Cover page

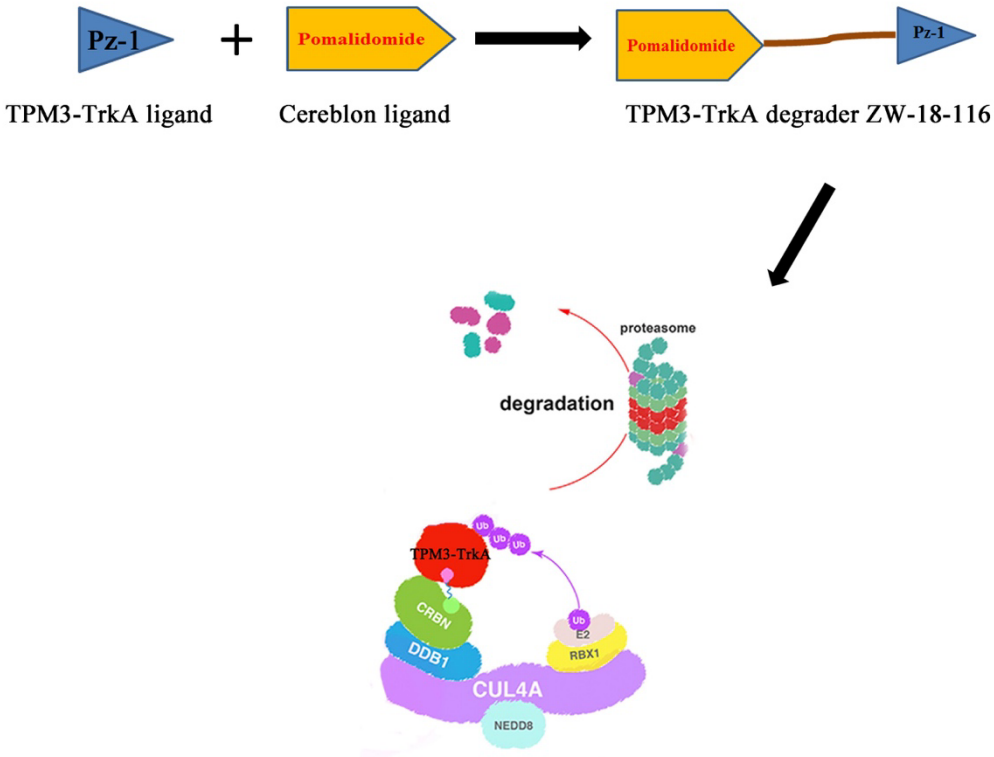
UNIVERSITY OF NAPLES FEDERICO II

DOCTORATE IN
MOLECULAR MEDICINE AND MEDICAL BIOTECHNOLOGY

XXXIV CYCLE

Lingzhi Zhang

Identification of Proteolysis Targeting Chimeras for TPM3-TrkA oncoprotein



2018-2022

Title page

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Identification of Proteolysis Targeting Chimeras for TPM3-TrkA oncoprotein

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List of abbreviations used

Abrreviations	Meaning
ALK	Anaplastic lymphoma kinase
BDNF	Brain-derived neurotrophic factor
BTK	Bruton's tyrosine kinase
CHX	Cycloheximide
CML	Chronic myeloid leukemia
CRBN	Cereblon
CRC	Colorectal cancer
CRLs	Cullin Ring ligases
DMSO	Dimethyl sulfoxide
EGFR	Epidermal growth factor receptor
FDA	Food and Drug Administration
IMiDs	Immunomodulatory drugs
NB	Neuroblastoma
NGF	Nerve growth factor
NHLs	non-Hodgkin lymphomas

NSCLC	Non-small cell lung cancer
NTRK 1/2/3	Neurotrophic tropomyosin receptor kinase 1/2/3
NTs	Neurotrophins
ORR	Objective response rate
POI	Protein of interest
PROTAC	Proteolysis-targeting chimeras
PTC	Papillary thyroid cancer
RING	Really interesting new gene
TKI	Tyrosine kinase inhibitor
TRK A/B/C	Tropomyosin receptor kinase A/B/C
Ub	Ubiquitin
VEGFR	Vascular endothelial growth factor receptor
VHL	Von Hippel-Lindau

Abstract

Tropomyosin receptor kinase A (TrkA) is a transmembrane tyrosine kinase receptor encoded by the neurotrophic tyrosine kinase receptor 1 (*NTRK1*) gene. *NTRK1* fusions have been identified in several cancers, including colorectal, lung, and thyroid carcinomas. The aim of this study was to explore a new strategy to target TrkA fusion proteins, based on Proteolysis Targeting Chimeras (PROTACs).

In conclusion, our study indicated that PROTAC-mediated degradation is a novel and efficient tool to intercept Trk kinase oncogenic signaling as an innovative strategy to treat cancer.

1. Introduction

Targeted cancer therapy

Huge progresses in targeted cancer therapy have been achieved during the past several decades, and several of targeted anticancer drugs have been approved for the treatment of different types of cancer. There are two main types of targeted therapies: small molecule inhibitors or monoclonal antibodies (mAb) (Baudino, 2015). Small molecule inhibitors are natural or artificial compounds of small molecular weight that interfere with the function of specific proteins; such molecules have a wide application in various types of diseases, including infectious diseases, autoimmune, as well as malignant disorders. Among the multiple targets for small molecule inhibitors, protein kinases have attractive characteristics. Protein kinases are enzymes involved in transferring the γ phosphate group from ATP to tyrosine, serine or threonine residues of specific proteins while phosphatases remove phosphate groups from proteins. These two enzymes modulate numerous activities of proteins within cells in accordance with external stimuli (Coussens et al., 1986). The human kinome is composed of 538 protein kinases (Manning et al., 2002). They control metabolism, transcription, cell division, motility, programmed cell death, and nervous system function (Alonso et al., 2004; Manning et al., 2002; Mueller, Mack and Teusch, 2005). Dysfunctional protein kinases have been found to associate with multiple human malignancies due to chromosomal rearrangement or point mutations (Bardelli et al., 2003; Grosveld and Hagemeijer, 1991). Small tyrosine kinase inhibitors (TKIs) block oncogenic kinase activity by inhibiting the transfer of the γ phosphate group from ATP to protein residues, resulting in the discontinuation of the downstream signaling pathways (Zhong et al., 2021). Generally, small molecule protein kinase inhibitors are divided into three groups: type I, type II, and type III kinase inhibitors. Type I inhibitors were defined as “small molecule that binds to the active conformation (Asp-Phe-Gly (DFG)-in) of a kinase in the ATP pocket”; the type II inhibitors were defined as “small molecule that binds to the inactive conformation (Asp-Phe-Gly (DFG)-out) of a kinase”, and type III inhibitors were defined as “non-ATP competitive inhibitor” or allosteric inhibitor (Dar and Shokat, 2011). Most of the current TKIs are multi-targeted and restrain a variety of protein kinases in a non-specific manner (Table 1). Gleevec (imatinib mesylate) is the first TKI approved by Food and Drug Administration (FDA) to treat patients with chronic myeloid leukemia (CML) driven by the *BCR-ABL* translocation (Cohen et al., 2002). Other approved anti-neoplastic small molecule inhibitors include: gefitinib and erlotinib as inhibitors of epidermal growth factor receptor (EGFR) for non-small cell lung cancer (NSCLC); lapatinib as inhibitor of EGFR/ERBB2 for breast cancer; sorafenib as inhibitor

of vascular endothelial growth factor receptor 2 (VEGFR2) kinase for renal cancer (Yap and Workman, 2012). Table 1 lists the complete compendium of FDA approved small molecules protein kinase inhibitors (Table 1).

Table 1. *FDA approved small molecule protein kinase inhibitors*

Drug (Code) Trade name	Primary targets[a]	Therapeutic indications[b]	Year approved
Abemaciclib (LY2835219) Verzenio	CDK4/6	Combination therapy with an (i) aromatase inhibitor or with (ii) fulvestrant or as a monotherapy for breast cancers	2017
Acalabrutinib (ACP-196) Calquence	BTK	Mantle cell lymphomas, CLL, SLL	2017
Afatinib (BIBW 2992) Tovok	ErbB1/2/4	NSCLC	2013
Alectinib (CH5424802) Alecensa	ALK, RET	ALK-positive NSCLC	2015
Axitinib (AG-013736) Inlyta	VEGFR1/ 2/3	RCC	2012
Avapritinib (BLU285) Ayvakit	PDGFRα	GIST with *PDGFRα* exon 18 mutations	2020
Baricitinib (LY 3009104) Olumiant	JAK1/2	Rheumatoid arthritis	2018
Binimetinib (MEK162) Mektovi	MEK1/2	Combination therapy with encorafenib for $BRAF^{V600E/K}$ melanomas	2018
Bosutinib (SKI-606)	BCR-Abl	CML	2012
Brigatinib (AP 26113) Alunbrig	ALK	ALK-positive NSCLC	2017
Cabozantinib (BMS-907351) Cometriq	RET, VEGFR2	Medullary thyroid cancers, RCC, HCC	2012
Capmatinib (INC-280) Tabrecta	c-MET	NSCLC with *MET* exon 14 skipping mutations	2020
Ceritinib (LDK378) Zykadia	ALK	ALK-positive NSCLC resistant to crizotinib	2014
Cobimetinib (GDC-0973) Cotellic	MEK1/2	BRAFV600E/K melanomas in combination with vemurafenib	2015

Crizotinib (PF 2341066) Xalkori	ALK, ROS1	ALK or ROS1-postive NSCLC	2011
Dabrafenib (GSK2118436) Tafinlar	B-Raf	BRAFV600E/K melanomas, BRAFV600E NSCLC, BRAFV600E anaplastic thyroid cancers	2013
Dacomitinib (PF-00299804) Visimpro	EGFR	EGFR-mutant NSCLC	2018
Dasatinib (BMS-354825) Sprycell	BCR-Abl	CML	2006
Encorafenib (LGX818) Braftovi	B-Raf	Combination therapy with binimetinib for BRAFV600E/K melanomas	2018
Entrectinib (RXDX-101) Rozlytrek	TrkA/B/C, ROS1	Solid tumors with NTRK fusion proteins, ROS1-positive NSCLC	2019
Erdafitinib (JNJ-42756493) Balversa	FGFR1/2/3/4	Urothelial bladder cancers	2019
Erlotinib (OSI-774) Tarceva	EGFR	NSCLC, pancreatic cancers	2004
Everolimus (RAD001) Afinitor	FKBP12/Mtor	HER2-negative breast cancers, pancreatic neuroendocrine tumors, RCC, angiomyolipomas, subependymal giant cell astrocytomas	2009
Fedratinib (TG101348) Inrebic	JAK2	Myelofibrosis	2019
Fostamatinib (R788) Tavalisse	Syk	Chronic immune thrombocytopenia	2018
Gefitinib (ZD1839) Iressa	EGFR	NSCLC	2003
Gilteritinib (ASP2215) Xospata	Flt3	AML	2018
Ibrutinib (PCI-32765) Imbruvica	BTK	CLL, mantle cell lymphomas, marginal zone lymphomas, graft vs. host disease	2013

Imatinib (STI571) Gleevec	BCR-Abl	Ph+ CML or ALL, aggressive systemic mastocytosis, chronic eosinophilic leukemias, dermatofibrosarcoma protuberans, hypereosinophilic syndrome, GIST, myelodysplastic/ myeloproliferative disease	2001
Lapatinib (GW572016) Tykerb	EGFR, ErbB2/ HER2	HER2-positive breast cancers	2007
Larotrectinib (LOXO-101) Vitrakvi	TrkA/B/C	Solid tumors with NTRK fusion proteins	2018
Lenvatinib (AK175809) Lenvima	VEGFR, RET	Differentiated thyroid cancers	2015
Lorlatinib (PF-06463922) Lorbrena	ALK	ALK-positive NSCLC	2018
Midostaurin (CPG 41251) Rydapt	Flt3	AML, mastocytosis, mast cell leukemias	2017
Neratinib (HKI-272) Nerlynx	ErbB2/HER2	HER2-positive breast cancers	2017
Netarsudil (AR11324) Rhopressa	ROCK1/2	Glaucoma	2018
Nilotinib (AMN107) Tasigna Ph+	BCR-Abl	CML	2007
Nintedanib (BIBF-1120) Vargatef	FGFR1/2/ 3	Idiopathic pulmonary fibrosis	2014
Osimertinib (AZD-9292) Tagrisso	EGFR T970M	NSCLC	2015
Palbociclib (PD-0332991) Ibrance	CDK4/6	Estrogen receptor- and HER2-positive breast cancers	2015
Pazopanib (GW786034) Votrient	VEGFR1/ 2/3	RCC, soft tissue sarcomas	2009
Pexidartinib (PLX3397) Turalio	CSF1R	Tenosynovial giant cell tumors	2019
Pemigatinib (INCB054828) Pemazyre	FGFR2	Advanced cholangiocarcinoma with a FGFR2 fusion or rearrangement	2020

Pralsetinib (Blu-667) Gavreto	RET	RET-fusion (i) NSCLC, (ii) medullary thyroid cancer, (iii)	2020
Ponatinib (AP 24534) Iclusig	BCR-Abl	Ph+ CML or ALL	2012
Regorafenib (GSK2118436) Tafinlar	VEGFR1/ 2/3	Colorectal cancers	2012
R406	Syk	Chronic immune thrombocytopenia	2018
Ribociclib (LEE011) Kisqali	CDK4/6	Combination therapy with an aromatase inhibitor for breast cancers	2017
Ripretinib (DCC-2618) Qinlock	Kit, PDGFRα	Fourth-line treatment for GIST	2020
Ruxolitinib (INCB-018424) Jakafi	JAK1/2/3, Tyk	Myelofibrosis, polycythemia vera	2011
Selpercatinib (CEGM9YBNG) Retevmo		RET fusion NSCLC and thyroid cancers and *RET* mutant medullary thyroid cancers	
Selumetinib (AZD6224) Koselugo	MEK1/2	Neurofibromatosis type I	2020
Sirolimus (AY 22989) Rapamycin	FKBP12/ mTOR	Kidney transplants, lymphangioleiomyomatosi	1999
Sorafenib (BAY 43–9006) Nexavar	VEGFR1/ 2/3	HCC, RCC, thyroid cancer (differentiated)	2005
Sunitinib (SU11248) Sutent	VEGFR2	GIST, pancreatic neuroendocrine tumors, RCC	2006
Temsirolimus (CCI-779) Torisel	FKBP12/ mTOR	RCC	2007
Tofacitinib (CP-690550) Tasocitinib	JAK3	Rheumatoid arthritis	2012
Vandetanib (ZD6474) Zactima	VEGFR2	Medullary thyroid cancers	2011
Vemurafenib (PLX-4032) Zelboraf	B-Raf	$BRAF^{V600E}$ melanomas	2011
Trametinib (GSK1120212) Mekinist	MEK1/2	$BRAF^{V600E/K}$ melanomas, $BRAF^{V600E}$ NSCLC	2013

Adapted from (R. Roskoski, Jr., 2021)

a. *Although many of these drugs are multikinase inhibitors, only the primary therapeutic targets are given here.*
b. *ALL, acute lymphoblastic leukemias; AML, acute myelogenous leukemias; CLL, chronic lymphocytic leukemias; CML, chronic myelogenous leukemias; ErbB2/HER2, human epidermal growth factor receptor-2; GIST, gastrointestinal stromal tumors; HCC, hepatocellular carcinomas; NSCLC, non-small cell lung cancers; Ph+, Philadelphia chromosome positive; RCC, renal cell carcinomas; SLL, small lymphocytic leukemias.*

Another way to specifically target a deregulated protein during tumorigenesis consists in using target specific monoclonal antibodies. Many monoclonal antibodies (mAbs) have been created to target various types of proteins including receptor tyrosine kinase (RTK) (Table 2). Current clinically available mAbs generally exploit a combination of mechanisms to promote cytotoxic effects in tumor cells. Thus, binding of mAbs on cell surface promotes both a cell-dependent cytotoxicity (ADCC) as well as a complement-dependent cytotoxicity (CDC). In addition, they can mediate target internalization, altering its half-life and intracellular signal transduction (Zahavi and Weiner, 2020). The humanized mAb trastuzumab (Herceptin®) directed against human epidermal growth factor receptor 2 (HER2) is the paradigm of monoclonal antibodies used for therapy of solid tumors. (Carter et al., 1992). Another important example is Avastin (bevacizumab, Genentech), a monoclonal antibody that by targeting vascular endothelial growth factor (VEGF) inhibits cancer-associated angiogenesis (Kim et al., 2014). Avastin was initially used to treat patients with metastatic colorectal cancer in combination with chemotherapy. Its applications nowadays include also metastatic breast cancer, NSCLC, glioblastoma, renal cell carcinoma, ovarian cancer and cervical cancer (Garcia et al., 2020). Rituxan (rituximab, Genentech) is a mAb that targets the B-lymphocyte antigen CD20 and causes cytotoxicity in both cancerous and normal B-cells (Plosker and Figgitt, 2003). It was approved by the FDA in association with other chemotherapeutic agents to treat patients with various CD20-expressing lymphoid malignancies, including indolent and aggressive forms of B-cell non-Hodgkin lymphoma (Jazirehi and Bonavida, 2005). Other example of mAbs used in cancer treatment is listed in Table 2.

