Review Article

Biology and impact of lineage plasticity in ALK-positive NSCLC: a narrative review

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Background and Objective: Lineage transformation is a known mechanism of acquired resistance to targeted therapies in non-small cell lung cancer (NSCLC). Transformation to small cell and squamous carcinoma and epithelial-to-mesenchymal transition (EMT) have all been identified as recurrent but rare events in ALK-positive NSCLC. However, centralized data informing our understanding of the biology and clinical implications of lineage transformation in ALK-positive NSCLC are lacking.

Methods: We performed a narrative review by searching the PubMed and clinicaltrials.gov databases for articles published in English from August, 2007 until October, 2022 and reviewing the bibliographies of key references to identify important literature related to lineage transformation in ALK-positive NSCLC.

Key Content and Findings: In this review, we aimed to synthesize the published literature describing the incidence, mechanism(s), and clinical outcomes of lineage transformation in ALK-positive NSCLC. Lineage transformation as a mechanism of resistance to ALK TKIs in ALK-positive NSCLC is reported at a frequency of <5%. Available data across molecular subtypes of NSCLC suggest that the process of lineage transformation is likely to be driven by transcriptional reprogramming rather than acquired genomic mutations. Retrospective cohorts including tissue-based translational studies together with clinical outcomes make up the highest level of evidence that exists to inform treatment approach for patients with transfomed ALK-positive NSCLC.

Conclusions: The clinicopathologic features of transformed ALK-positive NSCLC as well as the biologic mechanisms underling lineage transformation remain incompletely understood. Prospective data are needed to develop improved diagnostic and treatment algorithms for patients with ALK-positive NSCLC that undergo lineage transformation.

Keywords: Anaplastic lymphoma kinase (ALK); non-small cell lung cancer (NSCLC); small cell lung cancer (SCLC); histologic transformation

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Introduction

Background

Advanced non-small cell lung cancers (NSCLCs) harboring activating fusions in the anaplastic lymphoma kinase (ALK) are highly sensitive to treatment with ALK-targeted tyrosine kinase inhibitors (TKIs). Unfortunately, despite significant improvements in clinical outcomes, acquired resistance to ALK TKIs inevitably occurs (1-11).

Rationale and knowledge gap

Resistance is most often mediated by second-site mutations in the ALK driver fusion (2-4), resulting in a tumor with

sustained dependence on ALK signaling for proliferation and survival. Such ALK-mediated resistance can be identified by DNA sequencing of tissue or plasma and can be overcome by later generations of ALK TKIs (4). However, a rarer and less well understood mechanism of resistance that occurs in only about 1.2% of TKI-resistant ALK-positive tumors is lineage transformation, most commonly as a shift in histology from adenocarcinoma to neuroendocrine or squamous histology (12). Patients whose resistant tumors have undergone lineage transformation represent a clinical subset for whom we have limited data to recommend optimal treatment regimens.

While ALK-positive tumors make up approximately 3–7% of NSCLC (13,14), tumors harboring other mutually exclusive, targetable driver oncogenes such as EGFR (epidermal growth factor receptor), RET (rearranged tyrosine kinase gene during transfection), and ROS1 (ROS proto-oncogene 1) collectively comprise approximately 20% of lung adenocarcinomas (15). With some variation in overall response rates, each of these genomic subtypes follow the same basic paradigm of initial response to targeted therapies followed inevitably by treatment resistance and disease progression. As with ALK-positive NSCLC, lineage transformation is a rare but recurrent mechanism of treatment resistance across genomic subtypes (12,16-19).

Objective

Here we present a review of what is known of the biology and clinical implications of lineage transformation in NSCLC, drawing parallels across oncogenic driver subtypes but with a focus on ALK-positive lung adenocarcinoma. We present this article in accordance with the Narrative Review reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-867/rc).

Methods

We performed a search of the published literature using PubMed and clinicaltrials.gov databases between October 1, 2022 and November 30, 2022 (*Table 1*). Search terms used included ALK, EGFR, NSCLC, histologic transformation, TKI resistance, small cell lung cancer (SCLC), squamous, large cell neuroendocrine carcinoma (LCNEC), and epithelialal-to-mesenchymal transition (EMT). We included only English-language publications reporting original research regarding randomized controlled trials, prospective or retrospective cohort studies, case reports or series, case-control studies, translational preclinical studies, and relevant review articles. Included articles were selected and assessed by both authors.

ALK-positive NSCLC: clinical overview

Fusion rearrangements resulting in constitutive activation of the ALK tyrosine kinase domain were initially identified in NSCLC in 2007 and occur in approximately 3–7% of NSCLCs, most commonly in lung adenocarcinomas (13,14). *EML4-ALK* rearrangements represent the majority of oncogenic ALK fusions occurring in NSCLC; however, with application of more advanced next-generation sequencing platforms for tumor genotyping, approximately 90 total fusion partners for ALK have been identified in NSCLC to date (20). Activating ALK fusions in NSCLC are associated with adenocarcinoma histology, younger age and minimal smoking history as compared to NSCLCs that do not harbor ALK rearrangements (14).

After a decade of successful drug development in this area, there are five ALK TKIs now approved in the first-line setting as monotherapy for patients with stage IV ALK-positive lung adenocarcinoma (21-36). Crizotinib, a first generation ALK TKI, earned the first such approval in 2011 on the basis of overall response rates greater than 50% in early phase trials (21-23) and subsequent confirmatory phase III studies demonstrating superior outcomes compared to chemotherapy (24). The ability of investigators and regulatory entities to achieve clinical approval of crizotinib such a short time from discovery of ALK oncogenic fusions in NSCLC was due to the fact that crizotinib was initially developed as a MET (mesenchymal-epithelial transition factor) inhibitor but was subsequently found to have ALK inhibitory properties on kinase screens. For this reason it could be rapidly repurposed for clinical testing in ALK-positive NSCLC.

Second-generation ALK TKIs ceritinib and alectinib were FDA-approved for first-line treatment in 2017 and second-generation ALK TKI brigatinib was FDA-approved for first-line treatment in 2020 (1). Initially developed to overcome acquired resistance to crizotinib, these second-generation ALK TKIs proved to be more potent ALK inhibitors than crizotinib (2,37,38). Phase III trials comparing second-generation ALK TKIs head-to-head with crizotinib demonstrated a reproducible improvement in progression-free and overall survival as well as improved CNS outcomes for patients treated with second-generation ALK TKIs compared to crizotinib in the first-line setting (32-34).

