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Cancer therapies based on targeted protein degradation — lessons learned with lenalidomide

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Abstract

For decades, anticancer targeted therapies have been designed to inhibit kinases or other enzyme classes and have profoundly benefitted many patients. Novel approaches are required, however, to target transcription factors, scaffolding proteins and other proteins central to cancer biology that typically lack catalytic activity and have remained mostly recalcitrant to drug development. The selective degradation of target proteins is an attractive approach to expand the druggable proteome, and the selective oestrogen receptor degrader fulvestrant served as an early example of this concept. Following a long and tragic history in the clinic, the immunomodulatory imide drug (IMiD) thalidomide was discovered to act by a novel and unexpected mechanism of action: targeting proteins to an E3 ubiquitin ligase for subsequent proteasomal degradation. This discovery has paralleled and directly catalyzed myriad breakthroughs in drug development, leading to the rapid maturation of generalizable chemical platforms for the targeted degradation of previously undruggable proteins. Decades of clinical experience have established frontline roles for thalidomide analogues in the treatment of haematological malignancies. With a new generation of ‘degrader’ drugs currently in development, this experience provides crucial insights into class-wide features of degraders, including a unique pharmacology, mechanisms of resistance and emerging therapeutic opportunities. Herein, we review these past experiences and discuss their application in the clinical development of novel degrader therapies.

Introduction

Thalidomide was introduced to the clinic in the late 1950s as a sedative and antiemetic and was marketed as a treatment for morning sickness in pregnant women[1]. Tragically, in utero exposure to thalidomide caused an embryopathy characterized by foreshortened limb anomalies, known as phocomelia, in thousands of children worldwide[2,3]. In the early 1960s, Frances Kelsey at the FDA played a crucial part in preventing the marketing approval

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and thus the widespread use of thalidomide in the USA until the association of this drug with teratogenicity was publically exposed in 1962[4] and the agency's annual drug safety excellence award is named in her honour. Although broadly withdrawn from clinical use, thalidomide remained on the market for decades as a treatment for erythema nodosum leprosum (an immune-mediated complication of leprosy)[5,6]. In the 1990s, thalidomide was reconsidered as an anticancer agent[7]. Clinical testing revealed the efficacy and tolerability of thalidomide in patients with multiple myeloma (MM)[8–10], and this success spurred the development of chemically similar analogues, first lenalidomide and then pomalidomide, as cancer therapies.

In 2013, thalidomide analogues were discovered to exert their therapeutic effects by acting as a molecular glue to recruit disease-relevant neosubstrate proteins to an E3 ubiquitin ligase, resulting in neosubstrate ubiquitination and, ultimately, proteasomal degradation[11–13]. This molecular mechanism of action and the clinical experience with thalidomide analogues highlight unique features of 'degrader' therapeutics, including a distinct pharmacology, mechanisms of resistance and the integrated effect of degrading multiple substrates on efficacy and toxicity profiles.

Herein, we review the wide and expanding use of thalidomide analogues in the treatment of multiple cancers, including MM, non-Hodgkin lymphoma (NHL) and myelodysplastic syndrome (MDS). With novel degrader drugs poised to expand the druggable proteome, we also outline the lessons learned from thalidomide analogues — particularly lenalidomide — that serve as an essential guide to the clinical development of targeted protein degradation platforms.

Thalidomide analogues

Mechanism of action and chemistry

Thalidomide and its derivatives were used clinically for decades prior to elucidation of the molecular basis of their activity. In 2010, thalidomide was found to directly bind to the protein cereblon (CRBN)[14], which serves as a substrate receptor component of the cullin-RING E3 ubiquitin ligase complex 4 (CRL4) comprising cullin-4 (CUL4), DNA damage-binding protein 1 (DDB1) and E3 ubiquitin-protein ligase RBX1 (FIG. 1a,b and BOX 1). Subsequently, our group and others concurrently discovered that thalidomide and its analogues do not inhibit the enzymatic activity of $CRL4^{CRBN}$, but instead confer neomorphic activity on CRBN to mediate the selective ubiquitination and resultant proteasomal degradation of the transcription factors Ikaros family zinc finger protein 1 (IKZF1) and IKZF3 (Aiolos) in MM cells and T cells[11–13]. Similarly, in del(5q) MDS cells, lenalidomide mediates the CRBN-dependent ubiquitination and degradation of casein kinase I isoform α (CK1α)[15]. IKZF1, IKZF3 and CK1α are required for MM or MDS cell survival (BOX 2), thereby establishing selective degradation of disease-relevant proteins as the unexpected mechanism underlying the anticancer effects of thalidomide analogues[16].

The distinct activity of each thalidomide derivative reflects differences in the specific set of proteins that are targeted for degradation by each drug. All pharmacologically active derivatives of thalidomide contain a glutarimide ring that directly binds within a conserved

pocket on the surface of CRBN[17] (FIG. 1c). Each thalidomide analogue is distinguished by variations on a phthaloyl ring moiety that dictate binding to a limited number of neosubstrate proteins. Many of the degraded neosubstrates, such as zinc finger transcription factors, do not contain ligand-binding sites that could be targeted using traditional therapeutic agents. Developed from the lead compound thalidomide, lenalidomide and pomalidomide are more potent degraders of IKZF1 and IKZF3, and all of these agents have distinct patterns of neosubstrate degradation[18]. Thus, the activity and toxicity profiles of each thalidomide analogue are determined by their unique polypharmacology: the combinatorial degradation of multiple neosubstrates by the ubiquitin-proteasomal system (UPS; BOX 1 and BOX 2).

Thalidomide analogues in cancer therapy

Thalidomide, lenalidomide and pomalidomide are currently FDA approved in a variety of clinical settings, including MM, NHL and MDS as well as Kaposi sarcoma (KS)[19–22]. Next generation analogues that may overcome therapeutic resistance or that have activity in new disease indications are in clinical development.

Lenalidomide in MM.—In 2002, a phase I trial of lenalidomide monotherapy revealed promising signs of efficacy in patients with relapsed and/or refractory (R/R) MM, about 50% of whom had previously received thalidomide[23]. Lenalidomide doses of up to 50 mg daily were tested and no dose-limiting toxicities were observed, although all patients treated at this dose developed severe myelosuppression after 1–2 cycles and thus 25 mg daily was suggested as the maximum tolerated dose[23]Data from a subsequent phase II study established 30 mg daily as a safe and active dose of lenalidomide in patients with R/R MM, with a single-agent objective response rate (ORR) of 25%; a further 29% of patients responded following the addition of dexamethasone[19]Again, responses were seen even in patients who had disease progression on prior thalidomide therapy and the combination of lenalidomide and dexamethasone in patients with one or more prior lines of therapy was FDA approved in 2006[24,25].

