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## Induced protein degradation: an emerging drug discovery paradigm

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### Abstract

Small-molecule drug discovery has traditionally focused on occupancy of a binding site that directly affects protein function, which typically precludes targeting proteins that lack such amenable sites. Furthermore, high systemic drug exposures may be needed to maintain sufficient target inhibition *in vivo*, increasing the risk of undesirable off-target effects. Induced protein degradation is an alternative approach that is ‘event-driven’: upon drug binding, the target protein is tagged for elimination. Emerging technologies based on proteolysis-targeting chimeras (PROTACs) that exploit cellular quality control machinery to selectively degrade target proteins are attracting considerable attention in the pharmaceutical industry owing to the advantages they could offer over traditional small-molecule strategies. These advantages include the potential to reduce systemic drug exposure, the ability to counteract increased target protein expression that often accompanies inhibition of protein function and the potential ability to target proteins that are not currently therapeutically tractable, such as transcription factors, scaffolding and regulatory proteins.

### 1) Introduction

Although the genomic revolution has allowed a plethora of novel protein targets to be linked to disease states<sup>1–4</sup>, standard small-molecule drug discovery strategies are unable to exploit all of these opportunities because many of these potential targets do not have suitable binding pockets that directly modulate protein function<sup>5</sup>. Due to their intracellular location, such proteins are often not tractable targets for antibody therapeutics either. The challenge that this presents for drug discovery is highlighted by the fact that there are very few marketed drugs that target scaffolding proteins, transcription factors, and other non-enzymatic proteins inside the cell<sup>6,7</sup>. Furthermore, small-molecule therapeutic strategies that focus on occupation of a binding site on a protein may require high systemic drug exposures to achieve sufficient site-occupancy *in vivo*<sup>8</sup>, potentially increasing the risk of off-target adverse effects.

One method for modulating intracellular protein concentrations is through nucleic acid-based agents such as antisense oligonucleotides (ASOs) or agents that exploit RNA interference (RNAi), such as small interfering RNAs (siRNAs). More recently, the advent of

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CRISPR/Cas9 technology<sup>9,10</sup> offers the potential of modifying the genome itself to achieve gene knockout; however, the clinical potential of this technology is yet to be explored (see recent reviews<sup>11–13</sup>). While nucleic acid-based tools have proven useful in research, their development as drug candidates has faced many challenges: unmodified nucleotides are highly unstable in serum<sup>14</sup>, whereas modified nucleotides tend to accumulate in the kidney<sup>15,16</sup> and can be immunogenic<sup>17,18</sup>. Nucleic acid-based agents encapsulated by nanoparticles to improve their delivery properties are still captured in the liver<sup>19–22</sup>. Additionally, RNAi can engage off-target mRNA, leading to undesired effects<sup>23–25</sup>. Finally, efficacy is ultimately dependent on target protein half-life; thus, long-lived proteins are less affected by these approaches. For a more detailed analysis, the reader is referred to these excellent reviews<sup>26–28</sup>. Although significant progress has been made, as indicated by the many ongoing clinical trials, only two ASO therapeutics (fomirisen<sup>29</sup> and mipomersen<sup>30</sup>) that reduce the production of a particular protein have been approved in the United States<sup>31</sup>.

Small-molecule induced protein degradation is emerging as a strategy that also has the potential to target a broader range of proteins than standard small-molecule strategies that focus on binding site occupancy, yet maintain the pharmaceutical advantages of that such compounds typically have compared with nucleic acid-based agents. This shift from inhibition of the target protein to its removal permits the targeting of proteins that have generally been considered “undruggable”<sup>32,33</sup>. Furthermore, removing “druggable” targets from the system can be synergistic with current inhibitor-based regimens. For example, inhibition of certain cellular pathways promotes feedback-mediated increased expression of the target protein, leading to pharmacological insufficiency<sup>34,35</sup>. Induced protein degradation not only reduces the number of active proteins needed to be inhibited but also counteracts compensatory protein overexpression often observed upon loss of protein function. Additionally, many “druggable” targets are recognized to have “undruggable” scaffolding roles that contribute to resistance mechanisms<sup>36–42</sup>.

Induced protein degradation is not a recent concept. Inhibitors of the molecular chaperone Heat Shock Protein 90 (HSP90), which helps other proteins fold properly, first entered clinical trials in 1999. The initial excitement generated by HSP90 inhibitors was founded on the discovery that cancer cells upregulate HSP90 to drive proliferation and survival pathways<sup>43</sup> and are more sensitive to HSP90 inhibition due to an overactive conformation of HSP90 found in cancer cells<sup>44</sup>. All reported HSP90 inhibitors target the ATP-binding domain<sup>45–47</sup>, affecting HSP90 chaperone cycling and leading to degradation of HSP90 client proteins<sup>48,49</sup>. There are more than 30 ongoing clinical trials associated with HSP90 inhibitors, yet none has gained FDA-approval (Box 1). HSP90 inhibitors have been hindered by poor *in vivo* pharmacological properties and severe hepatotoxicity attributed to their benzoquinone moiety<sup>50–52</sup>. Furthermore, due to the diversity of HSP90 client proteins, which includes more than half the human kinome<sup>53–55</sup>, it has been difficult to determine the exact cellular context in which an oncological vulnerability can be exploited by a broad inducer of protein degradation, although attempts have been made to bias the degradation<sup>56,57</sup>. Several excellent reviews offer a more thorough survey of the HSP90 inhibitor literature<sup>58–60</sup>.

In contrast to the general client protein knockdown achieved with HSP90 inhibitors, selective estrogen receptor degraders (SERDs) and selective androgen receptor degraders (SARDs) exhibit high specificity in target protein degradation. Interestingly, these compounds were originally developed as modulators of protein function but were subsequently serendipitously discovered to be protein degraders<sup>61</sup>. Though the efficacy of these compounds has been impressive, this strategy is not generalizable to other targets: the compounds can only induce the degradation of estrogen and androgen receptors.

More recently, we and others have developed platform technologies that combine the modularity of nucleic acid-based strategies with the *in vivo* pharmacology of small-molecule therapeutics. Based on the concept of ‘event-driven’ pharmacology, proteolysis-targeting chimera (PROTAC) and hydrophobic tagging (HyT) technologies utilize bifunctional molecules, whereby one end binds to the protein of interest while the other hijacks cellular quality control mechanisms to induce the degradation of the target protein (Figure 1). Only a transient binding event is required for activity and thus, in contrast to the stoichiometric site-occupancy of the ‘occupancy-driven’ model, these ‘event-driven’ molecules can cycle through multiple rounds of activity, removing super-stoichiometric quantities of proteins. Additionally, compared to the dissociation kinetics of an inhibitor from an active site, restoration of protein function requires re-synthesis of the protein, providing a kinetic advantage. Furthermore, the protein ligand does not need to occupy a binding site that affects protein function (such as an enzyme active site) in order to function as a degrader — binding at any “nook” or “cranny” on a target could potentially induce degradation. This advantage could be exploited by utilizing thermal shift-, surface plasmon resonance (SPR)- or nuclear magnetic resonance (NMR)-based screens that focus on the identification of ligands that simply bind to a target, rather than screens that focus on identifying ligands that affect the function of the protein<sup>62–66</sup>. Moreover, by turning protein ligands into degraders, it might be possible to resurrect high-throughput screening (HTS) hits that bind to the desired targets but were set aside because they could not adequately block protein function<sup>67–69</sup>. Finally, several studies have determined that the *degradation* selectivity of PROTACs not only retains, but can also exceed, the *binding* selectivity of the protein ligand, such that PROTAC binding to off-target proteins does not necessarily lead to the degradation of the off-target protein; thus, PROTACs offer an added layer of specificity that can be modulated by which E3 ligase is recruited to the targets<sup>70–72</sup>.