Table 1 The search strategy summary

Items	Specification
Date of search	October 1, 2022 to November 30, 2022
Databases and other sources searched	PubMed and clinicaltrials.gov
Search terms used	ALK, EGFR, NSCLC, histologic transformation, TKI resistance, small cell lung cancer (SCLC), squamous, large cell neuroendocrine carcinoma (LCNEC), and epithelial-to-mesenchymal transition (EMT)
Timeframe	From August 2007 until October 2022
Inclusion and exclusion criteria	Inclusion criteria: • English-language articles • Article types were randomized controlled trials, prospective or retrospective cohort studies, case reports or series, case-control studies, translational preclinical studies, and relevant review articles Exclusion criteria: • Article not published in English • Article types were editorial comments, abstracts, conference materials, review articles, guidelines, consensus statements, or study protocol
Selection process	Study selection and full-text articles were assessed by CB Meador and Z Piotrowska

Finally, lorlatinib is a third generation ALK TKI designed to be highly potent and selective in order to effectively overcome a range of resistance mutations known to be acquired following treatment with first- and second-generation ALK TKIs (35,36,39,40). Lorlatinib was initially FDA approved in 2018 for patients following treatment with alectinib or ceritinib (either as second line or after both crizotinib and second-generation ALK TKI) based on demonstrated response rate of 40% and median progression-free survival (mPFS) 6.9 months in this setting (41). FDA approval for lorlatinib in the first-line setting came in 2021 after publication of the phase III trial comparing first-line treatment with lorlatinib versus crizotinib demonstrated significantly greater overall response rate (76% *vs.* 58%) and higher 12-month progression-free survival (78% *vs.* 39%) in the lorlatinib treatment arm (42). Of note lorlatinib was intentionally designed to achieve excellent CNS penetration and the superior CNS efficacy was evident in this trial which demonstrated a significantly longer time to CNS disease progression (42).

Mechanisms of resistance to ALK TKIs

Despite the impressive clinical responses seen in metastatic ALK-positive NSCLC in response to ALK TKIs, unfortunately, disease progression inevitably occurs (*Figure 1A*). Repeat biopsies of tumor tissue (and, more recently, plasma) at the time of TKI resistance have provided insights into the mechanisms of resistance to ALK targeted therapies and their relative frequency across multiple generations of ALK TKIs (2,3,6). While a comprehensive review of mechanisms of resistance to ALK TKIs is beyond the scope of this narrative review, we outline below a broad overview of the field in order to put our discussion of lineage transformation in context.

Broadly speaking, resistance mechanisms can be categorized into 'on-target' and 'off-target' biological processes. 'On-target' mechanisms of resistance are defined as ALK-dependent, or ALK-mediated, resistance mechanisms and are the most common type of resistance mechanism that develops in the face of ALK TKI treatment with a variety of ALK resistance mutations described to date (2). In the case of crizotinib, ALK-dependent acquired resistance often occurs as acquired ALK L1196M mutations or via ALK amplification suggesting insufficient ALK inhibition with crizotinib therapy (2). In general, the landscape of acquired 'on-target' ALK resistance mutations varies by specific inhibitor, but for second-generation ALK TKIs the G1202R ALK solvent-front mutation is among the most commonly identified at the time of resistance, and compound ALK mutations also occur with some frequency

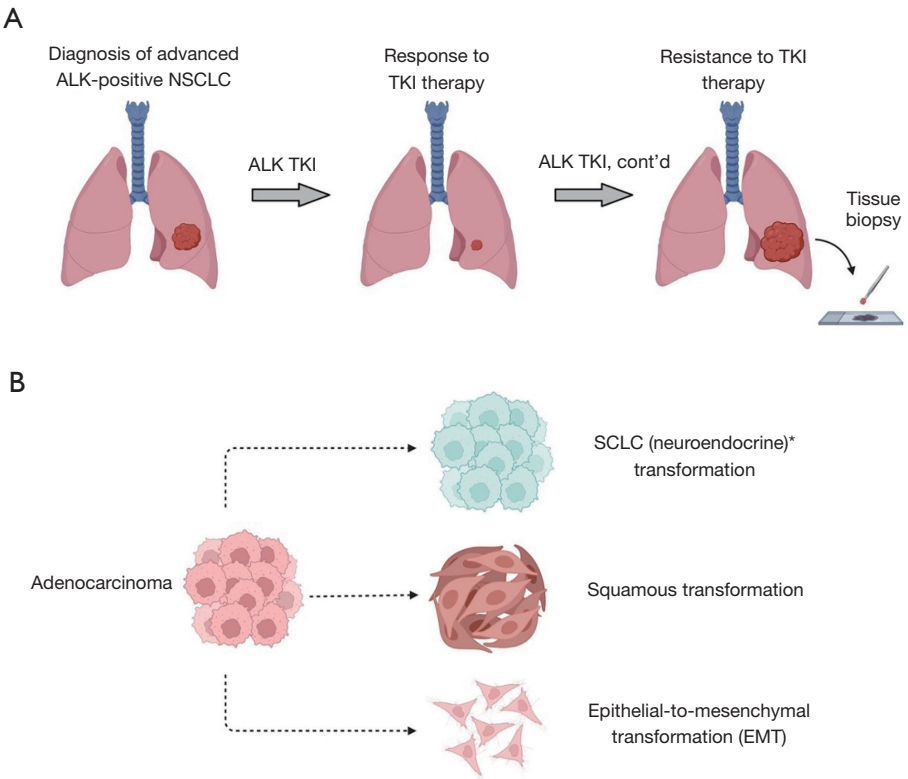

Figure 1 Lineage transformation as a mechanism of resistance to ALK TKIs. (A) Schematic representation of initial response of ALK-positive lung adenocarcinoma to treatment with ALK TKI, followed acquired resistance. Tissue biopsy at the time of acquired resistance is necessary for identification of lineage transformation. (B) Overview of the three commonly recognized categories of lineage transformation. *, though SCLC is the most common form of neuroendocrine transformation, other types of neuroendocrine histologies have been observed, such as LCNEC. Note that this overview excludes detailed representation of mixed histology and heterogeneity likely present in many cases (see text for details). Created with biorender.com. ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; SCLC, small cell lung cancer; LCNEC, large cell neuroendocrine carcinoma.

following resistance to both second-generation and third-generation ALK TKIs (2,6). Following acquired resistance to lorlatinib, the frequency of 'on-target' resistance mutations decreases to 25–30% frequency (from >50% frequency following second-generation inhibitors) and preclinical data suggest that this figure may be evolve even lower with use of lorlatinib in the first-line setting (3,4).