In patients with newly diagnosed MM, ORRs are even higher, with up to 90% of patients responding to the combination of lenalidomide and dexamethasone[26]. A randomized study comparing lenalidomide and dexamethasone to melphalan, thalidomide and prednisone in newly diagnosed patients demonstrated superior 4-year OS (59% versus 51%; HR 0.78, p=0.02) and led to FDA approval in 2015[27]. Subsequent research has focused on demonstrating the safety and efficacy of lenalidomide and dexamethasone in combination with various other anti-myeloma agents, leading to the establishment of a variety of triplet and quadruplet induction therapy regimens as the current standard of care for patients with newly diagnosed MM. In this population, lenalidomide and dexamethasone are typically combined with a proteasome inhibitor, such as bortezomib[28], and/or the CD38-targeting monoclonal antibody daratumumab[29]. For older patients and/or those unfit for autologous transplantation, the preferred induction approach remains lenalidomide in combination with dexamethasone with or without daratumumab, regimens that are effective and well tolerated in this patient population[27,29].

Maintenance treatment with lenalidomide.—Maintenance lenalidomide following induction therapy with or without autologous haematopoietic stem cell transplantation (auto-HSCT) is also a standard of care for patients with MM[30,31]. Such maintenance therapy was first attempted with thalidomide, resulting in an increase in complete remission (CR) rates (62% versus 43% without maintenance; $P<0.001$) and an event-free survival (EFS) benefit (56% versus 44% at 5 years; $P=0.01$), but no overall survival (OS) benefit (5-year OS ~65% in both groups; $P=0.90$)[32]. By contrast, lenalidomide has demonstrated a clear OS advantage (~88% versus 80% at 3 years) with a hazard ratio for death of approximately 0.60[30,31,33,34]. Patients with high-risk cytogenetic features might be the only exception, although they do derive a progression-free survival (PFS) benefit[34,35]. In this subset of patients, maintenance therapy with proteasome inhibitors alone or in combination with lenalidomide seems to be more effective than single-agent lenalidomide[34,35]. The most worrisome adverse effect observed in studies of lenalidomide maintenance therapy is an up to six-fold increase in the risk of second primary malignancies, in particular, MDS and acute myeloid leukaemia (AML): 5.3% versus 0.8% with placebo or observation[34]. Efforts to limit the risk of this toxicity have fueled debate about whether maintenance should continue until disease progression or be limited to 1–2 years. Meta-analyses suggest that indefinite maintenance therapy is superior in terms of survival outcomes[36], although the optimal maintenance duration is a subject of an ongoing randomized trial (NCT01863550).

Current roles of thalidomide analogues in R/R MM.—Patients who have disease progression while not on treatment with lenalidomide can often be re-treated effectively with this agent[37,38], sometimes multiple times, and typically in combination with proteasome inhibitors such as carfilzomib[39] and ixazomib[40] or with daratumumab[41]. The success of lenalidomide led to the development of pomalidomide, which has demonstrated efficacy in around a third of patients with lenalidomide-refractory MM[42–44]. **P**omalidomide is most often used in combination with dexamethasone, either alone or as part of triplet regimens with a variety of other anti-myeloma agents, including bortezomib[45], daratumumab[46] or elotuzumab[47].

Synergy with other anti-myeloma agents.—Response rates to single-agent lenalidomide have consistently been surpassed by those achieved with doublet or triplet combinations, which has been taken as evidence for synergistic activity of this agent with other anti-myeloma agents[48]. Data from a number of in vitro studies support the hypothesis that thalidomide analogues synergize with dexamethasone[49,50], proteasome inhibitors[51,52], histone deacetylase inhibitors[53,54] and a variety of other agents; however, whether such combinations have additive or synergistic clinical activity remains unclear.

The two drugs most commonly combined with thalidomide analogues, bortezomib and carfilzomib, are proteasome inhibitors. The discovery that thalidomide analogues induce proteasomal degradation of their target proteins is therefore surprising, given that proteasome inhibitors antagonize lenalidomide-induced protein degradation[11,12,55]. Indeed, the pharmacokinetic characteristics of these drug combinations emphasize independent actions, in keeping with the hypothesis that independent drug effects against differentially sensitive cell populations explain the efficacy of most combination cancer

therapies[56]. Specifically, whereas lenalidomide is dosed daily, bortezomib and carfilzomib are administered once or twice a week and are rapidly metabolized[57], resulting in a background of prolonged selective, lenalidomide-induced protein degradation punctuated by short periods of proteasome inhibition. Notably, targeted protein degraders have the pharmacological advantage of extended target suppression until protein re-synthesis (FIG. 2)[58] which might negate the effects of transient disruption of protein degradation and contribute to the efficacy of the counterintuitive combination of targeted protein degraders and proteasome inhibitors.

Immunomodulatory effects of thalidomide analogues.—A constellation of immunomodulatory effects resulting from IKZF1 and IKZF3 degradation have been attributed to thalidomide analogues[18] and are proposed to contribute to the activity of these agents. These effects include alterations of immune synapse formation[59], mimicry of interferon signaling in large B cell lymphoma cells[60], enhanced T cell co-stimulation[61], cytokine release and function, as well as inhibition of TNF secretion by stimulated human monocytes[62]. Indeed, early thalidomide analogues were developed for optimized TNF inhibition, thus leading to their classification as IMiDs[18]. These immunomodulatory effects have been hypothesized to have a major role in the therapeutic activity of thalidomide analogues[18]; however, in no malignancies are these agents active without evidence of direct cytotoxicity to the cancer cells. Notably, no relevant clinical activity has been observed in trials encompassing a variety of solid tumour types, including immunotherapy-sensitive tumours such as renal cell carcinoma[63] and melanoma[64]. Thus, despite clear effects on immune cell function and clinical activity against erythema nodosum leprosum and KS[22], no strong data support a primarily immunomodulatory mechanism of antitumour action of thalidomide analogues. This scenario might be explained by both immunostimulatory and immunosuppressive effects of these agents, which might balance each other in the setting of solid tumour biology.

