BRAF <sup>V600E</sup> mutations (stage IV) NSCLC	Previously treated with chemotherapy	78	33 (95%, 23–45)	5.5 months	35 (42%) of 84 patients reported serious AE, 77 (92%) reported drug-related AE; serious AE include: squamous cell carcinoma (n=10, 12%), asthenia (n=4, 5%); Basal cell carcinoma (n=4, 5%); other AE were related to skin toxicities (skin hyperkeratosis, skin papilloma, dry skin)
<b>Combinational studies</b>									
Planchard <i>et al.</i> , 2016 (26), NCT01336634	Phase II	Dabrafenib with trametinib	BRAF inhibitor with MEK inhibitor	BRAF <sup>V600E</sup> mutated (stage IV) NSCLC	Pre-treated metastatic stage IV with tumour progression after at least one previous platinum-based chemotherapy, with less than three previous systemic anticancer therapies, no previous treatment with BRAF or MEK inhibitor	57	63.2 (95%, 49.3–75.6)	11.6 months	32 (56%) of 57 patients reported serious AE, including pyrexia in 9 (16%), anaemia in 3 (5%), confusional state in 2 (4%), decreased appetite in 2 (4%), haemoptysis in 2 (4%), hypercalcaemia in 2 (4%), nausea in 2 (4%), and cutaneous squamous cell carcinoma in 2 (4%); neutropenia in 5 patients (9%), hyponatraemia in 4 (7%), and anaemia in 3 (5%)

NSCLC, non-small cell lung cancer; RR, response rate; PFS, progression-free survival; pt, patient; AE, adverse event; NGS, next-generation sequencing; PCR, polymerase chain reaction.

low, intermediate and high MET groups respectively when treated with crizotinib (40). Additional studies have since attempted to better delineate the patient population driven by MET amplification. Excluding patients with other co-existing oncogenes and using a ratio of >1.8, around 4.8% of patients remain MET-amplified (35). Ongoing studies of cMET tyrosine kinase inhibitors, e.g., INC280 Capmatinib (NCT02750215) and MGCD 265 Glesatinib (NCT02544633) are directed at defining clinically relevant thresholds that predict for efficacy to cMET inhibitors.

There is also accumulating data on the therapeutic tractability of cMET exon 14 skipping mutations. From a mechanistic viewpoint, these mutations result in a splicing defect resulting in exon 14 skipping, resulting in loss of a ubiquitination site, and hence constitutive over-expression of c-MET (32). The PROFILE 1001 study included a further cohort of patients with exon 14 skipping mutations that reported an ORR of 44% and median PFS of 7.36 months to crizotinib. Certain guidelines have also incorporated the use of a MET inhibitors in patients with MET activated tumors (40).

While exon 14 skipping mutations appear to be a defined molecular subset, the implications of the varying copy numbers in patients with MET amplification is still not well understood. The importance of stratification has been highlighted through preliminary data where imposing strict definitions of MET amplification—patients with a ratio of >5 yielded an ORR of 67% compared to 0% in patients with ratios of <2 (40). Trials of MET inhibitors enrolling patients with specific copy number gain thresholds will further our understanding of targeting the MET axis, although they also pose significant logistical and regulatory challenges. Here, the major concern is that stringency of enrolment criteria can directly impact on size of target population, which in turn can influence trial feasibilities and anticipated efficacy. In this instance, the availability of relevant historical controls in patients harbouring a similar molecular profile are useful to benchmark outcomes in the setting of a single-arm study.