'Off-target' mechanisms of resistance to ALK TKIs refer to acquired activation of ALK-independent pathways sustaining tumor growth in the setting of ongoing ALK inhibition. The most common form of 'off-target' resistance is bypass pathway signaling, including activation of either parallel receptor tyrosine kinase activation or downstream kinase signaling pathways. Bypass pathway resistance mechanisms identified at the time of crizotinib resistance include activation of signaling of receptor tyrosine kinases EGFR, HER2 (human epidermal growth factor receptor 2), KIT (proto-oncogene c-KIT) (43,44) and IGF-1R (insulin like growth factor 1 receptor) (45), downstream KRAS (kirsten rat sarcoma viral oncogene homolog) mutation (46), downstream YES1 (YES proto-oncogene 1) amplification (47), and DUSP6 (dual specificity phosphatase 6) loss (46). Bypass pathway activation reportedly conferring resistance to second- and third-generation ALK TKIs include MET amplification/mutation (48,49), RET rearrangement (50), HER2 amplification (51), and mutations or amplification in NF2 (neurofibromatosis type 2) (10), YES1 (47), BRAF (B-Raf Proto-Oncogene) (52), MAP2K1 (Mitogen-Activated Protein Kinase Kinase 1) (50,53), and PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha) (10).

Another form of 'off-target' resistance to ALK TKIs,

and the primary focus of this narrative review, is lineage transformation. Originally reported as a mechanism of resistance to EGFR TKIs in *EGFR*-mutant NSCLC (16,18,54,55), lineage transformation has been identified as a recurrent driver-independent mechanism of resistance to targeted therapies across genomic subtypes of NSCLC and even among other solid tumor types (56). Because it is a rare phenomenon, the exact frequency of lineage transformation in the context of ALK-positive NSCLC has not been well established. Studies suggest a rate of at least 1.2% of SCLC transformation following next-generation ALK TKIs (12), and case reports demonstrate the occurrence of not only SCLC transformation but also squamous transformation and epithelial-to-mesenchymal transformation (EMT) following treatment with first, second and third-generation ALK TKIs (2,10,57-77) (*Table 2* and *Table 3*).

A comprehensive understanding of the biology of lineage transformation in ALK-positive NSCLC is limited in part by its infrequent occurrence and lack of robust patient-derived tissue samples for study. In addition, the complexity of factors governing cellular differentiation make this an especially challenging biologic process to define. However, lineage state is clearly a critical component of our understanding of tumor initiation and evolution across the spectrum of malignancies. As alluded to above, within the scope of the lung cancer literature, insights into the biology of lineage transformation can be broadly grouped into three categories for discussion: SCLC histologic transformation, squamous histologic transformation, and EMT (*Figure 1B*).

SCLC histologic transformation

Incidence and diagnosis of SCLC transformation

Transformation from NSCLC to SCLC histology was initially reported as a mechanism of TKI resistance in *EGFR*-mutant NSCLC (54). As a result, much of our knowledge of the biology, risk factors, and clinical outcomes of SCLC transformation comes from the *EGFR*-mutant NSCLC literature. In initial studies of resistance mechanisms to early-generation EGFR TKIs that collectively included 192 patients, SCLC transformation was identified in 3–14% of TKI-resistant tumors (54,78). The incidence of SCLC transformation at the time of resistance to third-generation EGFR TKI osimertinib is estimated at 5–7% based on recent studies that included a total of 103 patients, though these estimates are limited by the fact that many of the largest series of osimertinib resistance have not evaluated tumor histology at the time of progression (17,19,79,80). There are also multiple case reports and case series demonstrating SCLC transformation following EGFR TKIs (58,81-95).

By comparison, available data suggest that SCLC transformation occurs less frequently in ALK-positive NSCLC than in *EGFR*-mutant NSCLC. The largest published studies analyzing repeat biopsies after ALK TKI resistance did not identify any cases of SCLC among 91 patients with resistance to crizotinib (2,12), a 0–0.08% frequency (out of 157 total patients) after resistance to second-generation ALK TKIs (2,12), and a 2.7% (out of 38 total patients) frequency of SCLC transformation after resistance to lorlatinib (12). Of the 9 published case reports of SCLC transformation as a mechanism of ALK TKI resistance, 3 occurred following crizotinib, 5 occurred following second-generation TKIs, and 1 occurred following lorlatinib (*Table 2*). Notably, while it is seen most commonly following resistance to EGFR and ALK targeted therapies, SCLC histologic transformation has been reported at the time of acquired resistance to targeted therapy in a NSCLC harboring a driver *ROS1* fusion (12) as well as following treatment with immune checkpoint inhibitors in NSCLC negative for oncogenic driver alterations (96-99). The relative incidence of SCLC transformation following different therapies, across genotypes and clinical presentation of NSCLC remains an area of ongoing study.

SCLC transformation is diagnosed by histopathologic examination of tissue biopsy at the time of acquired resistance to therapy. Morphologically and immunohistochemically, transformed SCLC mimics the appearance of *de novo* SCLC (classic, non-transformed SCLC), characterized by small cells with high nuclear-to-cytoplasmic ratio, high mitotic activity and positive staining for neuroendocrine markers such as chromogranin and synaptophysin (12,18,54,100). Genomically, the majority of transformed SCLC tumors harbor loss-of-function mutations in tumor suppressors *TP53* (tumor protein 53) and *RB1* (RB transcriptional corepressor 1), which is also characteristic of *de novo* SCLC tumors (12,18,54,100). In fact, an *EGFR*-mutant NSCLC with complete inactivation of p53 and RB1 at baseline is 43 times more likely to evolve to SCLC as a mechanism of resistance to TKI therapy compared to an *EGFR*-mutant NSCLC without these co-occurring alterations at baseline (101). Finally, while the original *EGFR* driver mutation is genomically preserved in the SCLC transformed tumor, loss of expression of EGFR is seen at the protein

