

PROTAC therapy as a new targeted therapy for lung cancer

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Despite recent advances in molecular therapeutics, lung cancer is still a leading cause of cancer deaths. Currently, limited targeted therapy options and acquired drug resistance present significant barriers in the treatment of patients with lung cancer. New strategies in drug development, including those that take advantage of the intracellular ubiquitin-proteasome system to induce targeted protein degradation, have the potential to advance the field of personalized medicine for patients with lung cancer. Specifically, small molecule proteolysis targeting chimeras (PROTACs), consisting of two ligands connected by a linker that bind to a target protein and an E3 ubiquitin ligase, have been developed against many cancer targets, providing promising opportunities for advanced lung cancer. In this review, we focus on the rationale for PROTAC therapy as a new targeted therapy and the current status of PROTAC development in lung cancer.

INTRODUCTION

There has been a reduction in new lung cancer diagnoses and lung cancer deaths due to decreases in smoking, early screening, and advances in treatment.^{1,2} However, lung cancer remains the leading cause of cancer deaths, contributing to almost 20% of all cancer deaths. Only 10% to 20% of all patients diagnosed with lung cancer have >5-year survival rate, and more people die of lung cancer than of breast cancer, colon cancer, and prostate cancer combined.¹

Lung cancer is classified into two major histological types: small cell lung cancer (SCLC), which makes up around 15% of all lung cancers, and non-small cell lung cancer (NSCLC), which makes up the majority at 85%.³ NSCLC includes three main histological subtypes: lung adenocarcinoma (around 40% of NSCLC), squamous cell carcinoma (25%), and large cell carcinoma (10%). The remaining types include adenosquamous carcinoma, sarcomatoid carcinomas, salivary gland type tumors, carcinoid tumors, and other unclassified carcinomas. Despite these subclassification efforts, molecular analyses within each of these histological lung cancer subtypes, such as adeno-, squamous, and small cell carcinoma, have shown a highly heterogeneous genetic landscape as revealed by the Cancer Genome Atlas.^{4–8} This heterogeneity continues to challenge the interpretation of large therapeutic trials that average clinical outcomes and, accordingly, may miss

important treatment opportunities that are limited to specific mutational profiles.

Therefore, it is increasingly recognized that both tumor, node, metastasis (TNM) staging and detailed genetics are important in guiding treatment of patients with lung cancer. Patients undergo biopsy, staging and next-generation sequencing to help guide lung cancer treatment, whether it be surgical resection, radiation, systemic chemotherapy, targeted therapy, and/or immunotherapy.^{3,9} For example, a subset of patients with NSCLC harboring actionable mutations, such as those in *EGFR*, *ALK*, *ROS1*, *BRAF*, or *MET*, receive targeted small molecule inhibitor therapy as the first-line treatment, whereas those without driver mutations may receive combinations of standard cytotoxic chemotherapy and immunotherapy or immunotherapy alone. However, most patients who present with advanced disease ultimately develop tumor progression through clonal selection of drug-resistant tumor cells and new strategies that overcome drug resistance are urgently needed to improve patient outcomes.

The concept of harnessing the endogenous ubiquitin-proteasome system to degrade pathogenic proteins through proteolysis targeting chimeras (PROTACs) was initially introduced and demonstrated in a proof-of-concept study in 2001.¹⁰ This technology has since emerged as a paradigm-shifting approach in small molecule drug discovery and has been driving unprecedented innovations in drug development in the past several years.^{11,12} Excitingly, multiple PROTACs are being evaluated in phase I and phase II clinical trials.^{12–15} With these advances, PROTAC technology is increasingly poised to lead to new targeted therapies that benefit patients with lung cancer. In this review, we introduce the PROTAC technology, summarize the progress of PROTAC development in lung cancer, and discuss the

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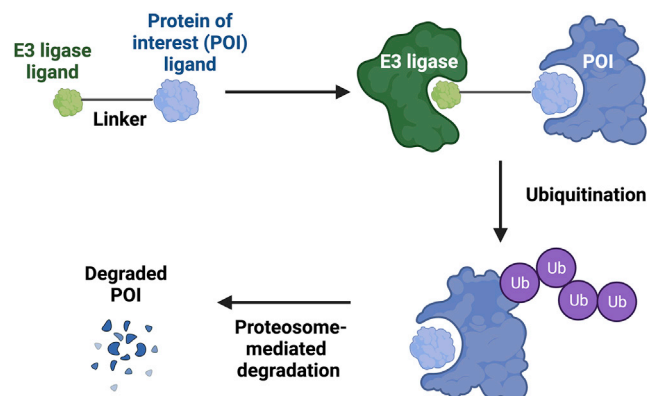


Figure 1. Schematic diagram of the PROTAC technology. A typical PROTAC molecule consists of two ligands, one binding to a protein of interest (POI) and the other recruiting an E3 ligase, which are held together via a chemical linker.