Table 2. *FDA approved mAbs for oncology*

Generic name (trade name)	Target	Indication	Year of FDA approval
Trastuzumab (Herceptin)	HER2/neu	Breast cancer	1998

Rituximab (Rituxan)	CD20	Lymphoma	1997
Cetuximab (Erbitux)	EGF receptor	Colorectal cancer	2004
Bevacizumab (Avastin)	VEGF	Colorectal, lung cancers	2004
Alemtuzumab (Campath-1H)	CD52	Chronic lymphocytic leukemia	2001

Adapted from Adams and Weiner, 2005

Differently from the conventional chemotherapeutics that inhibit cell proliferation of cancer as well as normal cells, causing off-target toxicities and side effects, targeted cancer therapeutics suppress cancer proliferation and progression through interacting with their targets that are indispensable for the cancer cells (yet not for normal cells). Accordingly, targeted cancer therapy should be more effective against cancer cells without being toxic for normal cells. However, it still poses undesired side effects due to a lack of selectivity issues. Either the drug itself is not completely specific for the target, which results in off-target effects on other proteins, or the target is not cancer-specific, which interferes with physiological functions in normal cells (Li and Song, 2020). Another important issue in the usage of small molecule-based or mAbs based therapies in the clinic is that cancer cells can develop resistance. This resistance can result from: the mutation in the interacting region between the drug and the target; the insensitivity to the drug by the overexpression of target or activation of an alternative signaling pathway that sustain cancer proliferation and progression (Li and Song, 2020). In addition, many oncogenic targets are intracellular proteins that cannot be blocked by mAbs. Considering these limitations, efforts have been made to develop a new and more effective strategy to target cancer drivers consisting in target degradation.

Proteolysis Targeting Chimeras technology (PROTACs)

Proteolysis Targeting Chimeras (PROTACs) represent a strategy used to target protein of interest (POI) to degradation by the ubiquitin-proteasome pathway. Structurally, PROTAC is a bi-functional molecule consisting of a ligand that binds to the POI, connected via a linker to another warhead able to recruit E3 ubiquitin ligases (Raina and Crews, 2017) (Figure 1 a). By hijacking the E3 ubiquitin ligase, PROTAC brings the POI to a close proximity with E3 ligase, thus leading POI poly-ubiquitination and degradation by the proteasome (Lai and Crews, 2017) (Figure 1 a). Meanwhile, the PROTAC can be recycled for subsequent rounds of degradation (Neklesa, Winkler, and Crews, 2017). Despite the fact that more than 600 E3 ubiquitin ligases are present in human

genome, only few of them have been exploited to design the PROTACs, including Skp1-Cullin-F box complex (SCF protein complex) (Deshaies, 1999), Von Hippel-Lindau tumor suppressor (VHL) (Kaelin, 2005), Cereblon (CRBN) (Shi and Chen, 2017), inhibitor of apoptosis proteins (IAPs) (Silke and Meier, 2013), and mouse double minute 2 homolog (MDM2) (Iwakuma and Lozano, 2003) (Figure 1 b). The polyubiquitination of a target protein usually involves three enzymes: an E1 activating enzyme, an E2 conjugating enzyme, and an E3 ubiquitin ligase. E1 enzymes activate the small protein ubiquitin (Ub) by binding it to one of its own cysteines, and then the activated Ub is transferred to the E2 ubiquitin-conjugating enzymes. Here again, the C-terminus of Ub is bound to the enzyme through a cysteine. Finally, E3 ubiquitin ligase enzymes transfer Ub to its final target protein. The C-terminus of Ub is covalently linked to the NH_2 side chain of a lysine in the target protein (Pickart, 2001; Weissman, 2001). Then, several ubiquitins are added in order to form a polyubiquitin chain that promotes target recognition by proteasome and later its degradation.

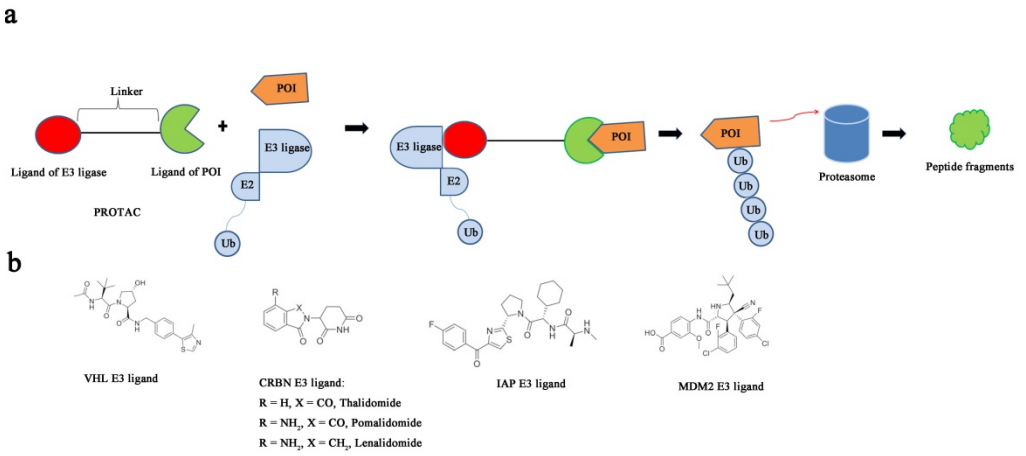


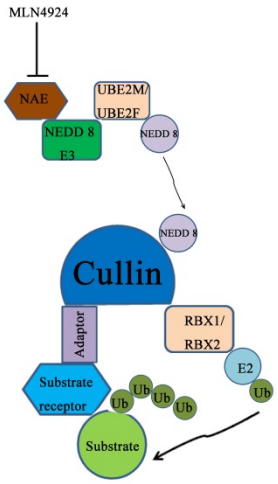
Figure 1. *a. Mechanism of PROTAC-mediated protein degradation. b. Representative small-molecule ligands of E3s for the development of PROTAC. Adapted from Li and Song 2020*

Structural basis of PROTAC-mediated protein degradation

Although there are more than 600 E3 ubiquitin ligase genes in human genome (George et al., 2018), only a tiny portion of them are utilized in PROTACs. E3 ubiquitin ligases are important for the selectivity towards target proteins (Hershko, 1983). E3 ligases are mainly categorized into three groups based on their specific structural motif: RING (really interesting new gene)-type E3s, HECT (homologous to the E6-AP carboxyl terminus) domain-containing E3s, and RBR (RING between RING)-type E3s. RING type E3s are the largest family, which can be further divided into several subgroups; one of these, the

Cullin-based E3 subgroup, is one of the largest classes of E3s (Weber, Polo, and Maspero, 2019). Cullin proteins bind to a RING protein, either RBX1 (RING box 1) or RBX2 (RING box 2), through their C-terminal. The N-terminal of Cullin proteins then binds to an adaptor and to a substrate receptor. Cells depend on Cullin-RING E3 ligases (CRLs)-mediated protein ubiquitination to eradicate several proteins (Lydeard, Schulman, and Harper, 2013), and the E3 ligase activity of CRLs is strictly controlled by the modification of Cullin via the ubiquitin-like protein NEDD8 (Duda et al., 2008) (Figure 2). Attachment of NEDD8 generates conformational changes and imparts the ability to recruit the other components of the complex to Cullins, allowing the formation of the Cullin-RING E3 ubiquitin ligases (CRLs) (Petroski and Deshaies, 2005). In particular, in the unneddylated condition, the Cullin C-terminal domain forms a groove in which the RING domain of the RING protein is embedded (Zheng et al., 2002). In this conformation, the movements of the RING protein are restrained, setting the ubiquitin E2 enzyme away from the substrates (Zheng et al., 2002). In accordance with this, inactivation of neddylation of CRLs by MLN4924, a neddylation inhibitor, causes the accumulation of various CRLs E3 substrates (Brownell et al., 2010) (Figure 2). Several CRL E3 enzymes composed of different Cullins, adaptors and substrate receptors have been identified (Fig 2b). Cereblon (CRBN) is the substrate receptor of the Cullin 4A-RBX1-DDB1-CRBN E3 ubiquitin ligase, also known as $CRL4A^{CRBN}$ (Ito et al., 2010) (Figure 2b). It is also a target of thalidomide and its analogs (lenalidomide and pomalidomide), which are known as immunomodulatory drugs (IMiDs) (Singhal et al., 1999). Upon binding to the Cullin 4A E3 ligase complex, CRBN is able to recruit substrate proteins, resulting in their ubiquitination by the CRL^{CRBN} and subsequent degradation by proteasome (Fischer et al., 2014). As a result, IMiDs are explored in the design of the PROTAC molecules in which they serve as ligands to recruit the $CRL4A^{CRBN}$ complex.

a



b

Cullin	Adaptor	Substrate adaptor
Cullin 1	SKP1	F-box protein
Cullin 2	ELONGIN B/C	VHL-box protein
Cullin 3	–	BTB protein
Cullin 4A/B	DDB1	DCAF、CRBN

Figure 2. *The Cullin family. a. Structures of cullin-RING E3 ligases (CRLs). The Cullin proteins bind to a RING protein, either RBX1 (RING box 1) or RBX2 (RING box 2), through their C-terminal. The N-terminal of Cullin proteins bind to an adaptor and a substrate receptor. E3 ligase activity is controlled by neddylation by the attachment of NEDD8 to Cullins. b. Components of different CRLs. There are four adaptor proteins for the Cullin proteins: SKP1 for CUL1, ELONGIN B/C for CUL2, and DDB1 for CUL4A/4B, whereas CUL3 does not have an adaptor protein. The substrate receptors are also distinct for different Cullin proteins as indicated. Abbreviations: CUL, cullin; DCAF, DDB1- and CUL4-associated factor 1; DDB1, damage-specific DNA-binding protein 1; E2, ubiquitin conjugating enzyme; E3, ubiquitin ligase; FBXW8, F-box and WD repeat domain-containing 8; NAE, NEDD8-activating enzyme; NEDD8, NEDD8 ubiquitin-like modifier; RBX1/2, RING-box 1/2; SKP1, S-phase kinase-associated protein 1; UBE2M, ubiquitin-conjugating enzyme E2M; UBE2F, ubiquitin-conjugating enzyme E2F. Adapted from Lu et al., 2021*

With respect to PROTACs mechanism of action, it is necessary to form a stable ternary complex between E3 ubiquitin ligase, the PROTAC, and the target protein to enable an efficient polyubiquitination of the target protein (Gadd et al., 2017). Thus, it was demonstrated that the factor that best correlates with the degradation potency of a given substrate protein by PROTACs is the ability to form a stable ternary complex among target protein, PROTAC and recruited E3 ubiquitin ligase (Bondeson et al., 2018). In previous studies, it was proved that regardless of the binding affinities to the PROTAC, a CRBN-based PROTAC with a promiscuous kinase ligand showed different targeting degradation profiles due to different stability of the ternary complex (Huang et al., 2018). These studies imply that weak interaction between target protein and PROTAC can be compensated by a strong stability of ternary complex, leading to efficient

degradation of the protein of interest. Recently, the studies about the structure of the ternary complex between VHL (E3 ligase), BRD4 (target protein), and a BRD4-degrading PROTAC shed light on the details of E3 ligases/target protein interaction (Gadd et al., 2017). For example, interfaces mediated by this PROTAC between VHL and BRD4 were not formed between VHL and BRD2 (despite the high homology of BRD2 to BRD4), explaining the selectivity of such PROTAC for BRD4. Once these BRD4-specific interfaces were mutated to the corresponding residues in BRD2, both the ternary complex formation and degradation ability were lost. Additional studies pointed out that the length of the linker may facilitate the formation of the ternary complex between E3 ubiquitin ligase and target protein (Bondeson et al., 2018). It is also important to consider that events downstream of ubiquitination may determine the degradation of the target protein. Although it was found that there is a direct correlation between degradation rates and ubiquitination rates (Riching et al., 2018), another rate-limiting process for degradation by the 26S proteasome is unfolding of the protein target (Bard et al., 2019). The unfolded or disordered region of a protein serves as an initiation site for unfolding and subsequent proteolysis by the proteasome. Thereby, an inefficient unfolding might limit the ability of the proteasome to degrade a specific ubiquitinated target (Prakash et al., 2004).

During the last decades, PROTAC-mediated protein degradation has become a new trend in the fields of pharmaceutical and biological discoveries. In many respects, PROTAC technology equivalents small interfering RNA (siRNA). Yet the former allows ablation of a protein at a post-translational level instead of silencing the gene at a post-transcriptional level. The small-molecule PROTACs are characterized with easy delivery and quick biodistribution, indicating a great interest from the pharmaceutical industry. Given the aforementioned advantages, PROTAC strategy is marching into the clinic for numerous applications. ARV-110, which is an orally available PROTAC targeting androgen receptor (AR), has entered clinical phase II trial (Petrylak et al., 2020); ARV-471, which is also an orally available PROTAC molecule targeting Estrogen receptor (ER), has completed its clinical trial, and data strongly support its potential to be developed as a treatment for breast cancer (Snyder et al., 2021). Apart from these, many proteins ranging from transcriptional factors, such as Androgen receptor (Raina et al., 2016), BRD2/4 (Noblejas-López et al., 2019), to kinases, such as AKT (You et al., 2020), HER2, EGFR, c-Met (Burslem et al., 2018), BCR-ABL (Lai et al., 2016) have been successfully selected as potential targets for PROTACs.

PROTAC-mediated degradation of protein kinases

EGFR (HER1)

Epidermal growth factor receptor (EGFR), also known as HER1, is a major member of the erythroblastosis oncogene B (ErbB) family, which belongs to transmembrane glycoproteins with tyrosine kinase activity (Takeuchi and Ito, 2011). The EGFR protein family consists of four members: EGFR (ErbB1, HER1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4) (Schlessinger, 2000). EGFR is the receptor for the epithelial growth factor (EGF). Overexpression or mutations of EGFR enhance tumor growth, invasion, and metastasis (Normanno et al., 2006), and play an important role in various malignant tumors, such as glioblastoma, non-small-cell lung cancer (NSCLC), head and neck, breast, colorectal, ovarian, prostate, and pancreatic cancer (Graves et al., 2013; Takeuchi and Ito, 2011). Therefore, EGFR has been an attractive target for the development of anti-cancer drugs. The first-generation EGFR tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib and erlotinib, were the first inhibitors to be approved for the treatment of NSCLC (Mok et al., 2009; Shepherd et al., 2005). However, drug resistance appeared rapidly. It was demonstrated that the T790M "gatekeeper" mutation in EGFR was the most frequently identified mechanism of the resistance to the first generation EGFR-TKIs (Chong and Jänne, 2013). To overcome the resistance, the second- and third-generation EGFR-TKIs, such as afatinib and osimertinib were developed (Sos et al., 2010). Besides being effective against T790M mutant, osimertinib was able to mitigate the side effects of first and second-generation inhibitors, and it was approved by the FDA in 2015 for the treatment of NSCLC patients harboring $EGFR^{T790M}$ (Greig, 2016). A phase III clinical trial conducted in advanced NSCLC patients with EGFR mutation showed increased longer progression-free survival (36.8 months, 95% confidence interval (CI), 34.5 to 41.8) than the non-treated group (31.8 months, 95% CI, 26.6 to 36.0) (Ramalingam et al., 2019).

Despite great progresses, the clinical application of third-generation EGFR-TKIs inevitably lead to new acquired resistance, reducing clinical response. Such resistance was mainly caused by mutations selected by prolonged medication (Lebraud et al., 2016; Sakamoto et al., 2001). Craig M. Crews and his colleagues reported some EGFR PROTAC degraders based on the kinase inhibitors: lapatinib, gefitinib, and afatinib (Burslem et al., 2018). They used VHL E3 ligase ligands to promote VHL recruitment and EGFR polyubiquitination. Compound 1 (a, Figure 3) was synthesized based on lapatinib and was able to induce degradation of both EGFR and an exon-20 insertion mutant form of EGFR (Yasuda et al., 2013). Switching to a warhead based on gefitinib, compound 3 (b, Figure 3) was synthesized, and shown to degrade exon-19 deletion EGFR as well as the $EGFR^{L858R}$ variant, while leaving the WT EGFR intact. The EGFR

scavanger based on afatinib was capable of degrading the gefitinib-resistant double mutants (L858R/T790M) (Yasuda et al., 2013). As lapatinib binds also strongly to HER2, the ability of compound 1 (a, Figure 3) to degrade this additional target was explored. Overexpression of HER2 is found to be present in about 30% of patients with breast and ovarian cancer (Bacus et al., 1990), and in more than 60% of patients with inflammatory breast cancer (score 3+ with herceptest). In addition, HER2 overexpression was also shown in Wilm's tumor (50%), and bladder cancer (44%) (Tagliabue et al., 1998). By modifying the linker of the compound 1, while leaving VHL ligase and lapatinib intact, they synthesized one compound, which could simultaneously degrade both EGFR and HER2 (Yasuda et al., 2013).

Recently, Zhang and his group also designed a series of EGFR degraders (Zhang et al., 2020). Compound 2 (c, Figure 3) and compound 10 (d, Figure 3) induced EGFR degradation in HCC827 ($EGFR^{Del19}$) cells. In addition, they also arrested HCC827 cells in G1 phase or induced apoptosis. By conjugating osimertinib with lenalidomide via different linkers, He and his team synthesized a number of EGFR degraders (He et al., 2020). Compound 16c (e, Figure3) strongly degraded EGFR in PC9 ($EGFR^{Del19}$) and H1957 ($EGFR^{L858R/T790M}$) cells.