Table 2 Published case reports of lineage transformation in ALK-positive NSCLC

Baseline histology	Trans histology	Age (years)	Sex	Smoking status	No. prior therapies	ALK TKI at trans	Time to trans (mos)	Co-occurring mutations or amplifications	Treatment post-trans	Response post-trans	OS (mos)	Ref
Adeno	SCLC	41	M	Never	4	Alectinib	72	Unk	Cisplatin + Irinotecan	PR	Unk	Yamagata (57)
Adeno	SCLC	35	F	Never	2	Crizotinib	Unknown	Unk	Ceritinib	PR	Unk	Yamagata (57)
Adeno	SCLC	35	M	Never	2	Lorlatinib	10	ALK G1202R	Carboplatin + Etoposide + Alectinib	PD	11	Ou (67)
Adeno	SCLC	53	F	Never	2	Ceritinib	14	PTEN T319del p53 V203M	Cisplatin + Etoposide	SD	Unk	Levacq (68)
Adeno	SCLC	67	F	Never	7	Alectinib	27	Unk	Irinotecan + Alectinib	PR	Unk	Fujita (69)
Adeno	SCLC	63	F	Never	2	Crizotinib	6	None	Alectinib	PR	Unk	Caumon (70)
Adeno	SCLC	72	M	Former (40 py)	2	Crizotinib	6	Not tested	Unk	Unk	Unk	Cha (71)
Adeno	SCLC	43	F	Unk	8	Alectinib	41.5	Not tested	None	N/A	Unk	Takegawa (72)
Adeno	SCLC	56	F	Never	4	Alectinib	47	Not tested	Cisplatin + Irinotecan	PD	Unk	Miyamoto (73)
Adeno	SCC	49	F	Former (5 py)	3	Brigatinib	60	MLH1 E37Q KMT2D L1461T KMT2D P647H NFE2L2 E82D	Lorlatinib	Unk	Unk	Ball (59)
Adeno	SCC	47	F	Never	3	Ceritinib	24	None	Anlotinib	SD	Unk	Zhang (60)
Adeno	SCC	53	F	Never	2	Ceritinib	4	Not tested	Chemotherapy, NOS	PD	6	Kaiho (62)
Adeno	SCC	60	F	Former (4 py)	3	Alectinib	43	None	Pembrolizumab	PD	48	Gong (63)
Adeno	SCC	52	F	Never	2	Alectinib	60	Unk	Repotrectinib	PR	Unk	Park (65)
Adeno	SCC	58	F	Never	9	Lorlatinib	138	MET amp	Unk	Unk	Unk	Ueda (74)
Adeno	LCNEC	74	F	Never	1	Alectinib	17	ALK 1202R ALK V1180L	Brigatinib	PR	Unk	Fares (61)
Adeno	LCNEC	54	F	Never	6	Brigatinib	75	None	Carboplatin + Pemetrexed	PD	81	Fares (61)
30% Adeno 70% LCNEC	N/A	73	F	Never	0	N/A	N/A	Not tested	Cisplatin + Pemetrexed + Alectinib	N/A (adjuvant)	12	Sim (66)
10% Adeno 90% SCLC	N/A	73	M	Former (40 py)	0	N/A	N/A	Not tested	None	N/A (adjuvant)	2	Sim (66)
10% Adeno 90% SCLC	N/A	64	M	Current (60 py)	0	N/A	N/A	Not tested	Cisplatin + Pemetrexed	N/A (adjuvant)	18	Sim (66)
Adeno	Focal SCLC	36	F	Never	1	Alectinib	8	Not tested	Cisplatin + Irinotecan	PR	Unk	Koyama (64)

Table 2 (*continued*)

Table 2 (*continued*)

Baseline histology	Trans histology	Age (years)	Sex	Smoking status	No. prior therapies	ALK TKI at trans	Time to trans (mos)	Co-occurring mutations or amplifications	Treatment post-trans	Response post-trans	OS (mos)	Ref
Adeno	EMT	36	F	Never	3	Alectinib	6.5	MET amp p53 R248Q	Alectinib + abraxane + Cisplatin	PR	19	Xie (76)
Adeno	EMT	Unk	Unk	Unk	4	Ceritinib	>26	ALK F1174C	None	N/A	Unk	Gainor (2)
Adeno	EMT	Unk	Unk	Unk	2	Ceritinib	18	None	Unknown (clinical trial)	Unknown	Unk	Gainor (2)
Adeno	EMT	Unk	Unk	Unk	2	Ceritinib	14	ALK L1196M	Chemotherapy, NOS	Unknown	Unk	Gainor (2)
Adeno	EMT	Unk	Unk	Unk	5	Ceritinib	>9	ALK L1196M	None	N/A	Unk	Gainor (2)
Adeno	EMT	Unk	Unk	Unk	2	Ceritinib	8	None	Chemotherapy, NOS	Unk	Unk	Gainor (2)
Adeno	EMT	Unk	F	Unk	1	Crizotinib	4	ALK L1196M	Unk	Unk	4	Fukuda (75)
Adeno	EMT	38	M	Never	4	Alectinib	36	BRAF V600E	Lorlatinib	PD	37	Urbansk (77)
Adeno	EMT	58	F	Never	3	Lorlatinib	64	ALK C1156Y	Lorlatinib*	N/A	96	Recondo (10)

*, Lorlatinib continued after cryoablation to transformed lesion. ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; Adeno, adenocarcinoma; SCLC, small cell lung cancer; SCC, squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma; EMT, epithelial-to-mesenchymal transition; trans, transformation; Unk, unknown; amp, amplification; py, pack years; OS, overall survival; PR, partial response; PD, progressive disease; SD, stable disease; N/A, not available; NOS, not otherwise specified.

level suggesting loss of dependence on EGFR oncogenic signaling for these transformed tumors (16,18,100).

In published cases of SCLC transformation of ALK-positive NSCLC, transformed SCLC is similarly reported to have classic SCLC morphology with acquired expression of neuroendocrine markers; however, expression of ALK protein is variable and not uniformly lost as with *EGFR*-mutant SCLC transformed tumors (57,67-73). Of the small overall number of published cases of SCLC transformation in ALK-positive NSCLC, only a handful report baseline next-generation panel-based sequencing data. As a result, there are not yet clearly established trends regarding rates of co-occurring mutations, for example loss-of-function mutations in p53 and/or RB1.

Of note, another histologic entity that falls under the spectrum of neuroendocrine histology but is distinct from SCLC is large cell neuroendocrine carcinoma (LCNEC). LCNEC is characterized by cytologic features of NSCLC with tissue architecture and IHC markers consistent with neuroendocrine histology (102). The largest published cohort of genomic and transcriptomic profiling of LCNEC tumors to date revealed two distinct genomic subgroups defined by bi-allelic *TP53* and *STK11* (serine/threonine kinase 11)/KEAP1 (Kelch Like ECH Associated Protein 1) alterations (type I) versus bi-allelic inactivating mutations in *TP53* and *RB1* (type II) (103). While LCNEC tumors are genomically similar to NSCLC, transcriptional profiling demonstrates that type I LCNEC resembles neuroendocrine-high SCLC, and type II LCNEC more closely resembles neuroendocrine-low SCLC (103). A full characterization of LNEC is outside the scope of this review, but the significant degree of histologic heterogeneity within LCNEC highlights the inherent lineage plasticity of this tumor subtype and suggests that improved understanding of the molecular features of LCNEC will lead to more precision in diagnosis and treatment decision-making.