The PROTAC molecules induce proximity between the POI and an E3 through the formation of ternary complexes, leading to poly-ubiquitin chain formation on the POI and ultimately proteasomal-mediated POI degradation. The figure was created with BioRender.

promises and challenges in moving PROTACs toward clinical applications in advancing lung cancer treatment.

THE PROTAC TECHNOLOGY OFFERS EXCITING OPPORTUNITIES FOR DEVELOPING NOVEL TARGETED CANCER THERAPEUTICS

PROTACs are hetero-bifunctional molecules that co-opt the intrinsic ubiquitin-proteasome system to specifically destroy proteins implicated in cancers and other diseases.^{10,12,14,16} PROTACs consist of two ligands, one that binds to a target protein of interest (POI) and the other that binds to an E3 ubiquitin ligase, with a linker connecting the two ligands (Figure 1). By forming a ternary complex with a POI and an E3 ligase, a PROTAC brings the POI to close proximity to the E3 ligase, inducing POI poly-ubiquitination and subsequent proteasome-mediated degradation. The PROTAC is then recycled to target and degrade another POI molecule.

The modular nature of PROTAC design offers great potential, flexibility, and speed in the development of novel PROTACs to target and degrade essentially any intracellular protein targets. In terms of ligands for POIs for degradation, small molecules that can bind to any surfaces of target proteins can potentially be used for PROTAC development, unlike the conventional small molecule inhibitors (SMIs) that require high affinity to block protein function. Available small molecule compounds such as those Food and Drug Administration (FDA)-approved small molecule targeted inhibitors, those that failed clinical trials, or tool compounds have been used in the design and development of many PROTACs. For example, as discussed below, the available small molecule epidermal growth factor receptor (EGFR) inhibitors and ALK inhibitors were used to develop PROTACs that target and degrade EGFR and ALK, respectively. With many available small molecule libraries, the identifica-

tion of new ligands that bind to many cancer targets will expand degradable POIs to those undruggable targets for PROTAC development.

Due to a very limited number of available E3 ligases, currently developed PROTACs mainly recruit E3 ligases cereblon (CRBN), von Hippel-Lindau (VHL), mouse double minute-2 (MDM2), and inhibitor of apoptosis proteins (IAPs), despite a collection of more than 600 E3 ligases encoded by the human genome. Most published PROTACs recruit ubiquitously expressed CRBN and VHL E3 ligases, which have the potential to cause on-target toxicity. PROTACs targeting the same POI, however, with either a CRBN or a VHL E3 ligase have demonstrated differing rates of degradation. Identification of tumor-specific/enriched E3 ligases and the development of their specific ligands will have great impact on enhancing tumor-specific/selective therapeutic effects.

The ligands for a POI and E3 ligase are held together through a chemical linker. Research has demonstrated that the type, the length, the rigidity, and the positions of linkage on the POIs and E3 ligands could affect PROTAC functions in terms of the induced ternary complex formation, the efficacy and selectivity of protein degradation, pharmacokinetics (PK), and bioavailability.¹⁶ Therefore, the optimization of the chemical linker is an important step for PROTAC development.

PROTACs offer several advantages in comparison with traditional SMIs.^{12,14,16,17} First, PROTACs degrade protein targets, instead of inhibiting the target activity. This likely achieves better inhibition in a scenario where oncogenic activity of a target can have activity independent of the enzymatic activity. For example, PROTACs can abrogate the scaffolding function of the target proteins to potentially override resistance and improve efficacy of the warheads. Second, PROTACs can be developed to degrade “difficult to drug” proteins that do not possess well-defined pockets, such as transcription factors (e.g., STAT3, MYC) and RAS (e.g., KRAS^{G12C}). It can also target and degrade multicomponent protein complexes, which are conventionally considered as “undruggable” because inhibition of one subunit does not necessarily remove the complex’s function. Third, PROTACs have the potential to overcome resistance by degrading upregulated POIs (induced by SMIs), or POIs that arise from mutations in the targets. Fourth, and perhaps most importantly, PROTACs have unique event-driven pharmacology and can mediate multiple rounds of degradation, unlike occupancy-driven pharmacology for SMIs. The unique mechanism of action allows one single PROTAC molecule to degrade multiple POIs, thus enabling the use of low drug concentrations to reach a desired therapeutic effect. Last, the modular nature of PROTACs enables relative ease and flexibility in the design and optimization.

