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Anthony Feral, Guillaume Laconde, Muriel Amblard, Nicolas Masurier. PROteolysis TArgetting Chimeras (PROTACs) strategy applied to kinases: recent advances. *Advanced Therapeutics*, 2020, 3 (11), pp.2000148. 10.1002/adtp.202000148 . hal-03080602

HAL Id: hal-03080602

<https://hal.science/hal-03080602>

Submitted on 17 Dec 2020

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PROteolysis TArgetting Chimeras (PROTACs)

strategy applied to kinases: recent advances.

Anthony Feral, Guillaume Laconde, Muriel Amblard, Nicolas Masurier*

IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

* *Corresponding author: Nicolas Masurier. Tel: + (33) 4 11 75 96 42, Fax: + (33) 4 11 75*

96 41, E-mail: nicolas.masurier@umontpellier.fr

Keywords: PROTAC, Kinase inhibitors, Proteasome, Ligase, Ubiquitin

Abstract:

Since the development of the first protein kinase inhibitor in the early 1980s, followed by the FDA approval of imatinib in 2001, kinases are one of the most intensively pursued targets in current medicinal chemistry research. These proteins are overrepresented in various diseases such as cancer, inflammation or autoimmune pathologies and play important roles in their physiopathogenic processes. Despite the development and approval of numerous potent kinase inhibitors, drug resistance and off-target side effects are commonly encountered with kinase inhibitors. Thus, development of novel strategies to overcome these problems is necessary. Since 2013, many research groups have proposed the conversion of potent kinase inhibitors into PROTAC compounds and shared relevant and encouraging results using this new technology which degrades proteins by employing the cellular machinery. Generally, this strategy brings enhancements in biological effects compared to the only use of the parent inhibitor. In this review article, recent findings related to the PROTAC technology applied to kinases are discussed, with a special focus on publications since 2018.

1. Introduction:

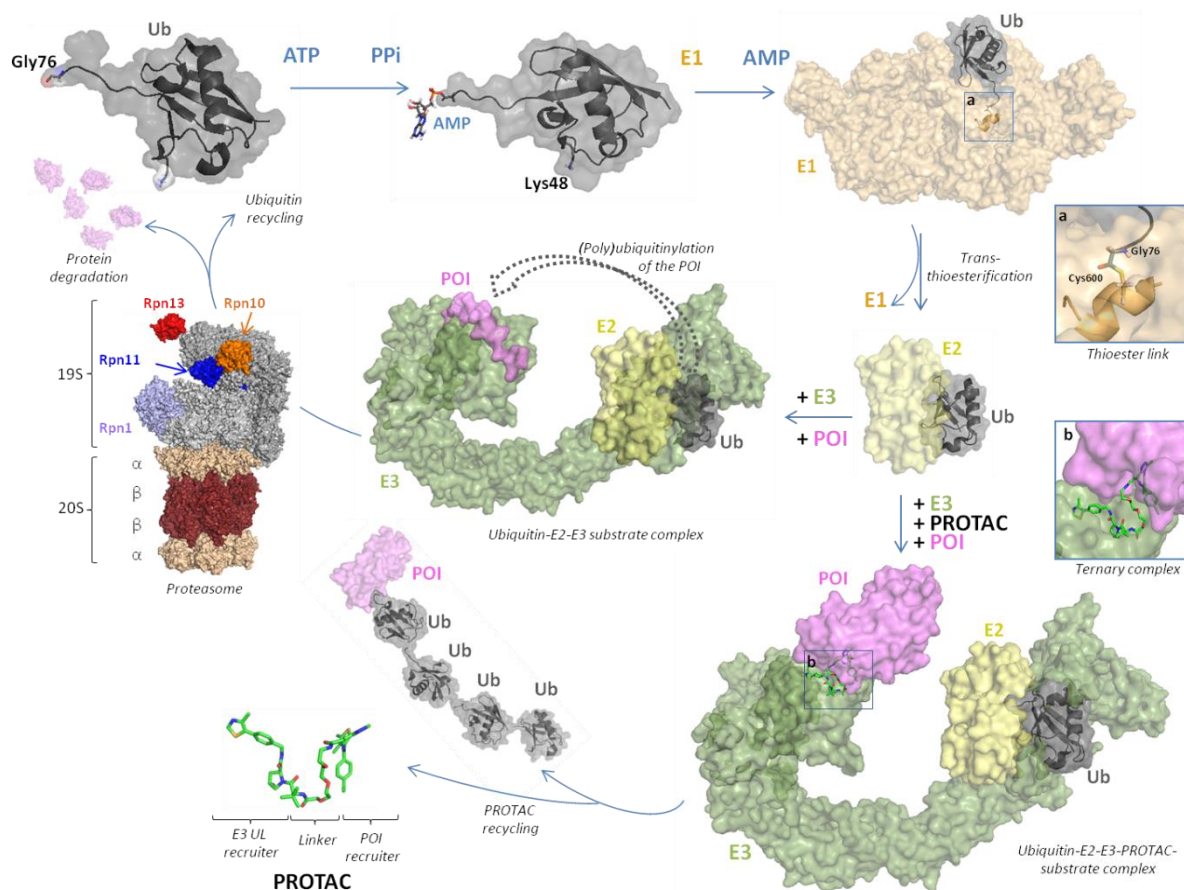
Phosphorylation is a crucial post-translational modification of proteins, which is involved in many cellular processes (such as differentiation, division, proliferation, apoptosis...). This chemical reaction is catalyzed by kinases, which transfer a gamma-phosphoryl group from ATP to an amino acid residue of the substrate protein (usually a serine, a threonine or a tyrosine). This chemical modification alters the properties of the protein and modifies its conformation, to activate or inhibit the enzyme, to allow interactions with other proteins, to modify its cellular localization, etc.^[1] As kinases constitute key actors in a large number of cellular processes, their deregulation often leads to pathological conditions such as cancer, but not exclusively. These enzymes constituted therefore priority targets in medicinal chemistry programs and intensive efforts have been made to develop kinase inhibitors.^{[2][3]} To date, the vast majority of kinase inhibitors are ATP-competitive, with direct interaction with the hinge region of the ATP-binding domain, and the limitations of such inhibitors are well known.^[4] In fact, this site is particularly conserved through the kinome and designing selective kinase inhibitors to reduce off-target-mediated toxicity have constituted a great challenge in medicinal chemistry. However, immense progress has been obtained in this field the last few years, in particular with the discovery of non-ATP competitive (allosteric) inhibitors.^[5] Another well-known limitation of kinase inhibitors is the development of resistance by cancer cells. Indeed, kinase inhibition triggers a strong discerning pressure for cells to acquire resistance to chemotherapy through kinase mutations or by bypassing signaling pathways, which lead to tumor escape. In this context, targeting protein kinase degradation instead of inhibiting the enzyme has emerged as a promising strategy to overcome these problems.^[6] For this reason, a lot of bispecific small molecule degraders, also known as PROTeolysis TArgeting Chimeras (PROTACs) have been developed recently against a large set of kinases, focusing more specifically in the field of oncology. PROTACs are bivalent compounds inducing the formation of a complex between a protein of interest (POI), here a kinase, and an E3 ubiquitin-ligase (UL). The formation of this complex allows poly-ubiquitination of the POI, which is then addressed to the Ubiquitin-Proteasome System (UPS) for subsequent degradation.^[7] Several groups highlighted this strategy in reviews.^{[3][8][9]} In view of the exponential number of studies reporting the use of the kinases-applied PROTAC strategy, we propose an update of these reviews to provide an overview of this field since 2018.

2. Concept and historical context:

In mammalian cells, the UPS constitutes a major intracellular pathway to degrade mutated, misfolded or unfolded proteins, that can be toxic and deleterious to cell integrity (**Figure 1**).^[10] It involves the hierarchical action of three general families of ubiquitin enzymes: namely E1 (ubiquitin-activating), E2 (ubiquitin-conjugating) and E3 (ubiquitin-ligase).^[11] In a first step, E1 activates ubiquitin, a 76 amino acid polypeptide, by adenylating (from ATP) the C-terminal glycine carboxylic group of ubiquitin (Gly76), followed by the formation of a thioester bond between the E1 catalytic cysteine residue and the C-terminus of ubiquitin. In a second step, this activated ubiquitin is transferred to the E2 catalytic cysteine. In a third step, E3 UL catalyzes, either directly or indirectly depending on the E3 UL involved, the transfer of activated ubiquitin on a lysine residue of the POI to generate an isopeptide bond. Ubiquitin itself can, in turn, be ubiquitinated on one or more of its seven surface lysine residues. In particular, the poly-ubiquitinylation of lysine-48 of the ubiquitin constitutes a cellular signal which is recognized by the 26S proteasome and leads to the degradation of the poly-ubiquitinated POI (PU-POI). Poly-ubiquitinylation of lysine-11^[12] or multi-ubiquitinylation^[13] were also reported to lead to protein degradation.

The 26S proteasome is a 2.5 MDa protein complex composed of several subunits which possess different functions involved into the degradation process (**Figure 1**). This ATP-dependent protease complex contains a 20S core particle, capped on one or both sides by a 19S regulatory particle. The 19S subunits Rpn1, Rpn10 and Rpn13 were identified as three high-affinity-ubiquitin-chain fixation domains, where the PU-POI can bind.^[14] After recognition and through a conformational change of the 19S particle,^[11] the ubiquitin chains are removed by proteasome-associated deubiquitinating enzymes (principally the metalloprotease Rpn11 and the cysteine proteases Usp14 and Uch37/UchL5). Ubiquitin is recycled whereas the protein substrate could enter into the 20S domain of the proteasome, where the degradation takes place. The 20S domain has a barrel-like structure and contains β 1, β 2, and β 5 catalytically active subunits, which display respectively caspase-like, trypsin-like, and chymotrypsin-like activity, that degrade the polypeptide chain into small peptides.^[15] Finally, these fragments, after leaving the proteasome, are degraded into amino acids by the cytosol's proteases.^[14]

Figure 1. The Ubiquitin-Proteasome System (UPS) and its hijacking using the PROTAC strategy. Shown are ubiquitin (Ub) and activated-Ub (PDB: 4NNJ), Ub-E1 (Uba1) complex (PDB: 4NNJ), ubiquitin-E2 (UBE2D2) complex (PDB: 6TTU), Ub-E2 E3-substrate complex (model composed of PDB: 1LQB, 5N4W and 6TTU), proteasome (model composed of PDB: 5L4G and 5A5B), Ub-E2-E3-PROTAC-substrate (model composed of PDB: 5T35, 5N4W and 6TTU).



The PROTAC technology hijacks the UPS system to degrade a specific target protein. PROTACs are composed of three moieties: the first one permits the recruitment of an E3 UL, the second one permits the recruitment of the POI and the last one is a linker which connects the first two moieties (**Figure 1**).^[16] E3 ULs are widely represented in the human body: in fact, more than 600 different ones have been identified so far, including 270 which have demonstrated their implication in the UPS.^[17] Currently, four E3 ULs are principally recruited by PROTACs, namely cereblon (CRBN), murine double minute 2 (MDM2), Von Hippel-Lindau (VHL) and cellular inhibitor of apoptosis protein-1 (cIAP1).^[18] It is important to note that PROTACs cannot be active if the targeted E3 UL is not present in the cell of interest, but it is not a really problematic issue because this type of enzyme, notably CRBN and MDM2, is widely expressed in a lot of tissues.^[19] The second part of PROTACs is the recruiting element

of the POI, which is usually a reversible inhibitor of the latter. Interestingly, the affinity of the inhibitor for the POI is not such a crucial parameter to generate an efficient PROTAC. Indeed, Crews and coworkers have shown that the ability of a PROTAC to induce the stable ternary complex (composed of the POI, the E3 UL and the PROTAC) efficiently degrade the POI.^[20] The chemical linker moiety also constitutes a key element for the elaboration of a PROTAC compound. The nature and the length of this linker are crucial parameters that will influence the stability of the ternary complex formed by the PROTAC and its targets.

Emerging in 2001, the PROTAC concept was firstly applied to a methionine aminopeptidase (POI), combining an inhibitor of this POI, a phosphopeptide as SCF^{β-TRCP} (E3 UL) recruiter and an alkyl linker to connect these moieties.^[7] This work constitutes the starting point of the first PROTACs generation called “Peptide-based PROTACs” for which the E3 UL recruiter is a short peptide sequence.^[21] This technology was applied for the first time to a kinase (phosphoinositide 3-kinase - PI3K) in 2013.^[22] However, this generation of PROTACs demonstrated weaknesses: low cell penetration, micromolar activity and high molecular weight, which make these compounds unusable for human clinical applications.^[23] In 2015, several groups described a new generation of PROTACs called “small molecule-based PROTACs” in which the E3 UL recruiter is a small molecule. These new PROTACs showed good potency to degrade the POI at concentrations below 100 nM. Indeed, after dissociation from the ubiquitinated POI, PROTAC is available again to interact with another molecule of POI and could act as a catalyst, inducing another cycle of ubiquitination and protein degradation. Thus, PROTACs can act at substoichiometric concentrations, this turnover contributing greatly to their efficacy. Moreover, their lower molecular weight than the previous generation led these compounds more attractive for in vivo applications. However, their non-optimal drug-like parameters still remains a challenging point to design optimal compounds for in vivo experiments and to envision their oral administration. In fact, as described in recent reviews^{[24][25]}, the optimization of ADME properties of such compounds has to be quite different compared to usual therapeutic molecules respecting the Lipinski’s rule of 5, due to their superior molecular weight (over 80%-120% of the recommended weight) and their extended Polar Surface Area (PSA) that could induce solubility and permeability problems, for example. Despite physicochemical properties that are more challenging to optimize for in vivo evaluations than small molecules, the PROTAC strategy offers greater opportunities than small molecule inhibitors. And this point is particularly true in the field of kinases, where the degradation of the protein not only remove the enzymatic

activity but also abolish its scaffolding properties, which could lead to better biological activities than the parent compound. These characteristics explained why PROTACs emerged as a promising new strategy in medicinal chemistry and led already to push several of these compounds in clinical trials.^[26] Recently, first Phase I results of two orally bioavailable PROTACs developed by Arvinas to degrade androgen or estrogen receptors showed that these compounds have been well tolerated by patients with no serious side effects.^[27] Future results are eagerly awaited to determine whether these compounds are of real clinical interest.

3. Receptor Tyrosine Kinase (RTKs) degraders

RTKs are cell-surface receptors having a similar molecular architecture. An RTK contains an extracellular-ligand-binding domain, a single transmembrane helix and a cytoplasmic region where the tyrosine kinase domain is located. Genetic mutations can alter the activity, abundance, cellular distribution or regulation of RTKs and can induce numerous diseases like cancers, diabetes, inflammation and severe bone or vascular disorders.^[28] Recently, three different RTKs were targeted using the PROTAC strategy: the FMS-Like Tyrosine kinase 3 (FLT3)^[29], the Tropomyosin receptor kinase C (TrkC)^[30] and the Anaplastic Lymphoma Kinase (ALK).^{[31][32]}

3.1 PROTAC for the degradation of FLT3

The FLT3 RTK is expressed in hematopoietic stem cells and plays an important role in normal hematopoiesis. Mutation of FLT3 is involved in Acute Myeloid Leukemia (AML), which is the most common form of acute leukemia. The insertion of a duplicated sequence, named internal tandem duplication (ITD), in the juxtamembrane domain region of the receptor leads to a constitutively active kinase and promotes cell proliferation.^{[33]–[35]} Thus, FLT3/ITD has been validated as a powerful therapeutic target for AML treatment^[35] but a maximum and sustained inhibition of FLT3/ITD signaling is essential to induce an effective clinical response.^[36]

In this context, Burlsem *et al.* have proposed a targeted protein degradation approach since small-molecule inhibitors cannot achieve a complete and sustained inhibition of FLT3/ITD signaling, while PROTAC compounds can.^[29] The authors described the conversion of quizartinib (AC220), a clinical candidate for the inhibition of FLT3^[37], into a PROTAC compound using a VHL ligand^[38] and an optimized PEG linker (compound **2a**, **Figure 2**). In vitro kinase activity assays showed that **2a** is 4 to 5-fold less potent than quizartinib to inhibit

both FLT3/ITD as well as wild-type FLT3 (IC_{50} ~50 nM for the PROTAC derivative against the mutated and the native kinases, compared to ~10 nM for quizartinib). It was also observed that **2a** showed a slightly better selectivity profile on a kinome inhibitory assay than quizartinib (24 kinases inhibited at 1 μ M vs. 57 for quizartinib). On MOLM-14 and MV4-11 cells, two leukemia cell lines expressing FLT3/ITD, the PROTAC derivative is >3.5-fold more potent than quizartinib to inhibit cell proliferation ($IC_{50} = 0.6 \pm 0.08$ nM vs. 1.87 ± 0.1 nM). Interestingly, low doses of **2a** led to a better apoptosis induction in leukemia cells than quizartinib, as shown by a greater activation of caspases 3/7 in the cells where the kinase has been degraded. Finally, in vivo assays were realized on athymic MV4-11 xenografted mice, treated once every 24 h for 3 days with 30 mg/kg compound (dose previously determined as providing an efficient plasmatic concentration to degrade the kinase). No adverse effects were observed, and a ~60% decrease of FLT3 levels were measured in tumors. This study showed that compound **2a** has a better anti-proliferative effect in cancer cells and is a more selective inhibitor than quizartinib, even if **2a** is a weaker inhibitor of the isolated kinase. This result supports that the degradation of FLT3/ITD by PROTACs allowed the access to more efficient bioactive molecules.