Substantial progress has recently been made, in particular with the development of PROTAC compounds demonstrating that PROTACs are not just useful chemical tools, but also viable therapeutic candidates. This progress also supports the idea that induced protein degradation is a novel drug discovery paradigm with the potential to target a broad range of proteins currently considered therapeutically intractable. In this review, we summarize the main approaches that have been investigated so far to induce degradation of targeted proteins, highlighting the progress that has been made and discussing the opportunities and challenges in establishing drug discovery platforms based on these approaches.

## 2) Selective hormone receptor degraders

The estrogen receptor (ER), a nuclear hormone receptor, is one of the few transcription factors amenable to modulation with small-molecule drugs. Upon binding to estrogens (17 $\beta$ -estradiol, estrone, and estriol), ER binds DNA to exert a cell-specific transcriptional response that is dependent on co-regulatory proteins present<sup>73</sup>; thus, estrogens have a diverse effect on the cardiovascular, musculoskeletal, immune and reproductive systems in both males and females<sup>74</sup>. As ER signaling is crucial for breast cancer progression, ER-positive breast carcinomas are responsive to hormonal therapy such as the selective estrogen receptor modulator (SERM) tamoxifen<sup>75</sup>. However, depending on the cellular context, SERMs can act as either an antagonist or agonist: in breast tissue, tamoxifen was found to act as an antagonist, whereas in the uterus, it was characterized as an agonist and led to an increased risk of endometrial cancers<sup>76</sup>. Furthermore, long-term estrogen deprivation by SERMs can lead to compensatory feedback signaling, resulting in pharmacological insufficiency<sup>77</sup>.

Addition of an alkylsulphonyl moiety to the endogenous ER ligand 17 $\beta$ -estradiol led to the development of fulvestrant (ICI82780, Faslodex; AstraZeneca)<sup>78,79</sup> that destabilizes the ER (Table 1)<sup>61</sup>. Fulvestrant binding to ER $\alpha$  induces a structural change that leads to increased surface hydrophobicity and subsequent degradation<sup>80,81</sup>. Fulvestrant-induced degradation affects the ligand-independent functions of ER<sup>82</sup> that are not readily addressed by tamoxifen<sup>83,84</sup> and likely has less risk of endometrial cancer than tamoxifen due to its pure anti-estrogen antagonism<sup>85</sup>. Thus, fulvestrant is the only FDA-approved SERD for ER-positive metastatic breast carcinomas that have progressed after tamoxifen therapy<sup>86</sup>. However, due to fulvestrant's lack of oral bioavailability and poor systemic exposure<sup>87,88</sup>, newer generation SERDs are in development<sup>89-92</sup>.

Despite its issues, fulvestrant's limited clinical success prompted the pursuit of an analogous approach to target the androgen receptor (AR) in prostate cancer, i.e., the development of selective androgen receptor degraders (SARDs). The AR is also a ligand-dependent transcription factor amenable to a traditional inhibitor approach. As a nuclear hormone receptor, AR responds to endogenous androgens including 5 $\alpha$ -dihydrotestosterone (5 $\alpha$ -DHT) and testosterone in order to mediate the development and maintenance of male reproductive organs<sup>93</sup>. For this reason, prostate cancer is also initially dependent on AR signaling; thus, antiandrogen therapy with flutamide or bicalutamide has demonstrated good clinical efficacy for prostate cancer<sup>94,95</sup>; however, resistance eventually develops, commonly through overexpression or amplification of the androgen receptor<sup>35,96</sup>, leading to castration-resistant prostate cancer (CRPC)<sup>97,98</sup>. A more potent androgen antagonist, enzalutamide (MDV3100, Xtandi; Medivation)<sup>99</sup>, established that a subset of CRPC is still dependent on androgen signaling. To address these resistance mechanisms, the first-in-class nonsteroidal SARD AZD3514<sup>100</sup> was developed and was shown to downregulate AR<sup>101-103</sup>. Despite promising phase I (NCT01162395) efficacy data<sup>103</sup>, the development of AZD3514 was discontinued due to tolerability issues<sup>104</sup>. Though much work<sup>105</sup> has been done in the field of SARDs, there are currently no known SARDs on the market.

The field of induced ER and AR degradation remains active, with multiple compounds in clinical trials. Targeted degradation not only combats compensatory upregulation associated

with inhibition of function but also affects functions not amenable to traditional inhibitors (i.e. ligand-independent function of ER $\alpha$ ). However, SARDs and SERDs are only capable of inducing the degradation of androgen and estrogen receptors; they cannot be repurposed to target other proteins for degradation.

### 3) Hydrophobic tagging (HyT)

To extend the concept of induced protein instability to a larger spectrum of protein targets, a new platform technology was recently developed by us and others: hydrophobic tagging (HyT). Similar to fulvestrant-induced surface hydrophobicity, this approach appends hydrophobic moieties (i.e., adamantane or Boc<sub>3</sub>-Arg) to the surface of target proteins in an attempt to mimic a partially unfolded protein state and co-opt the cytosolic unfolded protein response to degrade the target<sup>106</sup>. Compared to SARDs and SERDs, this approach is modular with respect to which proteins can be targeted as long as a ligand to the target can be identified.

The ability of Boc<sub>3</sub>-Arg to induce protein degradation was demonstrated in 2012, with the targeting of glutathione-S-transferase  $\alpha$ 1 (GST- $\alpha$ 1) through the covalent inhibitor etacrynic acid linked to Boc<sub>3</sub>-Arg at 80 $\mu$ M (Figure 2)<sup>107</sup>. Similarly, dihydrofolate reductase was degraded using micromolar concentrations of the non-covalent inhibitor trimethoprim coupled to Boc<sub>3</sub>-Arg. This induced target protein degradation pathway occurs via the proteasome in an ubiquitin- and ATP-independent manner, although the roles of chaperones and other quality control pathways remain unknown. Interestingly, it was recently found that the Boc<sub>3</sub>-Arg moiety can inhibit the translational machinery as mediated by the mammalian Target of Rapamycin Complex 1 (mTORC1) pathway<sup>108</sup>. This off-target effect of Boc<sub>3</sub>-Arg could potentially limit the clinical use of Boc<sub>3</sub>-Arg as a hydrophobic tag.

An earlier HyT strategy, the adamantane-based HyT strategy, has been applied to a broader range of targets. This technology also consists of a bifunctional molecule, with one end binding to a protein of interest and the other end engaging quality control mechanisms through a hydrophobic adamantyl moiety<sup>109</sup>. First reported in 2011, ectopically expressed cytosolic and transmembrane HaloTag fusion proteins<sup>110–112</sup> were shown to be susceptible to HyT-mediated degradation. Pharmacokinetic studies with adamantane-based HyT in mice demonstrated that 75% of the hydrophobic tag remained after 24h and the HyT compound was sufficient to suppress xenografted HaloTag-HRas<sup>G12V</sup>-driven tumor growth in mice by 80%<sup>110</sup>. Immunoprecipitation of HaloTag fusion protein tagged with the adamantyl group demonstrated increased association with HSP70, suggesting that HSP70 mediates adamantyl-mediated degradation<sup>113</sup>. Furthermore, higher molecular weight bands corresponding to ubiquitination were observed in the immunoprecipitates, suggesting that the ubiquitin-proteasome system (UPS) plays a role in adamantane-based HyT. In the same study, a second small-molecule ligand (HALTS1) was discovered to stabilize HaloTag fusion proteins, allowing for bidirectional control of protein levels (Box 2)<sup>113</sup>. Despite the promising introduction of HyT technology as a tool for modulation of intracellular protein levels, degradation of targets with HyT was only demonstrated via covalent interaction with the artificial HaloTag system at the time.