Synergistic immunomodulatory mechanisms have also been proposed for combinations of thalidomide analogues with monoclonal antibodies used in the treatment of MM. The anti-SLAMF7 antibody elotuzumab has no anti-myeloma activity as monotherapy[65], but the addition of this agent to lenalidomide or pomalidomide and dexamethasone improved ORRs, PFS and OS in patients with R/R MM[47,66,67]. Elotuzumab has been proposed to function at least partially through upregulation of IL-2 production by T cells and stimulation of natural killer (NK) cell-mediated antibody-dependent cellular cytotoxicity (ADCC)[68–70]. Lenalidomide also enhances T cell and NK cell functions, probably via degradation of IKZF3, raising the possibility of mechanistic synergy between these two agents. Similar mechanisms of synergy between lenalidomide and daratumumab have been proposed[71]; however, the combination of daratumumab with either lenalidomide or bortezomib results in similar ORRs[41,72], suggesting that potential mechanistic synergy does not translate into substantial clinical advantages.

Lenalidomide in B cell lymphomas and leukaemias.—In addition to MM, other mature B cell malignancies seem to depend upon IKZF1 and IKZF3 for maintenance of cancer cell phenotype and survival. In relapsed mantle cell lymphoma single agent

lenalidomide produced a significant increase in median PFS (8.7 versus 5.2 months; HR 0.61; p=0.004) compared to provider's choice chemotherapy in patients with relapsed or refractory disease, leading to its FDA approval in 2013[73,74]. Lenalidomide has been combined with the anti-CD20 antibody rituximab in the so-called 'R^2' regimen, a chemotherapy-free treatment, which was approved by FDA in 2019 for the treatment of R/R marginal zone lymphoma (MZL) or follicular lymphoma (FL)[75,76] based on high ORRs (65% in MZL and up to 80% in FL versus 55% and 44%, respectively with placebo plus rituximab) and a substantial improvement in PFS (39.4 months versus 14.1 months; HR 0.46; 95% CI 0.34–0.62; P <0.0001). Frontline treatment with this chemotherapy-free regimen has also produced promising results in patients with FL, with CR and 3-year PFS rates similar to those achieved with rituximab plus chemotherapy (48% versus 53% and 77% versus 78%, respectively) but with a lower incidence of grade 3–4 neutropenia (32% versus 50%)[75]. In patients with treatment-naive MCL, the R^2 regimen has produced an ORR of 92%, a CR rate of 64%, and 2-year PFS and OS of 85% and 97%, respectively[77]. In addition, patients with R/R chronic lymphocytic leukaemia (CLL) receiving R^2 have a ORR of >65%[78], and lenalidomide monotherapy triples PFS when used as a maintenance regimen following standard second-line chemotherapy (median PFS 33.9 months versus 9.2 months with placebo; HR 0.40, 95% CI 0.29–0.55; P <0.0001)[79]. Lenalidomide has also safely been combined with other anti-CD20 antibodies, such as obinutuzumab, in the treatment of B cell lymphomas (for example, R/R FL)[80]. Nevertheless, adoption of lenalidomide for the treatment of indolent lymphomas and CLL has been limited, largely owing to the availability of other highly active therapies for these diseases.

Lenalidomide has been less successful in the treatment of patients with aggressive lymphomas, such as diffuse large B cell lymphoma (DLBCL). Data from early phase clinical trials and in vitro models indicate that this agent might be active in patients with activated B cell-like DLBCL[81,82]; however, a phase III study showed no benefit of adding lenalidomide to chemoimmunotherapy with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) in newly diagnosed patients with this disease subtype[83]. Lenalidomide maintenance might have a role in patients with R/R DLBCL who are not fit for potentially curative treatments[84], such as auto-HSCT or chimeric antigen receptor (CAR) T cell therapies, and more potent, next-generation thalidomide analogues (such as avadomide) might have greater activity in this disease[85]. Lenalidomide is unlikely to have utility in patients with early B cell malignancies, such as pre-B cell acute lymphoblastic leukaemia (ALL), considering that deletions and loss of function mutations in *IKZF1* promote the development of these cancers[86,87].

Lenalidomide in T cell lymphomas.—In mice, both Ikzf1 and Ikzf3 are required for normal T cell development and maintenance of mature T cell populations[88,89], which provides a rationale for targeting these proteins using lenalidomide in patients with T cell malignancies. In early phase studies, single-agent lenalidomide typically induced responses in ~30% (ORRs of 22–42%) of patients with R/R peripheral T cell lymphomas[90–92], mycosis fungoides or Sézary syndrome[93]. Clinical studies combining lenalidomide with conventional CHOP chemotherapy (NCT01553786), antibody–drug conjugates (such as brentuximab vedotin; NCT03409432) or other, novel agents (such as histone deacetylase

inhibitors [NCT02232516] and immune-checkpoint inhibitors [NCT04038411]) in these diseases are ongoing.

Lenalidomide in myeloid malignancies.—Lenalidomide was first approved by the FDA in 2005 for the treatment of transfusion-dependent anaemia resulting from MDS with del(5q), an indication in which it is associated with ORRs of 76–83%, with 45–50% of patients also having a cytogenetic CR[21,94]. Del(5q) is the most common cytogenetic abnormality in MDS; patients with this genetic aberration often present with a consistent clinical phenotype, termed the '5q− syndrome', which is more common in women than in men and has a relatively indolent course characterized by hypoplastic anaemia[95]. Lenalidomide has activity in this disease by inducing degradation of CK1α, the protein product of the haploinsufficient gene *CSNK1A1* that is located on the common deleted region of chromosome 5q, leading to activation of p53 and subsequent apoptosis[15,96]. Accordingly, mutations in *TP53* lead to resistance to lenalidomide in patients with MDS[97,98] (FIG. 3). Co-occurring *TP53* mutations probably also explain why patients with del(5q) in combination with a complex karyotype or in the setting of AML do not respond to lenalidomide[99]. Of note, IKZF1 is expressed throughout haematopoiesis and regulates myeloid cell development and function[100]. Thus, altered regulation of gene expression as a result of IKZF1 degradation might also contribute to responsiveness of myeloid malignancies to lenalidomide[97,101].

Lenalidomide monotherapy has produced CR rates of almost 20% in the settings of both newly diagnosed and R/R AML[102,103]. In patients with R/R AML, the combination of high-dose lenalidomide (50 mg daily) with mitoxantrone, etoposide and cytarabine (MEC) resulted in a CR or CR with incomplete haematological recovery (CR/CRi) rate of 41%[104], which is considerably higher than expected with MEC alone (17–28%) based on data from historical cohorts[105,106]. A phase II study evaluating lenalidomide with MEC in this patient population is ongoing (NCT03118466). Lenalidomide provides limited benefit when combined with azacytidine in patients with newly diagnosed AML[107]; however, the results are more encouraging when this combination is used following disease relapse after allogeneic HSCT, with almost 50% of patients having a major clinical response, possibly owing to stimulation of graft versus leukaemia immune responses[108]. Lenalidomide also has activity in patients with myelofibrosis, with responses occurring in up to a third of patients when used in combination with prednisone, some of which have lasted as long as 9 years[109].