### ***HER2 mutations/overexpression/amplifications***

Mutation of the human epidermal growth factor 2 (HER2) is found in 1–4% of NSCLC patients, and in 6% of EGFR, ALK and KRAS negative individuals, with a preponderance for never smokers and women (41). The most common mutation of HER2 in NSCLC is an exon 20 in-frame insertion (A775\_G776insYVMA), leading to

constitutive kinase activity and downstream activation of AKT and MEK pathways (42). In addition to insertion at the kinase domain, point mutation of the extracellular domain (S310F) has also been reported (43). To date there have been limited number of clinical trials specifically targeting this molecular subset (*Table 2*). Earlier trials showed that the monoclonal antibody against HER2, trastuzumab in combination with chemotherapy failed to demonstrate any clinical benefit in NSCLC patients (49,50). A retrospective review of 65 HER2 mutation positive patients, representing 1.7% of 3,800 patients screened, for the first time reported an objective response rate of 50% with trastuzumab monotherapy and various combinations with chemotherapy (51). In an extended pan European cohort study (EUHer2), patients who underwent HER2-based treatment (trastuzumab, neratinib, afatinib and lapatinib), demonstrated an objective response rate of 50.9% and a PFS of 4.8 months (44). While these studies on combination chemotherapy and HER2-based treatment showed promising response rates, the actual contribution of HER2 targeting and relative efficacy of each individual anti-HER2 agent are difficult to ascertain from currently available data. Another prospective phase II study of dacomitinib (NCT00818441), an irreversible inhibitor of HER2, EGFR, and HER4 was reported in 30 patients, of which 26 harbored HER2 mutations (25 insertions and 1 missense mutation); 3 of the 26 patients responded [RR 12% (95% CI, 2–30%)] with no partial responses seen in the amplified cases (45). A recent retrospective study of HER2 mutant NSCLC treated with afatinib also showed activity of 15% in 4 out of 27 patients (48). Interestingly, there may be mutation specific drug sensitivities for HER2 exon 20 insertions, such as those that result in insertions of amino acids either before or after the C-helix, resulting in a change in conformational transitions between active and inactive states, and sensitivity to irreversible EGFR TKI (52).

More recently, two phase II studies highlighted the potential of trastuzumab emtansine (T-DM1) in HER2 mutant NSCLC (46,47). In the first study by Stinchcombe *et al.* (NCT02289833), 49 patients with HER2 overexpression (IHC2+ and above) received T-DM1. Patients with IHC2+ demonstrated an ORR of 0% and PFS of 2.6 months, while those with IHC3+ had an ORR of 20% and PFS of 2.7 months (46). Li *et al.*'s basket trial (NCT02675829) in 18 patients showed an ORR of 33% and PFS of 4 months (47). It is important to highlight that both studies employed different selection criteria for trial enrolment. The first study enrolled patients who were