In 2014, the HyT technology was further extended to endogenously expressed proteins with the degradation of erythroblastosis oncogene B3 (ErbB3/HER3), a pseudokinase considered to be “undruggable” by an ‘occupancy-driven’ approach<sup>114</sup>. Gray and co-workers discovered a selective ErbB3 covalent ligand from a HTS with an apparent IC<sub>50</sub> of 23 nM<sup>115</sup>; however, given that ErbB3 has minimal kinase activity, a small-molecule binder would have little consequence on the scaffolding role of ErbB3. Coupling adamantane to the ErbB3 ligand, Gray and co-workers converted the ligand into a HyT degrader termed TX2-121-1. Covalent binding of TX2-121-1 to ErbB3 led to the degradation of ErbB3 at 500nM, inhibiting downstream signaling and exhibiting an antiproliferative effect on ErbB3-dependent cell lines. Elimination of the covalent interaction reduced the efficacy of the adamantane-based HyT. The extension of the technology to the previously “undruggable” ErbB3 was a significant step in the development of HyT as a therapeutic strategy; however, the degradation of ErbB3 was still dependent on a covalent interaction, and so the degradation would be stoichiometric, rather than sub-stoichiometric.

Recently, our group has expanded the clinical potential of the HyT technology by targeting the androgen receptor (AR) with a non-covalent binder<sup>116</sup>. A high-affinity AR *agonist*, RU59063<sup>117</sup>, was coupled with an adamantyl moiety, thus converting an agonist into an antagonistic degrader. Upon incubation with LNCaP prostate tumor cells, AR degradation was observed in the sub-micromolar range, reducing the expression of AR target genes and leading to decreased cell proliferation. As the first demonstration of a successful adamantane-based HyT strategy with a non-covalent interaction, this study was a step towards expanding the target spectrum for this approach. Despite this success, more examples of targets being degraded through a *non-covalent* interaction need to be demonstrated before HyT becomes a potential therapeutic strategy. Indeed, the *in vivo* potential of adamantane-based and Boc<sub>3</sub>-Arg HyTs in a non-artificial system remains untested.

The HyT platform illustrates the potential of a modular post-translational protein knockdown strategy by targeting traditionally intractable targets (i.e. ErbB3) or by utilizing non-covalent protein ligands to induce protein degradation; however, there is some concern that hydrophobic moieties of such compounds can remain bound by serum proteins resulting in low free fraction of the compound available to induce degradation of target proteins. Moreover, it is unclear whether HyT-mediated degradation is dependent on recognition of the adamantane/Arg-Boc<sub>3</sub> moiety or on the induced destabilization of the protein. If the latter, HyT would be less effective against more stable proteins, limiting the targets that could be accessed for degradation. Additionally, the degradation pathways for adamantane- and Arg-Boc<sub>3</sub>-based HyT may be different. Thus, as the mechanism of HyT is unresolved, HyT technology remains a chemical tool for discovery biology rather than a viable therapeutic strategy. In contrast, PROTACs, which utilize small-molecule E3 ligase ligands to induce proximity-induced ubiquitination of a target protein, are much closer to clinical translation.

## 4) Proteolysis-targeting chimeras (PROTACs)

### Peptide-based PROTACs

In several proof-of-concept experiments published in 2001, our lab, in collaboration with Ray Deshaies, reported the first PROTACs —bifunctional molecules that harness the ubiquitin–proteasome system (UPS) by recruiting an E3 ligase to a protein of interest, leading to the proximity-induced ubiquitination and subsequent degradation of the protein (Box 3)<sup>118</sup>. These early PROTACs were composed of a phosphopeptide portion of I $\kappa$ B $\alpha$  to recruit the F-box protein  $\beta$ -TrCP, a component of the Skp1-Cullin1-F box E3 ligase complex (SCF $\beta$ -TrCP)<sup>119</sup>, and the angiogenesis inhibitor ovalicin to covalently bind the target protein, methionine aminopeptidase-2 (MetAP-2)<sup>120,121</sup>. Addition of this PROTAC to *Xenopus* egg extracts led to MetAP-2 degradation. The technology was further demonstrated in a cellular system to induce the degradation of AR by microinjecting a  $\beta$ -TrCP-based PROTAC with dihydroxytestosterone (DHT) as the protein ligand into HEK293 cells<sup>122</sup>. Though these were the first demonstrations of the PROTAC technology, there was much room for improvement: the potencies of these PROTACs were in the micromolar range and cellular permeability was very low; furthermore, the dependency on I $\kappa$ B $\alpha$  phosphorylation for E3 ligase recruitment was limiting given the action of cellular phosphatases.

The first improvement in peptidic PROTACs was a switch to a smaller E3 ligase recruiting peptide fragment that did not require phosphorylation for activity. The E3 ligase von Hippel-Lindau (VHL) recognizes a core hydroxylated proline in a seven-amino-acid recognition sequence of hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ )<sup>123,124</sup>. An AR-targeting PROTAC was developed using DHT conjugated to the HIF1 $\alpha$  peptide fragment with a poly-*D*-arginine tag appended for cell penetrance<sup>125</sup>. Addition of the PROTAC at 25 $\mu$ M led to significant AR depletion in HEK293 cells. Analogous PROTACs using estradiol demonstrated ER $\alpha$  degradation, leading to reduced proliferation of ER $\alpha$ -dependent breast cancer cell lines<sup>126–128</sup>. More recently, VHL-recruiting peptidic PROTACs were utilized to induce the degradation of Akt<sup>129</sup> and Tau<sup>130</sup>. The latter’s ability to induce degradation of a traditionally “undruggable” protein highlights the advantages of ‘event-driven’ over ‘occupancy-driven’ pharmacology.

The advantage of ‘event-driven’ pharmacology is further emphasized with the application of the PROTAC technology to the conditional removal of proteins. As the PROTAC technology works post-translationally (in contrast to nucleic acid-based approaches), there is a pharmacological opportunity to remove a select subset of proteins. Our group introduced a phospho-dependent PROTAC, whereby activation of the NGF receptor (TrkA) or ErbB3/HER3 was coupled to the post-translational degradation of downstream signaling components, i.e., fibroblast growth factor receptor substrate 2 $\alpha$  (FRS2 $\alpha$ ) and phosphatidylinositol-3-kinase (PI3K), respectively<sup>131</sup>. Upon growth factor stimulation, TrkA or ErbB3 phosphorylates a peptidic PROTAC, which is then recognized by either FRS2 $\alpha$  or PI3K. FRS2 $\alpha$  or PI3K, bound to their respective phosphorylated peptidic PROTAC, is then ubiquitinated by VHL. In a similar conditional knockdown study, the  $\beta$ -catenin binding motif of E-cadherin was fused to  $\beta$ -TrCP. The resulting fusion protein was capable of inducing the degradation of only the cytosolic population of  $\beta$ -catenin, sparing the

membrane-bound population<sup>132</sup>. However, the potencies of these peptidic PROTACs remain in the micromolar range, likely due to the poor cell penetration associated with peptide-based therapeutics. Fortunately, incremental improvements have led to the development of potent small-molecule PROTACs with *in vivo* stability.