Kaposi sarcoma.—In 2020, pomalidomide was approved by the FDA for the treatment of KS, an endothelial cell-derived solid tumour caused by human herpesvirus 8 (HHV8) infection[110], based on data from a phase I/II trial that demonstrated an ORR of 73% in patients with newly diagnosed KS[22]. The mechanistic basis underlying the efficacy of pomalidomide in this disease is not well characterized. Interestingly, two other HHV8-associated neoplasms, primary effusion lymphoma[111] and Castleman disease[112], have also been shown to be responsive to treatment with thalidomide analogues.

Next-generation thalidomide analogues

New thalidomide analogues are being developed both to overcome resistance by inducing deeper degradation of known targets and also to degrade new targets. These agents include avadomide, iberdomide, CC-92480 and CC-90009, which are rapidly making their way toward the clinic (TABLE 1).

Avadomide.—Avadomide (previously known as CC-122) is chemically similar to pomalidomide (FIG. 1c), and accordingly, has similar substrate specificity and potency of degradation[113]. Avadomide has been tested in clinical trials involving patients with R/R DLBCL, demonstrating promising single-agent activity (ORR 29% and CR rate 11%)[85]. This agent has also been tested in patients with a variety of solid tumours; although no objective responses were seen in an initial report[114], five of six patients with brain tumours had stable disease for at least 6 months. Additional clinical studies of avadomide are ongoing in other solid tumour settings, including advanced-stage melanoma and hepatocellular carcinoma, and in various combinations (TABLE 1).

Iberdomide.—Iberdomide (CC-220) is a thalidomide derivative with an increased binding affinity for CRBN and greater potency than pomalidomide in multiple myeloma cell lines, with activity observed against some pomalidomide-resistant cell lines[115,116]. Iberdomide has a distinct chemical structure compared with other thalidomide analogues (FIG. 1c) and might have a broader target specificity[117]. This agent has promising activity in combination with dexamethasone in patients with R/R MM, with an ORR of 31% and a disease-control rate of 88%[118], and its use in combination with other agents is being explored in this disease (TABLE 1). Iberdomide has also shown efficacy in models of systemic lupus erythematosus in vitro where treatment can decrease production of auto-antibodies, and trials are ongoing in this disease setting[119].

CC-92480.—CC-92480 is currently being tested in patients with MM (TABLE 1). This agent has activity across a variety of MM cell lines resistant to lenalidomide and pomalidomide, even those with low levels of CRBN expression, which has been proposed as a mechanism of drug resistance (FIG. 3)[120].

CC-90009.—CC-90009 differs from the other thalidomide analogues in that it targets the substrates G1 to S phase transition protein 1 homologue (GSPT1) and GSPT2, which are also known as eukaryotic peptide chain release factor GTP-binding subunits ERF3A and ERF3B, respectively, reflecting their roles as essential regulators of translation termination[113,121]. CC-90009 has been tested in patients with R/R MDS or AML and demonstrated promising single-agent activity: a total of 49 were treated, three of whom had a CRi or better with a morphological leukaemia-free state achieved in one additional patient[122]. Toxicities included manageable grade 3–4 hypotension (in 13% of patients) and on-target, drug-induced hypocalcaemia (in 22%)[122]. CC-90009 is currently being investigated as a single agent as well as in combination with hypomethylating agents, venetoclax or the FLT3 inhibitor gilteritinib in patients with MDS and AML (TABLE 1).

Clinical toxicities

Effects on haematopoiesis.—Thalidomide analogues often lead to peripheral blood cytopenias, most commonly neutropenia (28% grade 3/4) and thrombocytopenia (8% grade 3/4)[27]. Thalidomide itself is an exception, given that it rarely causes thrombocytopenia[10], making it a preferred option for some patients. Aside from neutropenia, data suggest that lenalidomide use is associated with limited immune dysfunction beyond that associated with the underlying malignancies. Both IKZF1 and CK1α have important roles in haematopoietic stem and progenitor cell (HSPC) function and differentiation, although the precise effects of thalidomide analogues on haematopoiesis have not been well studied. Lenalidomide therapy decreases the efficiency of growth factor-stimulated stem cell mobilization and collection for auto-HSCT, thus limiting its use prior to stem cell harvesting[123,124]. This issue can often be overcome using high-dose cyclophosphamide or the CXCR4 antagonist plerixafor[125].

Second primary malignancies.—Long-term use of lenalidomide, especially following high-dose melphalan and autologous stem cell rescue, has been associated with an increased incidence of second solid tumours and haematopoietic malignancies, primarily MDS and AML[34,126]. Effects on HSPC function probably contribute to the increased risk of secondary myeloid malignancies, although these effects might also underlie the activity of lenalidomide in these same malignancies. Solid tumours associated with lenalidomide use are most commonly non-melanoma skin cancers, which can usually be managed conservatively[127].

Thrombosis.—Thalidomide analogues are associated with an increased risk of primarily venous thromboembolism[128–130] (OR 3.51; $p<0.001$), although arterial events can also rarely occur[131]. Concomitant use of anti-platelet or anti-thrombotic agents is, therefore, a routine component of clinical care[132]. Whether the thrombotic phenotype occurs through on-target protein degradation remains unclear. The increased risk seen with concomitant use of corticosteroids, such as dexamethasone, and chemotherapeutic agents, such as anthracyclines, raises the possibility of thrombosis secondary to endothelial cell injury[133].

Teratogenicity.—The potential for teratogenic effects of analogues other than thalidomide has not been assessed. Nevertheless, this adverse effect is assumed to be common to all thalidomide analogues, and appropriate precautions are recommended for all drugs of this class.

Other adverse effects.—Other toxicities commonly associated with thalidomide analogues include rashes, fatigue and diarrhoea. The rash associated with lenalidomide use occurs in up to 30% of patients, is unpredictable and can occur at any time during treatment[134]. Moreover, this adverse effect can range in severity, from mild erythema to toxic epidermal necrolysis, but is often easily managed with corticosteroids and drug cessation[135]. Mechanistically, the rash might be related to immune activation. Most patients can be rechallenged with lenalidomide without recurrence of rash; in rare cases in which rashes recur, management with desensitization strategies involving gradual titration from low starting doses has been effective[136]. Fatigue is often a dose-limiting toxicity of thalidomide analogues, but its mechanism is unclear. Finally, long-term use of lenalidomide

can cause diarrhoea related to fat malabsorption, which can be effectively treated with the bile acid-binder colestipol[137].