3.2 PROTACs for the degradation of TrkC

TrkC is the high affinity receptor for the neurotrophin NT-3 growth factor. This latter is particularly involved in neuronal differentiation and survival in the peripheral and central neuronal systems. Overexpression of this RTK is reported in many human tumors^[39], in particular in neuroblastoma^[40] and glioblastoma^[41], but also in breast cancer^[42] and melanoma.^[43] Aberrant activation of this RTK induces cell growth and metastasis in some forms of tumorigenesis.^[44]

Zhao and Burgess reported in 2019 the first PROTAC against this RTK.^[45] This one was based on a small molecule, a bivalent peptide (Isoleucine-Tyrosine-Tyrosine-Isoleucine) mimic that binds TrkC with submicromolar affinity ($K_d = 112$ nM) and offers a good internalization into cells.^{[46][47]} In this study, they synthesized three different conjugates with this TrkC inhibitor. Two presented a nutlin moiety, as the MDM2 recruiting moiety, and a PEG linker with two different lengths; and one conjugated with pomalidomide as CRBN recruiter (compound **2b**, **Figure 2**). In vitro assays showed poor potency of the nutlin-based PROTACs, whereas the pomalidomide-based PROTAC **2b** showed potent degradation of TrkC at 1-10 μ M in Hs578t cells, a TrkC⁺ breast cancer cell line. The authors estimated a

50% protein degradation concentration (DC₅₀) using this conjugate at 0.1-1.0 μM.^[30] This study yielded to the first and potent TrkC PROTAC and clearly provides new therapeutic opportunities for TrkC-dependent diseases. It could be interesting to develop new TrkC degraders by using another TrkC ligand like larotrectinib, a Trk inhibitor approved in 2018 by the FDA^[48] with an IC₅₀ < 1 nM^[49], or entrectinib, a Trk inhibitor approved in 2019 by the FDA^[48] with an IC₅₀ = 0.1 nM^[50], to determine if, in this case, the use of some POI ligands with a better affinity for the target could produce more potent TrkC PROTACs. Indeed, even if the affinity of the POI recruiter is not a crucial parameter as previously reported (see part 2 and reference ^[20]), in some cases using a more active POI recruiter led to more active PROTACs (see part 5.1).

3.3 PROTAC and Gold NanoParticle-based PROTAC for the degradation of ALK

ALK is part of the insulin receptor family but the exact physiological function of this RTK is not totally understood in mammalian cells.^[51] However, it is known that various forms of ALK-containing fusion proteins result in kinases capable of driving oncogenesis in various cancers. For example, the NMP-ALK fused form is present in anaplastic large cell lymphoma (ALCL).^[52] Several studies have shown that inhibition of ALK activity can suppress proliferation of cancer cells that harboring ALK fusion protein.^{[53][54]} Thus, the development of PROTACs targeting ALK seems to be a good opportunity to improve clinical responses provide by the already approved ALK inhibitors.

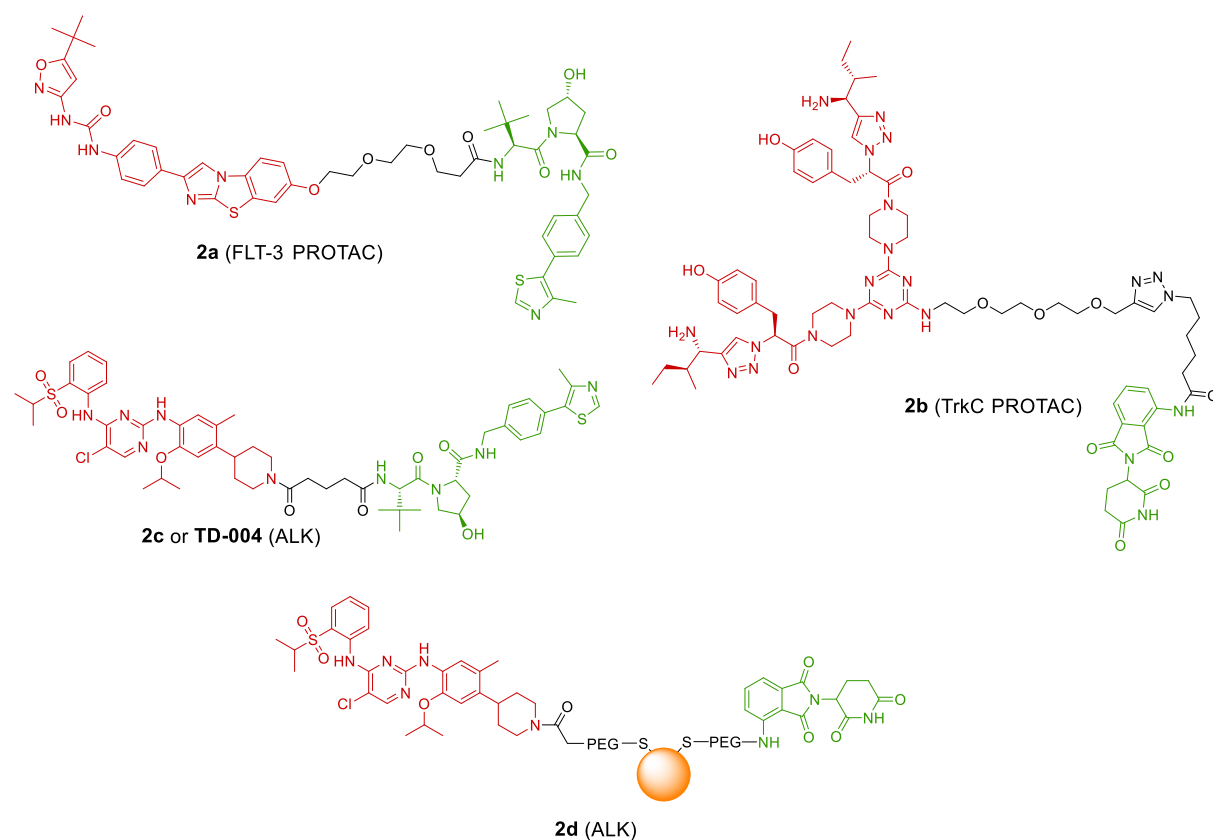
Here, Kang *et al.* reported the synthesis of a classical PROTAC using ceritinib, an ALK inhibitor approved by the FDA^[48], and a VHL ligand.^[31] Different linkers (composition and linkage) were tested and the compound bearing a propyl chain and an amide linkage (TD-004, **Figure 2**) showed the best results. In fact, TD-004 reduced over 90% the amount of NMP-ALK protein in SU-DHL-1 cells for a concentration of 1 μM. The authors demonstrated that TD-004 inhibited the cancer cell proliferation of SU-DHL-1 (IC₅₀ = 58 nM) and H3122 cells (IC₅₀ = 180 nM), which are both fusion ALK positive cancer cells, but TD-004 did not inhibit the A549 cell growth (IC₅₀ > 10 000 nM), a fusion ALK negative cancer cell line.^[31] These results clearly demonstrated the selectivity of TD-004 for ALK positive cancer cells. Once daily in vivo administration of TD004 (58 mg/kg) for 14 days to mice xenografted with H312 cells showed a significant diminution of the tumors size and no weight loss in mice. In summary, the authors found a PROTAC that induces a good degradation of ALK-fusion protein in vitro and in vivo, a good selectivity on ALK positive cancer cells and a good anti-

tumor efficacy on a mouse model. These results can be compared to other previously published results. In fact, Zhang *et al.*^[55] and Powell *et al.*^[56] also described ceritinib-based PROTACs for this kinase, but both with a CRBN E3 UL ligand (pomalidomide). PROTACs, which are described in these two previous studies, only differed by the length and the nature of their linkers. These two studies reported good anti-proliferative activities for these pomalidomide-based PROTACs (IC₅₀ 20-33 nM on SU-DHL-1 or H3122 cells), which were significantly better than the ones obtained with the VHL-based PROTAC. Unfortunately, in vivo results are not available for the PROTACs developed by Zhang *et al.* and Powell *et al.* However, comparison of these three studies highlights an important point on PROTACs development. In fact, as seen above, changing the nature or the length of the linker as well as the E3 UL recruited can deeply modify the biological activity of PROTAC compounds.

Another recent study, by Liu *et al.*, on an ALK fusion protein (EML4-ALK) degrader has been reported, but this time, a POI ligand was not connected to an E3 UL ligand. In fact, the authors described a multi-headed PROTAC by connecting several POI and E3 UL ligands on gold nanoparticles (GNP).^[32] There are multiple interests using this methodology, even if the efficacy of this first generation of GNP needs to be improved.^[57] Indeed, GNP could be easily functionalized due to the strong binding affinity of gold for thiol functions. Moreover the introduction of thiol-modified PEG chains, as spacers between the drug and GNP, increases the biocompatibility and the in vivo stability of the conjugates.^[58] It has been demonstrated that the presence of an excess of therapeutic ligands on particle surface can provide more advantages than monovalent ones. In the case of a multi-headed PROTAC, the multivalency could increase the probability of bringing the POI and the ligase in close proximity to form the ternary complex. To produce their GNP-based multi-headed PROTAC, the authors first prepared a modified ceritinib (ALK ligand) and a modified pomalidomide (CRBN ligand) by introducing on these two compounds a thiol-PEG chain. These two derivatives were then immobilized on GNP (**2d**, **Figure 2**). In cellulo assays demonstrated that **2d** co-localized in the cytoplasm with ALK proteins after 24 h incubation with NCI-H2228 cells (expressing EML4-ALK), and that the gold core remained intact with a good dispersion. They used the same cell line to examine the ability of their particles to induce EML4-ALK degradation. At a concentration of particles equivalent to 4 μM ceritinib, the authors observed that more than 80% of ALK-fusion proteins were degraded and described an inhibition of NCI-H2228 cell proliferation with an IC₅₀ = 4.8 μM. By testing their compound on an ALK-negative cell line (A549), they also found that their compound only induced minor off-target toxicity. However,

particles **2d** revealed to be less efficient to inhibit cell proliferation than ceritinib. Despite these mixed results, multivalency properties brought by GNP could offer a promising strategy to increase the probability of putting the POI and the E3 UL into close proximity, which consequently promotes the formation of the ternary complex.^[32] Further investigations, in particular in vivo evaluations, are necessary to evaluate the interest of such approach.

Figure 2. Structure of RTK degraders. POI-binding warheads appear in red, E3 UL recruiting moieties in green and linkers in black.



4. Non-Receptor Tyrosine Kinase (NRTK) degraders

NRTKs are cytoplasmic proteins playing an important role in the process of tumorigenesis. This family of enzymes structurally differs from each other, and their mechanism of activation is much more complex than the RTKs one.^[59] Recently, four NRTKs were targeted using the PROTAC technology: the Janus Kinases (JAKs)^[60], the Breakpoint Cluster Region-Abelson (BCR-ABL)^{[61][62]}, the Focal Adhesion Kinase (FAK)^{[63]–[65]} and the Bruton's Tyrosine Kinase (BTK).^{[66]–[72]}

4.1 PROTACs for the degradation of JAKs

The JAK-Signal Transducers and Activators of Transcription (JAK-STAT) signaling pathway is activated by diverse cytokines, interferons, growth factors and other molecules. This membrane-to-nucleus pathway is important to regulate different cell processes, and its dysfunction can cause several diseases. The JAKs family comprises four known isoforms (JAK1, JAK2, JAK3 and TYK2) which are proximal membrane-bounded.^[73] Targeting this family of enzymes by PROTACs to highjack JAK-STAT pathway may provide novel opportunities in therapeutic or biological applications.

Shah *et al.* developed a series of six PROTAC compounds capable of inducing JAK1/2 degradation.^[60] This series is the first example in literature that described JAKs degraders. The authors designed several PROTACs based on cIAP, CRBN and VHL ligands and using two POI inhibitors: a pyrimidine and a quinoxaline compounds, both of which inhibit several JAKs isoforms. For the quinoxaline derivative, the linker was introduced in the part of the molecule exposed to the solvent region of the kinase, whereas two sites of ligation were envisioned for the other inhibitor. A panel of alkyl and PEG linkers, from 10 to 20 atoms of length, was studied to determine the optimal linker for each compound. Biochemical assays on isolated JAK1-3 kinases showed that all constructs were able to inhibit the three isoforms efficiently. In most cases, pyrimidine derivatives were more active against JAK1, whilst quinoxaline showed a better potency against JAK2, as also observed with their respective parental compounds. In vitro assays using THP-1 cells, a human leukemia monocytic cell line expressing JAK1 and JAK2, showed thereafter that a significant degradation of JAK1/2 was only observed with compounds bearing a cIAP E3 UL ligand. Moreover, whereas the selectivity profile of quinoxaline-based PROTACs was similar to that of the parental compound (more active against JAK2 than against JAK1), surprisingly the pyrimidine-based PROTACs induced a greater JAK2 degradation compared to JAK1. For example, the quinoxaline JP-5 (compound **3a**, **Figure 3**) induced 85% of JAK2 degradation at a concentration of 5 μ M and 40-70% of JAK1 degradation at the same concentration. The pyrimidine JP-2 (compound **3b**, **Figure 3**) induced 55-95% of JAK2 degradation at 5 μ M and 40-70% of JAK1 degradation at the same concentration.^[60] Two hypotheses were proposed to explain this result. JAK2 could possess more (or more accessible) ubiquitylation sites than JAK1, contributing to its further degradation. Otherwise, since JAK2 has a longer half-life than JAK1, less resynthesis of this protein during the assay may have altered the final result. Anyway, this study demonstrates that PROTAC compounds could also be used for the

degradation of proximal membrane-bound proteins implicated in various diseases and thus increases applications and opportunities of the PROTAC technology. However, due to tremendous progress registered by targeting JAK kinases, the downstream effectors of JAKs, the STATs, might be more efficaciously targeted. It is for this reason that numerous efforts have been made to design effective PROTAC compounds targeting STAT3.^[74]

4.2 PROTACs for the degradation of BCR-ABL

BCR-ABL is a fusion protein essential for initiation, maintenance and progression of Chronic Myelogenous Leukemia (CML). Several BCR-ABL tyrosine kinase inhibitors (TKIs), including the well-known imatinib and dasatinib, have been approved by the FDA^[48] and are able to efficiently induce apoptosis of BCR-ABL mutated cells, with total remission for most treated patients. However, it had been proposed that primary CML stem cells could evade apoptosis despite inhibition of the kinase, which could contribute to the patient's relapse when their treatment is stopped. It had been demonstrated that the resistance to TKIs of CML stem cells is related to neither bcr-Abl gene amplification nor kinase domain mutation.^[75] Thus, non-kinase domains of the fusion protein, by their capability to recruit multiprotein signaling complexes (e.g. SHG, SHP2 and GAB2), were proposed to be involved in survival mechanisms.^[76] Since the BCR-ABL's scaffold protein function is not inhibited by TKIs, knockdown of BCR-ABL protein might be a good therapeutic strategy to remove persistent stem cells. Consequently, several groups reported the design of PROTAC compounds targeting this kinase.

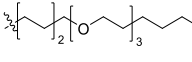
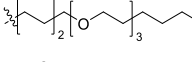
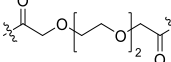
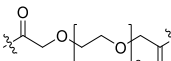
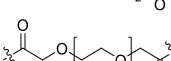
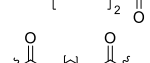
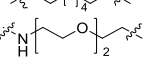
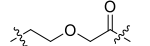
First works were performed using the competitive ATP inhibitor dasatinib as the POI recruiter. Several PROTACs were described by conjugating this TKI to CRBN, cIAP or VHL ligands.^{[77][78]} These PROTACs (**Table 1**), in particular the CRBN- and cIAP-based compounds, were able to efficiently degrade BCR-ABL, but the studies revealed that using dasatinib and a VHL ligand linked by a PEG chain was not suitable to obtain good BCR-ABL degradation profiles. In fact, only the non-fusion cABL protein was degraded by these VHL PROTACs. To solve this problem, Zhao *et al.* designed two series of VHL-based PROTAC compounds using dasatinib as the BCR-ABL ligand.^[61] These two series only differ by the nature of the linker. In a first series, the authors introduced a PEG linker, as already studied by Crews^[77] and Naito's groups.^[78] However, they were not able to enhance the activity of the previous reported VHL-based PROTACs. In fact, whereas some designed PROTAC showed good potency to inhibit leukemic cell proliferation, they were not able to efficiently

degrade BCR-ABL. Then, they turned their attention to various alkyl linkers and showed that an optimal alkyl linker length should comprise 5-10 atoms. SIAIS178, with an eight-carbon atom linker length (compound **3c**, **Figure 3** and **Table 1**) was the best degrader of BCR-ABL yielded in this study, with a DC₅₀ value of 8.5 nM in K562 cells. Concerning its selectivity, SIAIS178 showed a better profile than its parental compound on a kinome scan, with high binding affinity for BCR-ABL, c-ABL, ABL2, EPHB4, RIPK2, Src, and their cell levels were decreased in a dose-dependent manner. However, among all these proteins only BCR-ABL was the most severely degraded, showing the relatively good selectivity of this PROTAC compound. The authors also performed in vivo assays in a murine xenograft model with K562 cells. They demonstrated that SIAIS178 induced tumor regression, without any apparent toxicity or significant weight loss in mice. Although, this compound was not more effective than dasatinib in vivo,^[61] it maintained a longer cellular activity after its removal, that is not the case for dasatinib. Finally, this study reported the first efficient BCR-ABL degrader based on a VHL ligand, confirming that the nature and the length of the linker are two important parameters to access efficient PROTACs.