However, there are several challenges associated with the PROTAC drugs. PROTAC molecules are generally large, which leads to poor pharmaceutical properties such as bioavailability. It has been reported that some PROTACs have excellent activity in cellular models, yet have no activity in mouse models *in vivo* due to issues with PK and tissue penetration. In addition, protein degradation potentially confers

severe on-target toxicity as compared with the inhibition of protein function in certain settings. It is also difficult to achieve specific degradation of altered proteins in tumors, as a result, potential on- and off-target toxicity could arise, which should be carefully evaluated.

PROGRESS IN THE PROTAC DEVELOPMENT FOR LUNG CANCER TREATMENT

PROTACs are under rapid development as novel therapeutics of lung cancer and novel strategies to overcome drug resistance in the past few years. Multiple PROTAC molecules have been developed for validated therapeutic targets in NSCLC such as EGFR, KRAS, ALK, BRAF, and BCL-XL, with promising compounds showing antitumor efficacy in cell models and preclinical tumor models, which are summarized below (Table 1). Further efforts are required to optimize these PROTAC molecules and to conduct systematic preclinical evaluation before clinical studies.

EGFR-targeting PROTACs

The EGFR signaling pathway is critical in regulating cell growth, differentiation, and survival.^{9,63} EGFR is mutated in approximately 15% of patients with lung adenocarcinoma with more frequent mutation rates in lifelong nonsmokers, women, and patients of East Asian descent. EGFR tyrosine kinase inhibitors (EGFR-TKIs) are standard care for patients with NSCLC who harbor activating mutations in EGFR, based on the paradigm-shifting discovery that tumor responses are tightly linked with tumor EGFR mutational status.^{64–67} The EGFR activating mutations include exon 19 in-frame deletions (del19) in approximately 45% mutant EGFR lung cancer cases, point mutations in exon 21 such as L858R in another 40% to 45% cases, and assorted additional “atypical” mutations in 5% to 10% mostly localized within exon 18. To date, three generations of FDA-approved EGFR-TKIs have been developed with fourth-generation agents on the horizon.^{68–78} The first-generation EGFR-TKIs, gefitinib and erlotinib, which bind to the ATP site, showed good therapeutic effect on patients with EGFR 19del and EGFR L858R mutations. However, disease invariably progresses within 13 months, due to acquired resistance from new mutations such as T790M, a gatekeeper mutation that increases the affinity of ATP to the protein. The second-generation EGFR-TKIs, afatinib and dacomitinib, are irreversible inhibitors forming covalent bonds with C797, and were shown to be superior to gefitinib in terms of primary end-point and overall survival. However, they failed to overcome T790M resistance. The third-generation osimertinib and almonertinib demonstrated improved survival outcomes compared with first-generation EGFR-TKIs. However, almost all patients who initially respond to osimertinib again developed acquired resistance due to secondary mutations in EGFR, structural mutations, and amplification of MET. Around 40% to 50% of patients developed new EGFR mutations such as in C797, G796, and L718 residues, causing tumor relapse due to drug resistance. Overall, resistance limits the efficacy of the EGFR-TKIs in essentially all advanced-stage lung cancer patients, highlighting the need for new therapeutic strategies targeting acquired resistance mechanisms such as selection for new EGFR C797S and T790M mutations.

The PROTAC technology represents an effective strategy to overcome EGFR resistant mutations through targeted degradation of EGFR mutants. The first EGFR PROTACs that degraded EGFR mutant proteins were reported from the Crews group in 2018.¹⁸ A mutant EGFR-selective gefitinib-based, VHL-recruiting PROTAC molecule, Compound 3, was shown to effectively degrade EGFR mutant proteins in HCC827 cells carrying 19del and H3255 cells harboring L858R mutation at low nanomolar concentrations, while sparing wild-type EGFR. Another afatinib-based PROTAC molecule, Compound 4, degraded gefitinib-resistant L858R/T790M mutated EGFR in the H1975 cell line. This study demonstrated the feasibility of using PROTACs to specifically degrade membrane-bound mutated EGFR to overcome drug-resistant EGFR mutations.