The distinct pharmacology of degraders

Drugs that induce targeted protein degradation, such as thalidomide analogues, have a number of unique pharmacological features in comparison with conventional enzyme inhibitors[58,138–140]. These features can in turn provide unique clinically beneficial properties.

Event-driven vs occupancy-driven action

Classical inhibitors – covalent or non-covalent - must stoichiometrically occupy the binding site on the target enzyme, and therefore, sustained target inhibition requires maintenance of an effective drug concentration (FIG. 2a). By contrast to this occupancy-driven action, degrader pharmacology is event-driven; transient target–drug–ligase binding events mediate irreversible target degradation[138,139] (FIG. 2b). Moreover, a single degrader molecule can cycle through multiple binding and degradation events, enabling sub-stoichiometric activity[141]. Effective target protein suppression can be prolonged and is reversed only following protein re-synthesis, thereby uncoupling pharmacokinetic and pharmarcodynamic relationships. Furthermore, any noncatalytic, scaffolding functions of degraded proteins are also abolished, potentially broadening anti-cancer effects beyond enzymatic inhibitors that typically spare scaffolding functions (FIG. 2a).

Resistance mechanisms

Downregulation of E3 ligase activity.—CRBN expression is required for the anti-myeloma activity of lenalidomide[142]. In genome-wide genetic screens of MM cells and lymphoma cells, disruption of $CRL4^{CRBN}$ components and the associated cullin-RING ligase machinery was found to result in resistance to lenalidomide[143–145]. Mutations in *CRBN*, including missense mutations in the thalidomide analogue binding domain, are associated with progressive thalidomide analogue exposure, and ultimately occur in up to a third of patients with pomalidomide-refractory disease[146–148] (FIG. 3a). CRBN expression levels in patient samples has been positively associated with sensitivity to thalidomide analogues in some studies[149–154], but not in others[155,156]. Future studies in this area will benefit from the use of standardized, direct measurements of CRBN expression[156]. Data from our group[113] and others[157] suggest that overexpression of CRBN can sensitize previously resistant MM cell lines to thalidomide analogues. Therefore, efforts to modulate CRBN expression might yield therapeutics that enhance the activity of thalidomide analogues.

Targeted protein degradation is influenced by multiple layers of competition for UPS components. In a study using a quantitative multiplex mass spectrometry-based assay to quantitate multiple substrate proteins, a stereotyped order of polysubstrate degradation was identified for various thalidomide analogues[113], suggesting that substrates with higher affinity and/or greater abundance can outcompete others. Indeed, induced overexpression of one substrate reduced the degradation of other substrates and induced drug resistance

in cellular models[113]. Therefore, cancer cells might develop resistance by upregulating the expression of unrelated substrates, thereby stabilizing substrates critical for oncogenesis, or alternatively upregulating critical targets such as IKZF1/3 directly[158] (FIG. 3a); however, quantitative proteomic datasets from clinical specimens are required to confirm these hypothesis and are currently lacking. Beyond substrate competition for CRBN, CRBN itself competes with other substrate receptors for access to limiting concentrations of CRL4[159]. Thus, the expression profile of the entire set of substrates, substrate receptors and the CRL4 backbone probably determines the efficiency of drug-induced degradation (FIG. 3a), which might vary not only between different cell types and states but also between tumour subclones. Given this emerging portrait of the highly complex epigenetic determinants of degrader pharmacodynamics, efforts to develop biomarkers predictive of degrader activity remain in their infancy.

Escape downstream of neosubstrate protein degradation.—Del(5q) MDS HSPCs are typically sensitive to lenalidomide-mediated depletion of CK1α, which activates p53-dependent cell death[15,96]. Accordingly, loss of p53 leads to resistance of mouse HSPCs to Ck1α targeting with either lenalidomide, a direct inhibitor or genetic knockdown (FIG. 3b)[15,160]. Consistent with these experimental findings, resistance to lenalidomide and disease progression are associated with an increased frequency of *TP53* mutations in patients with del(5q) MDS[98,161,162]. Similarly, lenalidomide-mediated depletion of IKZF1 in del(5q) MDS cells drives RUNX1 upregulation and megakaryocyte differentiation, and sequencing of matched clinical specimens has identified *RUNX1* mutations that are associated with lenalidomide resistance[97]. Analogous mechanisms conferring resistance to degrader-mediated depletion of IKZF1 and IKZF3 in lymphoid malignancies are an active area of research.

Clinical pharmacodynamic monitoring

The detailed investigation of thalidomide analogue function in patients has been limited by the semi-quantitative nature of protein detection methodologies and limited deployment of such assays to clinical settings. In the past few years, our group and others have used targeted mass spectrometry to perform detailed quantitative analyses of thalidomide analogue neosubstrate proteins and the UPS[113,159]. These approaches have the advantages of enabling detection of target proteins from small sample inputs and rapid processing times that in turn enable high-resolution kinetic analyses. Such analyses have revealed the importance of competition among both substrates and E3 ubiquitin ligase complexes in determining neosubstrate abundance during thalidomide analogue therapy. Targeted proteomic assays have been translated to the clinical laboratory setting[163,164], and such assays present a potentially appealing approach for the development of clinical biomarkers of activity and resistance to degrader drugs.

Emerging opportunities with degraders

Over the decades, the story of thalidomide has traversed phases of tragic teratogenicity, followed by renewed optimism as an anticancer agent and the subsequent discovery of an unexpected mechanism of action. In parallel to the discovery that thalidomide

analogues act as ‘molecular glue’ degraders, heterobifunctional proteolysis targeting chimeras (PROTACs), which are based on a similar principle and consist of an E3 ubiquitin ligase-recruiting element linked to a target-binding moiety, have advanced from being proof-of-concept molecules to investigational anticancer drugs (BOX 3)[140]. The next phases of drug discovery focused on targeted protein degradation will benefit from mechanism-guided drug design and clinical development; next-generation degraders will be designed to more efficiently and selectively target novel disease-relevant neosubstrate proteins, and biomarkers of activity and resistance will be used to inform clinical decision-making. More broadly, lenalidomide and the other approved thalidomide analogues provide clinical validation for the therapeutic concept of targeted protein degradation, which has increased both interest and investment in a range of related technologies. In the following sections, we will highlight new paths to degrader therapeutics with potential clinical utility, as well as the possible use of degraders in synthetic biology approaches to controlling adoptive cell-based immunotherapies.