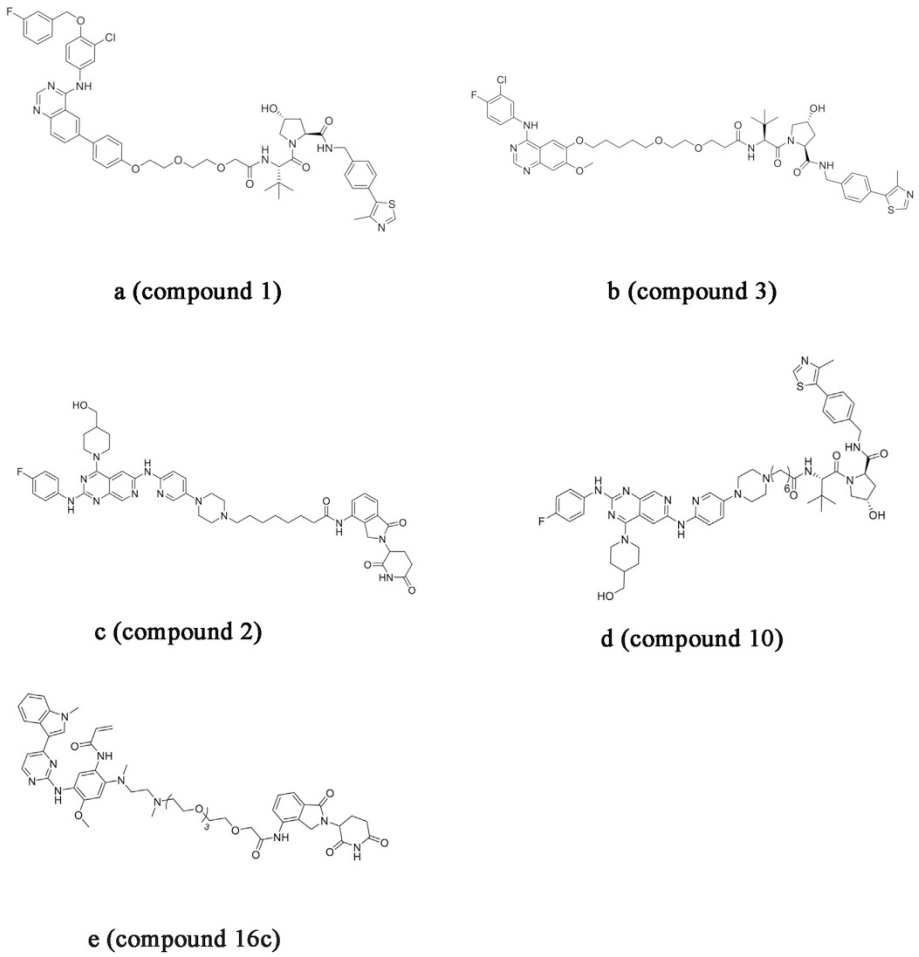


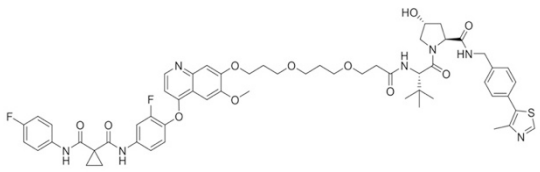
Figure 3. *EGFR PROTAC degraders*

c-Met

C-Met (mesenchymal-epithelial transition factor) is a receptor tyrosine kinase, which belongs to the MET family and is expressed on the surfaces of various cells (Birchmeier et al., 2003). Once bound to its ligand hepatocyte growth factor (also known as HGF or "scatter factor"), c-Met forms dimers and becomes auto-phosphorylated on tyrosine residues within kinase domain (Y1234 and Y1235) and on C-terminal docking domain (Y1313, Y1349, Y1356, and Y1365) (Organ and Tsao, 2011). Various protein adaptors, such as Gab1, Crk, Grb2, SHC and p85/PI3K can be recruited to the docking site, contributing to the activation of the downstream mitogenic pathways, such as RAS/MAPK or PI3K/AKT ones. Aberrant activation of c-Met can facilitate the development and progression of multiple cancers, including liver, kidney, lung, colon, breast, pancreatic, ovarian and prostate cancers. (Kimet et al., 2016; Szturz et al., 2017).

Even though c-Met inhibitors prevent the activation of MAPKs and AKT, the results from clinical trials for most of them were not fully satisfactory (Scagliotti et al., 2015; Sequist et al., 2011). Foretinib is an orally available multikinase inhibitor which targets c-Met, RON, Axl, and VEGFR and recently approved for treatment of a subset of renal cell carcinomas that display c-Met mutations (Eder et al., 2010).

Crew and his team reported the first c-Met PROTAC degrader, compound 7 (Figure 4), which incorporated foretinib as a c-Met recruiting element, with a VHL recruiting ligand (Figure 1) (Burslem et al., 2018). This c-Met PROTAC induced the degradation of c-Met in a dose- and time-dependent manner, promoting block of downstream signaling, such as Akt phosphorylation in a similar pattern.



1 (compound 7)

Figure 4. *c-Met degrader*

BCR-ABL

The translocation between the long arms of chromosome 9 and 22 gives rise to a shortened chromosome 22, commonly known as the Chromosome Philadelphia (Ph) (Figure 5). Chromosome Ph was identified in more than 90% of chronic myeloid leukemia (CML) patients (Nowell and Hungerford, 1961). The translocation results in the formation of the chimeric fusion gene BCR-ABL, which is the leading cause of the CML. Tyrosine kinase inhibitor (TKI) treatment is the current treatment of CML. Imatinib mesylate (IM, Gleevec), a BCR-ABL tyrosine kinase inhibitor has revolutionized the treatment of CML (Cohen et al., 2002), improving prognosis, response rate and overall survival in CML patients in comparison to previous therapeutics (Sacha, 2014). However, drug resistance inevitably occurred in patients receiving imatinib medication, mostly due to the mutations in the ABL kinase domain. The second-generation BCR-ABL TKIs (nilotinib and dasatinib) were able to circumvent some mutations and showed a good activity in clinical trials in patients resistant to imatinib therapy, but not in patients with the BCR-ABL T315I mutation (Hantschel, Rix, and Superti-Furga, 2008). Ponatinib is the third-generation of BCR-ABL TKI, which was approved to treat CML patients harboring BCR-ABL T315I mutation (Cortes et al., 2013).

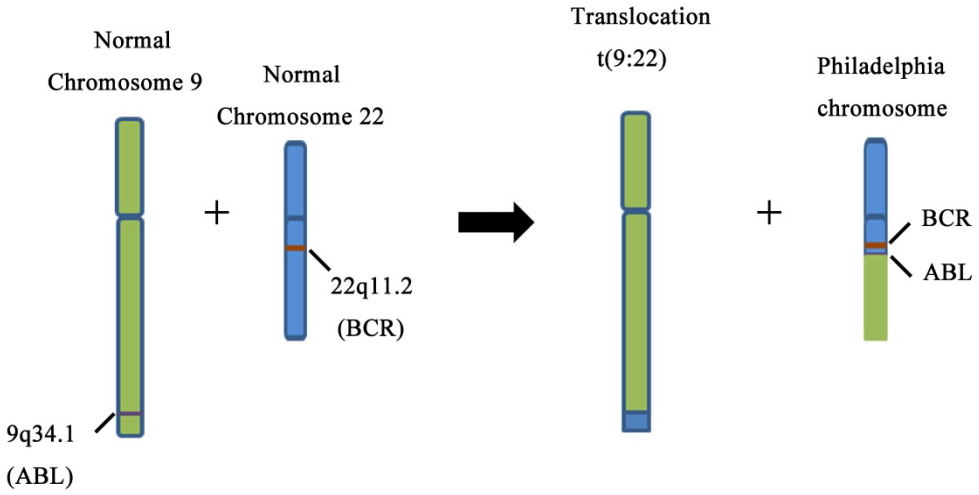


Figure 5. *Schematic of the Philadelphia chromosome formation*

The first BCR-ABL PROTAC degrader was developed by Crews's group. By incorporating three different BCR-ABL TKIs (imatinib, bosutinib and dasatinib) with either VHL or CRBN E3 ligase recruiting ligands, his team synthesized several BCR-ABL degraders composed of: imatinib-VHL-ligand; bosutinib-VHL/CRBN-ligand; dasatinib-VHL/CRBN ligand; (Lai et al., 2016). Dasatinib-VHL ligand (1, Figure 6) selectively induced the degradation of c-ABL (>65%, 1 μM, 24 h). Dasatinib-CRBN ligand (2, Figure 6) and bosutinib-CRBN ligand (3, Figure 6) were both able to degrade c-ABL and BCR-ABL proteins. Compared to untreated cells, dasatinib-CRBN ligand degraded more than 85% of c-ABL protein and over 60% of BCR-ABL protein with a concentration of 1 μM. Bosutinib-CRBN ligand exerted a more potent degradation, with a more than 90% decrease of c-ABL and over 80% degradation of BCR-ABL at a concentration of 2.5 μM.

In 2019, a group from China discovered another effective BCR-ABL degrader (Zhao et al., 2019). By connecting dasatinib with VHL E3 ligase ligand, the team synthesized several BCR-ABL degraders with varying lengths of the linkers. They optimized one BCR-ABL PROTAC degrader, SIAIS178 (4, Figure 6), which was able to degrade half of the protein after 4 h treatment with a concentration of 100 nM in K562 cell line. Consistent with the results from the K562 cells, *in vivo* study also confirmed that SIAIS178 degraded the BCR-ABL in K562 xenografed mice, reducing xenografts growth.

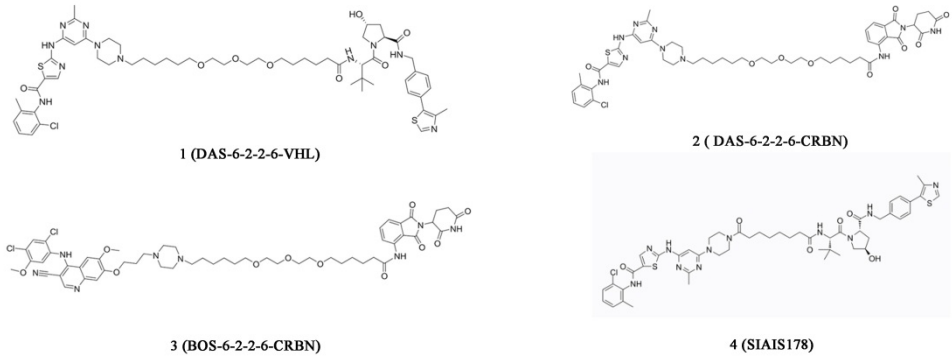


Figure 6. *Representatives of BCR-ABL PROTAC degraders*

Bruton's tyrosine kinase (BTK)

Bruton's agammaglobulinemia tyrosine kinase (BTK) is a non-receptor tyrosine kinase belonging to the Tec family of kinases (Gomez-Rodriguez et al., 2007; Smith et al., 2001). BTK is predominantly expressed in B lymphocytes, and it is very critical for B cell development, survival and differentiation (Tsukada et al., 1993; Vetrie et al., 1993). BTK-deficient B cell lymphocytes failed to reach mature state, leading to immunodeficiency (Pal Singh, Dammeijer, and Hendriks, 2018). Currently, a selective inhibitor, ibrutinib, has been approved for the treatment of mantle cell lymphoma (MCL), one of the subtypes of B-cell malignancies. MCL patients treated with ibrutinib developed drug resistance due to the selection of the BTK C481S mutation (Mohamed et al., 2009).

Aiming to develop multi-kinase PROTAC degraders, Huang and his colleagues synthesized a PROTAC by conjugating a well-characterized ALK inhibitor TAE684 with a cereblon-binding ligand (TL12-186) (1, Figure 7) (Huang et al., 2018). TAE684 dramatically downregulated the expression of 14 out of 7,599 identified proteins in acute myeloid leukaemia MOLM-14 cells. Notably, 12 out of 14 degradable proteins were kinases, which include AAK1, AURKA, AURKB, BTK, CDK12, FLT3, FES, FER, PTK2, PTK2B, ULK1, and TEC. In addition, they also synthesized two selective BTK PROTAC degraders: CJH-005-067 (2, Figure 7) and DD-04-015 (3, Figure 7), which effectively degrade BTK after 4-hr treatment in MOLM-14 cells. These two BTK degraders showed similar anti-proliferative potency in TMD8 cells, which is a diffuse large B cell lymphoma cell that is highly dependent on BTK activity for survival. Moreover, DD-04-015 exhibited prolonged pharmacodynamics effects compared to its parent tyrosine kinase inhibitor RN486, demonstrating a new pharmacological approach to target BTK in cancer.

In an attempt to explore PROTAC molecule to induce degradation of BTK, Rao and his group fused ibrutinib or spebrutinib (ligands of BTK) to pomalidomide (CRBN ligand) or RG-7112 (MDM2 ligand) (Sun et al., 2018). They synthesized several BTK-targeting PROTAC molecules, and found that CRBN-based PROTACs were usually more efficient in inducing the degradation of the target than MDM2-based ones. Among the CRBN-based PROTACs, P13I (4, Figure 7) with the conjugation of ibrutinib and pomalidomide demonstrated the most potent effect in degrading BTK. P13I showed high efficacy in degrading both wild type BTK and BTK C481S mutant form, with DC_{50} at 9.2 nM and 30 nM, respectively. Furthermore, P13I successfully circumvented the off-target effects, which occurred to the parent tyrosine kinase inhibitor ibrutinib.

Meanwhile, Crew and his team also developed a potent BTK PROTAC degrader, MT-802, a compound resulting from the conjugation of ibrutinib and pomalidomide (Buhimschi et al., 2018) (5, Figure 7). It induced potent degradation for both the wild type BTK and BTK C481S mutant form, with DC_{50} of 14.6 nM and 14.9 nM, respectively. As early as 4 h after treatment with MT-802, BTK protein was completely degraded at a concentration of 250 nM. By using KINOMEscan, a high-throughout, competition-based assay, they tested a panel of 468 kinases for binding to MT-802. They found that BTK was among the most strongly bound kinases by MT-802, indicating high specificity of MT-802 towards BTK.

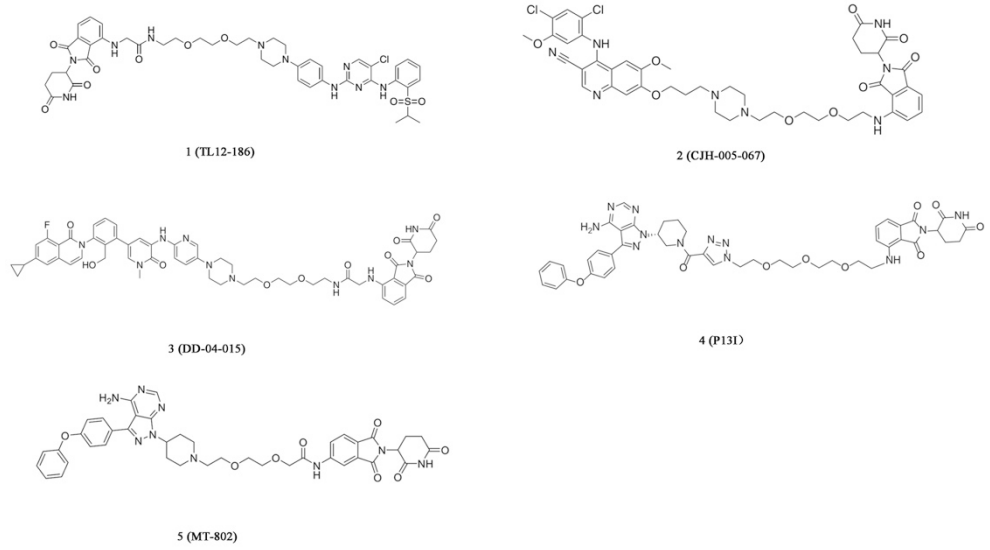


Figure 7. *Representatives of BTK PROTAC degraders*

ALK

The anaplastic lymphoma kinase (*ALK*) gene is a transmembrane tyrosine kinase receptor which belongs to insulin receptor (IR) superfamily (Morris et al., 1995). The most common genomic ALK aberrations in human cancer are chromosomal rearrangements, resulting in fusion proteins. Almost 30 different ALK fusion protein partners have now been described. Anaplastic large cell lymphomas (ALCL) is a T-cell non-Hodgkin's lymphoma, which displays ALK rearrangements in around 50% of all adult cases (Medeiros and Elenitoba-Johnson, 2007). The most frequent translocation in ALCL is *NPM1-ALK*, accounting for around 75%-80% of all ALK-positive ALCL (Pulford, Morris, and Turturro, 2004). Increased copy number and the presence of activating point mutations that result in kinase activation are also associated with oncogenic conversion of *ALK*. These oncogenic alterations are identified in multiple malignancies, including lung cancer, neuroblastoma, rhabdomyosarcoma, renal cell carcinoma, inflammatory myofibroblastic tumor, inflammatory breast cancer, and melanomas (Kelleher and McDermott, 2010; Webb et al., 2009). Crizotinib, ceritinib, and alectinib are first-generation ALK inhibitors approved by FDA to treat patients with metastatic ALK-positive NSCLC (Khozin et al., 2015; Larkins et al., 2016; Malik et al., 2014). Although crizotinib has shown excellent activity in patients with *ALK*-positive NSCLC, durable responses are rarely achieved due to the development of drug resistance.

Hwang and his co-workers designed ALK PROTAC degraders by conjugating ALK inhibitor certinib, via a linker to the VHL recruiting ligand (Kang et al., 2018). TD-004 (Figure 8) was able to degrade more than 90% NPM1-ALK protein in SU-DHL-1 cells (1 μM), which are anaplastic large cell lymphoma cell line with *NPM1-ALK* fusion. Consistently, the drug strongly inhibited proliferation of ALK fusion-positive cancer cells. TD-004 was also able to degrade echinoderm microtubule-associated protein-like 4 (EML4)-ALK fusion protein in a dose-dependent fashion in NCI-H3122 cell lines, which is an ALK positive NSCLC cell line. Moreover, it effectively suppressed the growth of tumors harboring ALK fusion protein *in vivo*, implying an alternative strategy to the treatment of ALK positive cancers.