Inspired by this exciting study, multiple groups have developed several novel EGFR PROTACs, with some showing *in vivo* antitumor efficacy in preclinical models.^{19–30,79} For example, the Zhang group recently reported an orally bioavailable EGFR PROTAC, HJM-561, as a promising therapeutic strategy for overcoming EGFR triple mutation-mediated drug resistance in NSCLC, because it specifically degraded EGFR mutant proteins and induced potent antitumor activity in EGFR Del19/T790M/C797S-driven Ba/F3 cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) models that were resistant to osimertinib treatment.²⁷ In another study,²⁸ the Zhu group generated novel potent covalent inhibitor dacomitinib-based EGFR degraders and identified a promising compound, 13, which potently induced degradation of EGFR(Del19) with low nanomolar DC(50) value in HCC827 cells, but not other EGFR mutant, wild-type EGFR proteins or HER2 and HER4 receptors from the EGFR family. This compound exhibited excellent antitumor efficacy with no observable toxic effects. The Li group also reported two highly potent and selective CRBN-recruiting EGFR(L858R/T790M) degraders, 13a and 13b, which effectively and selectively blocked the growth of the NCI-H1975 xenograft tumors with good PK properties *in vivo*.²⁹ Moreover, CFT8919, developed by C4 Therapeutics, is an orally bioavailable, allosteric, selective degrader of the EGFR L858R mutation, which accounts for more than a third of mutant EGFR lung cancer diagnoses. CFT8919 was reported to induce tumor regression in preclinical tumor models resistant to first-, second-, and third-generation EGFR-TKIs and has the potential to target CNS metastasis in the preclinical model.³⁰ Collectively, these promising EGFR PROTACs are excellent candidates that can be further developed and evaluated in clinical studies as novel therapeutics to overcome EGFR-TKI-induced resistance.

KRAS-targeting PROTACs

The GTPase KRAS is a signal transducer in the RAS/MAPK pathway with a crucial role in regulating multiple cellular processes such as cell growth and survival.⁸⁰ KRAS mutations have been observed in around 30% of lung adenocarcinomas, which cluster in several hot-spots such as residues 12, 13, 61, and 141.⁶ Specifically, KRAS^{G12C} mutation makes up around half of KRAS mutations found in patients with lung adenocarcinomas.

Table 1. A list of PROTACs that have been developed to target key cancer drivers and tested at different preclinical phases for lung cancer

Gene target (POI)	PROTAC name	Ligand for POI	E3 ligand	Specificity	Lung cancer type	Study type	<i>In vivo</i> lung cancer model	Ref.
EGFR	Compound 3	Gefitinib	VHL ligand	Del19; L858R	LUAD	<i>In vitro</i>	NT	Burslem et al. ¹⁸
	Compound 4	Afatinib	VHL ligand	L858R/T790M	LUAD	<i>In vitro</i>	NT	Burslem et al. ¹⁸
	MS39	Gefitinib	VHL ligand	Del19; L858R	LUAD	<i>In vitro</i>	NT	Cheng et al. ¹⁹
	MS154	Gefitinib	CRBN ligand	Del19; L858R	LUAD	<i>In vitro</i>	NT	Cheng et al. ¹⁹
	Compound 14o	XFT-262	VHL ligand	L858R/T790M	LUAD	<i>In vitro</i>	NT	Zhang et al. ²⁰
	DDC-01-163	JBj-07-149	CRBN ligand	L858R/T790M; L858R/T790M/C797S; L858R/T790M/L718Q	LUAD	<i>In vitro</i>	NT	Jang et al. ²¹
	SIAIS125;126	Canertinib	CRBN ligand	Del19; L858R/T790M	LUAD	<i>In vitro</i>	NT	Qu et al. ²²
	Compound P3	Compound F	VHL ligand	Del19; L858R	LUAD	<i>In vitro</i>	NT	Zhao et al. ²³
	CP17	Osimertinib	VHL ligand	Del19; L858R/T790M	LUAD	<i>In vitro</i>	NT	Zhao et al. ²⁴
	Compound 6h	Brigatinib analog	VHL ligand	Del19/T790M/C797S	LUAD	<i>In vitro</i>	NT	Zhang et al. ²⁵
KRAS	Compound 1q	CO-1686	CRBN ligand	Del19; L858R	LUAD	<i>In vitro</i>	NT	Li et al. ²⁶
	HJM-561	Brigatinib	CRBN ligand	Del19/T790M/C797S; L858R/T790M/C797S	LUAD	<i>In vitro</i> ; <i>In vivo</i>	PDX-LUAD (Del19/T790M/C797S)	Du et al. ²⁷
	Compound 13	Dacomitinib	VHL ligand	Del19	LUAD	<i>In vitro</i> ; <i>In vivo</i>	HCC827 xenograft	Shi et al. ²⁸
	13a;13b	Osimertinib	CRBN ligand	L858R/T790M	LUAD	<i>In vitro</i> ; <i>In vivo</i>	H1975 xenograft	Zhang et al. ²⁹
	CFT8919	EGFR allosteric inhibitor (not disclosed)	CRBN ligand	L858R; L858R/C797S; L858R/T790M; L858R/T790M/C797S	LUAD	<i>In vitro</i> ; <i>In vivo</i>	H1975 xenograft	C4 Therapeutics ³⁰
	XY-4-88	ARS-1620	CRBN ligand	GFP-KRAS	LUAD	<i>In vitro</i>	NT	Zeng et al. ³¹
	LC-2	MRTX849	VHL ligand	KRAS ^{G12C}	LUAD	<i>In vitro</i>	NT	Bond et al. ³²
	YF135	MRTX849	VHL ligand	KRAS ^{G12C}	LUAD	<i>In vitro</i>	NT	Yang et al. ³³
	Compound 9d	BI 1701963	VHL ligand	SOS1	LUAD	<i>In vitro</i> ; <i>In vivo</i>	NCI-H358 xenograft	Zhou et al. ³⁴
	Compound 9	TAE684	CRBN ligand	ALK or ALK fusions	LUAD	<i>In vitro</i>	NT	Powell et al. ³⁵
ALK	Compound 11	Ceritinib	CRBN ligand	ALK or ALK fusions	LUAD	<i>In vitro</i>	NT	Powell et al. ³⁵
	Compound 5;6	Ceritinib	CRBN ligand	EML4-ALK; NPM-ALK	LUAD	<i>In vitro</i>	NT	Zhang et al. ³⁶
	Opto-dALK	Ceritinib	CRBN ligand (opto-pomalidomide)	EML4-ALK; NPM-ALK	LUAD	<i>In vitro</i>	NT	Liu et al. ³⁷
	IAIS091; SIAIS001	Alectinib	CRBN ligand	NPM-ALK	NT	<i>In vitro</i>	NT	Ren et al. ³⁸
	SIAIS117	Brigatinib	VHL ligand	EML4-ALK	LUAD, SCLC	<i>In vitro</i>	NT	Sun et al. ³⁹
	TD-004	Ceritinib	VHL ligand	EML4-ALK	LUAD	<i>In vitro</i> ; <i>In vivo</i>	H3122 xenograft	Kang et al. ⁴⁰
	Compound 17	Alectinib	CRBN ligand	EML4-ALK	LUAD	<i>In vitro</i> ; <i>In vivo</i>	NT	Xie et al. ⁴¹