While these descriptions refer to *de novo* LCNEC tumors, histologic transformation from adenocarcinoma to LCNEC is also rarely identified as a mechanism of resistance to targeted therapies, including ALK TKIs (61,83,104-106) (*Table 2*). Molecular features associated with LCNEC transformation remain poorly defined, but there is variability in baseline p53/RB1 status and clinical outcomes among the few reported cases. Within *EGFR*-mutant NSCLC, there are reported cases of both TKI-sensitive and -resistant *de novo EGFR*-mutant LCNEC tumors (107-109), as well as published examples of histologic transformation

to LCNEC following treatment with EGFR-targeted therapies (83,106,110). Though the published examples of such cases are limited in number, the observed variability in responses to EGFR TKIs among *EGFR*-mutant tumors with LCNEC histology likely reflects the inherent molecular and histologic heterogeneity of LCNEC tumors more broadly.

Molecular features of SCLC transformation

As mentioned in the prior section, loss of function of tumor suppressors p53 and RB1 appears to be a critical biologic factor predisposing tumors to undergo SCLC transformation. The combined loss of these p53/RB1 function has been described as necessary but not sufficient for SCLC transformation in the context of *EGFR*-mutant NSCLC (101). This is consistent with the genomic landscape of *de novo* SCLC in which >95% of tumors are deficient in both p53/RB1 (111-113). Beyond p53/RB1 loss, however, multiple additional molecular mechanisms have been proposed as potentially important pathways in SCLC transformation. In the largest retrospective clinical cohort of transformed *EGFR*-mutant SCLC published to date, *PIK3CA* mutations were reported in 27% (n=14/52) of pre-transformed tumors (18) which was consistent with recurrent *PIK3CA* mutations observed in the initial report of SCLC transformation (54) and other subsequent case series (88). In another study comparing 7 pre-SCLC transformation tumors to 32 NSCLC tumors with baseline EGFR, *TP53*, and *RB1* mutations that did not go on to SCLC transformation, the pre-transformed tumors were enriched for mutations or amplifications in *SMYD1* (SET and MYND domain containing 1), *MYND* (Mynd Domain-Containing Protein), *NOTCH2* (neurogenic locus notch homolog protein 2), *PIK3CA*, *MYC* (Cellular Myelocytomatosis Oncogene), *CREBBP* (CREB-binding protein), *PTEN* (Phosphatase and TENsin homolog), *CCNE1* (Cyclin E1) and *ELF3* (E74 like ETS transcription factor 3) (16). Pre-transformed tumors were also enriched for whole-genome duplication (WGD) compared to *EGFR/TP53/RB1* co-mutated tumors that did not transform (16), and multiple studies have also identified APOBEC (Apolipoprotein B mRNA-Editing Enzyme, Catalytic Polypeptide) hypermutation signatures in pre-transformed *EGFR*-mutant NSCLC (16,101). Other case reports and case series of transformed SCLC tumors suggest that increased expression of FGF9 (fibroblast growth factor 9) (114), amplification of TERT (telomerase reverse transcriptase) (81), and overall increased burden of copy-number variants (115) may also play a role in SCLC transformation.

Mixed histology tumors have also become a valuable resource for comparing the biology of discordant histologic components of a single biopsy or resection specimen. In a series of 100 surgically resected cases of NSCLC, a component of SCLC or LCNEC histology was seen in 25% of cases (116). A recent report describing 11 *EGFR*-mutant SCLC transformed tumors from a retrospective cohort of 7282 cases of lung cancer demonstrated discernable foci of SCLC histology in 8/11 'pre-transformation' pathology specimens suggesting a true diagnosis of mixed histology at baseline (84). Finally, some component of mixed histology was present at the time of transformation in a subset of reported cases of *EGFR*-mutant NSCLC (54,92) and ROS1-rearranged transformed SCLC (12).

With increasingly powerful investigative tools such as spatial transcriptomics and single-cell sequencing technologies, we continue to gain more insights into the biology of these mixed histology tumors. A recent study comparing genomic, transcriptomic, and methylomic data from 11 mixed histology (adenocarcinoma/SCLC) tumors, 5 pre-transformed lung adenocarcinomas, and 3 post-transformed SCLCs (including one matched case) to never-transformed lung adenocarcinomas (n=15) and to *de novo* SCLCs (n=18) was arguably the most comprehensive analysis of transformed and mixed NSCLC/SCLC cases published to date (117). In it, the authors demonstrate consistent genomic findings in NSCLC and SCLC components as previously published; specifically, enrichment of both chromosomal 3p loss and APOBEC hypermutation signatures is demonstrated in the NSCLC portions of mixed histology tumors, and increased expression of genes related to the PRC2 (polycomb repressive complex 2) complex, PI3K/AKT (Protein Kinase B) signaling, and NOTCH signaling pathways is seen in SCLC components of transformed or mixed histology tumors (117). Interestingly but not surprisingly, transcriptional and methylation data reveal substantial overlap between the respective NSCLC and SCLC components of mixed histology tumors as compared to their never-transformed comparison cohorts (117). This finding affirms the hypothesis that these pre-/post-transformed and mixed histology tumors represent an intermediate state of lineage plasticity and further strengthens the view that the process of histologic transformation is likely one governed by transcriptional reprogramming. However, while there has

been work defining key transcriptional programs governing SCLC tumorigenesis (118), the mechanisms driving gene expression and methylation changes in the process of SCLC transformation remain incompletely understood.

While it's important to note that directionality of histologic transformation cannot be assumed in a mixed histology tumor, the available published data summarized above demonstrate clonality within mixed histology tumors. The presence of mixed histologic components prior to TKI or other treatment exposure raises the question of whether (or when) driver oncogene inhibition is required for the process of SCLC histologic transformation. While loss of EGFR expression in SCLC-transformed *EGFR*-mutant NSCLCs demonstrates a repression of driver oncogene pathway activity in SCLC transformed tumors (16,18,54), multiple studies investigating the clonal evolution of SCLC transformation suggest that, in some cases, the divergence of precursor SCLC clones occurs prior to TKI exposure (101,115).