Identification of thalidomide analogue neosubstrates

The full complement of proteins targeted for degradation by thalidomide analogues remains to be elucidated. Most known neosubstrate proteins have been identified through proteomic analyses of different cell lineages[11,15,165,166]. Each target protein has been found to contain a structural degron composed of a β-hairpin with a pinnacle glycine motif, most often in the form of a Cys2-His2 (C2H2) zinc finger domain, that binds to a composite thalidomide analogue–CRBN surface[17,117,121,167]. The approximately 700 C2H2 zinc finger-containing transcription factors constitute the largest class of transcription factors in the human genome[168], but have previously not been amenable to drug development. Functional genomics approaches coupled with computational docking and in vitro binding analyses have been used to interrogate all human C2H2 zinc finger domains in order to define the zinc finger ‘degrome’ of thalidomide analogues[117]. These studies resulted in the identification of 11 individual zinc finger domains and six full-length zinc finger-containing that are subject to drug-mediated degradation, as well as a larger number of zinc finger domains that are capable of binding to pomalidomide-engaged CRBN in vitro[117]. Notably, different thalidomide analogues mediate the degradation of overlapping but distinct sets of zinc finger domains[117], implying that further chemical diversification will provide unique opportunities to target additional members of this important class of once-undruggable proteins. Crystallographic and saturation mutagenesis studies have identified the amino acid residues of particular zinc finger domains that are crucial for their drug-induced recruitment to $CRL4^{CRBN}$ and subsequent degradation[117]; such insights might facilitate further cycles of systematic neosubstrate protein discovery.

Endogenous substrates of CRBN

CRBN was first described as a gene that, when mutated, causes a monogenetic intellectual disability[169]. A number of endogenous CRBN-interacting proteins have been reported, including MEIS2, AMPK, MCT1, and GS[17,170–172], some of which compete with thalidomide analogue-targeted neosubstrates for $CRL4^{CRBN}$-dependent ubiquitination and degradation. The full range of endogenous $CRL4^{CRBN}$ substrates and the relevance of

thalidomide analogue-induced modulation of these substrates for the efficacy and toxicities of such treatments are areas of active investigation.

Novel classes of degrader therapeutics

Two additional, entirely distinct classes of small-molecule molecular glue degraders have been discovered. The first class comprises aryl sulfonamides, including indisulam, tasisulam, E7820 and chloroquinoxaline, which to date have produced modest response rates as monotherapy in clinical trials involving patients with advanced-stage solid tumours[173–176]. Two independent groups identified RNA-binding motif protein 39 (RBM39, also known as CAPERα), a nuclear protein involved in pre-mRNA splicing, as the direct molecular target responsible for the antitumour effect of these sulfonamides, which recruit this splicing factor to the $CRL4^{DCAF15}$ E3 ubiquitin ligase for polyubiquitination and subsequent proteasomal degradation[177–179]. Kinetic and structural analyses revealed relatively weak drug–protein interactions that are stabilized by ternary docking across broad protein–protein interfaces, which results in highly selective drug-dependent protein degradation of RBM39 and its paralogue RBM23[179–181].

With regard to the second novel class of agents, three independent chemogenomic screening studies resulted in the identification of structurally diverse molecular glue degraders of cyclin K. Firstly, by mining correlations between drug sensitivity and the gene-expression profiles of E3 ubiquitin ligase components in hundreds of cell lines, it was discovered that CR8, which was originally developed as a cyclin-dependent kinase (CDK) inhibitor, acts as a first-in-class molecular glue degrader of cyclin K[182]. Secondly, comparative chemical screening of hyponeddylated and well-neddylated cell lines revealed three destabilizers of cyclin K[183]. Finally, a novel cyclin K-targeting cytotoxic compound, HQ461, was identified during high-throughput screening for chemical suppressors of NRF2 activity[184]. Remarkably, these structurally divergent small molecules all directly engage a substrate receptor-less CUL4 E3 ligase complex by binding to its substrate adaptor component, DDB1, whilst simultaneously interacting with and thereby recruiting CDK12 in complex with cyclin K. Thus, selective complementarity between the surfaces of DDB1 and CDK12 hold promise for the future development of cyclin K degrader therapeutics[182]. The discovery of multiple classes of molecular glue degraders raises the exciting possibility that additional existing drugs also work through this mechanism. Indeed, the full complement of molecular glue degraders is not yet known.

The molecular glue-based mechanism of action of thalidomide analogues and anticancer sulfonamides have inspired efforts to design molecular glue degraders prospectively. Attractive targets include oncogenic forms of β-catenin that harbor hotspot mutations in a phosphodegron sequence normally recognized by the E3 ligase $SCF^{β–TrCP}$, which result in the accumulation of β-catenin protein and thus constitutive Wnt pathway activation[185]. Simonetta and colleagues[186] prospectively identified and performed subsequent rational structure-guided optimization of drug-like small molecules that profoundly enhance the interaction between $SCF^{β–TrCP}$ and such mutant forms of β-catenin. Future efforts are expected to target otherwise undruggable oncoproteins with prospectively designed molecular glue degraders.

Beyond molecular glue degraders, drug-induced polymerization is an emerging mechanism of targeted protein degradation. The transcription factor BCL6 is a therapeutic target in certain B cell malignancies, and small-molecule screening for novel inhibitors of this protein resulted in the identification of BI-3802 as a compound that binds directly to the BTB dimerization domain of BCL6 and acts as a specific BCL6 degrader[187]. Cryoelectron microscopy revealed the polymerization of BCL6–BI-3802 complexes into helical filaments in vitro[188]. In cells, BI-3802 induced the formation of BCL6 protein foci that were then rapidly degraded by the UPS[188]. Drug-induced polymerization promoted BCL6 degradation by the E3 ubiquitin ligase SIAH1, which recognizes a linear degron sequence distal to the drug-binding domain[188]. Such small-molecule inducers of targeted protein degradation via polymerization present an exciting new approach to drugging previously undruggable proteins. Generalizable rules governing target protein polymerization remain to be defined.