(Continued on next page)

Table 1. Continued

Gene target (POI)	PROTAC name	Ligand for POI	E3 ligand	Specificity	Lung cancer type	Study type	<i>In vivo</i> lung cancer model	Ref.
	Compound B3	LDK378	CRBN ligand	ALK fusion	LUAD	<i>In vitro</i> ; <i>In vivo</i>	H3122 xenograft	Yan et al. ⁴²
BRAF	P4B	BI 882370	CRBN ligand	BRAF-V600E	LUAD	<i>In vitro</i> ; <i>In vivo</i>	NT	Posternak et al. ⁴³
	SJF-0628	Vemurafenib	VHL ligand	3 classes of BRAF mutations	LUAD	<i>In vitro</i> ; <i>In vivo</i>	NT	Alabi et al. ⁴⁴
	Compound 12	Vemurafenib	CRBN ligand	BRAF-V600E	LUAD	<i>In vitro</i>	NT	Han et al. ⁴⁵
	Compound 23	BI 882370	CRBN ligand	BRAF-V600E	LUAD	<i>In vitro</i>	NT	Han et al. ⁴⁵
BET	ARV-825	OTX015	CRBN ligand	BRD4	LUAD	<i>In vitro</i> ; <i>In vivo</i>	NT	Vartak et al. ⁴⁶ ; Lu et al. ⁴⁷
	dBET	JQ-1	CRBN ligand	BRD2, BRD3, BRD4	LUAD; SCC; SCLC	<i>In vitro</i> ; <i>In vivo</i>	NT	Zong et al.; Zhou et al.; Winter et al. ^{48–50}
	ZBC260	JQ-1	CRBN ligand	BRD2, BRD3, BRD4	LUAD; SCC; SCLC	<i>In vitro</i> ; <i>In vivo</i>	H1975 xenograft; lung PDX	Zong et al.; Zhou et al.; Winter et al. ^{48–50}
	CFT-2718	JQ-1 analog	CRBN ligand	BRD4	SCLC	<i>In vitro</i> ; <i>In vivo</i>	SCLC PDX	Zong et al. ⁴⁸ , Sun et al. ⁵¹
	QCA570	QCA276	CRBN ligand	BRD2, BRD3, BRD4	LUAD; LSCC	<i>In vitro</i> ; <i>In vivo</i>	HCC827/AR xenograft	Qin et al. ⁵² ; Liu et al. ⁵³
SHP2	SHP2-D26	Compound 5	VHL ligand	SHP2	LUAD; LSCC	<i>In vitro</i> ; <i>In vivo</i>	PC9 and PC9/AR xenografts	Wang et al. ⁵⁴ ; Deng et al. ⁵⁵
FAK	D-PROTAC	Defactinib	VHL ligand	FAK	LUAD	<i>In vitro</i> ; <i>In vivo</i>	A427 xenograft	Liu et al. ⁵⁶
	PROTAC B5	Compound 2	CRBN ligand	FAK	LUAD	<i>In vitro</i>	NT	Sun et al. ⁵⁷
	PROTAC-3	Defactinib	VHL ligand	FAK	NT	<i>In vitro</i>	NT	Cromm et al. ⁵⁸
STAT3	SD-36	SI-109	CRBN ligand	STAT3	NT	<i>In vitro</i> ; <i>in vivo</i>	NT	Bai et al. ⁵⁹
BCL-XL	DT2216	ABT263	VHL ligand	BCL-XL	LUAD; SCLC	<i>In vitro</i> ; <i>In vivo</i>	H358 and H146 xenografts	Khan et al. ^{60,61}
BCL-XL/2	753b	ABT263	VHL ligand	BCL-XL/2	SCLC	<i>In vitro</i> ; <i>In vivo</i>	H146 xenograft (unpublished)	Lv et al. ⁶²