Recently, Burslem *et al.* described a VHL-based PROTAC^[62] with an allosteric inhibitor of BCR-ABL, GNF2,^[79] as the POI recruiter. This POI recruiter has already been used by Shimokawa *et al.*^[80] in 2017, but in a cIAP-based PROTAC, to yield an efficient BCR-ABL degrader (**Table 1**) with a DC₅₀ of 30 nM in K562 cell assays. This compound was also able to inhibit K562 cell proliferation (IC₅₀ ~100 nM). In the case of Burslem *et al.*, a PEG linker was introduced on the primary amide function of GNF-2, which is exposed to the solvent region of the kinase. Then, replacement of the amide bond with an ether function as well as its displacement from the *meta* to the *para* position of the phenyl ring increased the activity of the VHL-based PROTAC compound (DC₅₀ decreased from 2 μM to 340 nM). Thus, GMB-475 (compound **3d**, **Figure 3** and **Table 1**) was able to inhibit the proliferation of mutated cells (on murine and human leukemic cell lines, but also on primary CML patient samples). Moreover, their compound did not display any toxicity against non-mutated cells up to 10 μM, thus showing a good selectivity. The authors also studied the impact of BCR-ABL degradation on key proteins which could be involved into the stem cell survival. They showed that the degradation of the targeted kinase, at the difference at its inhibition alone, led to a decrease of the phosphorylation of SGH, SHP2 and GAB2 proteins in leukemic K562 cells. This result demonstrated the great interest of the PROTAC approach over the use of conventional TKIs. Finally, the authors performed a co-treatment with GMB-475 and imatinib

on Ba/F3 mutated cells. They showed that this co-treatment caused an almost 3-fold decrease in IC_{50} of imatinib on the cell proliferation inhibition.^[62] Thus, the authors demonstrated for the first time the opportunity of using an allosteric PROTAC in concert with an orthosteric inhibitor to reduce the dose of this inhibitor. Such therapeutic approach could limit the side effects of TKI treatments. The PROTAC developed by Shimokawa *et al.* offered a better degradation potency on K562 cells than GMB-475 and has also shown a hijacking of the downstream signal proteins. Thus, we can reasonably assume that attractive results could be expected if the compound developed by Shimokawa *et al.* is tested in combination with a conventional TKI, as proposed by Burslem *et al.*

Table 1. Comparison of structure and activities of BCR-ABL PROTACs.

Cpd	POI recruiter	E3 UL recruiter	Linker	IC_{50} (nM) ^a	CC_{50} (nM) ^b	DC_{50} (nM)	Ref
DAS-CRBN 1	Dasatinib	Pomalidomide		0.60	4.4 ^c	200	[77]
DAS-VHL 1	Dasatinib	VHL ligand		0.92	NR ^d	>10 ⁴	[77]
DAS-CRBN 2	Dasatinib	Pomalidomide		NR	~10 ^c	~100	[78]
DAS-VHL 2	Dasatinib	VHL ligand		NR	>30 ^c	>300	[78]
DAS-IAP	Dasatinib	clAP ligand		0.54	8.60 ^c	20	[78]
SIAIS178 (3c)	Dasatinib	VHL ligand		NR	24 ^c	8.5	[61]
SNIPER-62	GNF2	clAP ligand		360	~100 ^c	~30	[80]
GMB-475 (3d)	GNF2	VHL ligand		NR	1.11 ^e	340	[62]

^a: Affinities for the ABL kinase domain

^b: cell viability

^c: K562 cells

^d: NR: not reported

^e: BCR-ABL1 transformed Ba/F3 cells

4.3 PROTACs for the degradation of FAK

Focal Adhesion Kinase (FAK), also called protein tyrosine kinase 2 (PTK2), is an NRTK implicated in many aspects of tumor growth, through kinase-dependent and kinase-independent mechanisms. Its increase in expression and/or activity is often associated with poor clinical outcomes. In fact, FAK comprises four important domains: a central domain displaying the kinase activity, an N-terminal four-point-one, ezrin, radixin, moesin (FERM) domain, a domain composed of many proline residues and a focal adhesion targeting (FAT) domain in C-terminus. This structuration allows FAK to have both tyrosine kinase and scaffolding activities. Several competitive ATP TKIs were developed against FAK, while

pharmacologic targeting of its scaffold function is at an early stage of development.^[81] Thus, the PROTAC technology seems to be a good answer to stop, at the same time, all the functions of FAK.

Popow *et al.* were the first to report a set of FAK-PROTACs recruiting VHL and CRBN,^[63] based on a close analog of an in-house selective and potent ATP-competitive inhibitor.^{[82][83]} They tested linker lengths up to five ethylene glycol units and identified, as best degraders, the compounds bearing a three ethylene glycol unit linker in the CRBN and VHL series: BI-3663 and BI-0319 respectively (compounds **3e** and **3f**, **Figure 3**). In vitro assays on human lung adenocarcinoma A549 cells showed that the CRBN-based PROTAC degraded FAK with a DC₅₀ value of 27 nM, whereas the VHL-based PROTAC was less potent with a DC₅₀ value of 243 nM after 16h treatment, despite their similar potency to inhibit FAK (IC₅₀ ≈ 18 nM). The authors demonstrated that their CRBN- and VHL-based PROTACs had high FAK-selectivity, because these compounds did not induce any significant changes in the abundance of other detectable kinases. BI-3663 and BI-0319 showed comparable degradation potency and efficacy in a set of 11 hepatocellular carcinoma cell lines but did not induce better anti-proliferative effects than the FAK inhibitor alone, even after 21 days of cell treatments.^[63]

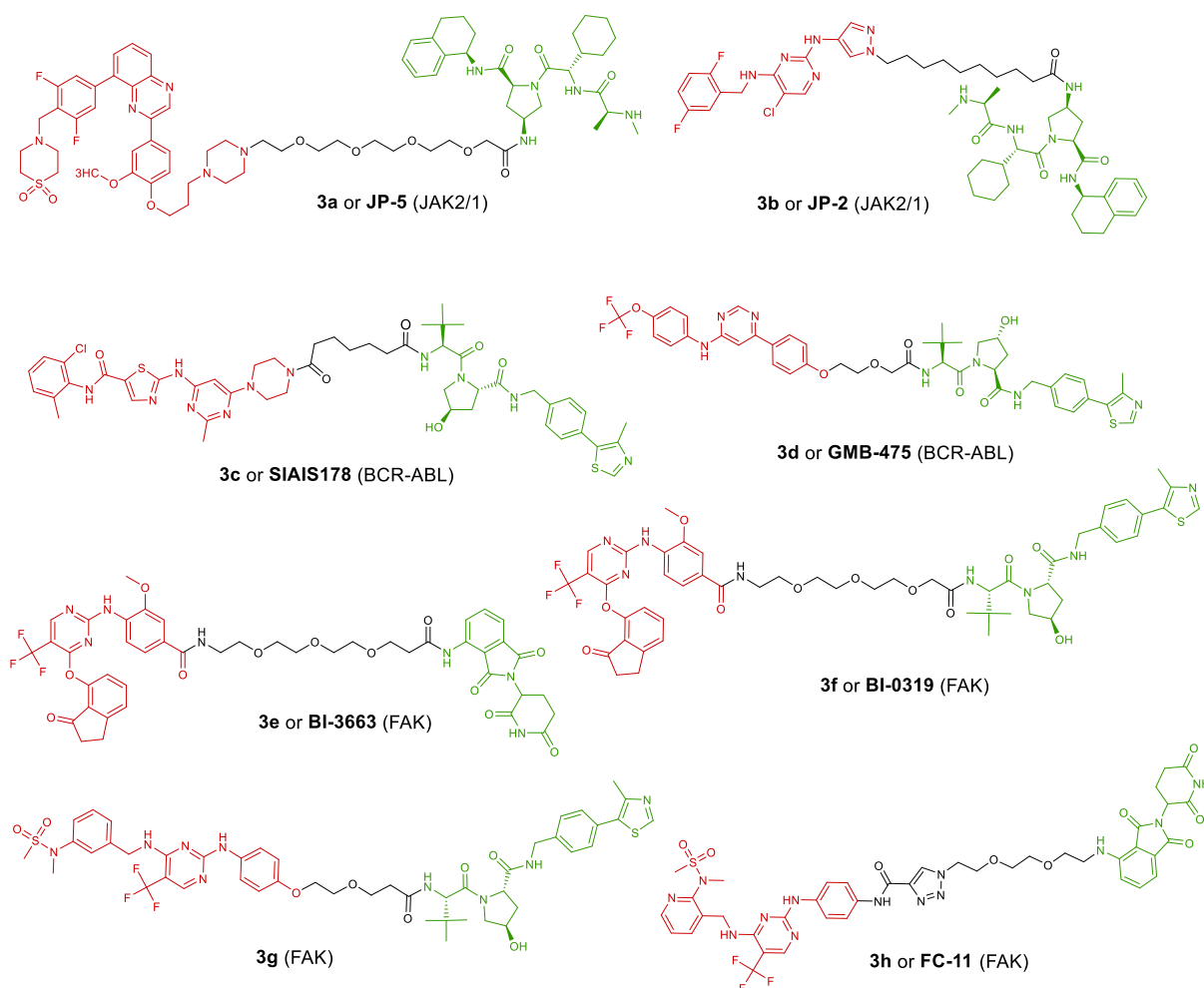
Crews' group has also designed several VHL- and CRBN-based FAK-PROTACs,^[64] using defactinib, a FAK inhibitor currently in clinical trials, as the POI recruiter. Defactinib was slightly modified to facilitate the anchoring of the linker, but also to reduce the multistep synthesis of the compounds. Six different PEG linkers were tested on in vitro and in cellulo (PC3 cells) assays. VHL-based PROTACs bearing a 6 or 7 atom length linker showed the best potency to degrade the targeted kinase. Interestingly, these assays highlighted the fact that PROTACs inhibition and degradation activity did not systemically correlate. In fact, one of the best FAK-inhibiting PROTAC of this study (IC₅₀ = 4.7 ± 0.3 nM) was one of the least potent degraders (DC₅₀ = 26.7 ± 14.8 nM), whereas the least potent FAK-inhibiting PROTAC of this study (IC₅₀ = 14.5 ± 0.6 nM) was one of the best potent degraders (DC₅₀ = 4.0 ± 2.2 nM). Finally, the authors described compound **3g** (**Figure 3**) as the best FAK-degrader of this study, with a DC₅₀ value of 3.0 ± 1.5 nM and demonstrated that their compound showed higher FAK selectivity than defactinib. In fact, at a 1 μM concentration, defactinib bound to about a hundred kinases on a kinome scan assay, while **3g** only bound to only 20 kinases under similar conditions. The authors also evaluated the effect of their compound on MDA-MB-231 cell migration, invasion and proliferation. No anti-proliferative effects were observed with the use of **3g** or defactinib. However, they found that **3g**, at low nanomolar

concentrations, showed significantly reduced cell invasion and migration, while defactinib showed no significant effects. These results suggested that cell invasion and migration depend on FAK's kinase-independent signaling. To the authors, their compound is the first degrader that outperforms an optimized kinase inhibitor and shows strong different biological results.^[64]

Gao *et al.* also designed several CRBN-based PROTACs by using two FAK inhibitors, VS6063 and PF562271,^[84] and tested different linkers, introduced by click chemistry.^[65] The authors, after testing the degradation potency of their PROTACs library, chose FC-11 (**Figure 3**), a PF562271-based PROTAC, as the more efficient degrader, with 90% of FAK degradation at a concentration of 1 nM in human ovarian PA1 cells, after 8 h treatment. This compound also showed high FAK degradation potency in a set of five normal and cancer cell lines, with subnanomolar DC₅₀. However, no selectivity profile of the PROTACs was provided, even if the authors assume their compounds were more selective than the small-molecule inhibitor. Unfortunately, like with other previous designed FAK-PROTACs, the anti-proliferative activity was not significantly better than that obtained with the parental FAK inhibitor in the tested cell lines.^[65]

A comparison of these three studies shows that, even if potent PROTACs against this kinase have been obtained, its degradation does not affect the proliferation of FAK-addicted cancer cells. More studies are expected to explain why depletion of FAK does not induce better anti-proliferative effect than small-molecule inhibitors, while several PROTACs can do it on other kinases. However, the conversion of a FAK TKI into a PROTAC compound could enhance the selectivity towards the targeted protein and also induce unobtainable effects on the scaffolding properties of the kinase, as for example, those involved in migration and invasion of cancer cells.

Figure 3. Structure of JAK, BCR-ABL and FAK degraders. POI-binding warheads appear in red, E3 UL recruiting moieties in green and linkers in black.



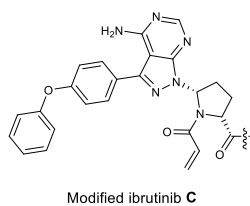
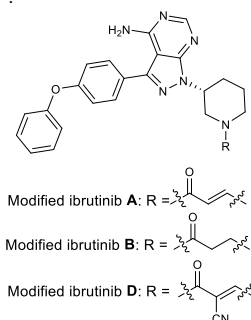
4.4 PROTACs for the degradation of BTK

BTK plays an important role in the viability of leukemic cells in many B cell malignancies and was also shown to be essential both for B cell development and function of mature B cells. Several small-molecule BTK inhibitors^[48] and, in particular the approved irreversible inhibitor ibrutinib, have shown excellent anti-tumor activity. The inhibition of BTK as an anti-cancer therapy has shown a considerable interest in the medicinal chemistry domain, not only in B cell malignancies but also in solid tumors.^[85] Reasonably, in this context, several BTK-PROTACs were developed (**Table 2**).

Table 2. Comparison of structure and activities of BTK PROTACs.

Cpd	POI binding warhead	NC/C/R C ^b	E3 UL recruiter	Bond ^d	Linker (type - number of atoms) ^e	IC ₅₀ (nM)	DC ₅₀ (nM)	Ref
4a	PF06250112	NC	Pomalidomide	Alkyl	PEG - 20	NR ^f	5.9 ^g	[66]
4e	Ibrutinib A ^a	C	clAP ligand	Amide	PEG - 22	26.5	ND ^h	[67]
4f	Ibrutinib B ^a	NC	clAP ligand	Amide	PEG - 22	58	200 ⁱ	[67]
4g	Ibrutinib C ^a	C	Pomalidomide	Amide	Alkyl - 9	186	< 300 ^j	[68]
4h	PLS-123	C	VHL ligand	Alkyl	PEG - 9	39	136 ^j	[68]
MT802 (4c)	Ibrutinib B	NC	Pomalidomide	Amide	PEG - 8	47	9.1 ^k	[69]
SJF620 (4d)	Ibrutinib B	NC	O-Lenalidomide ^c	Ether	PEG - 8	NR	7.9 ^k	[70]
SPB5808 (4b)	Ibrutinib B	NC	4-OH-thalidomide	Ether	PEG - 12	127	~ 250 ^l	[71]
4j	Ibrutinib B	NC	4-OH-thalidomide	Ether	PEG - 18	159	< 200 ^g	[72]
NC-1	Ibrutinib B	NC	4-OH-thalidomide	Ether	PEG - 20	NR	2.2 ^m	[86]
IR-2	Ibrutinib A	C	4-OH-thalidomide	Ether	PEG - 20	NR	1.9 ^m	[86]
RC-3	Ibrutinib D ^a	RC	4-OH-thalidomide	Ether	PEG - 20	NR	6 ^m	[86]

a:



b: Type of inhibition: C: covalent; NC: non covalent; RC: reversible covalent

c: The primary amine of lenalidomide was replaced by an OH

d: Nature of the bond between the linker and the UE3 recruiter

e: For more details, see Figure 4 (linker appears in black)

f: NR: not reported

g: Ramos cells

h: ND: not determined

i: THP-1 cells

j: K562 cells

k: NAMALWA cells

l: JeKo cells

m: Mino cells

Zorba *et al.* developed a set of 11 CRBN-based PROTACs composed of a BTK binding moiety, which is a non-covalent analog derived from the previously disclosed covalent PF06250112^[87], and a PEG-linker of different lengths.^[66] They also synthesized a small set of VHL-based and IAP-based PROTACs with the same POI recruiter and evaluated all these constructs to their capability to degrade BTK in Ramos cells. Optimal results were obtained within the CRBN series. More precisely, PROTACs with longer linker lengths (14 to 21 atoms) in comparison with shorter PROTACs (5 to 9 atoms) were able to potently degrade BTK. Compound **4a** (Figure 4), which selectively degraded 82% of BTK at 1 μ M in Ramos cells after 24 h of incubation, have been selected for further in vivo investigations. Rats treated subcutaneously with **4a** (175 mg/kg twice a day during 48 h) showed a drastic reduction of BTK levels in spleen but not in lungs, despite similar tissue uptake and plasma availability of the compound. These results demonstrated that their BTK-PROTAC is

delivered to the tissue and can degrade BTK in vivo, in a dose- and tissue-dependent manner. This study also displayed the fact that BTK knockdown seems to be E3 UL-dependent, as VHL-based and IAP-based PROTACs were ineffective.^[66]