Synthetic biology and cell therapies

Beyond direct antitumour effects, degrader therapeutics are under development as chemical biology tools to regulate engineered proteins in the context of genetically modified cell therapies. Such cellular immunotherapies are emerging as powerful anticancer agents, but these autonomous, ‘living drugs’ are capable of inducing severe toxicities[189]. For example, chimeric antigen receptor (CAR) T cells are highly efficacious in patients with B cell malignancies but can cause severe T cell hyperactivation-related toxicities[190]. Precision control is therefore required to enhance the safety, efficacy and accessibility of such therapies[191], and indeed small-molecule-regulated kill switches for cell therapies[192] and transcriptional control systems for gene vectors[193] have entered clinical testing for this purpose. Multiple chemical–genetic systems have been devised to enable tunable regulation of protein stability using small molecules[194], including FKBP12-based destabilization domains[195], auxin-inducible degrons[196] and small-molecule-assisted shutoff (SMASh)[197]. With each platform, a heterologous protein of interest is fused to a drug-regulated domain in order that the fusion protein can either be stabilized or degraded in the presence of the small-molecule controller. Likewise, fusion proteins incorporating the bacterial dehalogenase-derived HaloTag protein can be degraded upon addition of either hydrophobic tag ligands that mimic a partially denatured protein state[198] or HaloPROTACs that recruit VHL (which serves as a substrate receptor component of the CRL2 E3 ubiquitin ligase)[199]. In addition, Nabet and colleagues[200,201] have developed the dTag system that uses PROTACs composed of a CRBN or VHL ligand, a linker and an AP1867 derivative. AP1867 and $FKBP12^{F36V}$ are a bump-and-hole engineered small molecule–protein pair orthogonal to rapamycin and wild-type FKBP12[202]. Accordingly, AP1867-derivative PROTACs can mediate the selective degradation of heterologous $FKBP12^{F36V}$-tagged proteins, enforcing rapid and potent depletion in vitro and in vivo[200,201]. CARs fused to an additional domain allowing for PROTAC-mediated degradation have been designed as a reversible chemical safety switch[203]. In aggregate, these approaches to rapidly and reversibly tune protein stability are invaluable research tools. However, with the exception of newer generations of destabilization domains[204], these systems are currently difficult to directly translate into clinical cellular immunotherapies because they use non-human proteins, immunosuppressive controller drugs or non-approved small molecules.

A molecular switch consisting of a thalidomide analogue and a variable protein construct with a human zinc finger degron tag would probably be suitable for clinical use. Notably, Koduri and colleagues[205] have demonstrated inducible degradation of multiple fusion proteins linked to a 25-amino acid zinc finger degron tag derived from IKZF3 and a >50% reduction in luminescence from tumour cells expressing a degron-tagged luciferase transgene upon treatment with thalidomide analogues. Carbonneau and colleagues[206] fused a CAR with a 60-amino acid zinc finger degron derived from IKZF3, enabling reversible thalidomide analogue-mediated CAR depletion, partial inhibition of CAR T cell effector function in vitro, and suppression of anti-tumor function in vivo. To further develop this concept, 'super-degron' tags composed of hybrid zinc finger degrons have been engineered to enhance the efficiency of drug-mediated degradation[117,207]. These super-degron tags have been incorporated into thalidomide analogue-mediated off-switch CARs, which can be degraded and thus functionally inhibited at sub-therapeutic drug concentrations (FIG. 4)[207]. Furthermore, a thalidomide analogue-inducible dimerization domain has been generated from a fragment of CRBN and a zinc finger with all lysines substituted to arginines (K0), and with this system split CARs have been built that require drug treatment for dimerization and subsequent activation[207]. Such control systems might prove broadly useful to enhance the safety and the efficacy of future cellular immunotherapies, for example, by acting as safety switches or by enabling the measured use of therapeutic proteins that are toxic when constitutively expressed, such as the pro-inflammatory cytokine IL-12[208]. This early success in developing custom degrons suggests that further engineering of thalidomide analogue-based degron tags is possible using structure-guided or directed-evolution approaches. These efforts might greatly expand the toolkit of clinically suitable synthetic biology approaches for the design of next-generation cellular immunotherapies.

Conclusions

Targeted protein degradation in oncology has reached an extraordinary juncture. Further chemical diversification of thalidomide analogues will expand the range of degradable target proteins. Two decades of development have culminated in the clinical testing of the first of many small-molecule PROTAC drugs, ARV-110 (NCT03888612). Additional classes of molecular glue and heterobifunctional degrader molecules will continue to be designed and discovered, building on the experience gained with such agents to date and exploiting the hundreds of E3 ubiquitin ligases. Chemical switches engineered from an expanding list of degrader–substrate pairs will render gene therapies and cell therapies more controllable. As new generations of degrader therapeutics reach the clinic, the lessons learned with lenalidomide and other thalidomide analogues can serve as a guide. Once-undruggable proteins that are amenable to therapeutic degradation owing to cancer dependency, synthetic lethality and/or aneuploidy must be identified, prioritized and targeted. Extensive testing of novel preclinical degraders across multiple cell types will be required to elucidate target substrates with the potential for efficacy and toxicity. Clinical proteomics will enable the monitoring of substrate protein dynamics. Mechanisms of relapse to drugs leveraging E3 ubiquitin ligases need to be anticipated and counteracted. Ultimately, insights into molecular glue chemistry, structural biology and proteomics will prove essential to realizing

the extraordinary breadth of medical opportunities unlocked by the expanding range of degradable proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Competing interests

B.L.E. has received research funding from Celgene, Deerfield, and Novartis and consulting fees from GRAIL. He serves on the scientific advisory boards and holds equity in Skyhawk Therapeutics, Exo Therapeutics, and Neomorph Therapeutics. The other authors declare no competing interests.

Glossary terms

Molecular glue
A small molecule that directly bridges protein-protein interactions.

Neosubstrates
Proteins that are conditionally targeted to a ubiquitin ligase in the presence of a small molecule.

High-risk cytogenetic features
Chromosomal alterations that are associated with aggressive disease and unfavourable survival outcomes; in myeloma, these features include t(4;14), t(14;16) and del(17p) alterations.

Antibody-dependent cellular cytotoxicity (ADCC)
A mechanism of cell killing that requires recognition of the antibody-bound cell by immune effector cells, such as natural killer cells.

Activated B cell-like DLBCL
A subtype of DLBCL that is defined by a specific transcriptional signature and is associated with high risk of relapse following standard R-CHOP chemoimmunotherapy.