### Small-molecule PROTACs

The current pharmacopeia that targets intracellular proteins remains largely dominated by small-molecules, as such targets are not accessible to monoclonal antibodies and so far, few nucleotide-based therapies have been approved. Given the clinical limitations of peptide-based PROTACs, efforts have increasingly focused on the generation of small-molecule PROTACs, beginning in 2008 with the creation of a PROTAC utilizing nutlin-3a<sup>133</sup> to recruit the E3 ligase mouse double minute 2 homolog (MDM2; nutlin-3a IC<sub>50</sub> against MDM2 = 0.09μM) to the AR<sup>134</sup>. PROTAC-induced AR degradation was observed at 10μM in cells; however, the maximum knockdown of the AR was not as dramatic as with the peptidic VHL ligand. Despite the success of this initial foray into all-small-molecule PROTACs, subsequent medicinal chemistry efforts were unsuccessful at achieving more effective degradation of the targets using the E3 ligase MDM2. However, newer generations of nutlins, RG7112 and idasanutlin (RG7388; Genentech), have been developed with increased potency<sup>135,136</sup> and it remains to be determined if these can be successfully incorporated into PROTACs with improved potency and knockdown efficiency. Moreover, dose-limiting cytopenias and gastrointestinal effects reported in phase I trials with idasanutlin are possible concerns for any nutlin-based PROTAC drug candidates<sup>137</sup>.

Another small-molecule-based PROTAC approach for targeted protein degradation, termed Specific and Non-genetic Inhibitor-of-apoptosis proteins (IAPs)-dependent Protein Eraser (SNIPER), was reported in 2010 by the Hashimoto group<sup>138</sup>. Utilizing bestatin (ubemimex; Nippon Kayaku) esters<sup>139</sup> to repurpose the E3 ligase cellular inhibitor of apoptosis protein 1 (cIAP1), Hashimoto and co-workers demonstrated the degradation of cellular retinoic acid-binding proteins (CRABP-I and -II) with *all-trans* retinoic acid (ATRA) as the target ligand. Unfortunately, bestatin is not specific for cIAP1 as it inhibits both arginyl aminopeptidases and leukotriene A4 hydrolase<sup>139,140</sup>. Additionally, these bestatin esters also induced the autoubiquitination and degradation of the recruited E3 ligase cIAP1, limiting the full potential of the technology<sup>141</sup>. However, to overcome some of these limitations, medicinal chemistry efforts have reduced the autoubiquitination observed with bestatin<sup>142</sup> or have recruited related E3 ligases for SNIPER<sup>143</sup>. In addition to the CRABP family, cIAP-based PROTACs were also used to degrade estrogen receptor<sup>144</sup>, androgen receptor<sup>145</sup>, retinoic acid receptors<sup>145</sup> and transforming acidic coiled-coil-3<sup>146</sup>. Despite the range of targets degraded, SNIPER PROTACs are still maximally effective only in the micromolar range. Given the micromolar potencies of cIAP-based PROTACs and the fact that bestatin is not specific for cIAP1, SNIPER PROTACs have yet to be tested in mouse models.

Recent efforts have focused on identifying an appropriate small-molecule replacement for the HIF1α peptide fragment to provide an alternative E3 ligase for the all small-molecule PROTAC approach. The first small-molecule ligand for the VHL E3 ligase was identified through *in silico* and fragment-based screens in 2012 with a reported K<sub>d</sub> of 5.4μM<sup>147-149</sup>.



This affinity is more than 20-fold weaker than a 10-mer HIF1 $\alpha$  fragment interaction with VHL ( $K_d = 0.2\mu\text{M}$ ). Subsequent structure-activity relationship (SAR) efforts yielded a VHL ligand with a  $K_d$  of 185nM<sup>150</sup>. Utilizing this improved ligand, a VHL-based small-molecule PROTAC was described in 2015 with nanomolar potency for nearly complete degradation of estrogen-related receptor  $\alpha$  (ERR $\alpha$ ) and receptor-interacting serine-threonine kinase 2 (RIPK2) without stabilization of the endogenous target HIF1 $\alpha$  at concentrations of up to 30 $\mu\text{M}$  of the ERR $\alpha$ -targeted PROTAC<sup>70</sup>. *In vivo* degradation of ERR $\alpha$  was achieved with approximately 40% knockdown of protein in heart, kidney and tumor xenograft. Moreover, PROTACs demonstrated sub-stoichiometric activity where one PROTAC molecule was capable of inducing multiple rounds of target protein ubiquitination. Finally, through comparative proteomic studies, PROTACs were not only specific for the target protein but also had less off-target degradation than initially suggested by the ligand binding specificity. This study was the first demonstration of a small-molecule VHL-based PROTAC with sub-stoichiometric activity and activity in mouse models.

Concurrent with the establishment of VHL-based PROTACs, another PROTAC system based on a fourth small-molecule E3 ligase recruiting ligand was developed. In 2010, work from Handa and colleagues identified the E3 ligase Cereblon (CRBN) as the primary target of thalidomide<sup>151</sup>. In 2014, the Kaelin and Ebert groups discovered that phthalimides (thalidomide, lenalidomide, and pomalidomide) induced the degradation of the lymphoid transcription factors Ikaros Family Zinc Finger 1 and 3 (IKZF1 and IKZF3)<sup>152–154</sup> by binding to CRBN ( $K_d = 150–250\text{nM}$ )<sup>155</sup> and repurposing the E3 ligase to ubiquitinate the transcription factors. Further studies have found that not all of the phthalimides have the same substrate degradation spectrum and thus are utilized in different clinical situations: lenalidomide can induce the degradation of casein kinase I $\alpha$  (CKI $\alpha$ ); in contrast, thalidomide and pomalidomide are incapable of inducing CKI $\alpha$  degradation<sup>156–158</sup>. Recently, screening for CRBN ligands that degraded other targets led to the identification of CC-885, a phthalimide derivative that targets G1 to S phase transition protein 1 homologue (GSPT1; also known as ERF3A)<sup>159</sup>.

Considering the ability of phthalimide to bind CRBN, our group and others sought to utilize phthalimide as an E3 ligase recruiting ligand to hijack CRBN to degrade proteins of interest. Conjugation of a small-molecule BRD4 binding moiety (OTX015) to pomalidomide yielded a PROTAC capable of degrading the epigenetic regulator BRD4 at picomolar potencies<sup>160</sup>. In comparison to the BRD4 inhibitors JQ1 and OTX015, the CRBN-based PROTAC provided a more sustained suppression of c-myc levels by counteracting known increased BRD4 expression associated with BRD4 inhibition<sup>161</sup>. This sustained suppression translated into superior antiproliferative and apoptotic effects against Burkitt's lymphoma cell lines compared to the inhibitors. In a separate but parallel study, the BRD4 inhibitor JQ1 was conjugated to a thalidomide derivative<sup>72</sup>. The resulting PROTAC, termed dBET1, was capable of inducing BRD4 degradation at low nanomolar concentrations. Proteomic studies with this PROTAC determined that the compound significantly reduced c-myc, BRD2, BRD3, and BRD4 levels with minimal off-target degradation. This bifunctional compound was also found to be superior to JQ1 against human AML and lymphoma cell lines. Finally, dBET1 was investigated in a xenograft model of AML and a disseminated leukemia mouse model; the PROTAC was found to be active in the AML model and outperformed JQ1 in the

disseminated model. The superiority of ‘event-driven’ versus ‘occupancy-driven’ pharmacology in preclinical models is encouraging and sets the stage for clinical applications in the near future.

In addition to CRBN-based PROTACs, a picomolar VHL-based pan-BET-targeting PROTAC was also developed, which demonstrated superior anti-proliferative activity compared to BET inhibitors<sup>162</sup>. The pan-BET-targeting PROTAC also exhibited activity against a 22RV-1 CRPC mouse xenograft, thus expanding the application of PROTAC technology to solid tumor malignancies. Similarly, Ciulli and co-workers coupled the VHL recruiting ligand to JQ1 to create a PROTAC capable of differentially degrading BRD4 over BRD2 and BRD3<sup>163</sup>. Maximum degradation of BRD4 occurred at nanomolar concentrations while effects on BRD3 and BRD2 required low micromolar PROTAC concentrations. This selective degradation of BRD4 over BRD3 and BRD2 leads to a differential gene expression pattern between the parent inhibitor JQ1 and the BRD4-targeting PROTAC. This observation suggests that PROTACs can provide an added layer of specificity relative to an inhibitor: off-target proteins can be spared from PROTAC-induced degradation in spite of target binding.