Finally, while the vast majority of *de novo* SCLC tumors show RB1 deficiency, the ~5% of SCLC tumors that are RB1 proficient appear to represent a distinct subset characterized by decreased expression of classical neuroendocrine markers, distinct genomic alterations [CDKN2A (cyclin-dependent kinase inhibitor 2A) mutation, CCND1 (cyclin D1) amplification, KEAP1/STK11 mutations, FGFR1 (fibroblast growth factor receptor 1) amplification] and a more aggressive clinical phenotype (113). These RB1 proficient tumors also more commonly demonstrate features of SCLC/NSCLC mixed histology, suggesting that 'mixed histology' and 'neuroendocrine-low'/RB1-proficient SCLC tumors may describe some component of an overlapping histologic subtype. In the context of clinically observed histologic transformations from adenocarcinoma to SCLC histology, extrapolation of data from mixed histology tumors is, at a minimum, hypothesis-generating, but may also provide key insights into mechanisms of lineage plasticity. Taken together, the sum of the data from the *EGFR*-mutant NSCLC literature about SCLC transformation suggests that it is a biologic process driven primarily by transcriptional reprogramming rather than acquired genomic alterations.

Treatment approaches and clinical outcomes following SCLC transformation

As a field, we lack prospective data informing clinical treatment of patients with SCLC transformed tumors. The largest retrospective cohort published to date includes data from 58 patients with *EGFR*-mutant NSCLC across 8 institutions whose tumors underwent SCLC transformation as a mechanism of EGFR TKI resistance (18). In it, the authors demonstrated an overall response rate of 54% and mPFS of 3.4 months after treatment with platinum/etoposide-based chemotherapy. Taxane-containing chemotherapy regimens also conferred promising clinical benefit with a 50% overall response rate and mPFS of 2.7 months. No responses to immune checkpoint inhibitors were seen in this cohort. The median time to SCLC transformation was 17.8 months and the mOS was 31.5 months. Median OS from the time of SCLC transformation was 10.9 months, comparable to the expected survival of *de novo* SCLC from the time of initial diagnosis (119,120).

Notably, the response rate to platinum etoposide chemotherapy reported in this retrospective case series is somewhat lower than the historical average of 60–65% to first-line platinum/etoposide-based chemotherapy in *de novo* SCLC (121,122). Though direct comparisons cannot be drawn between this retrospective study and independent prospective clinical trials, these data suggest decreased sensitivity to platinum-based chemotherapy regimens in the setting of transformed SCLCs compared to *de novo* SCLCs. Interestingly, the response rate to platinum/etoposide in patients who were previously treated with platinum-based chemotherapy for pre-transformed adenocarcinoma was 80% (n=8/10) suggesting that, if the lower overall response rate reflects truly differing clinical rates of response, this is more likely due to the underlying biology of transformed SCLCs than a result of prior platinum-based chemotherapy exposure (18). A more recently published cohort of 29 patients with *EGFR*-mutant NSCLC whose tumors underwent SCLC transformation following treatment with EGFR TKIs demonstrated a median time to transformation of 27.5 months and mOS from the time of SCLC transformation of 14.8 months (82). The mPFS for patients who received EGFR TKI treatment concurrently with chemotherapy at the time of SCLC transformation was significantly longer than those who received chemotherapy alone (5.0 *vs.* 3.7 mos) but there was no significant difference between the two groups in mOS from the time of transformation.

No such large retrospective cohort analyses exist in the published literature for ALK-positive NSCLC following SCLC transformation, but data compiled from case series suggest an average time to SCLC transformation of

Table 3 Compiled clinical outcomes of reported cases of lineage transformation in ALK-positive NSCLC

Transformed histology (references)	SCLC (57,67-74)	SCC (59,60,62,63,65)	LCNEC (61)	EMT (2,10,75-77)	Mixed histology (64,66)
Total number	9	6	2	9	4
Age at diagnosis if known (years)*, median [range]	53 [35–72]	52.5 [47–60]	64 [54–74]	38 [36–58]	68.5 [36–73]
Smoking hx, n [%]					
Never	7 [78]	4 [67]	2 [100]	3 [33]	2 [50]
Former; Avg pack years	1 [11]; 40	2 [33]; 4.5	–	–	2 [50]; 50
Unknown	1 [11]	–	–	6 [67]	–
Time to transformation in mos**, median [range]	20.5 [6–72]	51.5 [4–138]	46 [17–75]	14 [4-64]	8 [8]
ALK TKI at the time of transformation^, n [%]					
Crizotinib	3 [33]	–	–	1 [11]	–
Alectinib	4 [44]	2 [33]	1 [50]	2 [22]	1 [25]
Ceritinib	1 [11]	2 [33]	–	5 [56]	–
Brigatinib	–	1 [17]	1 [50]	–	–
Lorlatinib	1 [11]	1 [17]	–	1 [11]	–
Treatment after transformation, n [%]					
ALK TKI monotherapy	2 [22]	1 [17]	–	2 [22]	–
Chemotherapy	3 [33]	1 [17]	2 [100]	2 [22]	3 [75]
ALK TKI + chemotherapy	2 [22]	–	–	1 [11]	–
Other/unknown	2 [22]	4 [67]	–	4 [44]	1 [25]
Response after transformation, n [%]					
PR	4 [44]	1 [17]	1 [50]	1 [11]	1 [25]
SD	1 [11]	1 [17]	–	–	–
PD	2 [22]	2 [33]	1 [50]	1 [11]	–
Unknown	2 [22]	2 [33]	–	7 [78]	3 [75]

*, 5 pts omitted as age not reported; **, 6 pts omitted as time to transformation not reported or not applicable (mixed histology at diagnosis); ^, 3 pts omitted as they were not treated prior to mixed histology biopsy. ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; Adeno, adenocarcinoma; SCLC, small cell lung cancer; SCC, squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma; EMT, epithelial-to-mesenchymal transition; TKI, tyrosine kinase inhibitor; PR, partial response; PD, progressive disease; SD, stable disease.

20.5 months (*Table 3*). The most commonly reported treatment regimens following SCLC transformation in ALK-positive lung cancer are platinum-based chemotherapy alone with or without ALK TKI (*Table 2*). Response data from case reports of SCLC transformed ALK-positive lung cancers following specific treatment regimens are detailed in *Table 2*. Of note, there is at least one reported case of successful retreatment with alectinib after eradication of a SCLC histologic clone following transformation (57).