Almost at the same time, Jin and his team also synthesized two ALK PROTAC degraders (MS4077 and MS4078) by linking ceritinib and pomalidomide with two different linkers (Zhang et al., 2018) (Figure 8). They tested degradation efficiency of them in SU-DHL-1 and NCI-H2228 cell lines, which are a human ALCL cell line expressing NPM1-ALK fusion protein, and a human NSCLC cell line expressing EML4-ALK fusion protein, respectively. Both ALK degraders could significantly reduce ALK fusion protein levels in these two cancer cell lines: SU-DHL-1 (MS4077: DC_{50}=3 ± 1 nM; MS4078:

DC_{50} =11 ± 2 nM) and NCIH2228 cells (MS4077: DC_{50}=34 ± 9 nM; MS4078: DC_{50}=59 ± 16 nM).

Nathanael S. Gray group synthesized two potent ALK PROTAC degraders (TL13-12 and TL13-112) by conjugating pomalidomide with two new ALK inhibitors TAE684 or LDK378 via linkers of different lengths (Powell et al., 2018) (Figure 8). These two compounds induced ALK degradation in various cancer cell lines, such as NSCLC cells H3122 (EML4-ALK), ALCL cells Karpas 299 (NPM-ALK), ALCL cells SU-DHL-1 (NPM-ALK) and NB cells (ALK F1174L or ALK R1275Q). In H3122, both compounds maintained potent degradation ability of ALK as long as 48 h after treatment, and they strongly inhibited the phosphorylation of the downstream transducer STAT3.

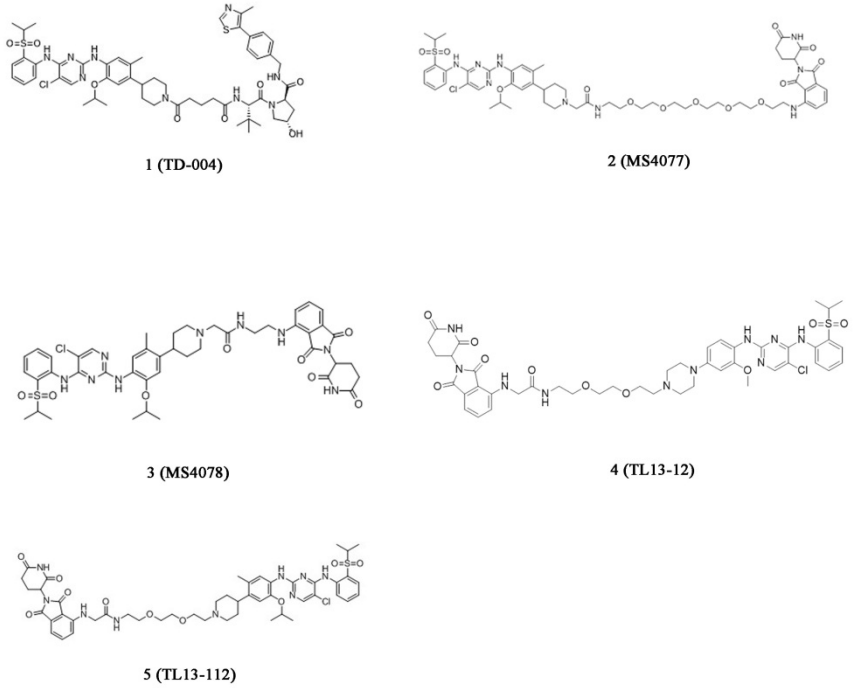


Figure 8. *Representatives of ALK PROTAC degraders*

Overview of neurotrophic tropomyosin receptor kinase (*NTRK*) gene family

Tropomyosin receptor kinase (Trk) family of receptors

Trk receptor family comprises three transmembrane proteins, including TrkA, TrkB, and TrkC. TrkA is encoded by *NTRK1* gene located on chromosome 1q21-22 (Weier et al., 1995). TrkB is encoded by *NTRK2* gene located on chromosome 9q22.1 (Nakagawara et al., 1995). TrkC is encoded by the *NTRK3* gene located on chromosome 15q25 (Valent, Danglot, and Bernheim, 1997). Each of the Trk receptor consists of an extracellular domain, a transmembrane

region and an intracellular region containing the tyrosine kinase domain. The extracellular domain includes two immunoglobulin-like (Ig1 and Ig2) and three leucine-rich 24-residue motifs (LRR1–3; Figure 9). The LRR1–3 motifs, flanked by two cysteine clusters (C1 and C2), are specific to Trk proteins and are not found in other receptor tyrosine kinases (Schlessinger, 2000; Snider, 1994).

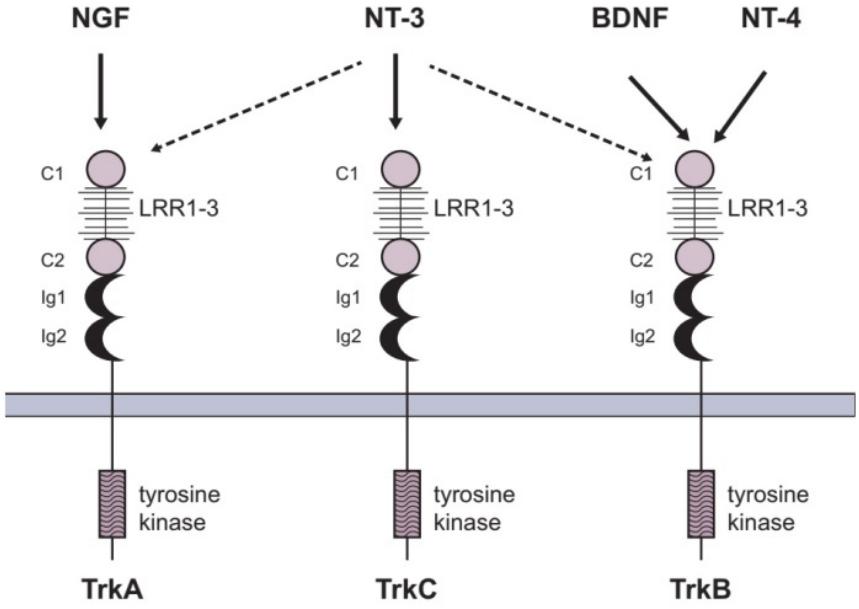


Figure 9. *Structure of Trk receptor family and interaction with ligand. Each neurotrophins display specific interactions with three receptors: nerve-growth factor (NGF) binds TrkA, brain-derived neurotrophic factor (BDNF) and neutrotrophin 3 (NT-4) binds TrkB, and NT-3 binds TrkC. NT-3 also has a lower binding affinity with TrkA and TrkB. C1/C2, cysteine-rich clusters; Ig1/Ig2, immunoglobulin-like domains; LRR1-3, leucine-rich repeats. Adapted from Skaper, 2008*

Trk receptors are expressed mainly in neuronal tissues, and play important roles in the development and function of the nervous system through their activation by neurotrophins (NTs) (Nakagawara, 2001). These specific ligands are known as nerve-growth factor (NGF) for TrkA, brain-derived neurotrophic factor (BDNF), and neurotrophin-4 (NT4) for TrkB, and NT-3 for TrkC, respectively (Huang and Reichardt, 2003). The ligand-receptor interaction results in the oligomerization of the receptor, which promotes autophosphorylation on specific tyrosine kinase residues and activation of the intracellular kinase domain. This event leads to the activation of various signaling transduction pathways that contribute to cell proliferation, differentiation and survival (Nakagawara, 2001) (Figure 10). The binding of

TrkA receptor by NGF causes the activation of the RAS/MAPK pathway, which leads to increased proliferation and differentiation through extracellular signal-regulated kinases (ERK) signaling (Amatu, Sartore-Bianchi, and Siena, 2016). The binding of BDNF or NT-4 to TrkB results in activation of the RAS-MAPK, PI3K and PLCγ pathway, leading to neuronal proliferation, differentiation and survival (Amatu et al., 2016). TrkC binding to NT-3 causes preferential activation of the PI3K/AKT pathway, inhibiting apoptosis and increasing cell survival (Amatu et al., 2016).

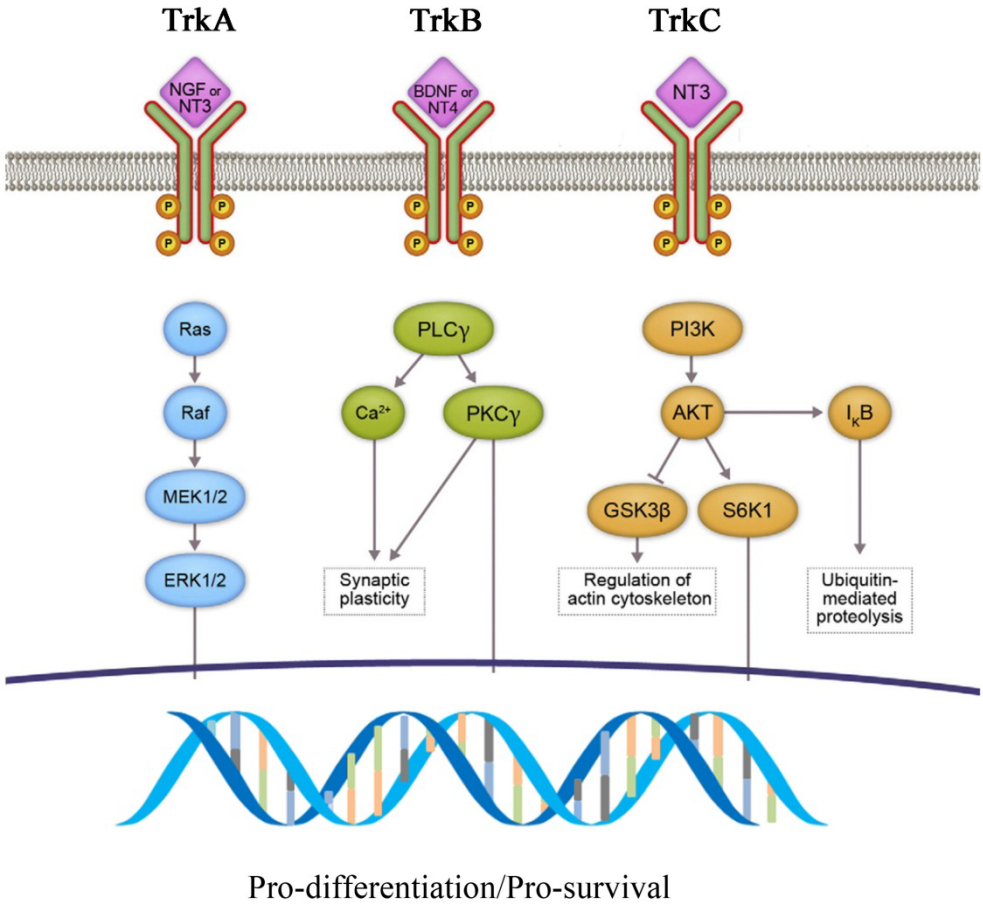


Figure 10. *Schematic view of Trk receptors signaling. AKT, v-akt murine thymoma viral oncogene homologue; ERK, extracellular signal-regulated kinase; GAB1, GRB2-associated-binding protein1; GRB2, growth factor receptor-bound protein2; IP3, inositol trisphosphate; MEK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinae C; PLC. Phospholipase C; RAF, rapidly accelerated fibrosarcoma kinase; RAS, rat sarcoma kinase. Adapted from Khotskaya et al., 2017*

Role in development and physiology

Trk receptors are predominantly expressed in neuronal tissues and play important roles in regulating the development and physiology of the nervous system (Barbacid et al., 1991; Reichardt, 2006). The activation of Trk receptors by neurotrophins has various effects on neuronal events, such as proliferation and survival, axonal and dendritic growth and modifications of synaptic functions (Amatu et al., 2019; Huang and Reichardt, 2003). Electrical activity, Ca^{2+} influx stimulate Trk receptors move from intracellular vesicles to neuron cell surface by exocytosis, allowing binding to neurotrophins and neuronal responses (Du et al., 2000; Meyer-Franke et al., 1998). As an example, several studies indicated that BDNF and NT3 produce rapid increase in synaptic strength in nerve muscle synapses, as well as increase in excitatory post-synaptic currents in hippocampal neurons (Bolton, Pittman, and Lo, 2000; Jacobi et al., 2009; Kang and Schuman, 1995). In summary, most survival-promoting and differentiation-promoting responses to neurotrophins require the presence of a Trk receptor on a neuron. Trk receptors and their respective neurotrophins have also been implicated in non-neuronal tissues including vessels, immune system, and ovaries (Coppola et al., 2004; Dissen et al., 1996; Kermani and Hempstead, 2007). Loss of function mutations in TrkA are involved in the pathogenesis of congenital insensitivity to pain with anhidrosis (CIPA), an autosomal recessive disorder characterized by a lack of pain sensation and anhidrosis (Mardy et al., 2001). Loss-of-function mutations in TrkB result in loss of appetite control and severe obesity (Xu and Xie, 2016). Mutations in TrkC are associated with susceptibility to congenital heart defects (Werner et al., 2014).

NTRK gene alterations in cancer

NTRK overexpression and splice variants

Trk overexpression has been implicated in a variety of cancers, including breast cancer (Lagadec et al., 2009), colon cancer (Zhang et al., 2010), bladder cancer (Lai, Chiu, and Huang, 2010), pancreatic cancer (Sclabas et al., 2005), and melanoma (Pasini et al., 2015). In breast cancer model, TrkA overexpression promoted cell growth, migration and invasion in MDA-MB-321 cells. Moreover, TrkA overexpression enhanced tumor growth, angiogenesis and metastasis of xenografted breast cancer cells in immunodeficient mice (Lagadec et al., 2009). TrkA amplification was also validated as a potential hotspot associated with tumor progression in multiple melanoma, correlating with worse clinical outcome (Pasini et al., 2015). In colon tumors, high expression of TrkB was observed and overexpression of TrkB was closely correlated with lymphatic vessel density and metastasis. TrkB inhibition by siRNA increased the apoptotic

rates *in vitro*, while the numbers of proliferative and invasive cells were decreased (Yu et al., 2010).

Aberrant activation of *NTRK1* due to the splice variants has been identified and characterized. In particular, the *NTRK1* splice variant TRKAIII and the in-frame deletion mutant (ΔTRKA) detected in neuroblastoma (NB) (Farina et al., 2018a; Farina et al., 2018b) and acute myeloid leukemia (Reuther et al., 2000), respectively, have been reported to be oncogenic. TRKAIII results in the omission of exons 6, 7, and 9, thus losing the functional Ig1 domain (Figure 11) (Arevalo et al., 2001). In contrast to fully spliced TrkA, which displays anti-tumour activity in NB and associates with good prognosis, TrkAIII was found to be associated with advanced stage metastatic disease, post therapeutic relapse and worse prognosis (Farina et al., 2018b). TrkAIII also induces tumorigenic transformation of NIH3T3 cells in nude mice (Tacconelli et al., 2004). ΔTRKA lacks 75 amino acids encompassing the ligand-binding Ig2 domain; this mutant led to constitutive activation of intracellular signaling pathways, transforming NIH3T3 fibroblasts *in vitro*, and enables tumor formation in nude mice (Reuther et al., 2000).

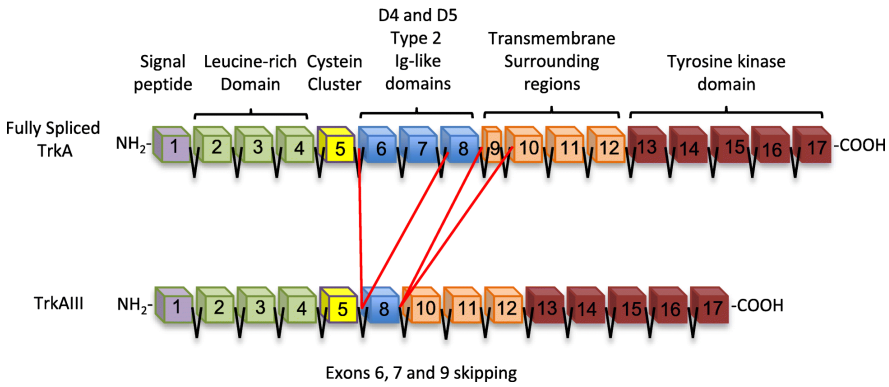


Figure 11. *Structure of the oncogenic neurotrophin receptor tropomyosin-related kinase variant, TrkAIII. Fully spliced TrkA has 17 exons, while exons 6, 7, and 9 are deleted in TrkAIII splice variant. Adapted from Farina et al., 2018b*

NTRK fusions in cancer

Fusions involving *NTRK1*, *NTRK2*, or *NTRK3* are the most common mechanisms of oncogenic Trk activation (Schram et al., 2017). Typically, *NTRK* oncogenic fusions arise from intra-chromosomal or inter-chromosomal rearrangements that juxtapose the 3' sequence of *NTRK* with 5' sequence of the partner gene (Figure 12 a). These fusions result in chimeric oncogenic proteins which are characterized by a ligand-independent constitutive activation of the tyrosine kinase domain (Cocco, Scaltriti, and Drilon, 2018). *NTRK* oncogenic conversion promotes the activation of receptor downstream signaling pathways

such as PI3K/AKT, RAS/MAPKs and PLC-gamma and promotes neoplastic transformation. Most of the partner genes contain an oligomerization domain, for instance, coiled-coil domain, zinc finger, or WD 40 repeat domain, and drive such ligand-independent dimerization of the tyrosine kinase domain of Trks (Coulier et al., 1989; Schram et al., 2017). However, it is worth noting that there exist cases where the dimerization domain of the fusion protein is missing. In these instances, the mechanism of the partner gene in promoting the ligand-independent activation of the tyrosine kinase remains unknown (Hechtman et al., 2017).