CRBN, cereblon; LSCC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; NT, not tested; PC9/AR and HCC827/AR, osimertinib-resistant LUAD cell lines; PDX, patient-derived xenograft; SCC, small cell lung cancer; VHL, von Hippel-Lindau.

KRAS is difficult to target due to the lack of deep binding pockets, high affinity to GTP and the abundant level of cellular GTP.^{80,81} The recent breakthrough involves the development of potent covalent KRAS^{G12C} inhibitors, leading to the first FDA-approved agent, sotorasib to treat patients with KRAS^{G12C}-mutated NSCLC in 2021.⁸² Adagrasib (MRTX849) is another oral, highly selective, covalent small molecule inhibitor of KRAS^{G12C} and has demonstrated clinical efficacy against KRAS^{G12C}-mutated NSCLC with an acceptable safety profile in phase 1 clinical trial.⁸³ However, similar to other targeted therapies, tumor cells acquire resistance to KRAS^{G12C} inhibition through multiple mechanisms, including selection of new KRAS mutations such as G12D/R/V/W, G13D, Q61H, R68S, H95D/Q/R, Y96C, and amplification of the KRAS^{G12C} allele as well as other KRAS-independent mechanisms such as MET amplification and activating mutations in NRAS, BRAF, MAP2K1, and RET.⁸⁴

KRAS PROTACs have been developed to overcome KRAS^{G12C} resistance and to target other KRAS mutants. The first KRAS PROTAC targeting KRAS^{G12C} was reported by the Gray group in 2020.³¹ The PROTACs used ARS-1620 that binds to KRAS^{G12C}, and thalidomide derivatives, thus targeting KRAS G12C mutant to CRBN E3-ligase for degradation. However, the lead degrader XY-4-88 failed to degrade endogenous KRAS^{G12C}, despite it being able to degrade GFP-KRAS fusion proteins. Subsequently, the Crews group designed VHL-based PROTACs using MRTX849 as the covalent KRAS^{G12C} warhead.³² Their lead molecule LC-2 induced KRAS degradation and impaired the downstream MAPK signaling, as demonstrated by reduced p-ERK level in several human KRAS^{G12C}-mutated lung cancer cell lines. However, this PROTAC did not induce better antiproliferative effects as compared with MRTX849, which is likely due to its covalent irreversible nature that compromises the catalytic mechanism of action typically associated with PROTACs. To reinstate the substoichiometric activity of PROTACs, the Lu group developed a reversible-covalent PROTAC that efficiently targeted endogenous KRAS^{G12C} degradation in KRAS^{G12C}-mutated H358 and H23 lung cancer cells.³³ To date, no KRAS PROTACs have been evaluated *in vivo*. Another strategy to indirectly treat KRAS-driven cancer is through targeted degradation of Son of Sevenless Homologue 1 (SOS1), the primary guanine nucleotide exchange factor for regulating the GDP-GTP cycle of KRAS. The Xu group developed the first-in-class agonist-based SOS1 PROTACs by connecting a VHL ligand to a SOS1 agonist.³⁴ They identified a compound that induced SOS1 degradation, displayed superior antiproliferative activity to the agonist itself in various KRAS-driven cancer cells, and showed antitumor potency against human lung cancer H358 xenograft models. Therefore, targeted SOS1 degradation represents a promising therapeutic strategy for overcoming KRAS-driven cancers.