While no prospective trial data are yet available to guide treatment of SCLC transformed tumors, the first generation of clinical trials designed for this specific patient population are open to enrollment (*Table 4*). To our knowledge, all three of these trials enrolling in the United States are open only to *EGFR*-mutant transformed SCLC, not ALK-positive transformed SCLC or other non-*EGFR*-mutant subsets. Extrapolating from the *de novo* SCLC literature (123), two trials are testing combination therapies with PD-L1 (programmed death-ligand 1) inhibitor durvalumab at the time of SCLC transformation, either

Table 4 Clinical trials for patients with NSCLC following lineage transformation

NCT identifier	Trial phase	Patient population	Intervention	Primary outcome(s)	Trial status
NCT04538378	Phase II	Stage IV *EGFR*-mutant NSCLC after transformation to SCLC or other NEC	Olaparib 300 mg bid plus durvalumab 1,500 mg monthly	Objective response rate	Recruiting
NCT03567642	Phase I	Stage IV *EGFR*-mutant NSCLC with co-occurring baseline *TP53* and *RB1* mutations	Addition of cisplatin (60 mg/m^2) or carboplatin (AUC 4–5) with etoposide (80–100 mg/m^2) starting C4D1 osimertinib 80 mg daily	Maximum tolerated dose (MTD)	Recruiting
NCT03944772	Phase II (Platform study)	Stage IV *EGFR*-mutant NSCLC after transformation to SCLC	Etoposide 80–100 mg/m^2 plus durvalumab 1,500 mg plus either cisplatin or carboplatin	Objective response rate	Recruiting

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; NEC, neuroendocrine carcinoma.

together with PARP (Poly-ADP Ribose Polymerase 1) inhibitor olaparib (NCT04538378) or with platinum/etoposide chemotherapy (NCT03944772; arm of the ORCHARD platform study). Interestingly, the third study open for this patient population is designed with the aim of delaying or preventing SCLC transformation by eradicating pre-existing SCLC subclones prior to the clinical emergence of transformation. This study enrolls patients with newly-diagnosed, high-risk *EGFR*-mutant NSCLC (those with concurrent *TP53/RB1* mutations at baseline) and prospectively adds four cycles of platinum/etoposide chemotherapy prior to transformation, after 12 weeks of standard-of-care osimertinib monotherapy (NCT03567642).

Of note, the list of trials described here does not comprehensively describe all clinical trial opportunities for these patients, as other trials designed specifically for *de novo* SCLC or other neuroendocrine cancers may allow transformed SCLC on a case-by-case basis. Thinking forward to additional therapeutics in this space, there are emerging data demonstrating rationale for other targeted therapies of potential biologic important in transformed SCLC. An example of one potential molecular target is the EZH2 (Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit) H3K27 methyltransferase, a catalytic subunit of the PRC2 complex which has been shown to be dysregulated in neuroendocrine transformation (117). Preclinical data have shown that inhibition of EZH2 can shift cells from neuroendocrine to non-neuroendocrine morphology and may play a role in SCLC lineage plasticity in NSCLC and other solid tumors (56,124). EZH2 inhibitors are currently being tested in early-phase clinical trials.

Finally, recent progress in translational studies of *de novo* SCLC has resulted in a novel classification system categorizing *de novo* SCLC into molecular subtypes based on gene expression of key transcription factors [ASCL1 (Achaete-Scute Homolog 1), NEUROD1 (neuronal differentiation 1), POU2F3 (POU Domain, Class 2, Transcription factor 3)] and immune-related genes (inflamed subtype). These classifications (SCLC-A, SCLC-N, SCLC-P, and SCLC-I) have been further shown in preclinical and retrospective studies to confer sensitivity to specific targeted therapies such as PARP inhibitors, AURK inhibitors, and BCL2 inhibitors (125); however they are not yet prognostic or predictive for patient outcomes. Initial data suggest representation of all transcription factor subtypes in transformed SCLC (117), though more investigation is required to understand the clinical relevance, if any, these subtypes will have in transformed SCLC.

Squamous histologic transformation

Incidence and diagnosis of squamous transformation

While SCLC transformation was the first identified category of lineage transformation and is the most comprehensively described to date, squamous transformation (from adenocarcinoma, within the NSCLC spectrum) is also a recurrent finding at the time of acquired resistance to targeted therapies in lung cancer. Characterized by changes in morphology and immunohistochemical staining from adenocarcinoma-associated markers (e.g., napsin A and TTF-1) to squamous-associated markers (e.g., p40 and p63) (126,127), it has been reported in at least six cases of resistance to ALK TKIs

(59,60,62,63,85,74) (*Table 2* and *Table 3*). Similar to SCLC transformation, squamous transformation is also a recurrent phenomenon in *EGFR*-mutant NSCLC (17,19,128,129) and squamous histology has also been rarely reported as a component of *EGFR*-mutant NSCLC histology at diagnosis (130,131). ALK fusions are an exceedingly rare occurrence in *de novo* squamous cell carcinoma tumors (132). Overall, the full clinical spectrum of squamous transformation from adenocarcinoma histology remains understudied, though it seems to represent a true recurrent adaptive mechanism for tumor evasion of targeted therapy.

Molecular features of squamous transformation

Similar to SCLC transformation, investigation of mixed adenosquamous tumors provides a compelling opportunity to learn about mechanisms of lineage transformation along the adenocarcinoma-squamous spectrum of NSCLC histology. Early studies performing molecular characterization of separate histologic compartments of surgically resected, macro-dissected mixed adenosquamous tumors demonstrated expected clonality between the adenocarcinoma and squamous tumor components (133). Specifically, whole-exome sequencing of three mixed adenosquamous tumors showed common genomic alterations in the identified driver mutation (*EGFR*, *BRAF*, and *MET* respectively) as well as shared loss of tumor suppressor *STK11* and chromosomal regions 3p, 15q, 19p. In addition to chromosomal amplification of 5p, focal amplification of *SOX2* (SRY-Box Transcription Factor 2) was also identified in all three tumors. *SOX2* amplification was previously shown to cause squamous differentiation in preclinical mouse models (134,135) and is amplified in 40% of squamous cell carcinomas but almost never in lung adenocarcinomas (126).

In a recent study published by Quintanal-Villalonga and colleagues, the authors performed genomic, epigenomic, and transcriptomic analysis of 11 micro-dissected adenosquamous tumors, 4 pre-transformation adenocarcinoma tumors, and 7 post-transformation squamous tumors compared to never-transformed lung adenocarcinomas and *de novo* lung squamous cell carcinomas (136). Common mutations present in both adenocarcinoma and squamous histologic compartments were in *EGFR*, *TP53*, *CDKN2A/B*, and *STK11*, but these alterations were not specifically enriched in the transformed or mixed histology tumors compared to controls (136). However, mutations in *TBX3* (T-Box transcription factor 3), *MET*, *RBM10* (RNA Binding Motif Protein 10) were enriched in pre-transformed lung adenocarcinomas compared to never-transformed lung adenocarcinomas, and increased expression of genes related to PI3K/AKT, MYC, and PRC2 signaling pathways was found in squamous transformed components of either mixed histology or post-transformation tumors (136). While further investigation is needed to confirm the role of these signaling pathways in squamous transformation, these data raise the hypotheses that squamous transformation may be primed by genomic alterations in *TBX3*, *MET*, *RBM10* and that the process of lineage transformation is driven by transcriptional reprogramming rather than further acquired genomic mutational changes.