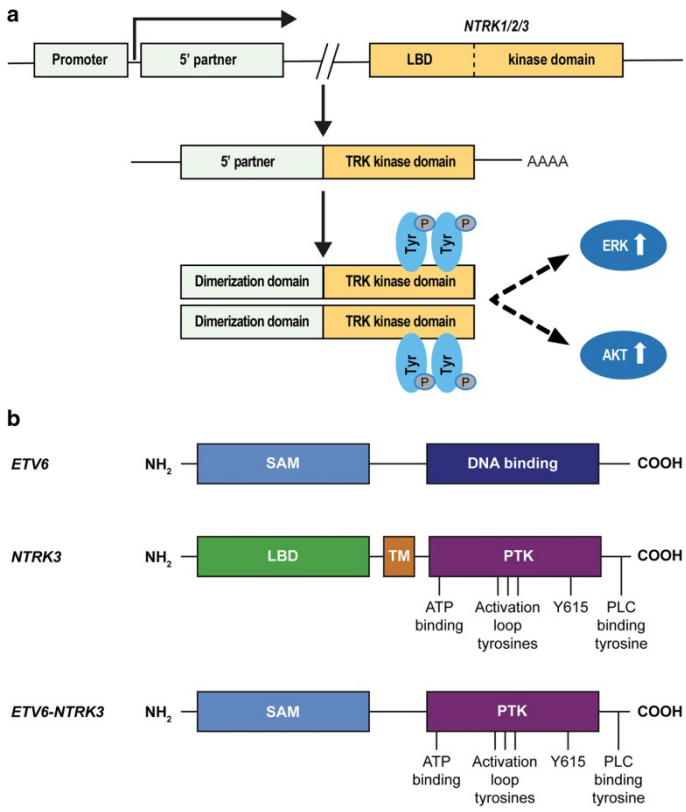


Figure 12. *Molecular mechanism of the NTRK oncogenic fusions. a. The structure of a representative NTRK fusion gene (ETV6-NTRK3). Most NTRK fusion partners are known to contain oligomerization domain (coiled coil, zinc finger, or WD domains,), conferring the ligand-independent activation of the tyrosine kinase. b. ETV6-NTRK3 gene fusion. LBD, ligand-binding domain; PTK, tyrosine kinase; TRK, tropomyosin receptor kinase; TM, transmembrane; SAM, sterile alpha motif. Adapted from Wong, Yip, and Sorensen, 2020*

NTRK rearrangements are fairly uncommon in cancers, being found at a low frequency, mostly ranging between 0.1% and 2% according to the tumor type

(Marino et al., 2020). At such low frequency, *NTRK* fusions can be detected in lung or pancreatic adenocarcinomas, head and neck squamous cell, bile duct, breast, colorectal, and renal cell carcinomas, melanomas, primary brain tumors of adulthood, and non-GIST soft-tissue sarcomas (Amatu et al., 2016; Drilon et al., 2018). On the contrary, some cancers such as papillary thyroid carcinomas (PTCs), spitzoid neoplasms, and certain pediatric gliomas harbor *NTRK* fusions with a high frequency (5-25%) (Amatu et al., 2016). High frequency of *NTRK* fusions has also been observed in some rare cancer types, such as secretory breast cancer and mammary analog secretory carcinoma (MASC) (Drilon et al., 2016; Krings et al., 2017) (Table 3). 100% of all MASC and 93% of secretory breast cancers exhibit the *ETV6-NTRK3* gene fusion (Vaishnavi, Le, and Doebele, 2015). *ETV6-NTRK3* fusion also occurs quite commonly in radiation-associated and pediatric PTCs, representing the prevalent gene rearrangement in this setting, whereas it is rare in adult sporadic thyroid cancer (Amatu et al., 2016). *ETV6-NTRK3* fusion has also been found in patient-derived acute promyelocytic leukaemia (APML) cell line AP-1060 (Chen et al., 2018); this is the only hematologic cancer model driven by endogenous *NTRK* translocation reported in the literature to date (Table 3).

In addition to *ETV6-NTRK3* fusion, *TPM3-NTRK1* is another frequently identified *NTRK* fusion. First discovered in 1986 through screening experiment performed with cDNA library derived from a colorectal cancer (CRC) biopsy (Martin-Zanca, Hughes, and Barbacid, 1986), *TPM3-NTRK1* was subsequently found in CRC, albeit with a rather low frequency (0.07%) (Clifton et al., 2018; Hechtman et al., 2017). Notably, in CRC, *NTRK* gene fusions seem to be associated with high mutation burden (Clifton et al., 2018), and microsatellite instability (MSI) (Kloosterman et al., 2017; Pietrantonio et al., 2017; Sartore-Bianchi et al., 2016). The prevalence of *NTRK1* rearrangement in PTC is around 12%, with the *TPM3-NTRK1* fusion being the most common event (Greco, Miranda, and Pierotti, 2010).

Table 3 summarizes the NTRK gene fusions and cancer in which they are present

Table 3. *NTRK gene fusion partners and corresponding cancers*

Cancer type	*NTRK1*	*NTRK2*	*NTRK3*
CRC	TPM3	—	—
	LMNA	—	—
	TPR	—	—
	SCYL3	—	—
NSCLC	CD74	—	—

	MPRIP	—	—
	SQSTM1	—	—
	—	TRIM24	—
GBM	ARHGEF2	—	—
	BCAN	—	—
	NFASC	—	—
	TPM3	—	—
	—	—	ET6
	—	VCL	—
	—	—	BTBD1
Pilocytic astrocytoma	—	NACC2	—
	—	QK1	—
Spitzoid melanoma	TP53	—	—
	TFG	—	—
	TPR	—	—
Thyroid carcinoma	PPL	—	—
Large cell neuroendocrine tumor (lung)	RFWD2	—	—
ICC	RABGAP1L	—	—
MASC	—	—	ETV6
SBC	—	—	ETV6
Infantile fibrosarcoma	LMNA	—	—
	—	—	ETV6
HNSCC	—	PAN3	—
Mesoblastic	—	—	ETV6
Low-grade glioma	—	AFAP1	—

Adapted from Jiang et al., 2021

Trk inhibitors

Several TKIs with diverse degrees of activity against TrkA, TrkB, and TrkC have been identified over the last years. Such inhibitors can be classified as multi-kinase inhibitors with activity against a variety of targets including Trks or as selective Trk inhibitors. The former includes merestinib, MGCD-516, PLX-7486, DS-6051b, DCC-2701, Cabozantinib and TSR-011 (Table 4). Entrectinib (RXDX-101) and larotrectinib (LOXO-101) are more selective drugs

and have been approved by FDA to treat solid tumors with *NTRK* fusion proteins (Roskoski, 2020). Thus, in 2018 the FDA granted accelerated approval of larotrectinib use in adults and children with solid tumors harboring an *NTRK* gene fusion. Later on, entrectinib, another potent inhibitor of TrkA, B, and C, ROS1 and ALK, was also approved by FDA to treat patients with *NTRK* fusion proteins (Marcus et al., 2021). By far, larotrectinib is the only highly selective pan-Trk inhibitor that has been developed for patients with Trk fusion proteins. Selitrectinib (LOXO-195) is a more recently identified Trk-selective inhibitor in clinical trial that will be discussed later (Table 4).

Table 4. *Trk inhibitors in clinical trials*

Trk inhibitor	Gene target	Company	Population	Disease
LOXO-195	*NTRK1/2/3*	Loxo oncology	Adult	Solid tumor
TSR-011	*NTRK1/2/3, ALK*	Tesaro	Adult	Solid tumor, lymphoma
PLX-7486	*NTRK1/2/3, CSF1R*	Plexxikon	Adult	Solid tumor
MGCD-516	*NTRK1/2/3, KDR, MET, KIT, PDGFR, DDR2*	Mirati	Adult	Solid tumor, urinary tract tumor, liposarcoma, NSCLC
DS-6051b	*NTRK1/2/3, ROS1*	DaiichiSankyo	Adult	Solid tumor
DCC-2701	*MET, NTRK, VEGFR2, TIE2*	Deciphera	Adult	Solid tumor
Cabozantinib	*NTRK2, RET, KIT,FLT1/3/4, MET, KDR, AXL*	Exelixis	Adult	NSCLC
Merestinib	*NTRK1/2/3, MET, AXL, ROS1, MKNK1/2, FLT3, TEK, DDR1/2*	Eli Lilly	Adult	Solid tumor

Abbreviations: ALK, anaplastic lymphoma kinase; CSF1R, colony-stimulating factor 1 receptor; DDR1/2, discoidin domain receptor tyrosine kinase 1/2; FLT1, fms related tyrosine kinase 1; KDR, kinase insert domain receptor; MKNK, mitogen-activated protein

kinase-interacting serine/threonine protein kinase; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; PDGFR, platelet-derived growth factor receptor; VEGFR2, vascular endothelial growth factor receptor 2; RET, rearranged during transfection; Adapted from Kheder and Hong, 2018

Acquired resistance to Trk inhibitors

As previously discussed, acquired resistance to TKI therapy can be attributed to on-target and off-target mechanisms. The on-target resistance is generally mediated by mutation of the kinase or overexpression. The off-target resistance, in turn, is mostly acquired via the activation of alternative pathways that can vicariate the function of the drug-targeted ones (Cocco et al., 2019). Also in the case of Trks, on-target resistance relies on *NTRKs* point mutations that, by sterically interfering with inhibitor binding, cause resistance to Trk inhibition (Drilon et al., 2017; Russo et al., 2016). Specifically, such mutations are located in three major regions of the kinase domain: the solvent front, the gatekeeper residue or the xDFG motif. Solvent front substitutions include $TrkA^{G595R}$ and $TrkC^{G623R}$ and they are paralogous to the ALK^{G1202R} and $ROS1^{G2032R}$, which are known to cause acquired resistance to TKIs in patients with *ALK*-rearranged and *ROS1*-rearranged lung cancers. Gatekeeper substitution includes $TrkA^{F589L}$, which is paralogous to ALK^{L1196M} and $ROS1^{L2026M}$ (Schram et al., 2017). xDFG motif substitutions include $TrkA^{G667C}$, and $TrkC^{G696A}$ that are paralogous to ALK^{G1269A} (Kim et al., 2013). However, *in vitro* kinase assays have indicated that $TrkA^{G595R}$ mutant showed increased affinity for ATP compared with that of the wild-type TrkA, implying that multiple factors might contribute to the resistant phenotype (Drilon et al., 2017). Interestingly, although the vast majority of the Trk mutants are resistant to many multi-kinase inhibitors, results in *in vitro* cell models demonstrated that some TKIs, including cabozantinib, ponatinib, foretinib, and nintedanib, retain strong inhibitory capacity against the G667C or G667S mutant (Fuse et al., 2017). The specific inhibitory profile of different TKI to different Trks mutants is listed in Figure 13.

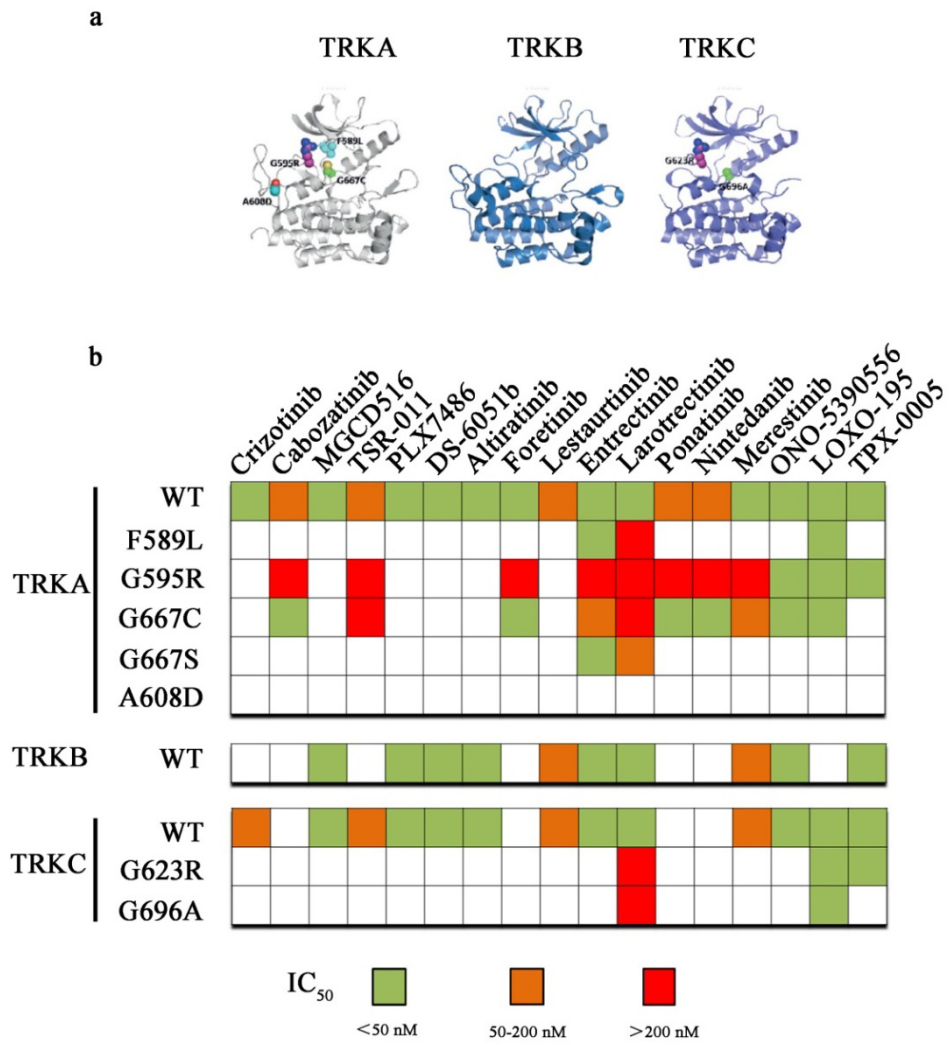


Figure 13. *Mechanisms of acquired resistance to Trk inhibition and profiles of Trk inhibitor activity. a. Structure of the tyrosine kinase domains of TrkA, TrkB, and TrkC showing amino acid substitutions resulting from somatic mutations in NTRK1, NTRK2, and NTRK3 fusions, respectively. b. A heat map of the half maximal inhibitory concentration (IC_{50}) values of kinase inhibitors is shown. Adapted from Cocco et al., 2018.*

*NTRK*s fusion-positive cancers can develop off-target resistance to tyrosine kinase inhibitor therapy. These mechanisms take the form of genomic alterations involving other receptor tyrosine kinases or downstream pathway mediators, including MET amplification, $BRAF^{V600E}$ mutation or mutations associated with KRAS (G12A, G12D). These resistant mutants have been found to be present in the circulating free DNA from patients with *NTRK* fusion cancers, who were previously treated with larotrectinib or entrectinib (Cocco et al., 2019). Combination therapy with RAF and MET inhibitors (dabrafenib and trametinib)

results in tumor regression. Triple therapy (larotrectinib, dabrafenib, and trametinib) was dramatically more effective at suppressing tumor growth (Cocco et al., 2019).

Next-generation Trk inhibitors

Fortunately, next-generation Trk inhibitors that overcome acquired resistance to first-generation TKIs are already in development. Specifically, LOX0-195 (Drilon et al., 2017), Repotrectinib/TPX-0005 (Drilon et al., 2018), and ONO-5390556 (Kozaki, Yoshizawa, Tsukamoto, Kato, and Kawabata, 2016) have shown strong activity against previously mentioned Trk mutants in the low nanomolar concentration range (Figure 13b). LOXO-195 is an orally available, highly potent, and selective Trk kinase inhibitor designed to overcome resistance mediated by acquired kinase domain mutations. One adult patient, with *LMNA-NTRK1* fusion-positive colorectal cancer and one pediatric patient, with *ETV6-NTRK3* fusion-positive recurrent infantile fibrosarcoma, both developed solvent mutation-mediated acquired resistance to larotrectinib. Consequently, LOXO-195 was administered accordingly, resulting in a rapid decrease in tumor burden, and showing a well-tolerated profile in both patients (Drilon et al., 2017). The safety and efficacy of LOXO-195 is being tested in a phase I/II trial involving patients with *NTRK*-rearranged cancers after prior treatment with other Trk inhibitors (NCT03215511). Recent preliminary data have shown that LOXO-195 has efficacy in patients with resistance to prior Trk inhibitors, with a 34% overall response rate (ORR) (Hyman et al., 2019).

Pz-1 is a newly identified type II tyrosine kinase inhibitor, which has shown strong inhibitory effect both on RET (rearranged-during transfection) oncogenic proteins and VEGFR2 with an IC_{50} value less than 1 nM (Frett et al., 2015). Pz-1 inhibited the formation of tumors induced by RET-transformed fibroblasts by restraining the phosphorylation of both RET and VEGFR2 in tumor tissue (Frett et al., 2015). Recently, our lab demonstrated that Pz-1 also showed a potent inhibitory effect for TrkA and its various mutants (Figure 14) (Moccia et al., 2021). In KM12 cells, which are a colon cancer-derived cell line endogenously harboring *TPM3-NTRK1* fusion, Pz-1 blocked TrkA phosphorylation and downstream signaling. In addition, Pz-1 maintained activity against TrkA G667C (which is resistant to entrectinib and larotrectinib) but not against the G595R mutant. Consistent with its potent inhibitory effect towards TrkA *in vitro*, Pz-1 was able to strongly hinder tumor growth of KM12 xenografts at the extremely low dosage of 0.3 mg/Kg (Moccia et al., 2021).