This idea was further studied by exploring VHL-based PROTACs and CRBN-based PROTACs in a head-to-head comparison<sup>71</sup>. Utilizing the protein tyrosine kinase inhibitors bosutinib (SK-606, Bosulif; Pfizer)<sup>164</sup> and dasatinib (BMS-354825, SPRYCEL; Bristol-Myer Squibb)<sup>165</sup>, ABL- and BCR-ABL-targeting PROTACs were generated via conjugation to either a VHL recruiting ligand or a CRBN recruiting ligand. Surprisingly, the degradation profile of ABL-targeting and BCR-ABL-targeting PROTACs depended on the identity of the inhibitor warhead (bosutinib or dasatinib) and on the E3 ligase recruited (VHL or CBN) but was independent of target engagement by the PROTACs. In this case, CRBN E3 ligase appeared more promiscuous in its degradation profile as compared to VHL, consistent with previous studies targeting BRD4. One explanation could lie within the unique flexibility of the CRBN E3 ligase complex containing Cullin 4A<sup>157,166–168</sup> compared to the VHL E3 ligase complex<sup>169,170</sup>, thus, allowing for a larger ubiquitination zone. These two studies into E3 ligase selectivity in the PROTAC system open up the possibility of converting a promiscuous *inhibitor* into a selective *degrader*.

With the progress made from the 2001 report of PROTAC-induced degradation in a *Xenopus* extract to the more recent targeted degradation achieved in cells and mouse models, interest in PROTACs has grown<sup>171</sup>. As a platform technology, PROTACs offer a modular ‘plug-and-play’ aspect that allows for general applicability: once a target protein ligand is identified, the ligand can be linked to an E3 ligase recruiting ligand for PROTAC development<sup>172</sup>. The removal of target proteins allows PROTACs to counteract compensatory accumulation that is often reported with inhibitors and provide a kinetic advantage similar to covalent inhibitors (i.e., restoration of protein function requires target protein re-synthesis). However, in contrast to the stoichiometric property of covalent ‘occupancy-driven’ inhibitors, the ‘event-driven’ catalytic nature of non-covalent PROTACs has the potential to require less total drug exposure. In addition, the possibility of conditional knockdowns and an increased layer of specificity hold the promise of minimizing off-target effects. Finally, proof-of-concept experiments in mouse tumor xenografts demonstrate that the *in vivo* efficacy of ‘event-

driven' targeted protein degradation can be superior to the 'occupancy-driven' pharmacology of the parent inhibitor compounds<sup>161,163</sup>.

The promise of induced protein degradation as a novel therapeutic modality has attracted the attention of the pharmaceutical industry<sup>173</sup> and led to the creation of companies focused on developing such technologies. Arvinas (based in New Haven, Connecticut, USA) was launched in 2013<sup>174</sup> and recently announced plans to advance a PROTAC-based drug candidate into Phase I clinical trials in 2017<sup>175</sup>, and C4 therapeutics (based in Cambridge, Massachusetts, USA) was launched in 2016. Either as tool compounds<sup>176,177</sup> or as a future drug discovery paradigm, the myriad of potential PROTAC applications has not yet been fully explored. However, from the few innovative reports to date, it is clear that induced protein degradation as a novel pharmaceutical paradigm holds much promise.

## 5) Challenges and Opportunities Ahead

With the progress made in the field of ASO and the more recent publications in the field of small-molecule induced degradation, a complementary approach to the traditional therapeutic strategies inhibiting protein function — the modulation of protein levels — has emerged (Figure 3). Nucleic acid-based therapeutics initially held much promise with their ease of synthesis and modularity; however, dependency on protein half-life and challenging *in vivo* pharmacological properties have hindered the broad development of nucleic acid-based clinical drug candidates. General inducers of degradation, such as HSP90 inhibitors, have suffered from challenging pharmacological properties, tolerability and the lack of a clear clinical indication; however, given the volume and range of ongoing clinical trials, HSP90 inhibitors may still find their way to the market. With the approval of fulvestrant, SERDs and SARDs remain another promising class of drugs that can address mechanisms of resistance arising from a traditional inhibitor approach. However, bioavailability and systemic exposure remain a challenge for this class of drugs. Additionally, these small-molecule approaches are not broadly applicable to other targets.

In theory, HyT technology can be broadly applied to targets yet the unknown mechanism of HyT might limit the adoption of the technology. By contrast, the PROTAC technology offers a modular approach to sub-stoichiometric targeted protein degradation, which only requires accessible surface lysines to be present on the target protein in order to harness the UPS (Box 3). However, PROTACs are not considered traditional "small" molecules since they do not follow Lipinski's rule of five<sup>178</sup> owing to their relatively large molecular mass. Unfortunately, this larger size of PROTACs provides more opportunity for metabolic modification of the compounds. First-pass metabolism could limit the oral delivery of PROTACs yet a lower bioavailability might not significantly hinder PROTAC efficacy given the sub-stoichiometric nature of the technology when using non-covalent ligands. In regards to future clinical application, the potencies achievable in cell culture as well as the efficacies observed in tumor xenografts are encouraging.

As the field of PROTAC-mediated target protein degradation matures, the idea of additional specificity could be explored further: is it possible to achieve tissue-specific or disease-specific induced protein degradation by utilizing tissue/disease-specific E3 ligases? Small-

molecule screens have already identified a number of candidate E3 ligase inhibitors that can potentially be utilized as recruiting ligands<sup>179–182</sup> and we have already found that different E3 ligases provide different degradation profile<sup>71</sup>. A more thorough understanding of E3 ligase tissue expression could potentially offer an increased therapeutic window with an appropriate E3 ligase-recruiting PROTAC. Similarly, subcellular localization and regulation of E3 ligases could offer opportunities for conditional removal of target proteins<sup>183</sup>; perhaps a nuclear localized E3 ligase would permit conditional degradation of activated nuclear receptors while sparing the cytoplasmic population. Additionally, as suggested by studies with peptidic PhosphoPROTACs, perhaps developing ‘state-specific’ protein ligands will permit conditional protein knockdown; on the other end of the PROTAC, we can also imagine a conditional unmasking of the E3 ligase recruiting ligand either by chemical or optical means to permit temporal and spatial control of target degradation analogous to the endogenous system of cryptic degrons. The concept of increased specificity is only the tip of the iceberg for this emerging technology.

Finally, PROTAC technology could have its biggest impact via the development of degrader molecules capable of targeting the “undruggable” proteome. Despite the recent success of small-molecule PROTACs, this technology has to date only been applied to classically “druggable” proteins. However, the ability to sub-stoichiometrically degrade transcription factors, scaffolding and other non-enzymatic proteins would greatly increase the current drug target space. One could envision a process in which appropriate protein binders are identified via screens and then conjugated to pre-made linkers attached to E3 ligase ligands to generate potent degrader compounds. In this way, PROTAC generation could become part of the routine strategy in drug discovery, thereby making the “undruggable” proteome finally pharmaceutically vulnerable.