**Table 2** Selected key studies or trials targeting HER2 alterations and overexpression

Trial	Trial type	Drug	Target	Molecular Selection	Patient population	N= respective HER2 status	RR	PFS	Common adverse events
EUHER2 study, Mazières et al., 2016 (44)	Retrospective	Trastuzumab emtansine, neratinib, afatinib, lapatinib; vinorelbine; docetaxel, paclitaxel, cisplatin	Different HER2 targeted drugs and chemotherapy	In frame insertion of exon 20	Advanced NSCLC failed on one line of systemic anticancer treatment	65 (trastuzumab [57], TDM1 [1], neratinib [14], afatinib [11], lapatinib [5]; vinorelbine [24], docetaxel [12], paclitaxel [12], cisplatin [7])	50% for trastuzumab with chemo; 18.2% for afatinib	5.1 months for trastuzumab with chemo; 3.9 months for afatinib	TRAE not provided
Kris et al., 2015 (45), NCT00818441	Prospective Phase II	Dacomitinib	Irreversible inhibitor of HER2	Pts with HER2 amplification or mutation tested using FISH or other sequencing techniques	Stage IIB/IV NSCLC	30, HER2-mutant (n=26, all in exon 20 including 25 insertions and 1 missense mutation); HER2-amplified lung cancers (n=4)	HER2 mutant cohort: 12 (95%, 2–30); HER2-amplified cohort: 0%	HER2-mutant cohort: 3 months; HER2-amplified cohort: 3 months	Diarrhea (90%; grade 3 (20%), grade 4 (3%); dermatitis (73%; grade 3 (3%) and fatigue (57%; grade 3 (3%))
Stinchcombe et al., 2017 (46), NCT02289833	Phase II	Trastuzumab emtansine (T-DM1)	HER2 targeted antibody drug conjugate	HER2 overexpression, classified as IHC2+ or IHC3+, screened via IHC	Locally advanced or metastatic NSCLC, previously treated with platinum-based therapy	49 (29 IHC2+, 20 IHC3+); 16 pts had HER2 amplification, (IHC2+, n=5; IHC3+ n=11),	IHC2+: no response (95% 0–11.9); IHC3+: 4 pts (20%) 20 (95, 5.7–43.7); HER2 amplification 3 (27.3%) of 16 responded <sup>†</sup>	2.6 months for IHC2+, 2.7 months for IHC3+	11 patients (22%) experienced grade 3–4 adverse events fatigue and dyspnea only two events that were seen in more than 1 patient (n=2 respectively)
Li et al., 2017 (47), NCT02675829	Phase II Basket Trial	Ado-trastuzumab emtansine (T-DM1)	HER2 targeted antibody drug conjugate	HER2 amplified or mutant only HER2 testing performed by NGS, with confirmation by FISH and IHC <sup>‡</sup>	Metastatic NSCLC	18; all negative for HER2 amplification according to NGS <sup>§</sup>	33% (5/15 confirmed, 95, 12–62) <sup>¶</sup>	4 months	Well-tolerated, toxicity mainly grade 1–2, including reaction, thrombocytopenia and transaminitis
Lai et al., 2017 (48)	Retrospective	Afatinib	Irreversible tyrosine kinase inhibitor of HER2	HER2-mutant	Advanced NSCLC	27; HER2 status not specified	15 (95%, 4–34)	PFS not provided, median overall survival: 23 months (95%, 18–62)	Not reported

<sup>†</sup>, HER2 status not reported; <sup>‡</sup>, of the HER2 amplification patients, the 3 patients all had IHC3+, <sup>§</sup>, 1 (8.3%) of 12 patients had HER2 amplification when tested with FISH. No IHC3+ detected in the 10 patients tested; <sup>¶</sup>, 3 patients with pending results. NSCLC, non-small cell lung cancer; ORR, objective response rate; PFS, progression-free survival; pt, patient; TRAE, treatment-related adverse event; NGS, next-generation sequencing IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

IHC2+ or IHC3+ on HER2 IHC, and showed that the latter group were more responsive to TDM-1, with 4 out of 20 patients exhibiting a partial response (20%), while none of the IHC2+ patients responded. Furthermore, amongst the 49 patients, 16 were detected to have HER2 amplification (by ISH), 5 were IHC2+ and 11 were IHC3+ patients. Among the IHC3+ patients who also had HER2 amplification, ORR was 27.3%—raising the possibility that IHC3+ patients with HER2 amplification may be more responsive to TDM-1 (46). The second study primarily evaluated HER2 mutations in the cohort, with no evidence of HER2 amplification by NGS or HER3+ expression in those with tissue available (47). Overall, the clinical experience to date supports the role of both kinase inhibitors and HER2 targeting antibodies in HER2 mutated or HER2 expressing NSCLC.