In 2018, Crews' group described a BTK degrader, MT802 (compound **4c**, **Figure 4**), consisting of a non-covalent analog of ibrutinib as the BTK ligand, a PEG linker and a pomalidomide-CRBN ligand.^[69] This compound revealed to be a potent and selective BTK degrader with a DC_{50} of 9.1 nM and a complete BTK knockdown at 250 nM in 4 h. Interestingly, this PROTAC was able to degrade both wild-type and C481S-mutated BTK, whereas ibrutinib was not active against the mutated kinase. However, this PROTAC did not show good pharmacokinetic parameters and thus was not suitable for in vivo studies. In fact, in mice, its clearance was too high and its half-life too short (1662 mL/min/kg and 0.119 h respectively). Concomitantly, the same team as well as another one reported thereafter very similar constructs to MT802 with better pharmacokinetic profiles. In 2020, Jaime-Fiueroa *et al.* studied different strategies to improve the pharmacokinetic properties of MT802 by modifying several parts of its structure.^[70] They first tried to change the CRBN moiety by two different VHL ligands. Despite the search of an optimized linker for these VHL-based compounds, the authors showed an important decreasing of potency compared to MT802. Then, they explored modifications of the CRBN ligand (a lenalidomide analog instead of pomalidomide) and its anchoring mode to the linker (an ether instead of an amide bond). These two modifications yielded to SJF620 (compound **4d**, **Figure 4**), which showed similar potency to MT802 (**Table 2**) and a better pharmacokinetic profile with a lower clearance and a longer half-life (40.8 mL/min/kg and 1.64 h respectively).^[70] In parallel, Liu *et al.* used also an ether bond to anchor the PEG linker to the thalidomide moiety and increased the linker length (12 atoms vs. 9 for MT802).^[71] SPB5208 (compound **4b**, **Figure 4**), was tested on the Mantle Cell Lymphoma (MCL) JeKo-1 cells^[88], known to express BTK, and the effect on cell proliferation was evaluated. The results showed a lower activity than ibrutinib itself on cell proliferation but the activity remained good ($IC_{50} \approx 0.1 \mu\text{M}$ vs. 2 nM). SPB5208 showed a maximal degradation of BTK (70%) at 500 nM after 24 h treatment. A selectivity experiment of their compound on five kinases showed that SPB5208 is more selective than ibrutinib for BTK, as already observed with MT802.^[69] The authors also tested the degradation induced by SPB5208 in vivo by treating ICR mice once a day for five days with a concentration of 15 mg/kg and 50 mg/kg. For these two concentrations, a degradation of more than 50% of BTK protein in the spleen of mice with no abnormality of mice's viscera were obtained.^[71] These

two studies pointed out that small modifications brought on PROTACs can provide access to compounds with suitable pharmacokinetic profiles for in vivo studies.

Whereas the previous studies explored non-covalent degraders, Tinworth *et al.* evaluated covalent BTK degraders and compared their activities to their non-covalent analogs.^[67] For that purpose, the authors designed two cIAP-based and two CRBN-based PROTACs. Each series consisted of one covalent binding PROTAC, using ibrutinib as the POI-binding warhead (compound **4e**, **Figure 4**) and one reversible PROTAC, using a non-covalent analog of ibrutinib (compound **4f**, **Figure 4**). The authors tested the cIAP series on THP-1 cells (cells known to express cIAP U3 ligase) and found that both **4e** and **4f** efficiently inhibited BTK. However, only the non-covalent PROTAC **4f** showed a degradation of BTK ($DC_{50} \approx 200$ nM), while the covalent one **4e** showed no degradation even at a concentration of 10 μ M. Similar results were obtained with their CRBN-based PROTACs. These results suggested that covalent cIAP- and CRBN-based PROTACs were not able to degrade BTK in cells. However, in another study, Xue *et al.* successfully developed PROTACs associating covalent analogs of ibrutinib and a CRBN- or a VHL-recruiting moiety, with good capabilities to degrade BTK.^[68] The CRBN-based PROTAC with PLS-123^[89] as the BTK-binding warhead and an alkyl linker (compound **4g**, **Figure 4**) showed a DC_{50} value less than 300 nM, after a 24 h treatment of leukemic K562 cells and a maximal BTK degradation (75%) at 1 μ M. The VHL-based PROTAC with a covalent analog of ibrutinib^[90] and a PEG linker (compound **4h**, **Figure 4**) showed a better DC_{50} value (136 nM) after 18 h of treatment and also a better maximal BTK degradation (88%) at 1 μ M. Finally, the authors turned these two previous covalent PROTACs into their non-covalent analogs, by reducing the reactive alkene moiety and then tested their activities. When the non-covalent compound maintained its affinity for BTK, as it was the case with the CRBN-based PROTAC, the compound showed similar degradation activity as its covalent analog. However, when the affinity for BTK decreased, as observed with the non-covalent VHL-based PROTAC, the degrader became less efficient. These results suggest that, whereas the turnovers (see paragraph 2) could compensate the moderate affinity of non-covalent PROTACs for their targets, covalent PROTACs, for which the recycling is abolished, need to possess a strong binding affinity to efficiently degrade the POI.^[68] Anyway, this study shows that it is possible to develop efficient BTK-covalent degraders. The results of Tinworth and Pan could be explained by the position of the covalent binding site of their respective PROTACs (**Figure 4** and **Table 2**). In fact, the alkene moiety, responsible for the covalent binding between the PROTACs and BTK, is not at the same place

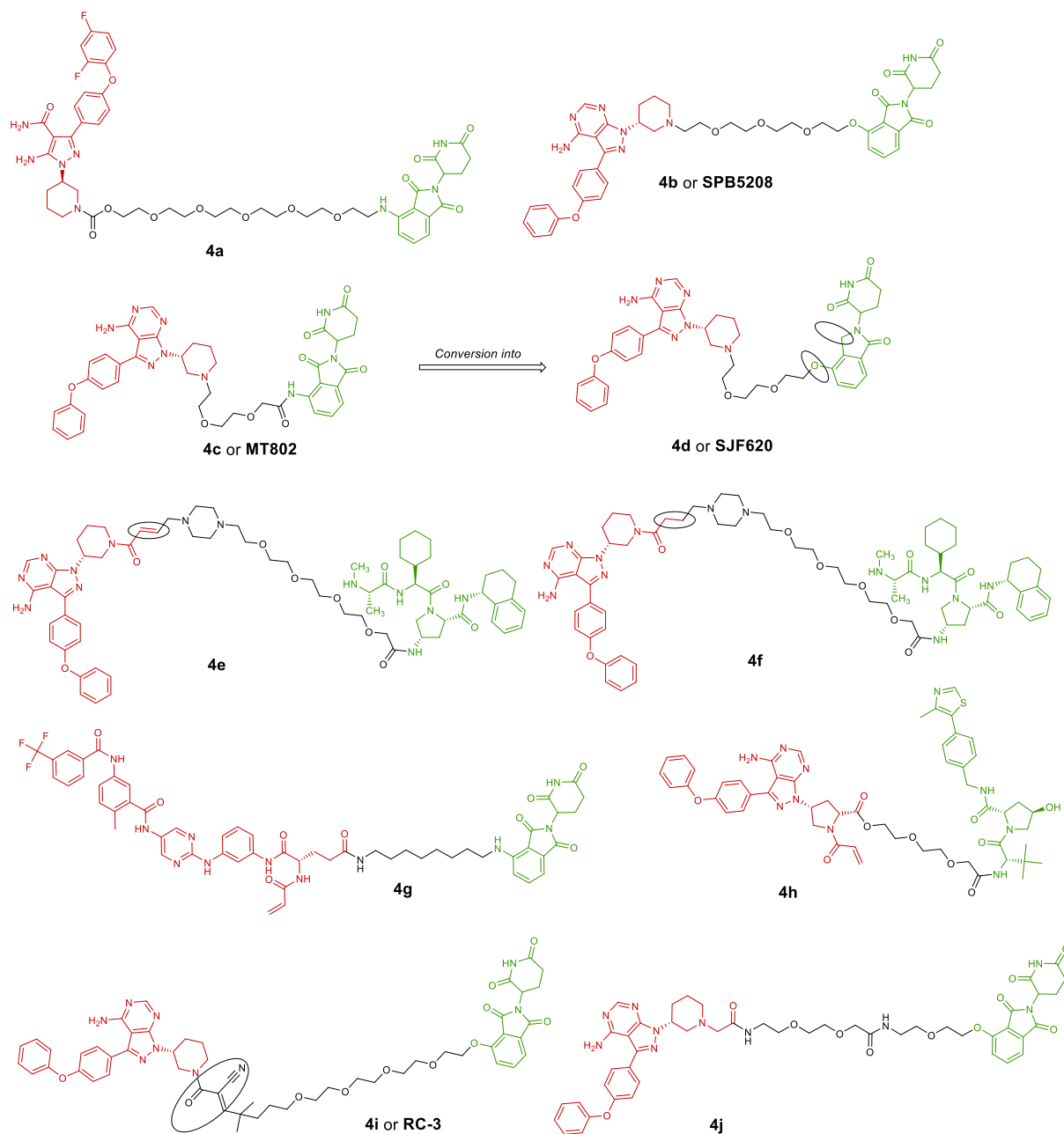
in the PROTAC molecule. In the first case, this function is part of the linker and possibly blocks the ternary complex into a position where the recruited E3 UL cannot execute the ubiquitination process. In the second case, the alkene function is farther from the linker and could probably allow the ternary complex to go into a favorable POI-ubiquitination position.

Recently, Gabizon *et al.* proposed to use reversible covalent PROTAC for BTK in order to combine the advantages of covalent inhibitors (enhanced potency, selectivity, and long duration of action) with those of a reversible mechanism due to the recycling of PROTACs by the cell.^[86] For that purpose, the authors used a cyanoacrylamide modified ibrutinib as the POI recruiter. This latter was previously developed by Bradshaw *et al.* by introducing an electrophile moiety into ibrutinib, namely a cyanoacrylamide function, to generate a kinase inhibitor^[91] with a prolonged on-target residence time. In fact, the introduction of a nitrile group converts an irreversible acrylamide Michael acceptor into an electrophile which could react with the cysteine thiol reversibly. The electron-withdrawing properties of the cyano group render the β -carbon more susceptible to nucleophilic attack by the thiol group, but also facilitate the thiol elimination by increasing the acidity of the α -proton.^[92] Based on this POI-binding warhead, the authors synthesized several CRBN-based PROTACs with various PEG-based linkers. Thanks to an assay on K562 or Mino cells, the authors determined an optimal five PEG unit linker for their compounds. They also synthesized two analogs of their reversible covalent PROTACs: a reversible non-covalent one and an irreversible PROTAC. The reversible non-covalent and the irreversible one (called NC-1 and IR-2 respectively in the study^[86]) were more potent than their best reversible covalent compound (RC-3; **Figure 4**) to degrade BTK into Mino cells and in primary cells from chronic lymphocytic leukemia patients. However, the three kinds of constructs were able to induce a strong degradation of the target (>85%) within 2-4 h. Moreover, RC-3 only induced the degradation of one off-target (BLK), while the others significantly degraded CSK, LYN and BLK. This enhanced selectivity was attributed to the reversible covalent mechanism of this PROTAC, but also to the introduction of a gem dimethyl group into the linker of RC-3. To demonstrate the reversible covalent profile of this latter, the authors performed several experiments. The first one evaluated their PROTACs on a mutated BTK, where the cysteine 481 known to react with ibrutinib was replaced by a serine residue. While the degradation induced by NC-1 was only mildly affected by the mutation, the degradation induced by RC-3 was severely impacted, showing the importance of this binding for the degradation. Another experiment consisted in incubating the compounds with wild-type BTK and then adding ibrutinib to evaluate the

reversibility of the binding and determine the dissociation kinetics. The displacement of NC-1 by ibrutinib was extremely rapid as expected, whereas the displacement of RC-3 by ibrutinib was slow (10-20 h). Taken together, these results confirmed the covalent reversibility of RC-3.^[86] Whereas some previous studies seemed to indicate that irreversible binding might be detrimental to the activity of covalent PROTACs, this study reported another example of efficient covalent PROTAC to degrade BTK. Moreover, this study also shows that the conversion of a “classic” reversible non-covalent PROTAC into a reversible covalent compound could enhance the selectivity of the degrader, despite a slight loss of potency.

Krajcovicova *et al.* have developed a new methodology to rapidly synthesized CRBN-based PROTACs and took as a proof of concept for their method, the synthesis of several BTK-PROTACs.^[72] This method consists of a preloaded resin with a thalidomide moiety and a small PEG linker which could be further acetylated to increase its length. Finally, several commercially available protein kinase inhibitors were introduced on the linker. The preparation of these compounds was really easy to set up. In fact, it consisted of a 6-16 h coupling time and a final 2 h cleavage of the resin with a trifluoroacetic acid/dichloromethane cocktail (1:1) at room temperature to get the final PROTAC. Further experiments were then realized with one of their compounds, PROTAC **4j** (**Figure 4**), possessing a very similar structure than MT802 reported by the Crews group.^[69] **4j** was tested on Ramos cells and showed an efficient degradation effect of BTK at a concentration of 200 nM for a 16 h treatment. An 85% BTK knockdown was achieved at a concentration of 2 μ M.^[72] With this new development tool in hands, the time production of CRBN-based PROTACs could considerably decrease and permit more rapid preliminary screening. This kind of tool for the synthesis of cIAP-, VHL- and MDM2-based PROTAC could enhance the time production of other categories of PROTACs.

Figure 4. Structure of BTK degraders. POI-binding warheads appear in red, E3 UL recruiting moieties in green and linkers in black. Key modifications (see the text) are circled in black.



5. Serine-Threonine Kinase (STKs) degraders

Most STKs are involved in RTKs downstream signaling pathways and are essential for cell proliferation and the onset of anti-apoptotic signaling. The deletion of negative regulators, such as the tumor suppressor PTEN (Phosphatase and TENsin homolog), combined with an enhanced activity of STKs are involved in various diseases.^[93] Important families of STKs, like the cyclin-dependent kinases (CDK) and the polo-like kinase (PLK), are key elements for

the regulation of the cell cycle progression and constitute attractive targets for cancer therapy. Other STKs are involved into signaling cascade leading to the production of pro-inflammatory cytokines. Thus, their inhibition could offer new ways to treat inflammatory or autoimmune diseases. Recently, several STKs were targeted using the PROTAC technology: the Interleukin-1 Receptor-Associated Kinase 4 (IRAK4)^[94], the Serum and Glucocorticoid-induced protein Kinase 3 (SGK3)^[95], the Polo-Like Kinase 1 (PLK1)^[96], several members of the Cyclin-Dependent Kinase (CDK) family^{[97]–[102]} and the B isoform of the Rapidly Accelerated Fibrosarcoma (BRAF).^[103]

5.1 PROTAC for the degradation of IRAK4

IRAK4 is an STK involved in transduction pathways stimulated by two types of receptors: the Toll-like receptors and the Interleukin-1 family of receptors.^[104] Its activation has been reported to be associated with various autoimmune diseases, due to its role in immune responses, and cancer.^{[105][106]} As seen with FAK and BCR-ABL, IRAK4 also has a non-kinase function (scaffolding protein) in several cell types, added to its kinase activity.^{[106][107]} Thus, the use of PROTACs to eliminate IRAK4 protein, instead of just inhibiting it, could constitute new therapeutic opportunities.