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## Glossary

### ‘Occupancy-driven’ Pharmacology

A drug discovery paradigm based on identifying compounds that modulate protein function through stoichiometric drug binding and occupation of the binding site to modulate protein function

### ‘Event-driven’ Pharmacology

A drug discovery paradigm based on identifying compounds that lead to the removal of the target protein from the system following drug binding

### Sub-stoichiometric

pertains to molecular compounds that have a greater effect than the 1:1 activity seen with a stoichiometric agent

**HaloTag**

a bacterial dehalogenase engineered by the Promega Corporation to covalently bind chloroalkanes and has been used primarily for protein purification and imaging purposes

**Erythroblastosis oncogene B3 (ErbB3/HER3)**

A member of the epidermal growth factor receptor (EGFR) family that has minimal kinase activity and acts primarily as a scaffold for signal transduction

**I $\kappa$ B $\alpha$** 

Inhibitor of NF- $\kappa$ B that contains a destruction motif that, when phosphorylated, is recognized by the E3 ligase substrate recognition component  $\beta$ -TrCP

**von Hippel-Lindau (VHL)**

the substrate recognition portion of the E3 ligase complex VHL-elongin B/C-cullin 2 (VHL-ELOB-CUL2) that mediates the transfer of ubiquitin onto hypoxia-inducible factor 1 $\alpha$  (HIF 1 $\alpha$ )

**Poly-D-arginine tag**

An arginine-containing peptide fragment based on the HIV Tat peptide that facilitates cellular uptake of proteins

**BRD4**

A member of the Bromodomain and Extra-Terminal (BET) family that recognizes acetylated lysines of histones to modulate the epigenetic code

**BCR-ABL**

an oncogenic fusion of the breakpoint cluster region protein (BCR) and the Abelson (ABL) tyrosine kinase leading to chronic myelogenous leukemia

**Rule of five**

the rule of five is a widely used guideline to predict the likelihood of poor absorption or permeability characteristics for small molecules. It is based on upper limits for physiochemical properties of the compounds, including molecular mass (for which 500 Da is the cut-off)

**Degron**

a peptide segment recognized by the cellular quality control system that influences the degradation rate of the full-length protein

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**Box 1****HSP90 Inhibitors as Broad Inducers of Protein Degradation**

Geldanamycin<sup>184,185</sup>, the first HSP90 inhibitor identified, exhibits low nanomolar activity against the NIH panel of 60 cancer cells lines<sup>186</sup>. However, as a clinical agent, geldanamycin has poor solubility, is readily metabolized and is hepatotoxic<sup>186</sup>. Second-generation compounds tanespimycin (17-AAG, KOS-953, CNF-101; Kosan Biosciences/Bristol-Myers Squibb) and alvespimycin (17-DMAG, KOS1022; Kosan Biosciences/Bristol-Myer Squibb) have improved solubility but remain hepatotoxic<sup>50–52</sup>.

Tanespimycin entered clinical trials in 1999; surprisingly, despite broad protein degradation, tanespimycin demonstrated good tolerability<sup>187</sup>. More recently in 2008, tanespimycin reported promising results from Phase II trials for breast carcinoma (NCT00773344)<sup>188</sup>. However, Phase III trials were not pursued by Bristol-Myer Squibb, likely due to production and patent issues<sup>58</sup>. A third natural HSP90 inhibitor, retaspimycin (IPI-504; Infinity Pharmaceuticals) entered Phase III trials for gastrointestinal stromal tumor (GIST) in 2008 (NCT00688766), which were terminated due to drug-related deaths<sup>189</sup>.

To address the solubility and hepatotoxicity issues of natural inhibitors, synthetic inhibitors were pursued. CNF2024/BIIB021 (Biogen Idec/Conforma Therapeutics)<sup>190</sup> was the first purine-based inhibitor to enter the clinic (NCT00345189) and was found to be relatively effective against GIST in Phase II trials (NCT00618319)<sup>191</sup>; however, Biogen halted its development after discontinuing its oncology pipeline<sup>192</sup>. One of the most potent HSP90 inhibitors, NVP-AUY922 (Vernalis/Norvatis)<sup>193</sup> entered multiple Phase II trials for non-small cell lung cancer (NSCLC), pancreatic cancer, GIST and breast cancer; however, Novartis recently halted all development of the compound. Ganetespib (STA-9090; Synta Pharmaceuticals)<sup>194</sup> also demonstrated some promise in a Phase II for NSCLC<sup>195</sup>; however, the following Phase III trial (NCT01798485) was recently terminated due to futility<sup>196</sup>. Identified through fragment-based drug discovery, onalespib (AT13387; Astex Pharmaceuticals)<sup>197</sup> has entered Phase I/II trials for NSCLC and breast cancer (NCT02535338) after achieving limited efficacy for GIST<sup>198</sup>. Although the development of synthetic inhibitors appears to have solved many of the pharmacological limitations that plagued the natural inhibitors, a clear clinical indication for HSP90 inhibitors has yet to be identified given the diversity of HSP90 client proteins.

**Box 2****Induced Protein Instability for Discovery Biology**

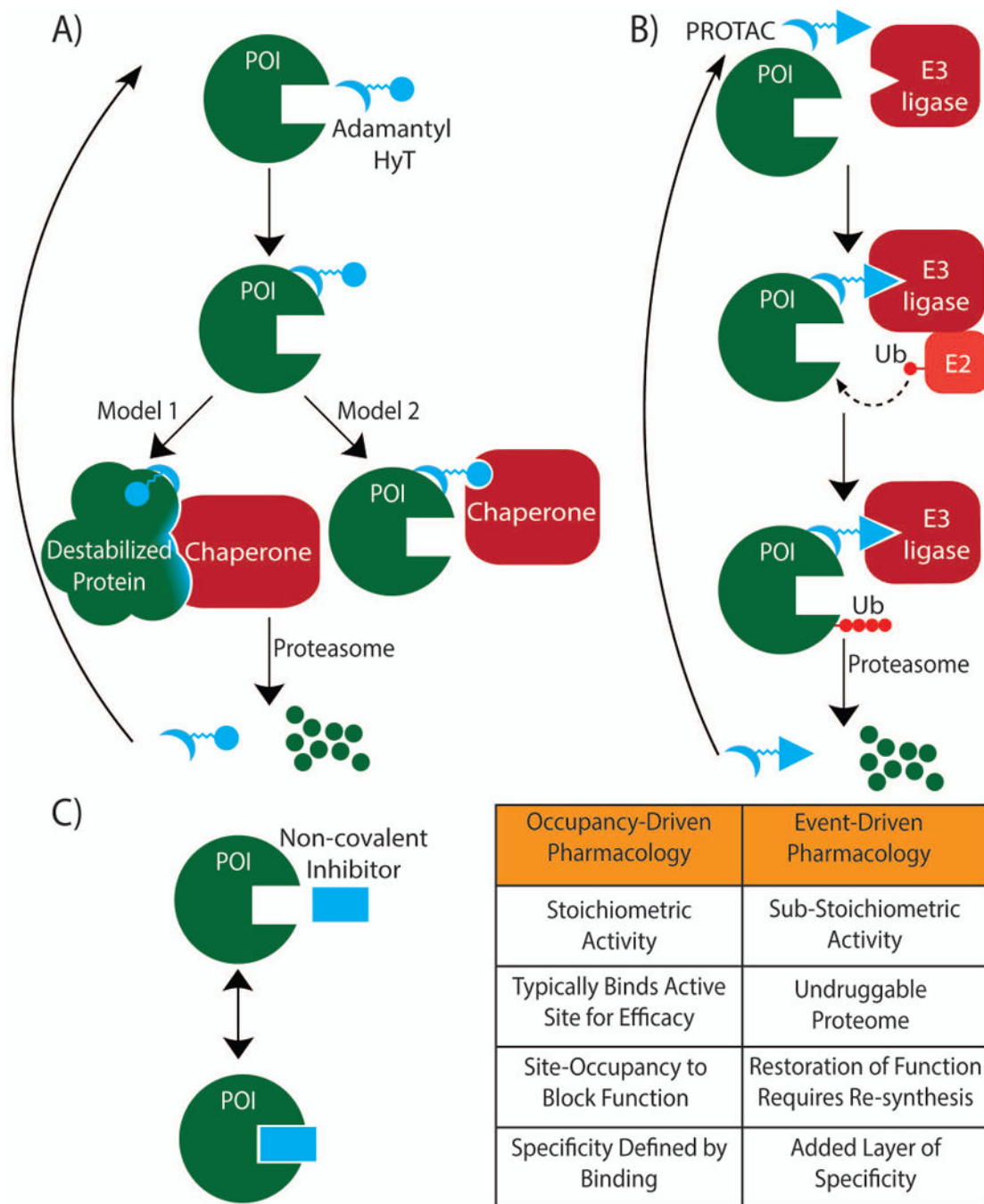
Several excellent reviews discuss the development of small-molecule induced protein instability as chemical biology tools<sup>172,176,199</sup>. However, two recent publications highlight the *application* of these chemical tools for discovery biology. In 2014, the Hydrophobic Tagging (HyT) technology was utilized to uncover an acute resolvable endoplasmic reticulum (ER) unfolded protein response (UPR) pathway that previously evaded analysis using earlier chemical and genetic tools<sup>200</sup>. Cells expressing ER-localized HaloTag fusion proteins were treated with adamantane-based HyT, leading to protein unfolding in the ER lumen. The ER stress induced by the HyT was transient, resolvable and non-apoptotic in nature in comparison to the pro-apoptotic chemical tools, thapsigargin<sup>201</sup> and tunicamycin<sup>202</sup>. This resolvable stress identified the estrogen signaling as a component of a late-stage ER stress resolution pathway.