### **RET rearrangements**

RET alterations account for 1–2% of NSCLC, and is especially prevalent in young, non-smokers with poorly differentiated tumours (53,54). While multi-kinase inhibitors potently targeting RET such as vandetanib (ZD 6474) and cabozantinib (XL184) have been approved by the FDA to treat advanced metastatic medullary thyroid cancer, its efficacy in NSCLC is currently still under investigation (55,56). Two phase II studies in Japanese and Korean population were recently reported. The LURET study (UMIN000010095) was a nationwide effort conducted in Japan, which investigated the role of vandetanib in 17 advanced NSCLC patients (57). The results were encouraging, nine (47%) of the 17 patients responded, while the median PFS was 4.7 months. Similarly, a Korean phase II study (NCT01823068) also investigated the vandetanib, demonstrating a response rate of 17%, in which three of the 18 patients responded, with a PFS of 4.6 months (58). A third study evaluated the role of cabozantinib in 25 patients (NCT01639508), showing an ORR of 28% and PFS of 5.5 months (59). Recently, an international global registry reported a modest role for current RET-targeting multi-kinase inhibitors in 53 RET rearranged NSCLC (which included cabozantinib, vandetanib, sunitinib, sorafenib, alectinib, lenvatinib, nintedanib, ponatinib and regorafenib). In this study, the PFS was reported to be 2.3 months and overall survival 6.8 months (60), underscoring the need for more specific and potent RET inhibitors. Although the small sample size of these studies no doubt contributed to the range of outcomes observed,

differences in pre-screening strategies can also impact on responses. Notably, the Japanese study enrolled patients from a national molecular screening program (LC-SCRUM) where patients underwent RT-PCR and were eligible only if confirmed by FISH (57), while the Korean study screened EGFR wild type, ALK non-rearranged patients with break-apart FISH, and the results were subsequently confirmed using one of the following tests, IHC, RT-PCR or targeted deep sequencing (58). In the Japanese study, the adjusted ORR following vandetanib was 83% among 6 patients with CCDC6-RET and 20% among 10 patients with KIF1B-RET fusion (57). Similarly the Korean study reported mixed clinical outcome with RET targeting, which was dependent on the underlying RET fusion partner (58), highlighting the potential implications of different RET fusions, as well as the importance of employing high precision biomarkers in clinical trials. A summary of these trials is presented in *Table 3*.

### **NTRK rearrangements**

Another emerging therapeutic target in NSCLC is the NTRK fusion protein. The NTRK genes (NTRK1, 2 and 3) encode for a family of receptor tyrosine kinase known as tropomyosin receptor kinase (TrkA, TrkB and TrkC), which plays a crucial role in neuronal development (61). Genetic rearrangements of the NTRK family have been reported in about 3% of NSCLC (62). Importantly, selective tyrosine kinase inhibitors against NTRK fusion proteins have demonstrated promising *in vitro* and *in vivo* anti-tumor activity in NSCLC (62–64).

### **Epigenetic targets in development for NSCLC**

While there have been significant advances in the various genomic targets described above, the role of epigenetic targets has been less well-defined. Nevertheless, given the important role in cancer development and drug resistance, there have been numerous forays into understanding the role and therapeutic tractability of DNA methylation and histone modifiers.

### **DNA methylation**

Genome wide DNA methylation studies have yielded differentially methylated genes in NSCLC compared to paired normal controls (65). Importantly, DNA methylation status of selected genes confers prognostic and predictive