ALK-targeting PROTACs

ALK fusion oncogenes are detected in about 3% to 5% of NSCLC, occurring in non or former smokers, and those of a younger age.^{85,86} EML4-ALK fusion is the most important ALK fusion and a validated oncogenic driver. ALK TKIs are first-line treatment in patients with ALK-rearranged lung cancer. To date, three generations

of ALK inhibitors have been approved by the FDA.⁶⁷ Second-generation ALK TKIs, such as alectinib and brigatinib, are preferred over first-generation ALK TKI crizotinib. Alectinib also showed improved outcomes in the treatment of brain metastases as compared with crizotinib. NSCLC develops resistance to ALK TKIs through acquiring secondary mutations within the ALK tyrosine kinase domain, amplification of the ALK fusion gene or activation of bypass signaling pathways.⁶⁷ Lorlatinib, a third-generation ALK TKI, is used for those patients who progress on previous generations. It has activity against most known ALK inhibitor resistance mutations such as G1202R. However, patients can also develop resistance to lorlatinib.⁶⁷

Currently, the PROTAC technology is being used to develop ALK degraders.^{35–39,41,42} In 2018, the Gray group reported the first ALK-targeting PROTAC molecules by conjugating known pyrimidine-based ALK inhibitors, TAE684 or LDK378, and the cereblon ligand pomalidomide.³⁵ They showed that one PROTAC, Compound 9, degraded ALK in EML4-ALK fusion-positive H3122 lung cancer cells at nanomolar concentrations and had antiproliferative activity *in vitro*; yet the compound non-specifically degraded other kinases as well. In the same year, the Hwang group reported a VHL-recruiting, ceritinib-based ALK PROTAC molecule, TD-004, which induced ALK degradation and blocked the proliferation of fusion-positive lung cancer cell line H3122 and lymphoma cell line SU-DHL-1.⁶⁶ Importantly, this PROTAC inhibited the growth of H3122 xenograft tumors with no significant effect on mouse body weight. In 2020, the Wei group reported a light-activated ALK-targeting PROTAC molecule.³⁷ When exposed to UV light irradiation, the photosensitive “cage” group on pomalidomide was removed from the PROTAC, allowing it to bind and degrade ALK proteins. In 2021, three new PROTACs with oral bioavailability, specific ALK degradation, and/or *in vivo* antitumor activities in a mouse model were reported from the Jiang, Li, and Xu groups.^{38,41,42} These PROTAC compounds are promising therapeutics that could delay the emergence of resistance mutations to improve patient outcomes.

Other PROTACs relevant to lung cancer

Many PROTACs currently under preclinical or clinical development for other protein targets or other cancer types have the potential to be used for lung cancer. BRAF mutations account for about 4% of NSCLC and the currently developed BRAF-targeting PROTACs^{43–45} could be evaluated in BRAF-mutated NSCLCs. The bromodomain and extraterminal domain (BET) proteins BRD2, BRD3, and BRD4 are epigenetic readers and critically regulate gene expression. High levels of BRD4 were associated with NSCLC progression, which is consistent with the finding that BRD4 PROTACs blocked 3D lung cancer cell growth *in vitro*.⁴⁶ Multiple BRD4 or pan-BRD PROTACs have been developed that showed promising antitumor efficacy in the xenograft tumors from EGFRi-resistant cell lines and lung cancer PDXs.^{46–53} The protein tyrosine phosphatase SHP2 is frequently activated in lung cancer to maintain a fully activating oncogenic signaling and thus serves as an attractive therapeutic target. A potent SHP2 PROTAC, D26, was developed⁵⁴ and shown to have therapeutic efficacy alone, or in combination with the third-generation EGFR

inhibitor, osimertinib, in blocking osimertinib-resistant lung cancer cells.⁵⁵ Focal adhesion kinase (FAK) is overexpressed in many types of tumors, including lung cancer. FAK-targeting PROTAC was reported to have antiproliferative effects in lung cancer cells *in vitro* or antitumor activity in KRAS mutant NSCLC A427 xenograft model.^{56–58} Up-regulation of total or phosphorylated STAT3 was associated with poor prognosis in patients with NSCLCs and thus STAT3 is an attractive therapeutic target.⁸⁷ The Wang group developed a potent and selective STAT3 PROTAC, which can be evaluated in lung cancer.⁵⁹ BCL-XL is a key antiapoptotic protein in the Bcl-2 family and its up-regulation is widely observed in human lung cancer cells. The Zhou and Zheng group developed BCL-XL PROTAC degrader DT2216,⁸⁵ which is currently in phase I clinical trials. They recently reported that DT2216 synergized with the FDA-approved KRAS^{G12C} inhibitor sotorasib to block tumor growth in preclinical models of KRAS^{G12C}-mutated cancers.⁶¹ This study thus identified a potential effective combination therapy for patients with KRAS^{G12C}-mutated NSCLC, although the safety of this combination requires further evaluation. Since many SCLC cells depend on BCL-XL and BCL-2, DT2216 in combination with ABT-199 (a BCL-2 selective SMI) was tested, which showed antitumor effect in an SCLC H146 xenograft model.⁶⁰ Furthermore, they recently developed a BCL-XL/BCL-2 dual degrader,⁶² which had antitumor efficacy as a single agent in SCLC xenograft tumors (unpublished). Collectively, PROTAC development has opened up new therapeutic opportunities in addition to alternative approaches to conventional chemical inhibition.