In 2015, a similar small-molecule induced protein instability technology elucidated a specific nuclear UPR distinct from the cytoplasmic heat shock response<sup>203</sup>. Furthermore, unfolding of a specific protein in the cytoplasm engaged a p53 transcriptional program that was not found in the general heat shock response. Cells primed with the inducible stress were more capable of surviving subsequent unfolded protein stress. These two studies highlight the advantages of chemical tools over genetic methods in terms of specificity, temporal control and dose-response for discovery biology.

**Box 3****The Ubiquitin-Proteasome System (UPS)**

The ubiquitin-proteasome system (UPS) is the primary mechanism for removing misfolded proteins from the cytosol and nucleus. The UPS consists of a coordinated enzymatic cascade that results in the covalent attachment of ubiquitin (a 76-amino-acid protein) to a lysine residue of a targeted protein, eventually leading to the removal of this protein substrate by the proteasome. This cascade begins with an ubiquitin-activating enzyme (E1)<sup>204</sup> creating a reactive thioester on ubiquitin in an ATP-dependent manner. The ubiquitin is then transferred to the ubiquitin-conjugating enzyme (E2)<sup>205</sup>. The last enzyme, ubiquitin ligase enzyme (E3)<sup>183</sup>, specifically binds the target protein and catalyzes the proximity-induced transfer of the activated ubiquitin from the E2 protein to the target protein.

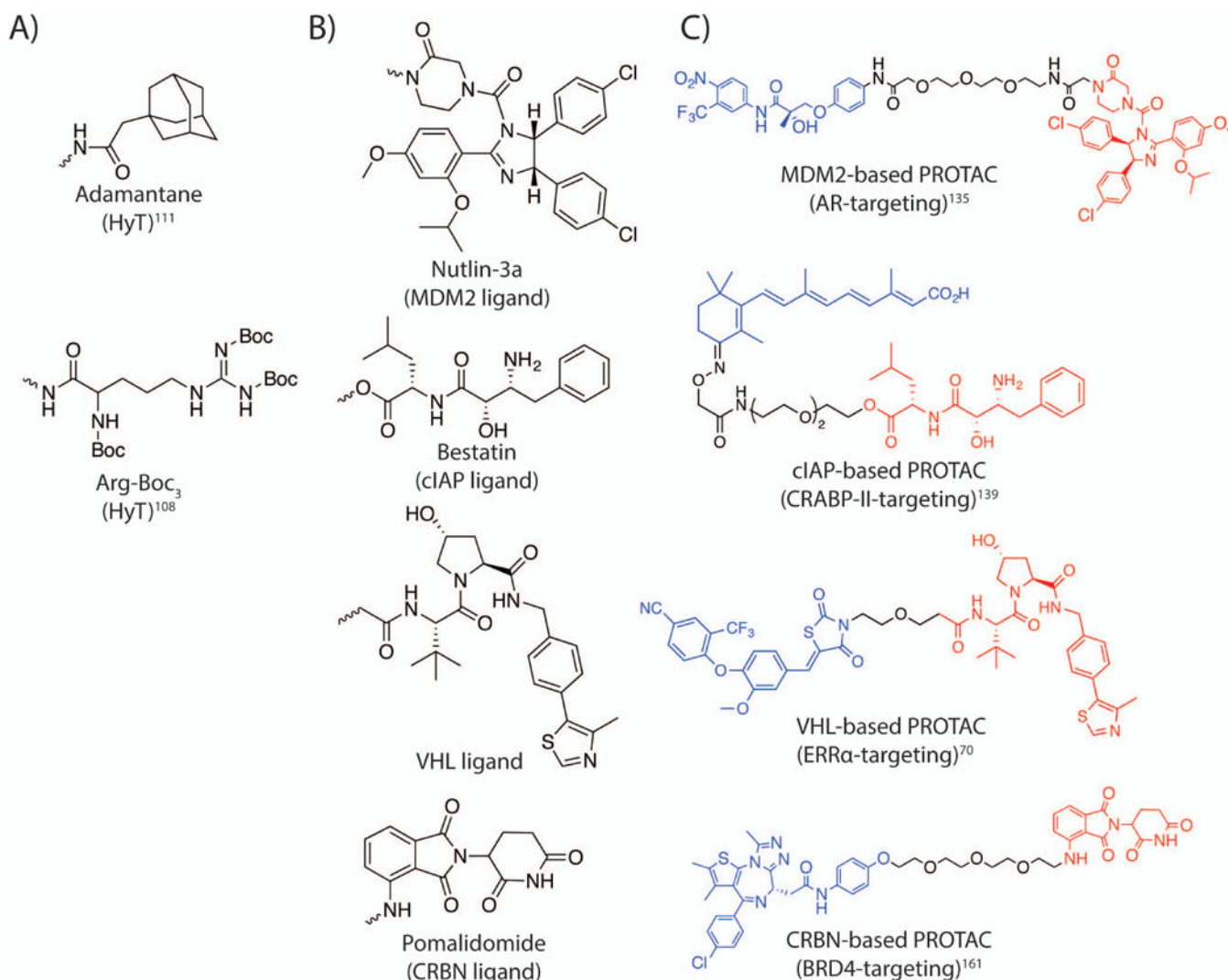
In order to regulate intracellular protein concentrations, E3 ligases recognize a number of degradation signals. For example, the E3 ligase von Hippel-Lindau (VHL) recognizes a core hydroxylated proline residue on hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ )<sup>124</sup> while  $\beta$ -TrCP binds specifically to phosphorylated residues on I $\kappa$ B $\alpha$  and  $\beta$ -catenin<sup>206</sup>. A variety of E3 ligases are utilized in PROTAC technology: CRBN, VHL and  $\beta$ -TrCP act as substrate recognition subunits in multi-subunit Cullin-Ring E3 ligase (CRL) complexes<sup>170,183</sup> while MDM2 forms homo- or hetero-oligomeric E3 ligase complexes for productive ubiquitination<sup>207,208</sup>. Since ubiquitin has seven lysines, substrate-linked ubiquitin can be further modified to produce polyubiquitin chains with unique topographies. Ubiquitin chains linked through Lys-48 have been classically associated with proteasomal degradation<sup>209,210</sup> while other chain types, such as Lys-63 and Lys-11, have been associated with subcellular localization, endocytosis and transcriptional regulation<sup>211,212</sup>. In contrast, a single ubiquitination event (monoubiquitination) has been recognized to play a part in transmembrane protein endocytosis and signaling<sup>213</sup>. PROTAC technology tethers an E3 ligase to a target protein with the goal of inducing polyubiquitination and subsequent removal of the target in the proteasome.