**Table 3** Selected key studies or trials targeting RET alterations

Trial	Trial type	Drug	Target	Molecular selection	Patient population	N= respective RET fusion partner	RR	PFS	Common adverse events
Yoh et al., 2017 (57) (UMIN000010095)	Phase II	Vandetanib	RET inhibitor	RET rearrangements detected by RT-PCR then confirmed using FISH	Advanced NSCLC without EGFR mutation who have failed at least one line of chemotherapy	19 pt (17 analyzed); 6 pts with CCDC6-RET, 10 with KIF5B-RET, 3 pts with unknown RET type	49% (9 out of the 17 pts); In CCDC6-RET cohort, five (83%) of 6 responded; KIF5B-RET cohort, two (20%) of 10 responded	4.7 months	Most frequently observed AE: hypertension (16, 84%), diarrhea (15, 79%), rash acneiform (12, 63%), dry skin (8, 42%), prolonged QT corrected interval (8, 42%), anorexia (6, 2%), and increased creatinine (6.32%); grade 3-4 TRAE included hypertension (58%), rash (16%), diarrhea (11%), and QTc prolongation (11%)
Lee et al., 2017 (58) NCT01823068	Phase II	Vandetanib	RET inhibitor	RET rearrangements detected by FISH then IHC or targeted deep sequencing or RT-PCR	Advanced NSCLC patients that have failed platinum-based chemotherapy with no other oncogenic drivers	18; only 10 screened 5 pts had KIF5B-RET, 2 pts with CCDC6-RET, 1 pt with MYO5C-RET	18% (3 out of 17 pts)	4.5 months	More than 70% of patients reported to have hypertension and skin rash of grade 1-2; Two grade 3 hypertension, two QT prolongation AEs, one AST/ALT elevation were reported
Drilon et al., 2016 (59) NCT01639508	Prospective Phase II	Cabozantinib	RET multikinase inhibitor	RET rearrangements detected by FISH and or NGS	Metastatic or unresectable lung cancer	26; 16 pts with KIF5B-RET, 1 pt with CCDC6-RET, 1 pt with MYO5C, 1 pt with TRIM33-RET, 1 pt with CLIP1-RET, 1 pt with ERC1-RET, 6 pt with unknown fusion type	28% (95, 12-49); Response only found in pts with KIF5B-RET fusion	5.5 months	Grade 3 treatment-related AE: lipase elevation (15%), increased alanine aminotransferase (8%), increased aspartate aminotransferase (8%), thrombocytopenia (8%), and hypophosphatemia (8%)
Gautschi et al., 2017 (60)	Retrospective study	Cabozantinib, vandetanib, sunitinib, sorafenib, alectinib, lenvatinib, nintedanib, ponatinib and regorafenib	RET multi-kinase inhibitor	RET rearrangement detected by molecular profiling (RT-PCR/FISH/NGS)	Any staged NSCLC	53 cabozantinib [21], vandetanib [11], sunitinib [10], sorafenib [2], alectinib [2], lenvatinib [2], nintedanib [2], ponatinib [2], and regorafenib [1]	37%, 18%, 22% (cabozantinib, vandetanib, sunitinib)	2.3 months (95, 1.6 to 5.0 months)	TRAE not reported

NSCLC, non-small cell lung cancer; ORR, objective response rate; PFS, progression-free survival; pt, patient; TRAE, treatment-related adverse event; FISH, fluorescence *in situ* hybridization; NGS, next-generation sequencing IHC, immunohistochemistry.



information on patient management. For example, the concurrent hypermethylation of four genes, CDKN2A, CDH13, RASSF1A and APC in early stage NSCLC correlated with early recurrence and death (66). Moreover, DNA methylation profile in a select panel of genes and hypomethylation of ERBB2 predict sensitivity to erlotinib (67), while methylation of IGFBP-3 is associated with resistance to cisplatin in NSCLC (68).

DNA methylation is mediated by a family of DNA methyltransferase (DNMT), namely DNMT1, DNMT3A and DNMT3B (69). 5-azacitidine and decitabine are two DNMT inhibitors that have demonstrated treatment efficacy in the realm of haematopoietic tumours (70). While clinical trials utilizing decitabine as monotherapy have yielded disappointing results (71,72), combination of DNMT inhibitor 5-azacitidine and histone deacetylase (HDAC) inhibitor entinostat showed objective response in patients with advanced NSCLC (73).