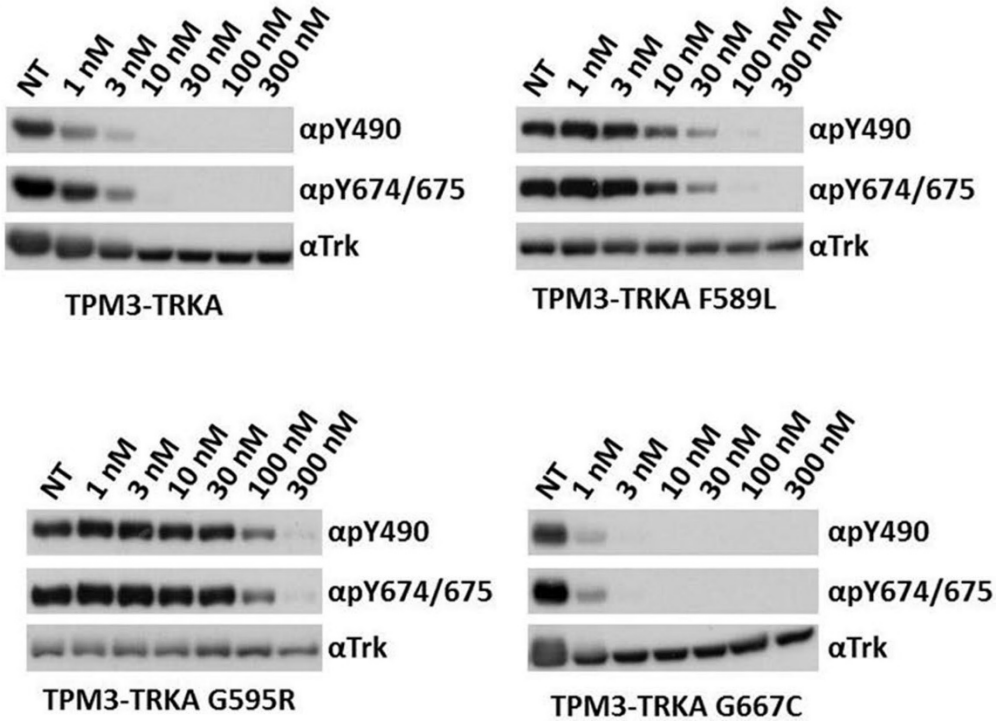


Figure 14. *Human HEK 293T cells were transfected with different TrkA mutants, and treated with Pz-1 for 2 h with the indicated concentrations. Total protein lysates were subjected to Western blotting with the indicated antibodies.*

2. Aim of the study

The aim of my PhD project was to expand the pharmacological tools to use as targeted therapy of *NTRK* fusions in cancer. Thereby, we explored the potentiality of targeting Trk oncogenic proteins using the PROTAC technology to promote their proteasome-dependent degradation.

3. Materials and Methods

3.1 Compound preparation

PROTAC compounds and Pz-1 were synthesized in the Li laboratory according to published procedures (Frett et al., 2015). All compounds were dissolved in DMSO, and were stored in -80 °C for future use. Final dosing solution was freshly prepared for each experiment from the stock solution; the equivalent amount of vehicle (DMSO) was used as control. Cycloheximide (C7698), pomalidomide (P0018) and MG132 (m8699) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The protein neddylation inhibitor MLN4924 (Pevonedistat) were purchased from Selleckchem (Huston, USA).

3.2 Cell lines

Human HEK 293 cells were from American Type Culture Collection (ATCC, Manassas, VA, USA) and were grown in EMEM (Lonza, Walkersville, MD USA) supplemented with 10% fetal bovine serum (FBS) (GIBCO, Thermo Fisher Scientific, Waltham, MA, USA). Human colorectal carcinoma (CRC) cell line KM12, kindly provided by A. Bardelli (Candiolo Cancer bInstitute, Torino, Italy), was cultured in RPMI-1640 medium (Life Technologies, Carlsbad, CA, USA) containing 10% FBS. All cell culture media were supplemented with 100 units/ml penicillin-streptomycin and 2 mM L-glutamine (Life Technologies). Cells were grown in a humidified incubator at 37℃ and with 5% CO_2. Authentication of the cell lines was performed at BMR genomics by DNA fingerprint (Aviano, Italy).

3.3 Cell transfection

Transient transfections of TPM3-TRKA and Cullin 4A mutant (NEDD8 truncted domain) vectors were carried out with Fugene according to the manufacturer's instructions (Promega, Milano, Italy). HEK 293 cells were allowed to adhere to the 100mm cell culture plate, and transfected with indicated plasmids when the 70%~80% of confluency was reached. 24 h after transfection, cells were divided into 60mm plate, and treated the next day.

3.4 Immunoblotting

Cells were all lysed in a buffer containing 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; pH 7.5), 1% (v/v) Triton X-100, 150 mM NaCl, 5 mM EGTA, 50 mM NaF, 20 mM sodium pyrophosphate, 1 mM sodium vanadate, 2 mM phenylmethanesulfonyl fluoride (PMSF), and 1 μg/mL aprotinin. Lysates were clarified at 12,7000 rpm for 30 min at 4℃ and protein concentration was evaluated using a modified Bradford assay (Bio-rad, Munich, Germany).

Samples were mixed with 5X loading buffer, which contains 10% SDS, 45% Glycerol, 312mM Tris-HCl, 5% β-mercaptoethanol and 0.05% bromophenol blue, and were denatured at 99℃ on a heat block for 10 min. Proteins were separated onto 8% polyacrylamide gel, and transferred to nitrocellulose membrane (4℃,overnight). The following day, the membranes were blocked at room temperature for 1 h with a buffer containing 5% BSA in TBS 1X (50 mM Tris-HCl pH 7.5, 150 mM NaCl), and then incubated with specific primary antibodies for 1 h at room temperature. After washing with TBST 1X (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1 % Tween 20), membranes were incubated with secondary antibodies for 35 min and then washed for 1 h. Immune complexes were visualized using Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific) and films were developed using a X-ray film developer.

3.5 Antibody

Antibodies for phospho-TrkA (Tyr 490) (#9141), phospho-TrkA (Tyr 674/675) (#4621), Trk (pan) (A7H6R) (#92991), phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (#4370), p44/42 MAPK (Erk1/2) (#4695) and phospho-SHC (Tyr317)(#2431) were from Cell Signaling Technology (Danvers, MA, USA). Anti-SHC (H-108) (#sc-1695) and Anti-Myc (9E10) (sc40) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-tublin (#T6074) was from Sigma-Aldrich. Secondary antibodies coupled to horseradish peroxidase were purchased from Bio-Rad.

3.6 Growth curves

KM12 cells were plated at a density of 10,000 cells/well in a 6-well plate, and allowed 24 hours to adhere to plate prior to treatment. The day after plating cells, the number of the cells for the zero point was counted; meanwhile, the medium was replaced with medium containing increasing concentration of compounds and refreshed every two days. KM12 cells were counted in triplicate every 2 days until the eighth day. The number of the cells obtained from the last day was used to evaluate growth inhibition.

3.7 Cycloheximide treatment

KM12 cells were plated in a 60mm dish with a 70%-80% confluency, allowed to adhere overnight. The next day, cells were pretreated with cycloheximide at 10 μg/ml for 20 minutes prior to adding selected compounds. Cells were harvested at the indicated timepoints.

3.8 Compound washout

KM12 cells were cultured in 60 mm plate, and were allowed to reach 70%~80% confluency. On the following day, KM12 cells were treated with selected compounds

3.9 Statistical analysis

IC_{50} doses for cell growth were calculated through a curve fitting analysis from last day of growth curves using the Prism software (Graphpad Software Inc). P values were statistically significant at < 0.5.

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5 References

Adams, G. P., and Weiner, L. M. (2005). Monoclonal antibody therapy of cancer. *Nat Biotechnol, 23*(9), 1147-1157.

Administration, US Food and Drug. (2018). FDA approves larotrectinib for solid tumors with NTRK gene fusions.

Alonso, A., Sasin, J., Bottini, N., Friedberg, I., Friedberg, I., Osterman, A., . . . Mustelin, T. (2004). Protein tyrosine phosphatases in the human genome. *Cell, 117*(6), 699-711.

Amatu, A., Sartore-Bianchi, A., Bencardino, K., Pizzutilo, E. G., Tosi, F., and Siena, S. (2019). Tropomyosin receptor kinase (TRK) biology and the role of NTRK gene fusions in cancer. *Annals of Oncology, 30*, viii5-viii15.

Amatu, A., Sartore-Bianchi, A., and Siena, S. (2016). NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. *ESMO Open, 1*(2), e000023.

An, J., Ponthier, C. M., Sack, R., Seebacher, J., Stadler, M. B., Donovan, K. A., and Fischer, E. S. (2017). pSILAC mass spectrometry reveals ZFP91 as IMiD-dependent substrate of the CRL4(CRBN) ubiquitin ligase. *Nat Commun, 8*, 15398.

Arevalo, J. C., Conde, B., Hempstead, B. I., Chao, M. V., Martín-Zanca, D., and Pérez, P. (2001). A novel mutation within the extracellular domain of TrkA causes constitutive receptor activation. *Oncogene, 20*(10), 1229-1234.

Bacus, S. S., Ruby, S. G., Weinberg, D. S., Chin, D., Ortiz, R., and Bacus, J. W. (1990). HER-2/neu oncogene expression and proliferation in breast cancers. *Am J Pathol, 137*(1), 103-111.

Bai, Longchuan, Zhou, Bing, Yang, Chao-Yie, Ji, Jiao, McEachern, Donna, Przybranowski, Sally, . . . Wang, Shaomeng. (2017). Targeted Degradation of BET Proteins in Triple-Negative Breast Cancer. *Cancer Research, 77*(9), 2476-2487.

Barbacid, M., Lamballe, F., Pulido, D., and Klein, R. (1991). The trk family of tyrosine protein kinase receptors. *Biochim Biophys Acta, 1072*(2-3), 115-127.

Bard, J. A. M., Bashore, C., Dong, K. C., and Martin, A. (2019). The 26S Proteasome Utilizes a Kinetic Gateway to Prioritize Substrate Degradation. *Cell, 177*(2), 286-298 e215.

Bardelli, A., Parsons, D. W., Silliman, N., Ptak, J., Szabo, S., Saha, S., . . . Velculescu, V. E. (2003). Mutational analysis of the tyrosine kinome in colorectal cancers. *Science, 300*(5621), 949.

Baudino, Troy A. (2015). targeted cancer therapy, the next generation of cancer treatment. *Current Drug Discovery Technologies, 12, No. 1*, 3-20.

Birchmeier, C., Birchmeier, W., Gherardi, E., and Vande Woude, G. F. (2003). Met, metastasis, motility and more. *Nat Rev Mol Cell Biol, 4*(12), 915-925.

Bolton, M. M., Pittman, A. J., and Lo, D. C. (2000). Brain-derived neurotrophic factor differentially regulates excitatory and inhibitory synaptic transmission in hippocampal cultures. *J Neurosci, 20*(9), 3221-3232.

Bondeson, D. P., Smith, B. E., Burslem, G. M., Buhimschi, A. D., Hines, J., Jaime-Figueroa, S., . . . Crews, C. M. (2018). Lessons in PROTAC Design from Selective Degradation with a Promiscuous Warhead. *Cell Chem Biol, 25*(1), 78-87 e75.

Brownell, J. E., Sintchak, M. D., Gavin, J. M., Liao, H., Bruzzese, F. J., Bump, N. J., . . . Dick, L. R. (2010). Substrate-assisted inhibition of ubiquitin-like protein-activating enzymes: the NEDD8 E1 inhibitor MLN4924 forms a NEDD8-AMP mimetic in situ. *Mol Cell, 37*(1), 102-111.

Buhimschi, A. D., Armstrong, H. A., Toure, M., Jaime-Figueroa, S., Chen, T. L., Lehman, A. M., . . . Crews, C. M. (2018). Targeting the C481S Ibrutinib-Resistance Mutation in Bruton's Tyrosine Kinase Using PROTAC-Mediated Degradation. *Biochemistry, 57*(26), 3564-3575.

Burslem, G. M., Smith, B. E., Lai, A. C., Jaime-Figueroa, S., McQuaid, D. C., Bondeson, D. P., . . . Crews, C. M. (2018). The Advantages of Targeted Protein Degradation Over Inhibition: An RTK Case Study. *Cell Chem Biol, 25*(1), 67-77 e63.

Carter, P., Presta, L., Gorman, C. M., Ridgway, J. B., Henner, D., Wong, W. L., . . . Shepard, H. M. (1992). Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc Natl Acad Sci U S A, 89*(10), 4285-4289.

Chamberlain, P. P., Lopez-Girona, A., Miller, K., Carmel, G., Pagarigan, B., Chie-Leon, B., . . . Cathers, B. E. (2014). Structure of the human Cereblon-DDB1-lenalidomide complex reveals basis for responsiveness to thalidomide analogs. *Nat Struct Mol Biol, 21*(9), 803-809.

Chen, S., Nagel, S., Schneider, B., Dai, H., Geffers, R., Kaufmann, M., . . . MacLeod, R. A. F. (2018). A new ETV6-NTRK3 cell line model reveals MALAT1 as a novel therapeutic target-a short report. *Cell Oncol (Dordr), 41*(1), 93-101.

Chong, C. R., and Jänne, P. A. (2013). The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat Med, 19*(11), 1389-1400.

Clifton, Katherine, Raymond, Victoria M., Dasari, A., Raghav, Kanwal Pratap Singh, Parseghian, Christine Megerdichian, Pereira, Allan Andresson Lima, . . . Morris, Van Karlyle. (2018). Actionable fusions in colorectal cancer using a cell-free circulating tumor DNA (ctDNA) assay. *Journal of Clinical Oncology, 36*(15_suppl), 3507-3507.

Cocco, Emiliano, Scaltriti, Maurizio, and Drilon, Alexander. (2018). NTRK fusion-positive cancers and TRK inhibitor therapy. *Nature Reviews Clinical Oncology, 15*(12), 731-747.

Cocco, Emiliano, Schram, Alison M., Kulick, Amanda, Misale, Sandra, Won, Helen H., Yaeger, Rona, . . . Scaltriti, Maurizio. (2019). Resistance to TRK inhibition mediated by convergent MAPK pathway activation. *Nature Medicine, 25*(9), 1422-1427.

Cohen, M. H., Williams, G., Johnson, J. R., Duan, J., Gobburu, J., Rahman, A., . . . Pazdur, R. (2002). Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. *Clin Cancer Res, 8*(5), 935-942.

Coppola, V., Barrick, C. A., Southon, E. A., Celeste, A., Wang, K., Chen, B., . . . Tessarollo, L. (2004). Ablation of TrkA function in the immune system causes B cell abnormalities. *Development, 131*(20), 5185-5195.

Cortes, J. E., Kim, D. W., Pinilla-Ibarz, J., le Coutre, P., Paquette, R., Chuah, C., . . . Kantarjian, H. (2013). A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med, 369*(19), 1783-1796.

Coulier, F., Martin-Zanca, D., Ernst, M., and Barbacid, M. (1989). Mechanism of activation of the human trk oncogene. *Mol Cell Biol, 9*(1), 15-23.

Coussens, L., Parker, P. J., Rhee, L., Yang-Feng, T. L., Chen, E., Waterfield, M. D., . . . Ullrich, A. (1986). Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science, 233*(4766), 859-866.

Dar, A. C., and Shokat, K. M. (2011). The evolution of protein kinase inhibitors from antagonists to agonists of cellular signaling. *Annu Rev Biochem, 80*, 769-795.

Deshaies, R. J. (1999). SCF and Cullin/Ring H2-based ubiquitin ligases. *Annu Rev Cell Dev Biol, 15*, 435-467.

Dissen, G. A., Hill, D. F., Costa, M. E., Les Dees, C. W., Lara, H. E., and Ojeda, S. R. (1996). A role for trkA nerve growth factor receptors in mammalian ovulation. *Endocrinology, 137*(1), 198-209.

Drilon, A., Laetsch, T. W., Kummar, S., DuBois, S. G., Lassen, U. N., Demetri, G. D., . . . Hyman, D. M. (2018). Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *N Engl J Med, 378*(8), 731-739.

Drilon, A., Li, G., Dogan, S., Gounder, M., Shen, R., Arcila, M., . . . Ho, A. L. (2016). What hides behind the MASC: clinical response and acquired resistance to entrectinib after ETV6-NTRK3 identification in a mammary analogue secretory carcinoma (MASC). *Ann Oncol, 27*(5), 920-926.

Drilon, Alexander, Nagasubramanian, Ramamoorthy, Blake, James F., Ku, Nora, Tuch, Brian B., Ebata, Kevin, . . . Hyman, David M. (2017). A Next-Generation TRK Kinase Inhibitor Overcomes Acquired Resistance to Prior TRK Kinase Inhibition in Patients with TRK Fusion–Positive Solid Tumors. *Cancer Discovery, 7*(9), 963-972.

Drilon, Alexander, Ou, Sai-Hong Ignatius, Cho, Byoung Chul, Kim, Dong-Wan, Lee, Jeeyun, Lin, Jessica J., . . . Shaw, Alice T. (2018). Repotrectinib (TPX-0005) Is a Next-Generation ROS1/TRK/ALK Inhibitor That Potently Inhibits ROS1/TRK/ALK Solvent- Front Mutations. *Cancer Discovery, 8*(10), 1227-1236.

Du, Jing, Feng, Linyin, Yang, Feng, and Lu, Bai. (2000). Activity- and Ca2+-Dependent Modulation of Surface Expression of Brain-Derived Neurotrophic Factor Receptors in Hippocampal Neurons. *Journal of Cell Biology, 150*(6), 1423-1434.