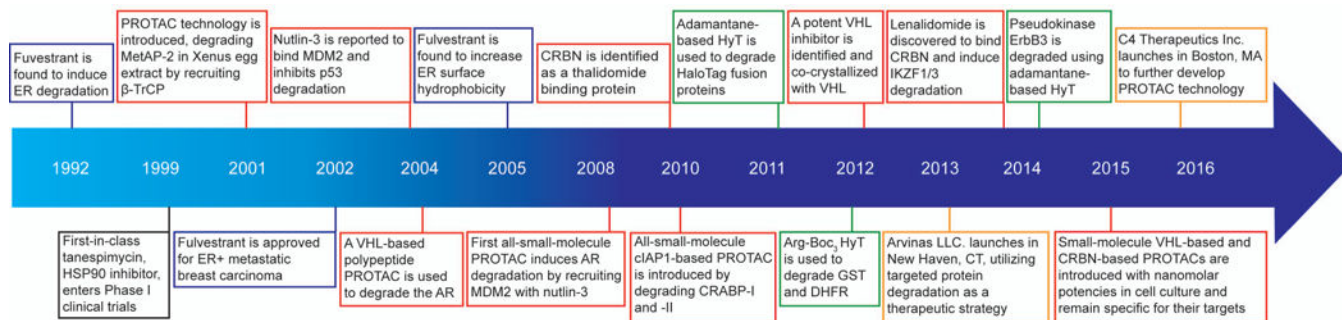
**Figure 1. Mechanism of induced protein degradation technologies**

A) Hydrophobic tagging (HyT) using bifunctional adamantane-based molecules. One proposal for the mode of action is that the hydrophobic tag destabilizes the protein of interest (POI), thereby recruiting endogenous chaperones to the unfolded protein. The protein is then shuttled for degradation to the proteasome. An alternative proposal is that chaperones recognize the hydrophobic tag directly and mediate the proteasomal degradation of the tagged protein. B) PROTAC technology works through active recruitment of an E3 ligase in order to tag proteins for disposal. Bifunctional PROTAC molecules bind to the POI

with one end while the other end binds an E3 ligase to form a ternary complex. The recruited E3 ligase then mediates the transfer of ubiquitin from an E2 enzyme to the POI. The ternary complex dissociates, the ubiquitinated POI is removed by the proteasome and the PROTAC can bind another POI (assuming the interaction with the POI is non-covalent). C) The traditional ‘occupancy-driven pharmacology’ model is predicated upon stoichiometric drug binding to the POI in order to modulate protein function. With non-covalent inhibitors, the drug is capable of dissociating from the POI, leading to restoration of protein function. Furthermore, inhibitors based on ‘occupancy-driven’ pharmacology typically bind to an active site on the POI (although allosteric inhibitors and inhibitors targeting protein–protein interactions are an exception). By contrast, induced protein degradation technologies are ‘event-driven’, and can potentially exploit binding anywhere on the POI in order to achieve degradation.



**Figure 2. Structures of compounds used for induced protein degradation technologies**  
 A) Hydrophobic tags (HyTs) utilized to recruit chaperones to a protein of interest (POI): adamantane and Arg-Boc<sub>3</sub>. B) E3 ligase recruiting ligands employed to hijack E3 ligases to ubiquitinate and degrade POIs. The wavy line illustrates the attachment point utilized in the studies discussed. C) The chemical structures of proteolysis-targeting chimeras (PROTACs) utilizing different E3 ligases to target the POIs for degradation. The POI ligands are shown in blue; the linkers are shown in black; and the E3 ligase recruiting ligands are shown in red.



**Figure 3. Timeline of the induced protein degradation field**

Selected events related to selective estrogen receptor degraders are highlighted in blue; proteolysis-targeting chimeras (PROTAC) technology in red; hydrophobic tagging (HyT) in green; and biotechnology company launches in yellow. MetAP-2, methionine aminopeptidase-2; ER, estrogen receptor; MDM2, mouse double minute 2 homolog; AR, androgen receptor; CRBN; Cereblon; cIAP1, cellular inhibitor of apoptosis protein 1; CRABP, cellular retinoic acid-binding protein; VHL, Von Hippel-Lindau; GST, glutathione-S-transferase; DHFR, dihydrofolate reductase; IKZF, Ikaros family zinc finger; ErbB3, erythroblastosis oncogene B3.

**Table 1**

## Proteins Successfully Targeted for Degradation

Target	Mechanism	Year
Akt	VHL (polypeptidic) <sup>129</sup>	2016
Androgen Receptor	$\beta$ -TrCP (polypeptidic) <sup>122</sup>	2003
	VHL (polypeptidic) <sup>125</sup>	2004
	MDM2 (nutlin-3) <sup>134</sup>	2008
	cIAP (small-molecule) <sup>145</sup>	2011
	HyT (adamantane) <sup>116</sup>	2015
Aryl Hydrocarbon Receptor	VHL (polypeptidic) <sup>214</sup>	2007
BCR-ABL	VHL (small-molecule) <sup>71</sup>	2016
	CRBN (small-molecule) <sup>71</sup>	2016
BET (BRD2/3/4)	VHL (small-molecule) <sup>162,163</sup>	2015
	CRBN (small-molecule) <sup>72,160</sup>	2015
CRABP-I and -II	cIAP (small-molecule) <sup>138</sup>	2010
DHFR	HyT (Arg-Boc <sub>3</sub> ) <sup>107</sup>	2012
ERR $\alpha$	VHL (small-molecule) <sup>70</sup>	2015
ErbB3	HyT (adamantane) <sup>114</sup>	2014
Estrogen Receptor	Fulvestrant <sup>78</sup>	1991
	$\beta$ -TrCP (polypeptidic) <sup>122</sup>	2003
	VHL (polypeptidic) <sup>127</sup>	2008
	cIAP (small-molecule) <sup>145</sup>	2011
FKBP12	CRBN (small-molecule) <sup>72</sup>	2015
FRS2 $\alpha$	VHL (polypeptidic) <sup>131</sup>	2013
GST	HyT (Arg-Boc <sub>3</sub> ) <sup>107</sup>	2012
HaloTag	HyT (adamantane) <sup>110</sup>	2011
	VHL (small-molecule) <sup>177</sup>	2015
	cIAP (small-molecule) <sup>215</sup>	2015
MetAP-2	$\beta$ -TrCP (polypeptidic) <sup>118</sup>	2001
PI3K	VHL (polypeptidic) <sup>131</sup>	2013
RAR	cIAP (small-molecule) <sup>138</sup>	2010
RIPK2	VHL (small-molecule) <sup>70</sup>	2015
TACC3	cIAP (small-molecule) <sup>146</sup>	2014
Tau	VHL (polypeptidic) <sup>130</sup>	2016

BCR-ABL, breakpoint cluster region-Abelson tyrosine kinase fusion protein; BET, bromodomain and extra-terminal; BRD4, bromodomain containing protein 4; CRABP, Cellular retinoic acid-binding protein; DHFR, dihydrofolate reductase; ErbB3, erythroblastosis oncogene B3; ERR $\alpha$ , estrogen-related receptor  $\alpha$ ; FKBP12, FK506 binding protein 12; FRS2 $\alpha$ , fibroblast growth factor receptor substrate 2 $\alpha$ ; GST, glutathione-S-transferase; MetAP-2, methionine aminopeptidase-2; PI3K, phosphatidylinositol-3-kinase; RAR, retinoic acid receptor; RIPK2, receptor-interacting serine-threonine kinase 2; TACC3, transforming, acidic coiled-coil containing protein 3.