#### ***Histone modification and chromatin organization***

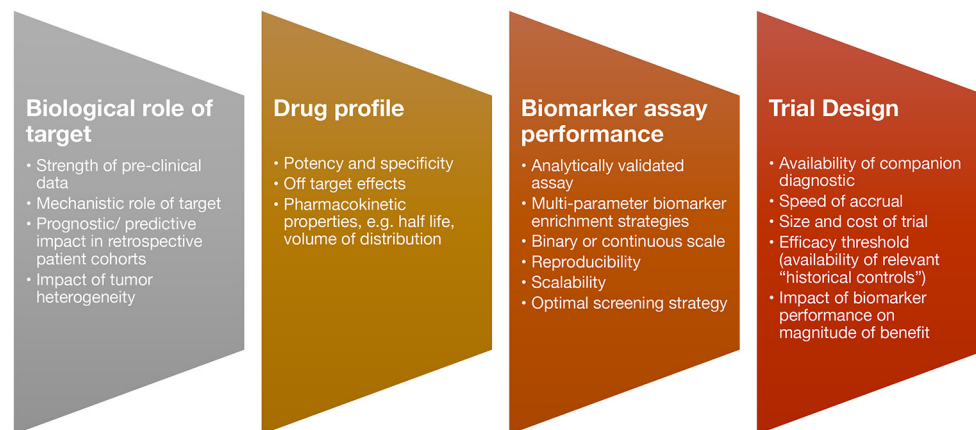
Post-translational modification of histones regulates chromatin structure and transcriptional activity. A number of histone post-translational modifications have been described to date, including methylation, acetylation, phosphorylation and ubiquitylation (74). These histone marks define functional regions of the epigenome. For example, H3K4me3 marks active promoters. Active enhancers are enriched for H3K27ac, while H3K27me3 is a repressive mark on enhancers. Early work showed that global levels of histone modifications by IHC in clinical samples of NSCLC confer prognostic information (75-77). Importantly, these histone marks are reversible and are tightly regulated by epigenetic regulators. Four classes of epigenetic regulators have been described, namely writers, erasers, readers and movers (74). Writers (histone acetyltransferase, histone methyl transferase) are enzymes that add a specific post-translational modification to histone, while erasers (HDAC, histone demethylase) do the opposite. Readers such as bromodomain and extra terminal domain (BET) family proteins, recognize specific histone marks and direct the appropriate transcriptional response. Movers (SWI/SNF complex) are ATP-dependent chromatin remodelers which reposition nucleosomes along the genome. Several large scale genomic studies in NSCLC have identified somatic mutations in epigenetic regulators, raising the notion of epigenetic deregulation as the eleventh hallmark of cancer (8).

EZH2, a subunit of the polycomb repressive complex 2 (PRC2) is a histone lysine methyltransferase that mediates H3K27 trimethylation to silence target genes (78). EZH2 is overexpressed in NSCLC and is associated with poor prognosis (79,80). Similarly overexpression of HDACs has been observed in NSCLC (81). It has been shown that EZH2 interacts with HDAC to repress transcription (82). Combined inhibition of EZH2 and HDAC has a synergistic antiproliferative effect in NSCLC cell lines (83). Inactivating mutations of MLL2, another histone lysine methyltransferase has also been reported in NSCLC (84).

BET family proteins (BRD2, BRD3, BRD4 and BRDT) are readers that recognize acetylated lysine residues in histones, which play an important role in transcription control (85). Translocation of BRD4, a well characterized member of the BET family, to the NUT gene with the resulting BRD4-NUT fusion protein defines a recently described entity known as NUT midline carcinoma (NMC). NMC is a poorly differentiated squamous cell carcinoma that occurs in the head and neck as well as the thoracic cavity (86). It shows marked sensitivity to BET targeting (87). Notably, BRD4 is also overexpressed in NSCLC, and correlates with poor prognosis in NSCLC patients (88). BET targeting has been shown to be effective in acute myeloid leukemia and multiple myeloma (89). A novel BET inhibitor, OTX015 exhibits *in vitro* anti-tumor activity against NSCLC cell lines harboring different oncogenic mutations (90).

SMARCA4/BRG1 is an ATP-dependent catalytic subunit of the SWI/SNF chromatin remodelling complex, best known for its role in malignant rhabdoid tumors. Loss of SMARCA4 has been reported in 5% to 20% of NSCLC, leading to epigenetic silencing of downstream genes, independent of DNA methylation (91,92). Interestingly, SMARCA4 deficient tumors are typically EGFR wild type and TTF-1 negative (93), suggesting that SMARCA4 loss could be a bona fide oncogenic driver event. Somatic inactivation of other members of the SWI/SNF complex, ARID1A and ARID2 has also been reported in NSCLC (94).