Treatment approaches and clinical outcomes following squamous transformation

There are unfortunately very little clinical data informing optimal treatment regimens for patients whose tumors undergo squamous transformation following resistance to targeted therapies. Without any published prospective data or clinical trials specifically designed for this patient population, decisions regarding treatment at the time of squamous transformation still rely on anecdotal evidence and expert opinion. In reported cases of ALK-positive and *EGFR*-mutant tumors undergoing squamous transformation, systemic therapy at the time of transformation has been variable—ranging from continued TKI to chemotherapy and immune checkpoint inhibitors (*Table 2*) (17,59,60,62,63,65,74,137). Unfortunately, the number of cases with reported clinical outcomes remains too few to inform prospective decision-making for patients currently on treatment. In general, similar principles apply as in SCLC transformation, including a shift to histology-based chemotherapy backbones (e.g., taxane-based regimens for squamous histology) and continuation of concurrent targeted therapy if safe and feasible versus shift to alternative targeted therapy if concurrent activation of bypass pathways are found on molecular analysis. Of note, while squamous transformation has been purported to be a primary and/or independent driver of TKI resistance, retrospective analyses in *EGFR*-mutant tumors with squamous transformation have shown concurrent known genomic mechanisms of resistance (17,137). It is not yet known whether these findings represent intra-tumoral heterogeneity in resistance mechanisms versus biologic association of these genomic changes with squamous transformation.

In the case of NSCLCs with baseline mixed adenosquamous histology, historical data suggest that clinical outcomes are overall poorer compared to baseline pure adenocarcinoma (138). However, the genomic context of a particular oncogenic driver alteration and associated available treatment regimens is likely to be important for interpretation of prognosis and expected clinical outcomes. In *EGFR*-mutant NSCLCs, some component of admixed squamous histology at diagnosis was reportedly associated with comparable clinical outcomes in a retrospective study of 12 patients treated with first-generation EGFR TKI erlotinib (130). However, other preliminary data from a retrospective analysis of *EGFR* adenosquamous or purely squamous NSCLC at diagnosis suggests that these patients have inferior clinical outcomes compared to those with adenocarcinoma histology (137). We lack comparable data regarding clinical outcomes of ALK-positive mixed adenosquamous NSCLCs.

Epithelial-to-mesenchymal transition (EMT)

EMT is another example of lineage plasticity that has been observed in ALK-positive lung adenocarcinoma following resistance to targeted therapies (2,10,75-77). Less diagnostically definitive than a complete SCLC or squamous histologic transformation, EMT is characterized by a morphologic and immunophenotypic shift of increased expression of mesenchymal markers (e.g., vimentin) and decreased expression of epithelial markers (e.g., e-cadherin) (2). Multiple rigorous preclinical studies have further defined subtypes or distinct 'modes' of EMT and signaling pathways thought to be primarily responsible for driving this form of cellular plasticity across solid tumor types (139-141). While a comprehensive summary of these preclinically-defined mechanisms of EMT is outside the scope of this narrative review, it is important to note that the biology of EMT—including predisposing factors, definitive diagnostic criteria, and clinical implications—remains an area of active study. Of note, characterization of the neuroendocrine-low morphologic subvariant of SCLC has revealed some molecular and phenotypic similarities to EMT (142), but the molecular relationship between EMT and SCLC transformation remains incompletely understood. EMT has been reported as a mechanism of resistance in at least 9 cases of acquired resistance to ALK TKIs in ALK-positive NSCLC (*Table 2* and *Table 3*) (2,10,75-77), though this is not a mechanism of resistance routine tested for or considered actionable on a clinical basis at this time.

Limitations and future directions

Lineage plasticity is an increasingly recognized but relatively rare mechanism of resistance to targeted therapies in ALK-positive NSCLC. Multiple forms of lineage plasticity have been implicated as methods of tumor cell evasion from the selective pressure of targeted therapies, and SCLC transformation, squamous transformation and EMT are the most well studied. This narrative review of the literature on lineage plasticity in ALK-positive NSCLC is limited in part by minimal available published data due to the rare occurrence of lineage transformation relative to other mechanisms of resistance to ALK TKIs. In order to address this limitation, we have referenced the relevant available literature about transformation in other genomic subtypes of NSCLC, such as *EGFR*-mutant NSCLC.

Distinct from genomic mechanisms of TKI resistance such as second-site oncogene mutations and bypass pathway amplification, lineage transformation does not have reliable genomic markers that can be assessed via DNA sequencing. This precludes diagnosis of lineage transformation by non-invasive plasma circulating tumor DNA sequencing assays, which are now commonly used to diagnose resistance mechanisms (143). As a result, lineage transformation can be easily missed if a repeat tissue biopsy is not obtained at the time of TKI resistance for the purpose of histologic analysis. At present, these tissue samples are also needed for ongoing translational studies to further understand the biology of lineage plasticity in NSCLC. In the future, non-invasive plasma-based diagnostic assays that could detect lineage plasticity would obviate the need for repeat tissue biopsies, but this technology is not yet readily available for clinical application.

We also still lack reliable predictive biomarkers identifying tumors predisposed to undergo lineage transformation. While dual *TP53/RB1* loss portends a higher likelihood of SCLC transformation in *EGFR*-mutant lung adenocarcinoma, we have yet to define any other biomarker of a pre-transformed tumor that could potentially be clinically actionable. Discovery of predictive markers of lineage transformation would potentially enable more adaptive trial designs aimed at preventing or delaying lineage transformation, hopefully improving patient outcomes. Finally, the role that timing and potency of oncogene inhibition plays in driving lineage transformation also remains largely unknown. Whether TKI inhibition of driver oncogene (e.g., shutting down oncogenic epithelial signaling pathways) is required for lineage transformation in pre-disposed tumors and/or whether increasingly specific

and potent TKIs will drive lineage transformation at higher frequencies compared to on-target resistance mechanisms remains unknown. Ongoing clinical and translational investigative efforts are needed to answer these questions more definitively and move clinical care forward for these patients.

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Footnote

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