Nunes *et al.* designed several PROTACs based on a simplified IRAK4 kinase ligand, PF-06650833, where the fluorine and the ethyl chain of the lactam moiety were removed from the inhibitor structure.^[94] These modifications led to a 10-fold less potent, but still highly active inhibitor (IC₅₀ of 2.1 nM *vs* 0.2 nM^[108]). The authors tested the degradation activity of CRBN-, VHL- and cIAP-based PROTACs with 12 atom PEG or alkyl linkers on Peripheral Blood Mononuclear Cells (PBMCs). They found out that only the VHL-compound with an alkyl linker led to the degradation of IRAK4 in this cell type. This PROTAC showed a maximal degradation of 50% at a concentration of 3 μM, after 24 h treatment. The PEG-linked VHL PROTAC did not induce any degradation due to probable weak cell permeability. The authors modified their degrader to increase its potency by converting the PF-06650833 analog warhead into the fully functionalized PF-06650833 compound (addition of the fluorine and the ethyl chain on the lactam moiety) and by modifying the 12-atom carbon linker to a more rigid spirocyclic pyrimidine. This new compound (**5b**, **Figure 5**) showed better degradation potency with a DC₅₀ of 151 nM in PBMCs and a good *in vitro* metabolic stability. The authors also tested their compound in human dermal fibroblasts and found out that it was efficient to degrade the kinase (DC₅₀ of 36 nM), but did not improve the blocking

secretion of inflammatory cytokines, compared to the parent inhibitor. Finally, the authors studied the potential modification of the scaffolding activity of the kinase. Whereas, the IRAK4 knockout in dermal fibroblasts^[109] have shown to totally block the secretion of IL-6 stimulated by IL-1 β , the IRAK4-PROTAC was not able to do the same. This last point suggests that more work is required to understand the biology of this target and its associated signaling network.^[94] Recently, Kymera Therapeutics have announced that an orally potent IRAK4-PROTAC (named KTX-582) was efficient to induce in vivo tumor regressions in lymphoma models and this degrader is expected to enter a phase I of clinical trials in the near future.^{[110][111]}

5.2 PROTAC for the degradation of SGK3

SGK3 is an isoform of the SGK family which are key downstream components of the Phosphoinositide-3-Kinase (PI3K)/mammalian Target Of Rapamycin (mTOR) pathway.^[112] This pathway, when hyperactivated, is a driver of tumor proliferation, survival and is represented in the majority of human cancers.^[113] As a target of PI3K, SGK3 has been found to be implicated in the regulation of several cellular processes and several studies reported its implication in the development and progression of several cancers, e.g. breast cancer, prostate cancer and melanoma.^[114] Thus, the inhibition of SGK3 could constitute a relevant therapeutic target in cancer treatment but, due to the high homology of the SGK ATP domain, it has not been possible to produce until now specific ATP competitive inhibitors that only targeting one isoform of the SGK family.^[115] In this context, the conversion into PROTACs of SGK inhibitors seems to be a reasonable solution to obtain selective compounds that only target one SGK isoform due to the potential increasing selectivity property of PROTAC compounds.

Tovell *et al.* described in their study the design of one VHL-based PROTAC using the Sanofi-308 compound^[116] as the POI-binding warhead. This small molecule, in its *R* configuration, is able to inhibit SGK3 ($IC_{50} = 5$ nM),^[95] and it was relatively selective on a panel of 140 kinases, even at a concentration of 1 μ M. Two other kinases, the isoform SGK1 and the off-target kinase S6K1, were also inhibited in a 1-10 nM IC_{50} range. This PROTAC composed of a three-PEG unit linker significantly reduced the SGK3 expression on HEK293 cells (48% at a concentration of 1 μ M and 69% at 10 μ M, after 48 h) without impacting the levels of SGK1, SGK2 or S6K1. The authors have then elaborated several analogs of the first identified PROTAC by shortening or extending the linker length. They found that four PEG units (13

atoms) was the optimal linker length. This new degrader displayed significantly improved potency, compared to its parental compound (reduction of 56% at a concentration of 1 μM and 80% at 10 μM after 48 h). After this first optimization, the authors changed the nature of the linker to increase the lipophilicity of this new degrader. The best activity was obtained with a 13-atom length linker composed of one hexyl chain and two-PEG units (compound **5a**, **Figure 5**). This compound reduced SGK3 levels by 65% at a concentration of 0.1 μM after 48 h, without affecting SGK1, SGK2 or S6K1 in HEK293 cells. A maximal degradation (70-80%) was obtained after 8h at a concentration of 0.3 μM . This selectivity was also observed in two SGK3 dependent breast cancer cell lines (CAMA-1 and ZR-75-1). The effect of **5a** on the proliferation of these cell lines was also studied. However, no effect on cell growth was observed.^[95] This study stresses that an increase in selectivity could be promoted by the conversion of inhibitors into PROTACs. It also offers to the community a selective and efficient chemical probe to further investigations on the SGK3 cell role.

5.3 Dual PROTAC for the degradation of PLK1 and bromodomain 4 (BRD4)

PLK1 overexpression is found in various malignancies such as Acute Myeloid Leukemia (AML). In this hematological malignancy, a member of the Bromodomain and Extra-Terminal (BET) family protein, BRD4, also plays an important role in regulating the expression of essential oncogenes.^[117] These two proteins constitute attractive therapeutic targets in AML,^{[118][119]} and inhibition of both targets have shown synergistic activities.^[120]

In this context, Mu *et al.* recently proposed the design of a dual PROTAC inducing the degradation of both PLK1 and BRD4 proteins.^[96] The authors used a dual BRD4/PLK1 inhibitor (BI2536^[121]) as the POIs recruiter and a pomalidomide moiety as CRBN ligand. They synthesized two PROTAC compounds only differing by one PEG-unit length linker. In a MV4-11 cell growth assay, the authors found that HBL-4 (**Figure 5**), with a two-unit PEG linker, as well as its three-unit PEG analog, showed good proliferation inhibitory activity, with IC_{50} values of 4.48 nM and 8.38 nM, respectively, while the parent inhibitor had a higher IC_{50} (88.5 nM). In MOLM-13 and KG1 cell growth assays, HBL-4 also exhibited potent anti-proliferative activities ($\text{IC}_{50} = 6.21$ nM and $\text{IC}_{50} = 6.94$ respectively). The degradation activity of this compound was then evaluated in MV4-11, MOLM-13 and KG1 cells. HBL-4 degraded BRD4 with a DC_{50} of 5 nM and PLK1 with a DC_{50} about 10-20 nM in MV4-11 cells. Thus, the dual PROTAC is more potent to degrade BRD4 protein than PLK1. In the two other cell types, HBL-4 nearly completely degraded BRD4 and PLK1 at 40 nM after 24 h. The authors

also demonstrated that their compound, at a 10 nM concentration in MV4-11 cells, induced a rapid and sustainable degradation of the POIs. In fact, after 6 h of treatment, more than 50% of the proteins were lost and the drop in their levels was maintained for 36 h, while BI2536 led to an increase of these proteins levels. In vivo assay on MV4-11 tumor xenograft model showed that HBL-4, even at six-fold lower concentration than BI2536 (respectively 5 mg/kg and 30 mg/kg three times a week for three weeks), effectively inhibited tumor growth better than BI2536 without any apparent toxicity or significant weight loss in mice. They also demonstrated that a single intravenous injection of HBL-4 (5 mg/kg) in MV4-11 tumors induced an important reduction of BRD4 and PLK1 levels after 1 h and this effect was maintained for at least 24 h.^[96] Thus this study reports the first potent dual kinase-bromodomain PROTAC compound and in vivo results that are promising for the treatment of AML.

5.4 PROTACs for the degradation of CDKs

Cyclin-dependent kinases (CDKs) are members of the STKs family but do not have any catalytic activity alone. Their activation depends on the binding of cyclins, explaining their name. The human genome encodes 21 CDKs and 15 cyclins both implicated in the cell cycle regulation but also in transcription processing. CDKs 1, 2, 3, 4 and 6 are the major protein kinases of this subfamily involved in the cell cycle regulation, while CDKs 7, 8, 9 and 11 are key players in RNA processing.^[122] Several PROTAC compounds were recently developed to degrade CDK9^[97], CDK6^{[98][100]}, CDK4/6 (a dual PROTAC)^[101] and CDK2.^[102]

CDK9 could interact with many transcription factors and plays an important role for the survival of cancer cells by regulating the expression of anti-apoptotic proteins.^[122] It has been proved that the inhibition or the degradation of CDK9 results in the rapid depletion of mRNAs of important survival proteins and oncogenes indicating that targeting selectively CDK9 could be an interesting new approach against cancer.^{[123][124]} However, due to the high sequence conservation of CDK9 compared to other members of the CDK subfamily, it is a real challenge to produce selective and potent CDK9 inhibitors. In this context, Polier *et al.* discovered that Wogonin, a natural product, has CDK9 and CDK7 inhibitory activity ($IC_{50} = 0.19 \mu\text{M}$ and $IC_{50} = 12.3 \mu\text{M}$ respectively) and could induce apoptosis in different cancer cell lines in vitro and in vivo.^[125] Thus, Bian *et al.* decided to design a Wogonin-based PROTAC using a pomalidomide moiety as the CRBN ligand to degrade CDK9.^[97] Eight different PROTAC compounds have been synthesized, that differ by the nature (alkyl- or triazole-

containing chains) and the length of the linker. They tested these compounds in MCF-7 cells and found that PROTACs incorporating a triazole moiety were significantly more efficient to degrade CDK9 than PROTACs with only alkyl chains. Compound **5d** (**Figure 5**) showed the best degradation activity of CDK9 ($DC_{50} = 1-10 \mu\text{M}$) and did not significantly modify the CDK2, 4, 5, 7 and 8 cellular levels even at a concentration of $30 \mu\text{M}$, indicating a good selectivity of this PROTAC. Anti-proliferative assays on MCF-7 cells demonstrated that **5d** was two-times more efficient than Wogonin ($IC_{50} = 17 \mu\text{M}$ vs. $30 \mu\text{M}$). However, this PROTAC did not show good results against the CDK9 low-expressed cell lines L02 (IC_{50} over than $100 \mu\text{M}$).^[97] This result suggests potential in vivo selectivity of this compound on cancer cells which overexpress CDK9. This study offers a selective and relatively potent CDK9 degrader based on a natural POI recruiter which could be a useful tool for further characterizations of CDK9 biological role in cells and organisms.

CDK6, as seen earlier, is an important STK involved in the regulation of the cell cycle. Rana *et al.* designed a set of five CRBN-based PROTACs using a pomalidomide moiety as the CRBN ligand and palbociclib as the CDK6-binding warhead.^[98] Palbociclib is a CDK4/6 inhibitor approved in 2015 by the FDA for cancer therapy.^[48] Interestingly, knock out studies on mammalian cells have shown that CDK4/6 may not be indispensable in normal cells but are critical for tumor growth,^[126] indicating that these CDKs are good therapeutic targets for cancer therapy. In this context, the authors designed a set of PROTACs to suppress both kinase-dependent and independent functions of CDK6. This set of compounds only differs by their linker length (8 to 17 atoms) and composition (alkyl or PEG). Their selectivity for CDK6 was evaluated in MiaPaCa2 cells, a pancreatic cell line, at a concentration of 500 nM. The more selective and potent compound highlighted by this assay was compound **5e** (**Figure 5**) which possessed the longer PEG linker (17 atoms). The other compounds exhibited only partial degradation of CDK6 and, among them, three also induced a degradation of CDK4. At a concentration of 100 nM, this PROTAC led to an almost quantitative degradation of CDK6 at 4 h, in the two tested human pancreatic cell lines. This effect was persistent until 24 h, without any modification of the CDK4 levels.^[98]

At the same time, Brand *et al.* described similar PROTACs, using a thalidomide moiety and a PEG linker with a length of 15 atoms.^[99] These PROTACs were tested on AML cell lines and the authors found a maximal and selective degradation of CDK6 at a concentration of 50 nM, whereas CDK4 was not impacted, even if the PROTACs possessed the same affinity for the two kinases. This difference in degradation activities is proved by the rapid ternary complex

formation with CDK6 but not with CDK4. Interestingly, the authors also showed that a slight modification in the linker structure increased the selectivity of the degrader. Indeed, when an amine bond was used to connect the ligase recruiter to the linker, some lymphoid transcription factors were also degraded by their PROTAC, due to the well-known capability of thalidomide-like compounds to degrade IKZF1 and IKZF3.^[127] Replacement of the amine by an ether bond was able to increase the selectivity of the degrader and thus to avoid the degradation of IKZF1 and IKZF3, two essential transcription factors for terminal differentiation of B and T cell lineages. A time-dependent experiment demonstrated that the most selective construct BSJ-03-123 (**Figure 5**) showed a maximal degradation of CDK6 at 200 nM after 3h of treatment. However, BSJ-03-123 did not show better anti-proliferative effects than palbociclib in several leukemia cell lines.

One year after, Steinebach *et al.* employed four different E3 recruiters (CRBN, VHL, cIAP and MDM2) for the design of potent and selective CDK6 PROTACs also using palbociclib as the POI recruiter.^[100] These four series of PROTACs were tested to determine an optimal linker in each series. Within the CRBN series, compounds with shortest linkers (7 to 15 atoms) showed the best potency to degrade both CDK4 and CDK6 in the multiple myeloma cell line MM.1S. However, the authors observed a decreased selectivity compared to BSJ-03-123, because their compounds induced a significant degradation of the IKZF1 transcription factor. The cIAP series was able to degrade CDK6 but also CDK4 in the same proportion, even if, compared to the CRBN compound, the authors observed a loss of potency (optimal activity at 1 μ M against 0.1 μ M for CRBN derivatives). The MDM2-based compound was simply unable to induce both CDK4 and CDK6 degradation. To the authors, the high lipophilicity of this PROTAC could hamper its cellular effects. Concerning the VHL series, the authors designed two subseries, differing by the anchoring position of the linker into the structure of the VHL recruiter. The first one consisting in the use of a classic VHL ligand bound to the linker through an amide bond with the *tert*-leucine residue (amide subseries). In this group, a PROTAC bearing an alkyl linker was able to degrade both CDKs. Switching from an alkyl linker to a PEG one led to more selective CDK6 degraders and yielded CST620 (**Figure 5**) as a potent and selective CDK6 VHL-degrader (98% at 0.1 μ M). In a second subseries, the linker was introduced on the phenyl ring through an ether bond (phenoxy subseries) and several modulations into the VHL ligand were studied. In this group, CST651 (**Figure 5**) showed a similar potency to degrade CDK6 but a best selectivity profile than CST620. Further characterizations of CST651 in MM.1S cells showed DC₅₀ values of 5.1 nM

for CDK6 and 20 nM for CDK4 after 16 h of incubation. This compound also showed a maximal CDK6 degradation over 95% at a concentration of 100 nM. The authors compared BSJ-03-123 with their best compound and observed that, still in MM.1S cells, a recovering of CDK6 levels began after 24 h with BSJ-03-123, whereas CST651 and CST620 induced a persistent degradation of CDK6 even after 96 h. These compounds also showed very efficient degradation of this kinase in a broad range of different human and murine cancer cells. Moreover, after a washout step, BSJ-03-123 kept CDK6 protein levels below 50% for a maximum of 6 h while the two VHL-compounds induced low and stable levels of CDK6 for up to 72h.^[100] This study confirmed that a CRBN-based PROTAC is not the only way forward to access efficient degraders. Moreover, whereas the nature of the linker has one more time demonstrated a critical effect on the efficacy and selectivity of the PROTACs, the structure of the E3 UL recruiter could also play an important role in these fields. Thus, deep modulations on PROTACs are crucial to have a more complete vision of their activities.