Duda, D. M., Borg, L. A., Scott, D. C., Hunt, H. W., Hammel, M., and Schulman, B. A. (2008). Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. *Cell, 134*(6), 995-1006.

Eder, Joseph Paul, Shapiro, Geoffrey I., Appleman, Leonard J., Zhu, Andrew X., Miles, Dale, Keer, Harold, . . . LoRusso, Patricia M. (2010). A Phase I Study of Foretinib, a Multi-Targeted Inhibitor of c-Met and Vascular Endothelial Growth Factor Receptor 2. *Clinical Cancer Research, 16*(13), 3507-3516.

Farina, A. R., Cappabianca, L., Gneo, L., Ruggeri, P., and Mackay, A. R. (2018a). TrkAIII signals endoplasmic reticulum stress to the mitochondria in neuroblastoma cells, resulting in glycolytic metabolic adaptation. *Oncotarget, 9*(9), 8368-8390.

Farina, A. R., Cappabianca, L., Ruggeri, P., Gneo, L., Pellegrini, C., Fargnoli, M. C., and Mackay, A. R. (2018b). The oncogenic neurotrophin receptor tropomyosin-related kinase variant, TrkAIII. *J Exp Clin Cancer Res, 37*(1), 119.

Fischer, E. S., Böhm, K., Lydeard, J. R., Yang, H., Stadler, M. B., Cavadini, S., . . . Thomä, N. H. (2014). Structure of the DDB1-CRBN E3 ubiquitin ligase in complex with thalidomide. *Nature, 512*(7512), 49-53.

Frett, B., Carlomagno, F., Moccia, M. L., Brescia, A., Federico, G., De Falco, V., . . . Li, H. Y. (2015). Fragment-Based Discovery of a Dual pan-RET/VEGFR2 Kinase Inhibitor Optimized for Single-Agent Polypharmacology. *Angew Chem Int Ed Engl, 54*(30), 8717-8721.

Fuse, Miho J., Okada, Koutaroh, Oh-hara, Tomoko, Ogura, Hayato, Fujita, Naoya, and Katayama, Ryohei. (2017). Mechanisms of Resistance to NTRK Inhibitors and Therapeutic Strategies in NTRK1-Rearranged Cancers. *Molecular Cancer Therapeutics, 16*(10), 2130-2143.

Gadd, M. S., Testa, A., Lucas, X., Chan, K. H., Chen, W., Lamont, D. J., . . . Ciulli, A. (2017). Structural basis of PROTAC cooperative recognition for selective protein degradation. *Nat Chem Biol, 13*(5), 514-521.

Garcia, J., Hurwitz, H. I., Sandler, A. B., Miles, D., Coleman, R. L., Deurloo, R., and Chinot, O. L. (2020). Bevacizumab (Avastin®) in cancer treatment: A review of 15 years of clinical experience and future outlook. *Cancer Treat Rev, 86*, 102017.

George, A. J., Hoffiz, Y. C., Charles, A. J., Zhu, Y., and Mabb, A. M. (2018). A Comprehensive Atlas of E3 Ubiquitin Ligase Mutations in Neurological Disorders. *Front Genet, 9*, 29.

Gomez-Rodriguez, J., Readinger, J. A., Viorritto, I. C., Mueller, K. L., Houghtling, R. A., and Schwartzberg, P. L. (2007). Tec kinases, actin, and cell adhesion. *Immunol Rev, 218*, 45-64.

Graves, L. M., Duncan, J. S., Whittle, M. C., and Johnson, G. L. (2013). The dynamic nature of the kinome. *Biochem J, 450*(1), 1-8.

Greco, A., Miranda, C., and Pierotti, M. A. (2010). Rearrangements of NTRK1 gene in papillary thyroid carcinoma. *Mol Cell Endocrinol, 321*(1), 44-49.

Greig, S. L. (2016). Osimertinib: First Global Approval. *Drugs, 76*(2), 263-273.

Hantschel, O., Rix, U., and Superti-Furga, G. (2008). Target spectrum of the BCR-ABL inhibitors imatinib, nilotinib and dasatinib. *Leuk Lymphoma, 49*(4), 615-619.

He, K., Zhang, Z., Wang, W., Zheng, X., Wang, X., and Zhang, X. (2020). Discovery and biological evaluation of proteolysis targeting chimeras (PROTACs) as an EGFR degraders based on osimertinib and lenalidomide. *Bioorg Med Chem Lett, 30*(12), 127167.

Hechtman, J. F., Benayed, R., Hyman, D. M., Drilon, A., Zehir, A., Frosina, D., . . . Jungbluth, A. A. (2017). Pan-Trk Immunohistochemistry Is an Efficient and Reliable Screen for the Detection of NTRK Fusions. *Am J Surg Pathol, 41*(11), 1547-1551.

Hershko, A. (1983). Ubiquitin: roles in protein modification and breakdown. *Cell, 34*(1), 11-12.

Hon, W. C., Wilson, M. I., Harlos, K., Claridge, T. D., Schofield, C. J., Pugh, C. W., . . . Jones, E. Y. (2002). Structural basis for the recognition of hydroxyproline in HIF-1 alpha by pVHL. *Nature, 417*(6892), 975-978.

Huang, E. J., and Reichardt, L. F. (2003). Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem, 72*, 609-642.

Huang, Eric J., and Reichardt, Louis F. (2003). Trk Receptors: Roles in Neuronal Signal Transduction. *Annual Review of Biochemistry, 72*(1), 609-642.

Huang, H. T., Dobrovolsky, D., Paulk, J., Yang, G., Weisberg, E. L., Doctor, Z. M., . . . Gray, N. S. (2018). A Chemoproteomic Approach to Query the Degradable Kinome Using a Multi-kinase Degrader. *Cell Chem Biol, 25*(1), 88-99 e86.

Hyman, David, Kummar, Shivaani, Farago, Anna, Geoerger, Birgit, Mau-Sorensen, Morten, Taylor, Matthew, . . . Hong, David. (2019). Abstract CT127: Phase I and expanded access experience of LOXO-195 (BAY 2731954), a selective next-generation TRK inhibitor (TRKi). *Cancer Research, 79*(13 Supplement), CT127-CT127.

Ito, T., Ando, H., Suzuki, T., Ogura, T., Hotta, K., Imamura, Y., . . . Handa, H. (2010). Identification of a primary target of thalidomide teratogenicity. *Science, 327*(5971), 1345-1350.

Iwakuma, T., and Lozano, G. (2003). MDM2, an introduction. *Mol Cancer Res, 1*(14), 993-1000.

Jacobi, S., Soriano, J., Segal, M., and Moses, E. (2009). BDNF and NT-3 increase excitatory input connectivity in rat hippocampal cultures. *Eur J Neurosci, 30*(6), 998-1010.

Jazirehi, A. R., and Bonavida, B. (2005). Cellular and molecular signal transduction pathways modulated by rituximab (rituxan, anti-CD20 mAb) in non-Hodgkin's lymphoma: implications in chemosensitization and therapeutic intervention. *Oncogene, 24*(13), 2121-2143.

Jiang, T., Wang, G., Liu, Y., Feng, L., Wang, M., Liu, J., . . . Ouyang, L. (2021). Development of small-molecule tropomyosin receptor kinase (TRK) inhibitors for NTRK fusion cancers. *Acta Pharm Sin B, 11*(2), 355-372.

Kaelin, W. G. (2005). The von Hippel-Lindau tumor suppressor protein: roles in cancer and oxygen sensing. *Cold Spring Harb Symp Quant Biol, 70*, 159-166.

Kang, C. H., Lee, D. H., Lee, C. O., Du Ha, J., Park, C. H., and Hwang, J. Y. (2018). Induced protein degradation of anaplastic lymphoma kinase (ALK) by proteolysis targeting chimera (PROTAC). *Biochem Biophys Res Commun, 505*(2), 542-547.

Kang, H., and Schuman, E. M. (1995). Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science, 267*(5204), 1658-1662.

Kawakami, T., Chiba, T., Suzuki, T., Iwai, K., Yamanaka, K., Minato, N., . . . Tanaka, K. (2001). NEDD8 recruits E2-ubiquitin to SCF E3 ligase. *EMBO J, 20*(15), 4003-4012.

Kelleher, F. C., and McDermott, R. (2010). The emerging pathogenic and therapeutic importance of the anaplastic lymphoma kinase gene. *Eur J Cancer, 46*(13), 2357-2368.

Kermani, P., and Hempstead, B. (2007). Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. *Trends Cardiovasc Med, 17*(4), 140-143.

Kheder, Ed S., and Hong, David S. (2018). Emerging Targeted Therapy for Tumors with NTRK Fusion Proteins. *Clinical Cancer Research, 24*(23), 5807-5814.

Khotskaya, Y. B., Holla, V. R., Farago, A. F., Mills Shaw, K. R., Meric-Bernstam, F., and Hong, D. S. (2017). Targeting TRK family proteins in cancer. *Pharmacol Ther, 173*, 58-66.

Khozin, S., Blumenthal, G. M., Zhang, L., Tang, S., Brower, M., Fox, E., . . . Pazdur, R. (2015). FDA approval: ceritinib for the treatment of metastatic anaplastic lymphoma kinase-positive non-small cell lung cancer. *Clin Cancer Res, 21*(11), 2436-2439.

Kim, B., Jung, N., Lee, S., Sohng, J. K., and Jung, H. J. (2016). Apigenin Inhibits Cancer Stem Cell-Like Phenotypes in Human Glioblastoma Cells via Suppression of c-Met Signaling. *Phytother Res, 30*(11), 1833-1840.

Kim, N. K., Park, J. K., Shin, E., and Kim, Y. W. (2014). The combination of nuclear factor kappa B, cyclo-oxygenase-2 and vascular endothelial growth factor expression predicts poor prognosis in stage II and III colorectal cancer. *Anticancer Res, 34*(11), 6451-6457.

Kim, S., Kim, T. M., Kim, D. W., Go, H., Keam, B., Lee, S. H., . . . Heo, D. S. (2013). Heterogeneity of genetic changes associated with acquired crizotinib resistance in ALK-rearranged lung cancer. *J Thorac Oncol, 8*(4), 415-422.

Kloosterman, W. P., Coebergh van den Braak, R. R. J., Pieterse, M., van Roosmalen, M. J., Sieuwerts, A. M., Stangl, C., . . . Voest, E. E. (2017). A Systematic Analysis of Oncogenic Gene Fusions in Primary Colon Cancer. *Cancer Res, 77*(14), 3814-3822.

Kozaki, Ryohei, Yoshizawa, Toshio, Tsukamoto, Kohki, Kato, Hikaru, and Kawabata, Kazuhito. (2016). Abstract 2954A: A potent and selective TRK inhibitor ONO-5390556, shows potent antitumor activity against both TRK-rearranged cancers and the resistant mutants. *Cancer Research, 76*(14 Supplement), 2954A-2954A.

Krings, G., Joseph, N. M., Bean, G. R., Solomon, D., Onodera, C., Talevich, E., . . . Chen, Y. Y. (2017). Genomic profiling of breast secretory carcinomas reveals distinct genetics from other breast cancers and similarity to mammary analog secretory carcinomas. *Mod Pathol, 30*(8), 1086-1099.

Kurimchak, Alison M, Shelton, Claude, Duncan, Kelly E, Johnson, Katherine J, Brown, Jennifer, O'Brien, Shane, . . . Duncan, James S. (2016). Resistance to BET Bromodomain Inhibitors Is Mediated by Kinome Reprogramming in Ovarian Cancer. *Cell Reports, 16*(5), 1273-1286.

Lagadec, C., Meignan, S., Adriaenssens, E., Foveau, B., Vanhecke, E., Romon, R., . . . Le Bourhis, X. (2009). TrkA overexpression enhances growth and metastasis of breast cancer cells. *Oncogene, 28*(18), 1960-1970.

Lai, A. C., and Crews, C. M. (2017). Induced protein degradation: an emerging drug discovery paradigm. *Nat Rev Drug Discov, 16*(2), 101-114.

Lai, A. C., Toure, M., Hellerschmied, D., Salami, J., Jaime-Figueroa, S., Ko, E., . . . Crews, C. M. (2016). Modular PROTAC Design for the Degradation of Oncogenic BCR-ABL. *Angew Chem Int Ed Engl, 55*(2), 807-810.

Lai, P. C., Chiu, T. H., and Huang, Y. T. (2010). Overexpression of BDNF and TrkB in human bladder cancer specimens. *Oncol Rep, 24*(5), 1265-1270.

Larkins, E., Blumenthal, G. M., Chen, H., He, K., Agarwal, R., Gieser, G., . . . Pazdur, R. (2016). FDA Approval: Alectinib for the Treatment of Metastatic, ALK-Positive Non-Small Cell Lung Cancer Following Crizotinib. *Clin Cancer Res, 22*(21), 5171-5176.

Lebraud, H., Wright, D. J., Johnson, C. N., and Heightman, T. D. (2016). Protein Degradation by In-Cell Self-Assembly of Proteolysis Targeting Chimeras. *ACS Cent Sci, 2*(12), 927-934.

Lee, D. H., and Goldberg, A. L. (1998). Proteasome inhibitors: valuable new tools for cell biologists. *Trends Cell Biol, 8*(10), 397-403.

Lee, S. J., Li, G. G., Kim, S. T., Hong, M. E., Jang, J., Yoon, N., . . . Lee, J. (2015). NTRK1 rearrangement in colorectal cancer patients: evidence for actionable target using patient-derived tumor cell line. *Oncotarget, 6*(36), 39028-39035.

Li, Xin, and Song, Yongcheng. (2020). Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. *Journal of Hematology and Oncology, 13*(1).

Liu, Y., Huang, X., He, X., Zhou, Y., Jiang, X., Chen-Kiang, S., . . . Xu, G. (2015). A novel effect of thalidomide and its analogs: suppression of cereblon ubiquitination enhances ubiquitin ligase function. *FASEB J, 29*(12), 4829-4839.

Lu, J., Qian, Y., Altieri, M., Dong, H., Wang, J., Raina, K., . . . Crews, C. M. (2015). Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4. *Chem Biol, 22*(6), 755-763.

Lydeard, J. R., Schulman, B. A., and Harper, J. W. (2013). Building and remodelling Cullin-RING E3 ubiquitin ligases. *EMBO Rep, 14*(12), 1050-1061.

Malik, S. M., Maher, V. E., Bijwaard, K. E., Becker, R. L., Zhang, L., Tang, S. W., . . . Pazdur, R. (2014). U.S. Food and Drug Administration approval: crizotinib for treatment of advanced or metastatic non-small cell lung cancer that is anaplastic lymphoma kinase positive. *Clin Cancer Res, 20*(8), 2029-2034.

Manning, G., Whyte, D. B., Martinez, R., Hunter, T., and Sudarsanam, S. (2002). The protein kinase complement of the human genome. *Science, 298*(5600), 1912-1934.

Marcus, L., Donoghue, M., Aungst, S., Myers, C. E., Helms, W. S., Shen, G., . . . Pazdur, R. (2021). FDA Approval Summary: Entrectinib for the Treatment of NTRK gene Fusion Solid Tumors. *Clin Cancer Res, 27*(4), 928-932.

Mardy, Sek, Miura, Yuichi, Endo, Fumio, Matsuda, Ichiro, and Indo, Yasuhiro. (2001). Congenital insensitivity to pain with anhidrosis (CIPA): effect of TRKA (NTRK1) missense mutations on autophosphorylation of the receptor tyrosine kinase for nerve growth factor. *Human Molecular Genetics, 10*(3), 179-188.

Martin-Zanca, D., Hughes, S. H., and Barbacid, M. (1986). A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. *Nature, 319*(6056), 743-748.

Matyskiela, M. E., Lu, G., Ito, T., Pagarigan, B., Lu, C. C., Miller, K., . . . Chamberlain, P. P. (2016). A novel cereblon modulator recruits GSPT1 to the CRL4(CRBN) ubiquitin ligase. *Nature, 535*(7611), 252-257.

Medeiros, L. J., and Elenitoba-Johnson, K. S. (2007). Anaplastic Large Cell Lymphoma. *Am J Clin Pathol, 127*(5), 707-722.

Meyer-Franke, A., Wilkinson, G. A., Kruttgen, A., Hu, M., Munro, E., Hanson, M. G., Jr., . . . Barres, B. A. (1998). Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. *Neuron, 21*(4), 681-693.

Moccia, M., Yang, D., Lakkaniga, N. R., Frett, B., McConnell, N., Zhang, L., . . . Carlomagno, F. (2021). Targeted activity of the small molecule kinase inhibitor Pz-1 towards RET and TRK kinases. *Sci Rep, 11*(1), 16103.

Mohamed, A. J., Yu, L., Bäckesjö, C. M., Vargas, L., Faryal, R., Aints, A., . . . Smith, C. I. (2009). Bruton's tyrosine kinase (Btk): function, regulation, and transformation with special emphasis on the PH domain. *Immunol Rev, 228*(1), 58-73.

Mok, T. S., Wu, Y. L., Thongprasert, S., Yang, C. H., Chu, D. T., Saijo, N., . . . Fukuoka, M. (2009). Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med, 361*(10), 947-957.

Morris, S. W., Kirstein, M. N., Valentine, M. B., Dittmer, K., Shapiro, D. N., Look, A. T., and Saltman, D. L. (1995). Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science, 267*(5196), 316-317.

Mueller, B. K., Mack, H., and Teusch, N. (2005). Rho kinase, a promising drug target for neurological disorders. *Nat Rev Drug Discov, 4*(5), 387-398.