CONCLUSION AND PERSPECTIVE: PROTAC THERAPY AS A PROMISING TARGETED STRATEGY FOR LUNG CANCER TREATMENT

PROTACs have the potential to be developed as new lung cancer therapeutics. Currently, two PROTACs, ARV-471, which targets the estrogen receptor (ER), and ARV-110, which targets the androgen receptor (AR), have been advanced into phase II clinical trials.^{12–15} Multiple other PROTACs are currently in phase I clinical trials, including NX-2127 (a Bruton Tyrosine Kinase, BTK degrader), KT474 (an IRAK4 degrader), and DT2216 (a BCL-XL degrader). Significant progress has been made in the development of PROTAC molecules for validated therapeutic targets in NSCLC such as EGFR, KRAS, and ALK fusions, leading to promising compounds with antitumor efficacy in preclinical models. These important advances demonstrate the feasibility and exciting potential of PROTACs as new therapeutics to improve lung cancer outcomes.

Lung cancer represents a highly heterogeneous collection of tumors, each carrying different combinations of genetic alterations for which there are only limited SMIs in the current clinical toolbox. In addition, even when highly specific SMIs are available, the invariable selection of tumor cells with acquired resistance is an ongoing challenge for patients and their clinicians. Unlike SMIs, PROTACs have the capacity to degrade oncogenic proteins, including resistant mutants, with very low intracellular concentration requirements due to the ability of single molecules to undergo multiple rounds of degradation.

Thus, PROTACs have the potential to overcome current treatment barriers in lung cancer and fulfill the promise of personalized therapy.

However, to expand the therapeutic landscape to more patients, it is crucial that ligands for the full oncogenic landscape of POI targets are available for development. PROTAC research has been focused, to date, on those cancer targets with available small molecule compounds, such as previous FDA-approved molecules and those that have entered clinical trials. It will be important to expand and evaluate small molecules that can be used to bind and degrade conventionally “undruggable” oncogenic proteins. In addition, Schneider et al created a library with a systematic assessment of >1,000 potential drug targets to guide researchers in further PROTAC development.⁸⁸

It is also important to design PROTACs to recruit an E3 ligase that is minimally expressed in normal tissues to reduce toxicity. There are currently more than 600 E3 ligases, and research is warranted to identify tumor-specific or tissue-specific E3 ligases and their ligands that could be used for PROTACs. Event-driven PROTAC molecules do not necessarily need high-affinity ligands for activity, thus, low micromolar E3 recruiting elements identified from screens could be linked to already existing high-affinity ligands to develop next-generation PROTACs. Further efforts should also be made to control PROTAC activity in degrading proteins on demand. Novel approaches have been explored in activating PROTACs with light (opto-PROTAC)³⁷ or in response to tumor hypoxic environment.⁸⁹

PROTACs as a class have poor physicochemical properties that pose challenges in drug development. For example, PROTACs may have poor penetration to the disease tissue. Thus, for the treatment of lung cancer, it is critical to evaluate the tissue distribution of the PROTACs to the lung. In addition, PROTACs are known to have pharmacodynamic (PD) effects beyond their PK, which would require the use of proper PD biomarkers to determine dosing regimen. Since the PROTAC technology is new, more work is also needed to understand how to integrate these investigational agents with other standard anti-cancer therapeutics to optimize clinical benefit. New biomarker assays designed to monitor PROTAC activity both *in vitro* and *in vivo* using lung cancer xenograft models and immunocompetent model models are needed in preclinical phases. More importantly, careful evaluations for PK and PD, toxicity profiles, and efficacy in human clinical studies are needed in order to move this new class of drugs to patient treatment.

For the past two decades, lung cancer has been at the forefront of molecular oncology therapeutics. New preclinical and early clinical development testing of an expanding portfolio of PROTAC candidates will continue the promise of personalized and targeted therapy to improve the outcome of patients with advanced lung cancer.

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AUTHOR CONTRIBUTIONS

L.W. and F.J.K. designed the manuscript. J.W.L. drafted the manuscript and prepared the figure. L.W., F.J.K., G.Z., and J.W.L. edited the manuscript. All authors approved the final version of the manuscript.

DECLARATION OF INTERESTS

G.Z. is co-founder of and has equity in Dialectic Therapeutics, which develops BCL-XL/2 PROTACs as cancer therapeutics.

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