Zhao and Burgess also reported a CRBN-based CDK4/6 degrader using palbociclib and pomalidomide, but with an inverse selectivity compared to the previous reported palbociclib-based PROTACs.^[101] This degrader, compound **5i** (**Figure 5**), contains a triazole alkyl chain as the linker and was reported to degrade more efficiently CDK4 than CDK6 (DC₅₀ about 15 nM vs. 100 nM respectively) in breast cancer MDA-MB-231 cells. Thus, the structure of the linker is crucial for the selectivity between the two CDKs. The authors equally showed that, for a concentration of 100 nM, **5i** induced an optimal degradation of CDK4 after 4 h, while a maximal degradation of CDK6 took 6 h or more. For a concentration of 300 nM, both CDK4 and CDK6 were degraded faster and, after a washout step, the levels of CDK4/6 were recovered within 24 h. The authors also tested their compound in two other cell lines (MCF-7, a breast cancer cell line, and U87, a glioblastoma cell line), but the degradation activity was lower. Finally, the capability to inhibit the proliferation of MDA-MB-231 cells were found to be modest with an estimated IC₅₀ of 10-50 μM.^[101]

CDK4/6 are known to be key actors to drive cell proliferation by their capabilities when associated with cyclin D to phosphorylate the retinoblastoma transcription factor (Rb). CDK2 is also able, upon binding to cyclin E, to further phosphorylate Rb to allow cell cycle entry into S phase.^[128] Previous studies revealed that ubiquitination and degradation of CDK2 could suppress tumor cell growth^[129] and could restore the differentiation of myeloblasts in patient-derived AML samples.^[130] In this context, a CDK2-based PROTAC could be of great interest. Zhou *et al.* proposed CRBN-based PROTACs to degrade CDK2,^[102] using two pan CDK

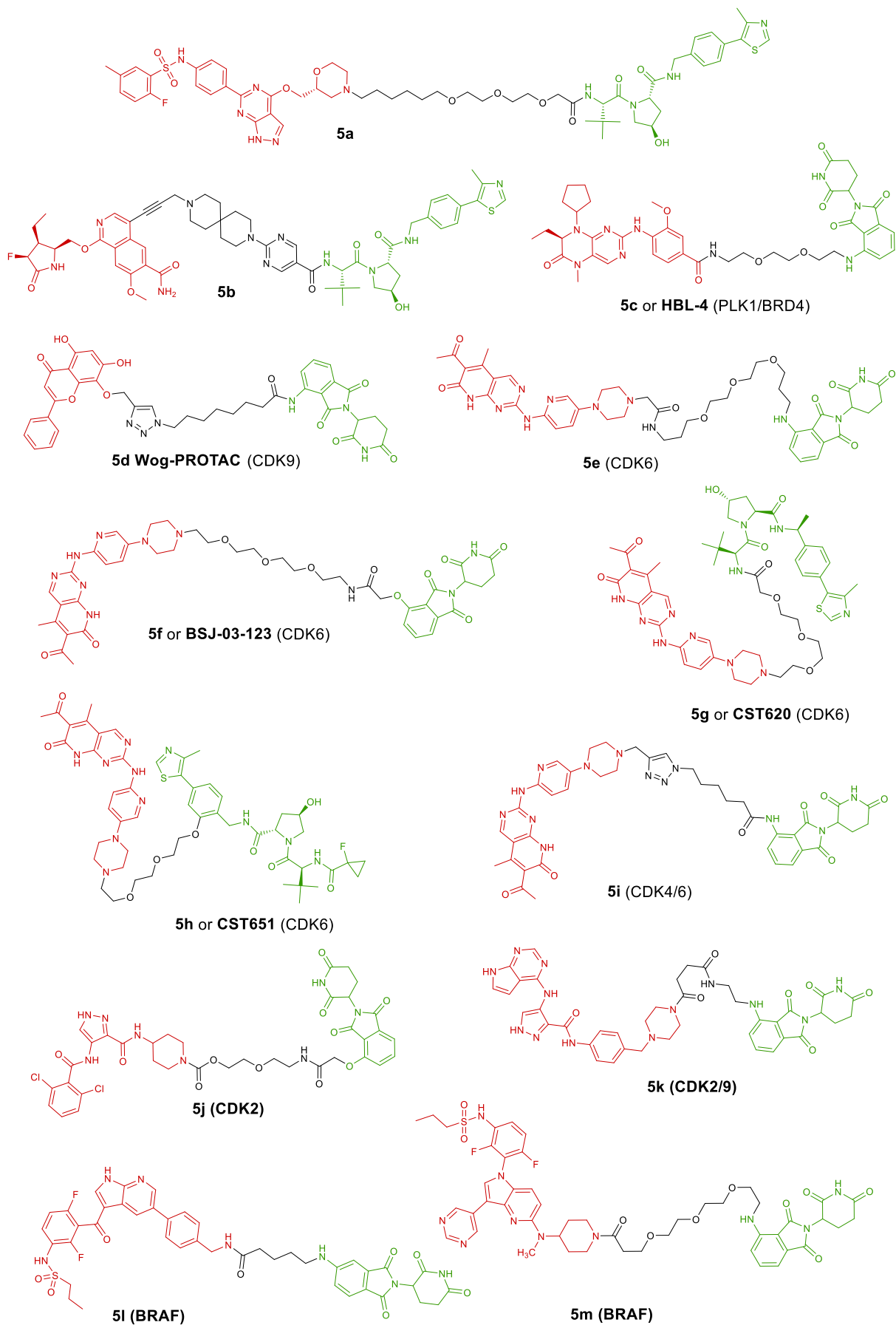
inhibitors, AT7519^[131] and FN-1501^[132]. With the two inhibitors, they tested several linkers to determine their optimal lengths and compositions. A first screening of 20 compounds in PC-3 cells showed that two compounds in the AT7519 series induced a selective degradation of CDK2 at 1 μ M for 12 h. However, compound **5j** (**Figure 5**) was selective for CDK2 at 5 μ M, while the other also degraded CDK9 at the same concentration. This screening also showed that three compounds achieved dual CDK2/9 degradation at 1 μ M but only compound **5k** (**Figure 5**) induced a potent degradation at 50 nM. All compounds were then evaluated for their potential anti-proliferative activities on PC-3 cells. Each best degrader of the two series induced the highest activity on this cell line, with IC₅₀ values of 0.84 μ M and 0.12 μ M for **5j** and **5k** respectively. Of note, the selective CDK2 degrader showed comparable potency with its parent inhibitor AT7519. Further characterizations of **5k** revealed that this compound had weak activity against non-tumor LO2 cells but also that its anti-proliferative activity in PC-3 cells was due to the degradation of the CDKs but not to their inhibition. Indeed, an analog of this PROTAC, unable to bind to CRBN, did not show any anti-proliferative activities. This compound also provided an effective degradation of CDK2/9 in MCF-7, HCT-116 and 22Rv1 cells, however, as no anti-proliferation assays were reported on these cell lines it is not possible to determine if the critical role of CDK2/9 for the PC-3 cells survival can be generalized or not.

5.5 PROTACs for the degradation of BRAF-V600E

BRAF is one of the three isoforms of the Rapidly Accelerated Fibrosarcoma (RAF) family which are involved in the mitogen-activated protein kinase (MAPK) cascade that supports proliferation and survival in mammalian cells.^[133] The mutation of BRAF converting the valine residue at codon 600 into a glutamic acid residue (V600E) represents 90% of the mutations of BRAF inducing a self-activation of this enzyme and thus an uncontrollable proliferation of cells.^{[134][135]} This mutation is overrepresented in melanoma (~50%) and small compounds inhibiting the RAF/MEK pathway have greatly improved median overall survival of mutated BRAF metastatic melanoma patients.^{[48][136]} However, the rapid emergence of drug resistance to initially responsive cancers has led to only marginal patient benefit. Although much progress has been made to increase patient responses (use of immunotherapies and/or drugs combination), development of new compounds or strategies to limit the occurrence of resistance in melanoma cell lines is still in demand.^{[137]–[139]}

In this context, Ran *et al.* elaborated two series of PROTACs^[103]: one based on vemurafenib, the first approved BRAF inhibitor for the treatment of metastatic melanoma^[136], and the other based on BI882370 which is a more potent pan-RAF kinase inhibitor.^[140] Influence of the nature (PEG or alkyl) and the length of the linker (3 to 17 atoms) were evaluated, as well as the E3 UL recruiter (CRBN or VHL). A screening assay in A375 cells, a melanoma cell line expressing BRAF-V600E, showed that CRBN-based PROTACs were more efficient to degrade the kinase (more than 85% of degradation at 500 nM compared to 54% for the best VHL-based PROTAC). Thus, vemurafenib-based PROTAC **5l** (**Figure 5**), where the 5-aminothalidomide moiety was attached to a pentanoyl linker, and the BI882370-based PROTAC **5m** (**Figure 5**), with an 11-atom PEG linker attached to the pomalidomide moiety were selected to further studies. During this assay, the authors observed, in a 16 h experiment with various concentrations of degraders, that the reduction of BRAF-V600E levels and the inhibition of ERK phosphorylation only occurred at concentrations over 12 nM. Interestingly, in a time-dependent experiment, **5m** (500 nM) only induced significant degradation of BRAF-V600E after 16 h of treatment, whereas a reduction in the phosphorylation of ERK occurred 2 h after incubation with cells. These results suggested that the binding of this PROTAC compound to BRAF-V600E did not induce a systematic degradation, possibly due to some difficulties in the formation of productive ternary complexes, according to the authors. They also evaluated the capacity of their degraders to induce the depletion of wild-type BRAF and did not observe any significant degradation, indicating an increased selectivity compared to the parent inhibitors. Concerning their activity in melanoma cell growth, the authors found that, compared to their respective parent inhibitors, the PROTACs did not show better results. The same observation was reported in the BRAF-V600E-dependent colon cancer cell line HT-29.^[103] This study proposed two prototypes of BRAF-V600E degraders and shows for the first time that it is possible to produce potent and selective PROTACs for this kinase. However, weaker activities of the developed PROTACs on melanoma cells viability than the parent inhibitor and a time of latency, before the degradation occurred, indicates that further optimizations are necessary to increase the biological activity of BRAF-targeted PROTACs.

Figure 5. Structure of STK degraders. POI-binding warheads appear in red, E3 UL recruiting moieties in green and linkers in black.



6. Conclusions

Since the first reported PROTAC targeting a kinase in 2013, many research groups have proposed the conversion of potent kinase inhibitors into their respective PROTACs. This new technology which degrades proteins by employing the cellular machinery often offered relevant and encouraging results, which pushed several of these compounds far in drug development processes. Such technology brings several advantages to increase the efficacy of therapeutic compounds. Indeed, due to the catalytic turnover mechanism of PROTACs and the persistence in time of the biological effects due to the degradation of the targeted protein, PROTACs could lead to better biological results than with the parent inhibitor. For example, FLT3 PROTAC led to a better apoptosis induction in leukemia cells than its parent inhibitor (see part 3.1) and BCR-ABL degraders maintained in cells a longer low level of BCR-ABL and its associated downstream signaling than dasatinib (see part 4.2). Moreover, in the kinase field, the PROTAC strategy is particularly attractive to exceed the main limits of ATP-competitive kinase inhibitors, which could possess moderate selectivity and/or lead to resistance development. Thus, several examples of PROTACs showed an increased selectivity compared to their parent inhibitors (see for example SIAIS178 in part 4.2 or **5a** in part 5.2). PROTAC could also modify the selectivity when several kinases are equipotently inhibited by the small-molecule inhibitor (see PROTACs developed to target CDK4/6, part 5.4 or to target JAK1/2, part 4.1). Using allosteric inhibitors as the POI recruiter could also increase the selectivity of PROTACs and reduce the side effects of parent inhibitors (see part 4.2). Although this increase of selectivity brought by the PROTAC strategy is already interesting, efforts are also made currently to identify tissue or disease-specific E3 ligases. Thus, it could be postulated that degraders recruiting such ligases could have even greater selectivity in the future.

The PROTAC technology also provides essential tools in the characterization and the determination of the role of various downstream or associated proteins in signaling pathways during knockout experiments. This tool could also reveal if the knockout of a kinase of interest induces an alternative downstream pathway resulting in a resistance process of cancer or other pathogenic cells against a protein degradation therapy. In particular, the degradation of the kinase abolishes its scaffolding functions contrary to its only inhibition and thus enhanced our knowledge on the implication of the corresponding protein in signaling networks. For example, the BCR-ABL degrader, GMB-475, modified some proteins involved in the formation of multiprotein signaling complexes (see paragraph 4.2). Some scaffolding

properties of kinases were described to be involved in cell resistance. Thus, the use of this type of compound was proposed to overcome such resistance induced by the use of classic inhibitors. However, further proof, especially through in vivo experiments, will be necessary to demonstrate such benefit. Degradation rather than inhibition could also provide a slightly different mechanism of action or reveal additional biological effects, such as a decrease in cancer cells migration that is not observed by the only use of the parent inhibitor (see compound **3g**, part 4.3).

Whereas PROTAC combines several attractive advantages compared to small-molecule inhibitors, the development of potent and in-vivo efficient PROTACs remains a challenge for medicinal chemists. Even if PROTAC compounds could be seen trivially as the combination in a same compound of an E3 UL and a POI recruiter linked together, access to a potent degrader is not so simple. The chemical composition and length of the linker deeply affect the binding affinity or permeability of PROTACs, as exemplified throughout this review. Another sticking point is the great influence of the linker into the ternary complex formation. Even if the PROTAC possesses a good affinity for his POI, it will be totally inefficient if it is unable to recruit the ligase. For example, palbociclib-based PROTAC incorporating an alkyl linker led to a better degradation of CDK4, whereas switching to a PEG linker modified the selectivity towards CDK6, even if the two PROTACs maintained a good affinity for the two kinases (see part 5.4). Modifying the nature of the anchoring of the linker with the E3 UL recruiter has also a great impact into the selectivity and the activity of the PROTAC (see for example, part 5.4 with VHL ligands). Thus, intensive structure-activity relationship studies are needed to discover the most active construct, which could be time consuming. In that context, chemical tools facilitating the synthesis of a large number of analogs may be of interest, as proposed by Krajcovicova *et al.* (see part 4.4).

Another key point concerns the in vivo evaluation of these compounds. As any compound of biological interest, PROTACs needs to share key physicochemical features to be evaluated in animal models. However, due notably to their high molecular weight and their great flexibility, PROTACs fall outside the traditional small-molecule property space. Moreover, their linker is a vulnerable structurally element that could render PROTACs susceptible to rapid metabolism and decreased half-lives. An example is the optimization of MT802 by Crews and coworkers. By modifying the linker and the CRBN recruiting moiety, they obtained a new derivative with a better pharmacokinetic profile (see part 4.4). Thus, chemical optimizations could give access to clinically compatible PROTACs, but also to the

identification of orally efficacious degraders, such as the selective and potent oral IRAK4 degrader developed by Kymera Therapeutics.^[141] As previously mentioned, in 2019 the first PROTACs entered the clinic and several others, including some targeting kinases are expected to join them in the near future.^[142]

As seen throughout the review of the recent literature, the use of thalidomide and its analogs to recruit E3 ligase remains the most frequent way followed, as these molecules are small and easy to handle. However, it is known that such compounds also lead to the degradation of lymphoid transcription factors, such as Ikaros (IKZF1) and Aiolos (IKZF3). Since depletion of its factors is especially harmful for hematological cancers, their degradation in another context is more questionable. Another limitation of the CRBN-based PROTACs is that CRBN is dispensable for most cancer cell lines and mutations of this ligase could lead to the development of resistance to this kind of PROTACs. Thus, expand the panel of E3 ligases and ligase recruiters could be helpful for further PROTACs development.

Another point is that the biological activities of PROTACs are not always fully understood, which require a deeper understanding of cellular mechanisms. For example, as seen in the Focal Adhesion Kinase part (4.3), despite the degradation of FAK, no anti-proliferative effect was observed in several cell types indicating that something is missing in our understanding of FAK-addicted cancer cells. Moreover, as described with IRAK4-PROTAC, the degradation of a kinase could, in certain cases, not be compared to a knockout of this kinase (see part 5.1).

To conclude, the PROTAC technology is a suitable tool and can offer active therapeutic compounds, principally in anti-cancer therapy. Presently, the majority of PROTAC programs reported in the literature are based on already validated clinical targets and often used FDA approved drugs, severely limiting the full potential of this technology. It is highly likely that this strategy will also be used in the coming years to target unexplored or undruggable kinases, but could also contribute to revealing new clinical targets. Expanding the toolbox of PROTACs, by using for example multi-headed PROTAC (see part 3.3) or covalent and reversible PROTACs (see part 4.4) could also reveal unprecedented therapeutic opportunities brought by this technology. Thus, this booming domain of research is still in the early stage but provides encouraging and promising results for our biological understanding and for the future of medicinal chemistry.

References:

- [1] F. Ardito, M. Giuliani, D. Perrone, G. Troiano, L.L. Muzio, *Int. J. Mol. Med.* **2017**, *40*, 271.
- [2] J. Jeon, A. Sparreboom, S. Baker, *Clin. Pharmacol. Ther.* **2017**, *102*, 726.
- [3] F.M. Ferguson, N.S. Gray, *Nat. Rev. Drug Discov.* **2018**, *17*, 353.
- [4] K.S. Bhullar, N.O. Lagarón, E.M. McGowan, I. Parmar, A. Jha, B.P. Hubbard, H.P.V. Rupasinghe, *Mol. Cancer* **2018**, *17*.
- [5] X. Lu, J.B. Smaill, K. Ding, *Angew. Chem. Int. Ed.* **2019**, *n/a*.
- [6] J.C. Groppe, *Expert Opin. Drug Discov.* **2019**, *14*, 1237.
- [7] K.M. Sakamoto, K.B. Kim, A. Kumagai, F. Mercurio, C.M. Crews, R.J. Deshaies, *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 8554.
- [8] L. Tan, N.S. Gray, *Chin. J. Chem.* **2018**, *36*, 971.
- [9] L.H. Jones, *Cell Chem. Biol.* **2018**, *25*, 30.
- [10] A. Hershko, A. Ciechanover, *Annu. Rev. Biochem.* **1998**, *67*, 425.
- [11] G. Kleiger, T. Mayor, *Trends Cell Biol.* **2014**, *24*, 352.
- [12] W. Mansour, M.A. Nakasone, M. von Delbrück, Z. Yu, D. Krutauz, N. Reis, O. Kleifeld, T. Sommer, D. Fushman, M.H. Glickman, *J. Biol. Chem.* **2015**, *290*, 4688.
- [13] K. Haglund, S. Sigismund, S. Polo, I. Szymkiewicz, P.P. Di Fiore, I. Dikic, *Nat. Cell Biol.* **2003**, *5*, 461.
- [14] G.A. Collins, A.L. Goldberg, *Cell* **2017**, *169*, 792.
- [15] Y. Leestemaker, H. Ovaa, *Drug Discov. Today Technol.* **2017**, *26*, 25.
- [16] Y. Zou, D. Ma, Y. Wang, *Cell Biochem. Funct.* **2019**, *37*, 21.
- [17] L. Liu, D.R. Damerell, L. Koukouflis, Y. Tong, B.D. Marsden, M. Schapira, *Bioinforma. Oxf. Engl.* **2019**, *35*, 2882.
- [18] M. Scheepstra, K.F.W. Hekking, L. van Hijfte, R.H.A. Folmer, *Comput. Struct. Biotechnol. J.* **2019**, *17*, 160.
- [19] M. Schapira, M.F. Calabrese, A.N. Bullock, C.M. Crews, *Nat. Rev. Drug Discov.* **2019**, *18*, 949.
- [20] D.P. Bondeson, B.E. Smith, G.M. Burslem, A.D. Buhimschi, J. Hines, S. Jaime-Figueroa, J. Wang, B.D. Hamman, A. Ishchenko, C.M. Crews, *Cell Chem. Biol.* **2018**, *25*, 78.
- [21] J.S. Schneekloth, F.N. Fonseca, M. Koldobskiy, A. Mandal, R. Deshaies, K. Sakamoto, C.M. Crews, *J. Am. Chem. Soc.* **2004**, *126*, 3748.
- [22] J. Hines, J.D. Gough, T.W. Corson, C.M. Crews, *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 8942.
- [23] Y. Itoh, *Chem. Rec. N. Y. N* **2018**, *18*, 1681.
- [24] C. Cantrill, P. Chaturvedi, C. Rynn, J. Petrig Schaffland, I. Walter, M.B. Wittwer, *Drug Discov. Today* **2020**.
- [25] H.J. Maple, N. Clayden, A. Baron, C. Stacey, R. Felix, *MedChemComm* **2019**, *10*, 1755.
- [26] S. An, L. Fu, *EBioMedicine* **2018**, *36*, 553.
- [27] Y. Wang, X. Jiang, F. Feng, W. Liu, H. Sun, *Acta Pharm. Sin. B* **2020**, *10*, 207.
- [28] M.A. Lemmon, J. Schlessinger, *Cell* **2010**, *141*, 1117.
- [29] G.M. Burslem, J. Song, X. Chen, J. Hines, C.M. Crews, *J. Am. Chem. Soc.* **2018**, *140*, 16428.
- [30] B. Zhao, K. Burgess, *Mol. Pharm.* **2019**, *16*, 4313.
- [31] C.H. Kang, D.H. Lee, C.O. Lee, J. Du Ha, C.H. Park, J.Y. Hwang, *Biochem. Biophys. Res. Commun.* **2018**, *505*, 542.
- [32] Y. Wang, L. Han, F. Liu, F. Yang, X. Jiang, H. Sun, F. Feng, J. Xue, W. Liu, *Colloids Surf. B Biointerfaces* **2020**, *188*, 110795.
- [33] F. Birg, M. Courcoul, O. Rosnet, F. Bardin, M.J. Pebusque, S. Marchetto, A. Tabilio, P. Mannoni, D. Birnbaum, *Blood* **1992**, *80*, 2584.
- [34] B. Deschler, M. Lübbert, *Cancer* **2006**, *107*, 2099.
- [35] C.C. Smith, Q. Wang, C.-S. Chin, S. Salerno, L.E. Damon, M.J. Levis, A.E. Perl, K.J. Travers, S. Wang, J.P. Hunt, P.P. Zarrinkar, E.E. Schadt, A. Kasarskis, J. Kuriyan, N.P. Shah, *Nature* **2012**, *485*, 260.

- [36] K.W. Pratz, J. Cortes, G.J. Roboz, N. Rao, O. Arowojolu, A. Stine, Y. Shiotsu, A. Shudo, S. Akinaga, D. Small, J.E. Karp, M. Levis, *Blood* **2009**, *113*, 3938.
- [37] P.P. Zarrinkar, R.N. Gunawardane, M.D. Cramer, M.F. Gardner, D. Brigham, B. Belli, M.W. Karaman, K.W. Pratz, G. Pallares, Q. Chao, K.G. Sprankle, H.K. Patel, M. Levis, R.C. Armstrong, J. James, S.S. Bhagwat, *Blood* **2009**, *114*, 2984.
- [38] C. Galdeano, M.S. Gadd, P. Soares, S. Scaffidi, I. Van Molle, I. Birced, S. Hewitt, D.M. Dias, A. Ciulli, *J. Med. Chem.* **2014**, *57*, 8657.
- [39] W. Jin, *Cancers* **2020**, *12*.
- [40] M. Rydén, R. Sehgal, C. Dominici, F.H. Schilling, C.F. Ibáñez, P. Kogner, *Br. J. Cancer* **1996**, *74*, 773.
- [41] Y. Wang, C. Hagel, W. Hamel, S. Müller, L. Kluwe, M. Westphal, *Acta Neuropathol. (Berl.)* **1998**, *96*, 357.
- [42] M.J. Blasco-Gutiérrez, I.J.S. José-Crespo, E. Zozaya-Alvarez, R. Ramos-Sánchez, N. García-Atarés, *Cancer Invest.* **2007**, *25*, 405.
- [43] X. Xu, S.R. Tahan, T.L. Pasha, P.J. Zhang, *J. Cutan. Pathol.* **2003**, *30*, 318.
- [44] W. Jin, G.M. Kim, M.S. Kim, M.H. Lim, C. Yun, J. Jeong, J.-S. Nam, S.-J. Kim, *Carcinogenesis* **2010**, *31*, 1939.
- [45] B. Zhao, K. Burgess, *Mol. Pharm.* **2019**, *16*, 4313.
- [46] D. Chen, F. Brahim, Y. Angell, Y.-C. Li, J. Moskowicz, H.U. Saragovi, K. Burgess, *ACS Chem. Biol.* **2009**, *4*, 769.
- [47] Z. Jiang, Z. Yang, F. Li, Z. Li, N. Fishkin, K. Burgess, *Bioconjug. Chem.* **2018**, *29*, 2920.
- [48] R. Roskoski, *Pharmacol. Res.* **2020**, *152*, 104609.
- [49] A. Drilon, R. Nagasubramanian, J.F. Blake, N. Ku, B.B. Tuch, K. Ebata, S. Smith, V. Lauriault, G.R. Kolakowski, B.J. Brandhuber, P.D. Larsen, K.S. Bouhana, S.L. Winski, R. Hamor, W.-I. Wu, A. Parker, T.H. Morales, F.X. Sullivan, W.E. DeWolf, L.A. Wollenberg, P.R. Gordon, D.N. Douglas-Lindsay, M. Scaltriti, R. Benayed, S. Raj, B. Hanusch, A.M. Schram, P. Jonsson, M.F. Berger, J.F. Hechtman, B.S. Taylor, S. Andrews, S.M. Rothenberg, D.M. Hyman, *Cancer Discov.* **2017**, *7*, 963.
- [50] S. Siena, A.E. Drilon, I.S.-H. Ou, A.F. Farago, M. Patel, T.M. Bauer, D. Hong, S.V. Liu, J. Lee, R. Patel, L. Schechet, D. Luo, E.C. Maneval, P.S. Multani, F.G.D. Braud, *Eur. J. Cancer* **2015**, *51*, S724.
- [51] B. Hallberg, R.H. Palmer, *Nat. Rev. Cancer* **2013**, *13*, 685.
- [52] K. Pulford, L. Lamant, S.W. Morris, L.H. Butler, K.M. Wood, D. Stroud, G. Delsol, D.Y. Mason, *Blood* **1997**, *89*, 1394.
- [53] J.G. Christensen, H.Y. Zou, M.E. Arango, Q. Li, J.H. Lee, S.R. McDonnell, S. Yamazaki, G.R. Alton, B. Mroczkowski, G. Los, *Mol. Cancer Ther.* **2007**, *6*, 3314.
- [54] M. Soda, Y.L. Choi, M. Enomoto, S. Takada, Y. Yamashita, S. Ishikawa, S. Fujiwara, H. Watanabe, K. Kurashina, H. Hatanaka, M. Bando, S. Ohno, Y. Ishikawa, H. Aburatani, T. Niki, Y. Sohara, Y. Sugiyama, H. Mano, *Nature* **2007**, *448*, 561.
- [55] C. Zhang, X.-R. Han, X. Yang, B. Jiang, J. Liu, Y. Xiong, J. Jin, *Eur. J. Med. Chem.* **2018**, *151*, 304.
- [56] C.E. Powell, Y. Gao, L. Tan, K.A. Donovan, R.P. Nowak, A. Loehr, M. Bahcall, E.S. Fischer, P.A. Jänne, R.E. George, N.S. Gray, *J. Med. Chem.* **2018**, *61*, 4249.
- [57] Y. Ding, Z. Jiang, K. Saha, C.S. Kim, S.T. Kim, R.F. Landis, V.M. Rotello, *Mol. Ther.* **2014**, *22*, 1075.
- [58] S. Aryal, J.J. Grailer, S. Pilla, D.A. Steeber, S. Gong, *J. Mater. Chem.* **2009**, *19*, 7879.
- [59] A. Golonko, H. Lewandowska, R. Świśtocka, U.T. Jasińska, W. Priebe, W. Lewandowski, *Eur. J. Med. Chem.* **2019**, *181*, 111512.
- [60] R.R. Shah, J.M. Redmond, A. Mihut, M. Menon, J.P. Evans, J.A. Murphy, M.A. Bartholomew, D.M. Coe, *Bioorg. Med. Chem.* **2020**, *28*, 115326.
- [61] Q. Zhao, C. Ren, L. Liu, J. Chen, Y. Shao, N. Sun, R. Sun, Y. Kong, X. Ding, X. Zhang, Y. Xu, B. Yang, Q. Yin, X. Yang, B. Jiang, *J. Med. Chem.* **2019**, *62*, 9281.

- [62] G.M. Burslem, A.R. Schultz, D.P. Bondeson, C.A. Eide, S.L.S. Stevens, B.J. Druker, C.M. Crews, *Cancer Res.* **2019**, *79*, 4744.
- [63] J. Popow, H. Arnhof, G. Bader, H. Berger, A. Ciulli, D. Covini, C. Dank, T. Gmaschitz, P. Greb, J. Karolyi-Özguer, M. Koegl, D.B. McConnell, M. Pearson, M. Rieger, J. Rinnenthal, V. Roessler, A. Schrenk, M. Spina, S. Steurer, N. Trainor, E. Traxler, C. Wieshofer, A. Zoephel, P. Ettmayer, *J. Med. Chem.* **2019**, *62*, 2508.
- [64] P.M. Cromm, K.T.G. Samarasinghe, J. Hines, C.M. Crews, *J. Am. Chem. Soc.* **2018**, *140*, 17019.
- [65] H. Gao, Y. Wu, Y. Sun, Y. Yang, G. Zhou, Y. Rao, *ACS Med. Chem. Lett.* **2019**.
- [66] A. Zorba, C. Nguyen, Y. Xu, J. Starr, K. Borzilleri, J. Smith, H. Zhu, K.A. Farley, W. Ding, J. Schiemer, X. Feng, J.S. Chang, D.P. Uccello, J.A. Young, C.N. Garcia-Irrizary, L. Czabaniuk, B. Schuff, R. Oliver, J. Montgomery, M.M. Hayward, J. Coe, J. Chen, M. Niosi, S. Luthra, J.C. Shah, A. El-Kattan, X. Qiu, G.M. West, M.C. Noe, V. Shanmugasundaram, A.M. Gilbert, M.F. Brown, M.F. Calabrese, *Proc. Natl. Acad. Sci.* **2018**, *115*, E7285.
- [67] C.P. Tinworth, H. Lithgow, L. Dittus, Z.I. Bassi, S.E. Hughes, M. Muelbaier, H. Dai, I.E.D. Smith, W.J. Kerr, G.A. Burley, M. Bantscheff, J.D. Harling, *ACS Chem. Biol.* **2019**, *14*, 342.
- [68] G. Xue, J. Chen, L. Liu, D. Zhou, Y. Zuo, T. Fu, Z. Pan, *Chem. Commun.* **2020**, *56*, 1521.
- [69] A.D. Buhimschi, H.A. Armstrong, M. Toure, S. Jaime-Figueroa, T.L. Chen, A.M. Lehman, J.A. Woyach, A.J. Johnson, J.C. Byrd, C.M. Crews, *Biochemistry* **2018**, *57*, 3564.
- [70] S. Jaime-Figueroa, A.D. Buhimschi, M. Toure, J. Hines, C.M. Crews, *Bioorg. Med. Chem. Lett.* **2020**, *30*, 126877.
- [71] S. Liu, Y. Da, F. Wang, R. Yan, Y. Shu, P. Lin, J. Lin, *Med. Chem. Res.* **2020**.
- [72] S. Krajcovicova, R. Jorda, D. Hendrychova, V. Krystof, M. Soral, *Chem. Commun.* **2019**, *55*, 929.
- [73] J.J. O'Shea, D.M. Schwartz, A.V. Villarino, M. Gadina, I.B. McInnes, A. Laurence, *Annu. Rev. Med.* **2015**, *66*, 311.
- [74] L. Bai, H. Zhou, R. Xu, Y. Zhao, K. Chinnaswamy, D. McEachern, J. Chen, C.-Y. Yang, Z. Liu, M. Wang, L. Liu, H. Jiang, B. Wen, P. Kumar, J.L. Meagher, D. Sun, J.A. Stuckey, S. Wang, *Cancer Cell* **2019**, *36*, 498.
- [75] A. Hamilton, G.V. Helgason, M. Schemionek, B. Zhang, S. Myssina, E.K. Allan, F.E. Nicolini, C. Müller-Tidow, R. Bhatia, V.G. Brunton, S. Koschmieder, T.L. Holyoake, *Blood* **2012**, *119*, 1501.
- [76] R. Ren, *Nat. Rev. Cancer* **2005**, *5*, 172.
- [77] A.C. Lai, M. Toure, D. Hellerschmied, J. Salami, S. Jaime-Figueroa, E. Ko, J. Hines, C.M. Crews, *Angew. Chem. Int. Ed.* **2016**, *55*, 807.
- [78] N. Shibata, K. Shimokawa, K. Nagai, N. Ohoka, T. Hattori, N. Miyamoto, O. Ujikawa, T. Sameshima, H. Nara, N. Cho, M. Naito, *Sci. Rep.* **2018**, *8*, 1.
- [79] J. Zhang, F.J. Adrián, W. Jahnke, S.W. Cowan-Jacob, A.G. Li, R.E. Iacob, T. Sim, J. Powers, C. Dierks, F. Sun, G.-R. Guo, Q. Ding, B. Okram, Y. Choi, A. Wojciechowski, X. Deng, G. Liu, G. Fendrich, A. Strauss, N. Vajpai, S. Grzesiek, T. Tuntland, Y. Liu, B. Bursulaya, M. Azam, P.W. Manley, J.R. Engen, G.Q. Daley, M. Warmuth, N.S. Gray, *Nature* **2010**, *463*, 501.
- [80] K. Shimokawa, N. Shibata, T. Sameshima, N. Miyamoto, O. Ujikawa, H. Nara, N. Ohoka, T. Hattori, N. Cho, M. Naito, *ACS Med. Chem. Lett.* **2017**, *8*, 1042.
- [81] B.Y. Lee, P. Timpson, L.G. Horvath, R.J. Daly, *Pharmacol. Ther.* **2015**, *146*, 132.
- [82] U.A. Hirt, I.C. Waizenegger, N. Schweifer, C. Haslinger, D. Gerlach, J. Braunger, U. Weyer-Czernilofsky, H. Stadtmüller, I. Sapountzis, G. Bader, A. Zoephel, B. Bister, A. Baum, J. Quant, N. Kraut, P. Garin-Chesa, G.R. Adolf, *Oncogenesis* **2018**, *7*, 1.
- [83] S. Tiede, N. Meyer-Schaller, R.K.R. Kalathur, R. Ivanek, E. Fagiani, P. Schmassmann, P. Stillhard, S. Häfliger, N. Kraut, N. Schweifer, I.C. Waizenegger, R. Bill, G. Christofori, *Oncogenesis* **2018**, *7*, 1.
- [84] A. Schultze, W. Fiedler, *Expert Opin. Investig. Drugs* **2010**, *19*, 777.
- [85] S.P. Singh, F. Dammeijer, R.W. Hendriks, *Mol. Cancer* **2018**, *17*, 1.