Nakagawara, A. (2001). Trk receptor tyrosine kinases: a bridge between cancer and neural development. *Cancer Lett, 169*(2), 107-114.

Nakagawara, A., Liu, X. G., Ikegaki, N., White, P. S., Yamashiro, D. J., Nycum, L. M., . . . Brodeur, G. M. (1995). Cloning and chromosomal localization of the human TRK-B tyrosine kinase receptor gene (NTRK2). *Genomics, 25*(2), 538-546.

Neklesa, Taavi K., Winkler, James D., and Crews, Craig M. (2017). Targeted protein degradation by PROTACs. *Pharmacology and Therapeutics, 174*, 138-144.

Noblejas-López, M. D. M., Nieto-Jimenez, C., Burgos, M., Gómez-Juárez, M., Montero, J. C., Esparís-Ogando, A., . . . Ocaña, A. (2019). Activity of BET-proteolysis targeting chimeric (PROTAC) compounds in triple negative breast cancer. *J Exp Clin Cancer Res, 38*(1), 383.

Normanno, N., De Luca, A., Bianco, C., Strizzi, L., Mancino, M., Maiello, M. R., . . . Salomon, D. S. (2006). Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene, 366*(1), 2-16.

Nowell, P. C., and Hungerford, D. A. (1961). Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. *J Natl Cancer Inst, 27*, 1013-1035.

Organ, S. L., and Tsao, M. S. (2011). An overview of the c-MET signaling pathway. *Ther Adv Med Oncol, 3*(1 Suppl), S7-S19.

Pal Singh, S., Dammeijer, F., and Hendriks, R. W. (2018). Role of Bruton's tyrosine kinase in B cells and malignancies. *Mol Cancer, 17*(1), 57.

Pasini, L., Re, A., Tebaldi, T., Ricci, G., Boi, S., Adami, V., . . . Quattrone, A. (2015). TrkA is amplified in malignant melanoma patients and induces an anti-proliferative response in cell lines. *BMC Cancer, 15*, 777.

Petroski, M. D., and Deshaies, R. J. (2005). Function and regulation of cullin-RING ubiquitin ligases. *Nat Rev Mol Cell Biol, 6*(1), 9-20.

Petrylak, Daniel Peter, Gao, Xin, Vogelzang, Nicholas J., Garfield, Mary Harlow, Taylor, Ian, Moore, Marcia Dougan, . . . III, Howard A. Burris. (2020). First-in-human phase I study of ARV-110, an androgen receptor (AR) PROTAC degrader in patients (pts) with metastatic castrate-resistant prostate cancer (mCRPC) following enzalutamide (ENZ) and/or abiraterone (ABI). *Journal of Clinical Oncology, 38*(15_suppl), 3500-3500.

Petzold, G., Fischer, E. S., and Thomä, N. H. (2016). Structural basis of lenalidomide-induced CK1α degradation by the CRL4(CRBN) ubiquitin ligase. *Nature, 532*(7597), 127-130.

Pickart, C. M. (2001). Mechanisms underlying ubiquitination. *Annu Rev Biochem, 70*, 503-533.

Pietrantonio, F., Di Nicolantonio, F., Schrock, A. B., Lee, J., Tejpar, S., Sartore-Bianchi, A., . . . Cremolini, C. (2017). ALK, ROS1, and NTRK Rearrangements in Metastatic Colorectal Cancer. *J Natl Cancer Inst, 109*(12).

Plosker, G. L., and Figgitt, D. P. (2003). Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. *Drugs, 63*(8), 803-843.

Powell, Chelsea E., Gao, Yang, Tan, Li, Donovan, Katherine A., Nowak, Radosław P., Loehr, Amanda, . . . Gray, Nathanael S. (2018). Chemically Induced Degradation of Anaplastic Lymphoma Kinase (ALK). *Journal of Medicinal Chemistry, 61*(9), 4249-4255.

Prakash, S., Tian, L., Ratliff, K. S., Lehotzky, R. E., and Matouschek, A. (2004). An unstructured initiation site is required for efficient proteasome-mediated degradation. *Nat Struct Mol Biol, 11*(9), 830-837.

Pulford, K., Morris, S. W., and Turturro, F. (2004). Anaplastic lymphoma kinase proteins in growth control and cancer. *J Cell Physiol, 199*(3), 330-358.

Raina, K., and Crews, C. M. (2017). Targeted protein knockdown using small molecule degraders. *Curr Opin Chem Biol, 39*, 46-53.

Raina, K., Lu, J., Qian, Y., Altieri, M., Gordon, D., Rossi, A. M., . . . Coleman, K. G. (2016). PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. *Proc Natl Acad Sci U S A, 113*(26), 7124-7129.

Ramalingam, Suresh S., Vansteenkiste, Johan, Planchard, David, Cho, Byoung Chul, Gray, Jhanelle E., Ohe, Yuichiro, . . . Soria, Jean-Charles. (2019). Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. *New England Journal of Medicine, 382*(1), 41-50.

Read, M. A., Brownell, J. E., Gladysheva, T. B., Hottelet, M., Parent, L. A., Coggins, M. B., . . . Palombella, V. J. (2000). Nedd8 modification of cul-1 activates SCF(beta(TrCP))-dependent ubiquitination of IkappaBalpha. *Mol Cell Biol, 20*(7), 2326-2333.

Reichardt, Louis F. (2006). Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*(1473), 1545-1564.

Reuther, G. W., Lambert, Q. T., Caligiuri, M. A., and Der, C. J. (2000). Identification and characterization of an activating TrkA deletion mutation in acute myeloid leukemia. *Mol Cell Biol, 20*(23), 8655-8666.

Riching, K. M., Mahan, S., Corona, C. R., McDougall, M., Vasta, J. D., Robers, M. B., . . . Daniels, D. L. (2018). Quantitative Live-Cell Kinetic Degradation and Mechanistic Profiling of PROTAC Mode of Action. *ACS Chem Biol, 13*(9), 2758-2770.

Roskoski, R., Jr. (2021). Properties of FDA-approved small molecule protein kinase inhibitors: A 2021 update. *Pharmacol Res, 165*, 105463.

Roskoski, Robert. (2020). Properties of FDA-approved small molecule protein kinase inhibitors: A 2020 update. *Pharmacological Research, 152*, 104609.

Russo, Mariangela, Misale, Sandra, Wei, Ge, Siravegna, Giulia, Crisafulli, Giovanni, Lazzari, Luca, . . . Bardelli, Alberto. (2016). Acquired Resistance to the TRK Inhibitor Entrectinib in Colorectal Cancer. *Cancer Discovery, 6*(1), 36-44.

Sacha, T. (2014). Imatinib in chronic myeloid leukemia: an overview. *Mediterr J Hematol Infect Dis, 6*(1), e2014007.

Sakamoto, K. M., Kim, K. B., Kumagai, A., Mercurio, F., Crews, C. M., and Deshaies, R. J. (2001). Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc Natl Acad Sci U S A, 98*(15), 8554-8559.

Sartore-Bianchi, A., Ardini, E., Bosotti, R., Amatu, A., Valtorta, E., Somaschini, A., . . . Siena, S. (2016). Sensitivity to Entrectinib Associated With a Novel LMNA-NTRK1 Gene Fusion in Metastatic Colorectal Cancer. *J Natl Cancer Inst, 108*(1).

Scagliotti, G., von Pawel, J., Novello, S., Ramlau, R., Favaretto, A., Barlesi, F., . . . Schwartz, B. (2015). Phase III Multinational, Randomized, Double-Blind, Placebo-Controlled Study of Tivantinib (ARQ 197) Plus Erlotinib Versus Erlotinib Alone in Previously Treated Patients With Locally Advanced or Metastatic Nonsquamous Non-Small-Cell Lung Cancer. *J Clin Oncol, 33*(24), 2667-2674.

Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. *Cell, 103*(2), 211-225.

Schram, A. M., Chang, M. T., Jonsson, P., and Drilon, A. (2017). Fusions in solid tumours: diagnostic strategies, targeted therapy, and acquired resistance. *Nat Rev Clin Oncol, 14*(12), 735-748.

Sclabas, G. M., Fujioka, S., Schmidt, C., Li, Z., Frederick, W. A., Yang, W., . . . Chiao, P. J. (2005). Overexpression of tropomysin-related kinase B in metastatic human pancreatic cancer cells. *Clin Cancer Res, 11*(2 Pt 1), 440-449.

Sequist, L. V., von Pawel, J., Garmey, E. G., Akerley, W. L., Brugger, W., Ferrari, D., . . . Schiller, J. H. (2011). Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. *J Clin Oncol, 29*(24), 3307-3315.

Shepherd, F. A., Rodrigues Pereira, J., Ciuleanu, T., Tan, E. H., Hirsh, V., Thongprasert, S., . . . Seymour, L. (2005). Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med, 353*(2), 123-132.

Shi, Q., and Chen, L. (2017). Cereblon: A Protein Crucial to the Multiple Functions of Immunomodulatory Drugs as well as Cell Metabolism and Disease Generation. *J Immunol Res, 2017*, 9130608.

Silke, J., and Meier, P. (2013). Inhibitor of apoptosis (IAP) proteins-modulators of cell death and inflammation. *Cold Spring Harb Perspect Biol, 5*(2).

Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., . . . Barlogie, B. (1999). Antitumor activity of thalidomide in refractory multiple myeloma. *N Engl J Med, 341*(21), 1565-1571.

Skaper, S. D. (2008). The biology of neurotrophins, signalling pathways, and functional peptide mimetics of neurotrophins and their receptors. *CNS Neurol Disord Drug Targets, 7*(1), 46-62.

Smith, C. I., Islam, T. C., Mattsson, P. T., Mohamed, A. J., Nore, B. F., and Vihinen, M. (2001). The Tec family of cytoplasmic tyrosine kinases: mammalian Btk, Bmx, Itk, Tec, Txk and homologs in other species. *Bioessays, 23*(5), 436-446.

Snider, W. D. (1994). Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell, 77*(5), 627-638.

Snyder, Lawrence B., Flanagan, John J., Qian, Yimin, Gough, Sheryl M., Andreoli, Monica, Bookbinder, Mark, . . . Taylor, Ian. (2021). Abstract 44: The discovery of ARV-471, an orally bioavailable estrogen receptor degrading PROTAC for the treatment of patients with breast cancer. *Cancer Research, 81*(13 Supplement), 44-44.

Sos, M. L., Rode, H. B., Heynck, S., Peifer, M., Fischer, F., Klüter, S., . . . Rauh, D. (2010). Chemogenomic profiling provides insights into the limited activity of irreversible EGFR Inhibitors in tumor cells expressing the T790M EGFR resistance mutation. *Cancer Res, 70*(3), 868-874.

Soucy, T. A., Smith, P. G., Milhollen, M. A., Berger, A. J., Gavin, J. M., Adhikari, S., . . . Langston, S. P. (2009). An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature, 458*(7239), 732-736.

Sun, Y., Zhao, X., Ding, N., Gao, H., Wu, Y., Yang, Y., . . . Rao, Y. (2018). PROTAC-induced BTK degradation as a novel therapy for mutated BTK C481S induced ibrutinib-resistant B-cell malignancies. *Cell Res, 28*(7), 779-781.

Szturz, P., Raymond, E., Abitbol, C., Albert, S., de Gramont, A., and Faivre, S. (2017). Understanding c-MET signalling in squamous cell carcinoma of the head and neck. *Crit Rev Oncol Hematol, 111*, 39-51.

Tacconelli, A., Farina, A. R., Cappabianca, L., Desantis, G., Tessitore, A., Vetuschi, A., . . . Mackay, A. R. (2004). TrkA alternative splicing: a regulated tumor-promoting switch in human neuroblastoma. *Cancer Cell, 6*(4), 347-360.

Tagliabue, E., Pilotti, S., Gianni, A. M., Ménard, S., and Colnaghi, M. I. (1998). Target molecules for immunotherapy of inflammatory breast carcinomas. *Eur J Cancer, 34*(12), 1982-1983.

Takeuchi, K., and Ito, F. (2011). Receptor tyrosine kinases and targeted cancer therapeutics. *Biol Pharm Bull, 34*(12), 1774-1780.

Tsukada, S., Saffran, D. C., Rawlings, D. J., Parolini, O., Allen, R. C., Klisak, I., . . . et al. (1993). Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell, 72*(2), 279-290.

Vaishnavi, A., Le, A. T., and Doebele, R. C. (2015). TRKing down an old oncogene in a new era of targeted therapy. *Cancer Discov, 5*(1), 25-34.

Valent, A., Danglot, G., and Bernheim, A. (1997). Mapping of the tyrosine kinase receptors trkA (NTRK1), trkB (NTRK2) and trkC(NTRK3) to human chromosomes 1q22, 9q22 and 15q25 by fluorescence in situ hybridization. *Eur J Hum Genet, 5*(2), 102-104.

van der Plas, D. C., Grosveld, G., and Hagemeijer, A. (1991). Review of clinical, cytogenetic, and molecular aspects of Ph-negative CML. *Cancer Genet Cytogenet, 52*(2), 143-156.

Vetrie, D., Vorechovský, I., Sideras, P., Holland, J., Davies, A., Flinter, F., . . . et al. (1993). The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. *Nature, 361*(6409), 226-233.

Webb, T. R., Slavish, J., George, R. E., Look, A. T., Xue, L., Jiang, Q., . . . Morris, S. W. (2009). Anaplastic lymphoma kinase: role in cancer pathogenesis and small-molecule inhibitor development for therapy. *Expert Rev Anticancer Ther, 9*(3), 331-356.

Weber, J., Polo, S., and Maspero, E. (2019). HECT E3 Ligases: A Tale With Multiple Facets. *Front Physiol, 10*, 370.

Weier, H. U., Rhein, A. P., Shadravan, F., Collins, C., and Polikoff, D. (1995). Rapid physical mapping of the human trk protooncogene (NTRK1) to human chromosome 1q21-q22 by P1 clone selection, fluorescence in situ hybridization (FISH), and computer-assisted microscopy. *Genomics, 26*(2), 390-393.

Weissman, A. M. (2001). Themes and variations on ubiquitylation. *Nat Rev Mol Cell Biol, 2*(3), 169-178.

Werner, Petra, Paluru, Prasuna, Simpson, Anisha M., Latney, Brande, Iyer, Radhika, Brodeur, Garrett M., and Goldmuntz, Elizabeth. (2014). Mutations in NTRK3 Suggest a Novel Signaling Pathway in Human Congenital Heart Disease. *Human Mutation, 35*(12), 1459-1468.

Wong, D., Yip, S., and Sorensen, P. H. (2020). Methods for Identifying Patients with Tropomyosin Receptor Kinase (TRK) Fusion Cancer. *Pathol Oncol Res, 26*(3), 1385-1399.

Xu, B., and Xie, X. (2016). Neurotrophic factor control of satiety and body weight. *Nat Rev Neurosci, 17*(5), 282-292.

Yap, T. A., and Workman, P. (2012). Exploiting the cancer genome: strategies for the discovery and clinical development of targeted molecular therapeutics. *Annu Rev Pharmacol Toxicol, 52*, 549-573.

Yasuda, H., Park, E., Yun, C. H., Sng, N. J., Lucena-Araujo, A. R., Yeo, W. L., . . . Costa, D. B. (2013). Structural, biochemical, and clinical characterization of epidermal growth factor

receptor (EGFR) exon 20 insertion mutations in lung cancer. *Sci Transl Med, 5*(216), 216ra177.

You, I., Erickson, E. C., Donovan, K. A., Eleuteri, N. A., Fischer, E. S., Gray, N. S., and Toker, A. (2020). Discovery of an AKT Degrader with Prolonged Inhibition of Downstream Signaling. *Cell Chem Biol, 27*(1), 66-73 e67.

Yu, Y., Zhang, S., Wang, X., Yang, Z., and Ou, G. (2010). Overexpression of TrkB promotes the progression of colon cancer. *APMIS, 118*(3), 188-195.

Zahavi, D., and Weiner, L. (2020). Monoclonal Antibodies in Cancer Therapy. *Antibodies (Basel), 9*(3).

Zhang, C., Han, X. R., Yang, X., Jiang, B., Liu, J., Xiong, Y., and Jin, J. (2018). Proteolysis Targeting Chimeras (PROTACs) of Anaplastic Lymphoma Kinase (ALK). *Eur J Med Chem, 151*, 304-314.

Zhang, H., Zhao, H. Y., Xi, X. X., Liu, Y. J., Xin, M., Mao, S., . . . Zhang, S. Q. (2020). Discovery of potent epidermal growth factor receptor (EGFR) degraders by proteolysis targeting chimera (PROTAC). *Eur J Med Chem, 189*, 112061.

Zhao, Q., Ren, C., Liu, L., Chen, J., Shao, Y., Sun, N., . . . Jiang, B. (2019). Discovery of SIAIS178 as an Effective BCR-ABL Degrader by Recruiting Von Hippel-Lindau (VHL) E3 Ubiquitin Ligase. *J Med Chem, 62*(20), 9281-9298.

Zheng, N., Schulman, B. A., Song, L., Miller, J. J., Jeffrey, P. D., Wang, P., . . . Pavletich, N. P. (2002). Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. *Nature, 416*(6882), 703-709.

Zhong, L., Li, Y., Xiong, L., Wang, W., Wu, M., Yuan, T., . . . Yang, S. (2021). Small molecules in targeted cancer therapy: advances, challenges, and future perspectives. *Signal Transduct Target Ther, 6*(1), 201.

Zito Marino, F., Pagliuca, F., Ronchi, A., Cozzolino, I., Montella, M., Berretta, M., . . . Franco, R. (2020). NTRK Fusions, from the Diagnostic Algorithm to Innovative Treatment in the Era of Precision Medicine. *Int J Mol Sci, 21*(10).