To summarize, epigenetic deregulation is involved, at least in part in the pathogenesis of NSCLC. Importantly selective inhibitors against a number of these epigenetic regulators are currently in various stages of trial and their potential role either as single agents or in combination with other targeted therapies are currently under investigation. We anticipate that evaluation for aberrations in these regulators would be critical in selecting patients for the appropriate epigenetic therapy.



**Figure 1** Key considerations in drug and biomarker development and impact on trial design.

### Expanding the biomarker repertoire for actionable targets

Although traditional platforms e.g., IHC, FISH, Sanger sequencing, have been adequate in the era of single biomarkers for a limited number of drugs, the lack of scalability and precision for emerging targets is a disadvantage (95). Further, given the low frequency of some alterations, tissue attrition remains a challenge if testing for multiple separate assays especially with needle biopsies frequently employed (96). Given the diverse targets that are rapidly approaching the clinic, there is a need to continue development of biomarker platforms capable for broad unbiased tumor profiling to capture the relevant actionable alterations.

One significant advance is the introduction of next generation sequencing in the clinic (97). At a genomic level, we have already described a range of genetic lesions that need to be tested concurrently—including somatic mutations, chromosomal rearrangements, amplification and loss of function. Somatic mutations are often responsible for the activation of the EGFR oncogene, KRAS, BRAF, while chromosomal rearrangement drives both ALK and ROS1 (3,39). Amplification and deletions can also lead to gain of function and loss of function with associated expression changes of certain genes (98). As alluded to in the previous sections there may also be genetic changes that overlap e.g., MET exon 14 skipping mutations and amplification, or HER2 mutations and amplifications, as well as co-alterations. Eliciting the function in the setting of complex genetic profiles where there may be two or more druggable alterations, remains a key clinical priority. Further, as we

improve our understanding on epigenetic mechanisms that drive tumorigenesis and progression, it might become increasingly feasible to develop biomarker assays that can be adopted in the clinic. Given the diverse mechanisms of epigenetic regulation, such advances will allow improved selection and stratification for specific epigenetic approaches, as well as facilitate combinatorial approaches. Moving ahead, functional evaluation, and unbiased RNA-seq complemented by high throughput epigenetic assays may be implemented.

As discussed amply in the earlier sections, the molecular screening methodology and understanding the genomic context of any biomarker can play a critical role in the eventual trial design. Some relevant factors for consideration are summarized in *Figure 1*. Ultimately the ability to calibrate the precision of biomarkers that exist on a continuous scale e.g., MET copy gain and PD-L1, will have significant implications on trial design, feasibility and outcomes. Given the ability to define small molecular subsets, and the complexity and dynamic nature of drug resistance, it is conceivable that there will be a diminishing role for large randomized trials. Furthermore, the expanding ability to interrogate individual tumors across multiple-dimensions, will place increasing emphasis on comprehensive biomarker compendiums that enable precise stratification of individual patient cohorts according to likelihood of benefit.

### Conclusions

Given the exciting developments with targeted therapies beyond EGFR and ALK, various guidelines now emphasize

the importance of broader profiling. For example, the National comprehensive cancer network (NCCN) guidelines in 2017, not only is screening for EGFR and ALK a part of the standard treatment for NSCLC, but testing for ROS1, RET, BRAF, HER2 and MET alterations are also recommended. Through broader profiling panels, we will continue to learn more about a range of genetic abnormalities that will provide a blueprint to understanding the life histories of individual cancers and potential therapeutic vulnerabilities. Other targets such as KRAS and PIK3CA will be more readily identified and present enhanced opportunities for clinical trials to better understand the mechanisms of response and resistance to targeted approaches. With further advances in technologies and declining sequencing costs, it is anticipated that the scale and breadth of tumor profiling will enable implementation of highly nuanced stratification approaches that ultimately aim to deliver cost-effective precision cancer therapies.

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### Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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