- [86] R. Gabizon, A. Shraga, P. Gehrtz, E. Livnah, Y. Shorer, N. Gurwicz, L. Avram, T. Unger, H. Aharoni, S. Albeck, A. Brandis, Z. Shulman, B.-Z. Katz, Y. Herishanu, N. London, *J. Am. Chem. Soc.* **2020**.
- [87] A.L. Rankin, N. Seth, S. Keegan, T. Andreyeva, T.A. Cook, J. Edmonds, N. Mathialagan, M.J. Benson, J. Syed, Y. Zhan, S.E. Benoit, J.S. Miyashiro, N. Wood, S. Mohan, E. Peeva, S.K. Ramaiah, D. Messing, B.L. Homer, K. Dunussi-Joannopoulos, C.L. Nickerson-Nutter, M.E. Schnute, J. Douhan, *J. Immunol.* **2013**, *191*, 4540.
- [88] K. Hagiwara, T. Tokunaga, H. Iida, H. Nagai, *Anticancer Res.* **2015**, *35*, 6679.
- [89] N. Ding, X. Li, Y. Shi, L. Ping, L. Wu, K. Fu, L. Feng, X. Zheng, Y. Song, Z. Pan, J. Zhu, *Oncotarget* **2015**, *6*, 15122.
- [90] J. Chen, X. Wang, F. He, Z. Pan, *Bioconj. Chem.* **2018**, *29*, 1640.
- [91] J.M. Bradshaw, J.M. McFarland, V.O. Paavilainen, A. Bisconte, D. Tam, V.T. Phan, S. Romanov, D. Finkle, J. Shu, V. Patel, T. Ton, X. Li, D.G. Loughhead, P.A. Nunn, D.E. Karr, M.E. Gerritsen, J.O. Funk, T.D. Owens, E. Verner, K.A. Brameld, R.J. Hill, D.M. Goldstein, J. Taunton, *Nat. Chem. Biol.* **2015**, *11*, 525.
- [92] S. Chackalamanni, D.P. Rotella, S.E. Ward, *Comprehensive Medicinal Chemistry III*, **2017**.
- [93] A. Levitzki, in *Handb. Cell Signal.* (Eds: R.A. Bradshaw, E.A. Dennis), Academic Press, Burlington **2003**, 451.
- [94] J. Nunes, G.A. McGonagle, J. Eden, G. Kiritharan, M. Touzet, X. Lewell, J. Emery, H. Eidam, J.D. Harling, N.A. Anderson, *ACS Med. Chem. Lett.* **2019**, *10*, 1081.
- [95] H. Tovell, A. Testa, H. Zhou, N. Shpiro, C. Crafter, A. Ciulli, D.R. Alessi, *ACS Chem. Biol.* **2019**, *14*, 2024.
- [96] X. Mu, L. Bai, Y. Xu, J. Wang, H. Lu, *Biochem. Biophys. Res. Commun.* **2020**, *521*, 833.
- [97] J. Bian, J. Ren, Y. Li, J. Wang, X. Xu, Y. Feng, H. Tang, Y. Wang, Z. Li, *Bioorganic Chem.* **2018**, *81*, 373.
- [98] S. Rana, M. Bendjennat, S. Kour, H.M. King, S. Kizhake, M. Zahid, A. Natarajan, *Bioorg. Med. Chem. Lett.* **2019**, *29*, 1375.
- [99] M. Brand, B. Jiang, S. Bauer, K.A. Donovan, Y. Liang, E.S. Wang, R.P. Nowak, J.C. Yuan, T. Zhang, N. Kwiatkowski, A.C. Müller, E.S. Fischer, N.S. Gray, G.E. Winter, *Cell Chem. Biol.* **2019**, *26*, 300.
- [100] C. Steinebach, Y.L.D. Ng, I. Sosič, C.-S. Lee, S. Chen, S. Lindner, L.P. Vu, A. Bricelj, R. Haschemi, M. Monschke, E. Steinwarz, K.G. Wagner, G. Bendas, J. Luo, M. Gütschow, J. Krönke, *Chem. Sci.* **2020**.
- [101] B. Zhao, K. Burgess, *Chem. Commun.* **2019**, *55*, 2704.
- [102] F. Zhou, L. Chen, C. Cao, J. Yu, X. Luo, P. Zhou, L. Zhao, W. Du, J. Cheng, Y. Xie, Y. Chen, *Eur. J. Med. Chem.* **2020**, *187*, 111952.
- [103] X.-R. Han, L. Chen, Y. Wei, W. Yu, Y. Chen, C. Zhang, B. Jiao, T. Shi, L. Sun, C. Zhang, Y. Xu, M.R. Lee, Y. Luo, M.B. Plewe, J. Wang, *J. Med. Chem.* **2020**, *63*, 4069.
- [104] A.A. Brzezinska, J.L. Johnson, D.B. Munafo, B.A. Ellis, S.D. Catz, *Immunology* **2009**, *127*, 386.
- [105] V.N. Ngo, R.M. Young, R. Schmitz, S. Jhavar, W. Xiao, K.-H. Lim, H. Kohlhammer, W. Xu, Y. Yang, H. Zhao, A.L. Shaffer, P. Romesser, G. Wright, J. Powell, A. Rosenwald, H.K. Muller-Hermelink, G. Ott, R.D. Gascoyne, J.M. Connors, L.M. Rimsza, E. Campo, E.S. Jaffe, J. Delabie, E.B. Smeland, R.I. Fisher, R.M. Braziel, R.R. Tubbs, J.R. Cook, D.D. Weisenburger, W.C. Chan, L.M. Staudt, *Nature* **2011**, *470*, 115.
- [106] E.Y. Chiang, X. Yu, J.L. Grogan, *J. Immunol.* **2011**, *186*, 1279.
- [107] J. Qin, Z. Jiang, Y. Qian, J.-L. Casanova, X. Li, *J. Biol. Chem.* **2004**, *279*, 26748.
- [108] K.L. Lee, C.M. Ambler, D.R. Anderson, B.P. Boscoe, A.G. Bree, J.I. Brodfuehrer, J.S. Chang, C. Choi, S. Chung, K.J. Curran, J.E. Day, C.M. Dehnhardt, K. Dower, S.E. Drozda, R.K. Frisbie, L.K. Gavrin, J.A. Goldberg, S. Han, M. Hegen, D. Hepworth, H.R. Hope, S. Kamtekar, I.C. Kilty, A. Lee, L.-L. Lin, F.E. Lovering, M.D. Lowe, J.P. Mathias, H.M. Morgan, E.A. Murphy, N. Papaioannou, A. Patny, B.S. Pierce, V.R. Rao, E. Saiah, I.J. Samardjiev, B.M. Samas, M.W.H.

- Shen, J.H. Shin, H.H. Soutter, J.W. Strohbach, P.T. Symanowicz, J.R. Thomason, J.D. Trzuppek, R. Vargas, F. Vincent, J. Yan, C.W. Zapf, S.W. Wright, *J. Med. Chem.* **2017**, *60*, 5521.
- [109] L. Cushing, W. Stochaj, M. Siegel, R. Czerwinski, K. Dower, Q. Wright, M. Hirschfield, J.-L. Casanova, C. Picard, A. Puel, L.-L. Lin, V.R. Rao, *J. Biol. Chem.* **2014**, *289*, 10865.
- [110] Kymera Therapeutics. IRAKIMiDs: Protein Degraders Targeting Both IRAK4 and IMiD Substrates Show Combinatorial Effects Leading to Broad Activity with Durable Regressions in MYD88 Mutant Lymphoma Xenografts In Vivo [internet]. [updated 2020 Jul 30]. Available from: <http://www.kymeratx.com/wp-content/uploads/2020/07/AACR-IRAKIMiD-Poster-5222.pdf>
- [111] N. Mainolfi, N. Ji, A.F. Kluge, M.M. Weiss, Y. Zhang, X. Zheng (KYMERA THERAPEUTICS, INC. US), WO/2020/113233, **2020**.
- [112] K.M. Vasudevan, D.A. Barbie, M.A. Davies, R. Rabinovsky, C.J. McNear, J.J. Kim, B.T. Hennessy, H. Tseng, P. Pochanard, S.Y. Kim, I.F. Dunn, A.C. Schinzel, P. Sandy, S. Hoersch, Q. Sheng, P.B. Gupta, J.S. Boehm, J.H. Reiling, S. Silver, Y. Lu, K. Stemke-Hale, B. Dutta, C. Joy, A.A. Sahin, A.M. Gonzalez-Angulo, A. Lluch, L.E. Rameh, T. Jacks, D.E. Root, E.S. Lander, G.B. Mills, W.C. Hahn, W.R. Sellers, L.A. Garraway, *Cancer Cell* **2009**, *16*, 21.
- [113] K. Sheppard, K.M. Kinross, B. Solomon, R.B. Pearson, W.A. Phillips, *Crit. Rev. Oncog.* **2012**, *17*.
- [114] H. Cao, Z. Xu, J. Wang, A. Cigliano, M.G. Pilo, S. Ribback, S. Zhang, Y. Qiao, L. Che, R.M. Pascale, D.F. Calvisi, X. Chen, *BMC Cancer* **2019**, *19*, 343.
- [115] G.Q. Gong, K. Wang, X.-C. Dai, Y. Zhou, R. Basnet, Y. Chen, D.-H. Yang, W.-J. Lee, C.M. Buchanan, J.U. Flanagan, P.R. Shepherd, Y. Chen, M.-W. Wang, *Acta Pharmacol. Sin.* **2018**, *39*, 1902.
- [116] M. Nazaré, N. Halland, F. Schmidt, H.-W. Kleemann, T. Weiss, J. Saas, C. Struebing, *N-(4-(Azaindazol-6-yl)-Phenyl)-Sulfonamides and Their Use as Pharmaceuticals*, WO2014140065.
- [117] J. Lovén, H.A. Hoke, C.Y. Lin, A. Lau, D.A. Orlando, C.R. Vakoc, J.E. Bradner, T.I. Lee, R.A. Young, *Cell* **2013**, *153*, 320.
- [118] A.G. Renner, C. Dos Santos, C. Recher, C. Bailly, L. Créancier, A. Kruczynski, B. Payrastre, S. Manenti, *Blood* **2009**, *114*, 659.
- [119] H. Herrmann, K. Blatt, J. Shi, K.V. Gleixner, S. Cerny-Reiterer, L. Müllauer, C.R. Vakoc, W.R. Sperr, H.-P. Horny, J.E. Bradner, J. Zuber, P. Valent, *Oncotarget* **2012**, *3*, 1588.
- [120] U. Tontsch-Grunt, D. Rudolph, I. Waizenegger, A. Baum, D. Gerlach, H. Engelhardt, M. Wurm, F. Savarese, N. Schweifer, N. Kraut, *Cancer Lett.* **2018**, *421*, 112.
- [121] P. Ciceri, S. Müller, A. O'Mahony, O. Fedorov, P. Filippakopoulos, J.P. Hunt, E.A. Lasater, G. Pallares, S. Picaud, C. Wells, S. Martin, L.M. Wodicka, N.P. Shah, D.K. Treiber, S. Knapp, *Nat. Chem. Biol.* **2014**, *10*, 305.
- [122] Y.A. Sonawane, M.A. Taylor, J.V. Napoleon, S. Rana, J.I. Contreras, A. Natarajan, *J. Med. Chem.* **2016**, *59*, 8667.
- [123] U. Lücking, A. Scholz, P. Lienau, G. Siemeister, D. Kosemund, R. Bohlmann, H. Briem, I. Terebesi, K. Meyer, K. Prella, K. Denner, U. Bömer, M. Schäfer, K. Eis, R. Valencia, S. Ince, F. von Nussbaum, D. Mumberg, K. Ziegelbauer, B. Klebl, A. Choidas, P. Nussbaumer, M. Baumann, C. Schultz-Fademrecht, G. Rühler, J. Eickhoff, M. Brands, *ChemMedChem* **2017**, *12*, 1776.
- [124] C.M. Olson, B. Jiang, M.A. Erb, Y. Liang, Z.M. Doctor, Z. Zhang, T. Zhang, N. Kwiatkowski, M. Boukhali, J.L. Green, W. Haas, T. Nomanbhoy, E.S. Fischer, R.A. Young, J.E. Bradner, G.E. Winter, N.S. Gray, *Nat. Chem. Biol.* **2018**, *14*, 163.
- [125] G. Polier, J. Ding, B.V. Konkimalla, D. Eick, N. Ribeiro, R. Köhler, M. Giaisi, T. Efferth, L. Desaubry, P.H. Krammer, M. Li-Weber, *Cell Death Dis.* **2011**, *2*, e182.
- [126] M. Malumbres, R. Sotillo, D. Santamaría, J. Galán, A. Cerezo, S. Ortega, P. Dubus, M. Barbacid, *Cell* **2004**, *118*, 493.

- [127] J. Krönke, N.D. Udeshi, A. Narla, P. Grauman, S.N. Hurst, M. McConkey, T. Svinkina, D. Heckl, E. Comer, X. Li, C. Ciarlo, E. Hartman, N. Munshi, M. Schenone, S.L. Schreiber, S.A. Carr, B.L. Ebert, *Science* **2014**, *343*, 301.
- [128] T. Akiyama, T. Ohuchi, S. Sumida, K. Matsumoto, K. Toyoshima, *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 7900.
- [129] W. Chen, J. Lee, S.Y. Cho, H.A. Fine, *Cancer Res.* **2004**, *64*, 3949.
- [130] M. Ying, X. Shao, H. Jing, Y. Liu, X. Qi, J. Cao, Y. Chen, S. Xiang, H. Song, R. Hu, G. Wei, B. Yang, Q. He, *Blood* **2018**, *131*, 2698.
- [131] P.G. Wyatt, A.J. Woodhead, V. Berdini, J.A. Boulstridge, M.G. Carr, D.M. Cross, D.J. Davis, L.A. Devine, T.R. Early, R.E. Feltell, E.J. Lewis, R.L. McMenemy, E.F. Navarro, M.A. O'Brien, M. O'Reilly, M. Reule, G. Saxty, L.C.A. Seavers, D.-M. Smith, M.S. Squires, G. Trewartha, M.T. Walker, A.J.-A. Woolford, *J. Med. Chem.* **2008**, *51*, 4986.
- [132] Y. Wang, Y. Zhi, Q. Jin, S. Lu, G. Lin, H. Yuan, T. Yang, Z. Wang, C. Yao, J. Ling, H. Guo, T. Li, J. Jin, B. Li, L. Zhang, Y. Chen, T. Lu, *J. Med. Chem.* **2018**, *61*, 1499.
- [133] D.E. Durrant, D.K. Morrison, *Br. J. Cancer* **2018**, *118*, 3.
- [134] K.T. Flaherty, I. Puzanov, K.B. Kim, A. Ribas, G.A. McArthur, J.A. Sosman, P.J. O'Dwyer, R.J. Lee, J.F. Grippo, K. Nolop, P.B. Chapman, *N. Engl. J. Med.* **2010**, *363*, 809.
- [135] T. Murphy, S. Hori, J. Sewell, V.J. Gnanapragasam, *Int. J. Cancer* **2010**, *127*, 2491.
- [136] G. Bollag, J. Tsai, J. Zhang, C. Zhang, P. Ibrahim, K. Nolop, P. Hirth, *Nat. Rev. Drug Discov.* **2012**, *11*, 873.
- [137] A. Gallud, O. Vaillant, L.T. Maillard, D.P. Arama, J. Dubois, M. Maynadier, V. Lisowski, M. Garcia, J. Martinez, N. Masurier, *Eur. J. Med. Chem.* **2014**, *75*, 382.
- [138] V. Bellet, L. Lichon, D.P. Arama, A. Gallud, V. Lisowski, L.T. Maillard, M. Garcia, J. Martinez, N. Masurier, *Eur. J. Med. Chem.* **2017**, *125*, 1225.
- [139] P.L. Baccon-Sollier, Y. Malki, M. Maye, L.M.A. Ali, L. Lichon, P. Cuq, L.-A. Vincent, N. Masurier, *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 935.
- [140] I.C. Waizenegger, A. Baum, S. Steurer, H. Stadtmüller, G. Bader, O. Schaaf, P. Garin-Chesa, A. Schlattl, N. Schweifer, C. Haslinger, F. Colbatzky, S. Mousa, A. Kalkuhl, N. Kraut, G.R. Adolf, *Mol. Cancer Ther.* **2016**, *15*, 354.
- [141] V. Campbell, J. Kelleher, J. Chen, J. Gollob, N. Ji, C. Klaus, C. Loh, M. Mayo, A. McDonald, H. Rong, S. Rusin, K. Sharma, M. Weiss, K. Yuan, D. Walker, X. Zheng, A. Slavin, N. Mainofi, *Arthritis Rheumatol* **2019**, *71* (supp 10).
- [142] A. Mullard, *Nat. Rev. Drug Discov.* **2019**, *18*, 237.

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Figure 1. The Ubiquitin-Proteasome System (UPS) and its hijacking using the PROTAC strategy. Shown are ubiquitin (Ub) and activated-Ub (PDB: 4NNJ), Ub-E1 (Uba1) complex (PDB: 4NNJ), ubiquitin-E2 (UBE2D2) complex (PDB: 6TTU), Ub-E2 E3-substrate complex (model composed of PDB: 1LQB, 5N4W and 6TTU), proteasome (model composed of PDB: 5L4G and 5A5B), Ub-E2-E3-PROTAC-substrate (model composed of PDB: 5T35, 5N4W and 6TTU).

Figure 2. Structure of RTK degraders. POI-binding warheads appear in red, E3 UL recruiting moieties in green and linkers in black.

Figure 3. Structure of JAK, BCR-ABL and FAK degraders. POI-binding warheads appear in red, E3 UL recruiting moieties in green and linkers in black.

Figure 4. Structure of BTK degraders. POI-binding warheads appear in red, E3 UL recruiting moieties in green and linkers in black. Key modifications (see the text) are circled in black.

Figure 5. Structure of STK degraders. POI-binding warheads appear in red, E3 UL recruiting moieties in green and linkers in black.

Table 1. Comparison of structure and activities of BCR-ABL PROTACs.

Table 2. Comparison of structure and activities of BTK PROTACs.

TOC

Cellular degradation of kinases instead of their “simple” inhibition constitutes an attractive new strategy to increase the efficacy and the selectivity of inhibitors used in the clinic. However, the development of potent PROTACs remains a challenge for medicinal chemists. This review discusses the recent developments in this field with particular attention to the opportunities and the current limitations of this technology applied to kinases.