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Box 1 |

An overview of the ubiquitin-proteasome system

- The ubiquitin-proteasome system (UPS) is a major cellular regulator of protein turnover and quality control[209].
- Ubiquitin is a 76-amino-acid protein that is activated and covalently linked to proteins in the following steps: 1) ATP-dependent conjugation of ubiquitin to an E1 ubiquitin-activating enzyme; 2) transfer of the activated ubiquitin to an E2 ubiquitin-conjugating enzyme; and 3) E3 ubiquitin ligase-mediated transfer of ubiquitin from the E2 enzyme to the substrate protein.
- The human genome contains two genes encoding E1 enzymes, 41 genes encoding E2 enzymes and >600 genes encoding E3 ubiquitin ligases.
- The various E3 ubiquitin ligases mediate selective protein ubiquitination by recognizing particular degradation signals in their substrate proteins, termed ‘degron’ motifs, which are most commonly linear sequences or, alternatively, conformational shapes. Each ligase might recognize multiple substrate proteins[210].
- A single ubiquitin protein can be linked at its C-terminus to lysine residues or the N-terminal methionine of substrate proteins, either at one target site (monoubiquitination) or successively at multiple sites (multimonoubiquitination).
- Successive ubiquitination can also form ubiquitin–ubiquitin linkages to generate polyubiquitin chains that can be differentiated according to which lysine residue (K6, K11, K27, K29, K33, K48 or K63) or N-terminal methionine of each ubiquitin monomer is covalently attached to the C-terminus of the subsequent monomer.
- Ubiquitination can be reversed by deubiquitination enzymes (DUBs). The human genome encodes approximately 100 DUBs.
- Patterns of ubiquitination constitute an extraordinarily complex ‘ubiquitin code’ and act as signals to elicit diverse functional outcomes, including protein transport, signal transduction or protein degradation[211]. For example, K11-linked and K48-linked polyubiquitin chains target proteins for proteasomal degradation.
- The 26S proteasome is the major machinery for selective, ubiquitin-mediated protein degradation. The proteasome is a large enzymatic protein complex that first removes the attached ubiquitin groups and then unfolds the target protein into a linear polypeptide chain, which is successively fed into the barrel-shaped core of the proteasome for processive proteolysis.

Box 2 |

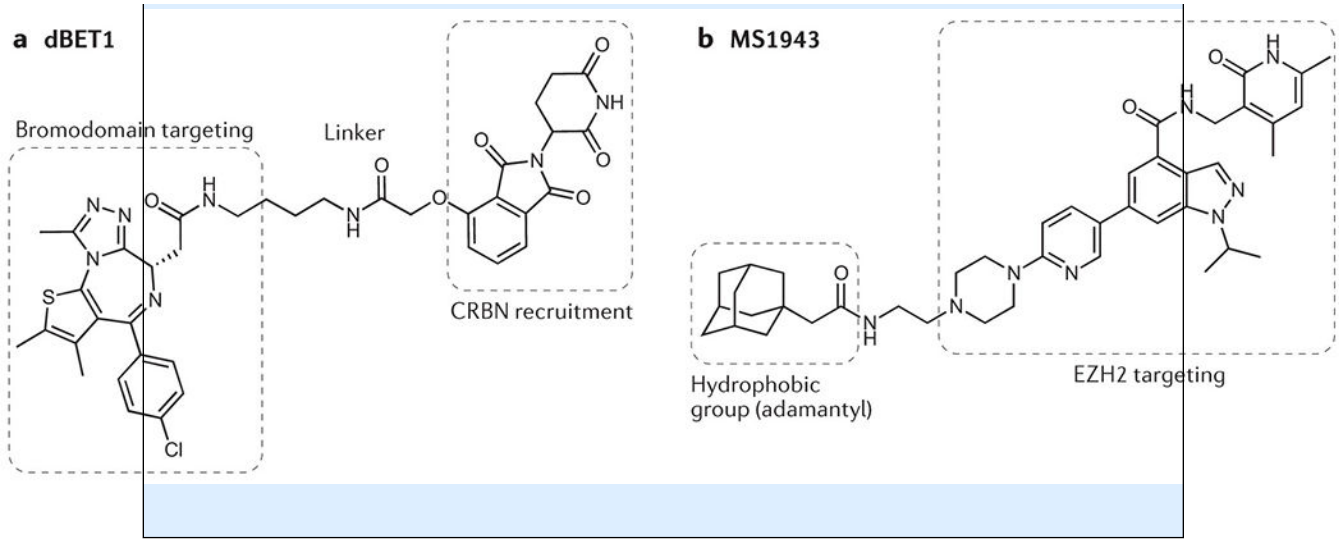
Target proteins implicated in thalidomide analogue efficacy and toxicities

- The proteasomal degradation of specific neosubstrate proteins underlies the clinical efficacy of thalidomide analogues in the treatment of cancer[16,212]. Various neosubstrate proteins, although unrelated in sequence, contain a structurally conserved degron motif composed of a β-hairpin with a pinnacle glycine residue, most often as part of a Cys2His2 (C2H2) zinc finger domain. This degron motif can bind to a composite thalidomide analogue–cereblon (CRBN) surface, ultimately resulting in neosubstrate ubiquitination and degradation.
- IKZF1 and IKZF3 are pan-thalidomide analogue targets and zinc finger-containing transcription factors that have crucial roles in B cell, T cell and plasma cell development[88,213,214]. The anti-myeloma effects of lenalidomide are mediated by depletion of IKZF1 and IKZF3[11,12], which in turn downregulates the expression of IRF4, an oncogenic transcription factor that drives multiple myeloma and other mature B cell neoplasms[81,215]. In T cells, IKZF3 directly binds to and represses the *IL2* promoter[216,217], and thus thalidomide analogues mediate immunomodulatory effects in part by derepression of IL-2 expression[11,13].
- *CSNK1A1* is located in the chromosomal region commonly deleted in del(5q) myelodysplastic syndrome (MDS) and encodes the serine/threonine kinase CK1α, which is a lenalidomide target. Haploinsufficiency of *CSNK1A1* provides haematopoietic stem and progenitor cells (HSPCs) with a clonal advantage via derepression of β-catenin[96], whereas homozygous loss of *CSNK1A1* induces p53 and HSPC apoptosis[96,218]. Thus, CK1α is the primary target for lenalidomide-mediated activity in del(5q) MDS[15]. Indeed, lenalidomide is the first clinically approved drug exploiting a therapeutic opportunity presented by haploinsufficiency of a protein[219], via selective induction of p53 in del(5q) cells haploinsufficient for *CSNK1A1*[15].
- GSPT1 (eRF3a) is a target of thalidomide analogues CC-885 and the related compound CC-90009 and is a translation termination release factor required for the G1 to S phase cell cycle transition[220,221]. Hence, GSPT1 is an essential protein, depletion of which is especially toxic to acute myeloid leukaemia cells[121].
- SALL4 is a pan-thalidomide analogue target and a developmental zinc-finger transcription factor implicated in thalidomide embryopathy[165,222]. Indeed, germline mutations in *SALL4* cause syndromes with multifaceted phenotypic overlap with thalidomide syndrome[222,223].

Box 3

Prospective development of targeted protein degraders

- The development of proteolysis targeting chimeras (PROTACs) over the past two decades has been expertly reviewed elsewhere[58,138–140,224], particularly for a drug-discovery audience.
- PROTACs leverage the concept of using bifunctional small molecules to bring proteins into close proximity[225,226].
- PROTACs are composed of three parts: an E3 ubiquitin ligase-recruiting ligand, a linker and a target-binding ‘warhead’ (see example of dBET1 in figure part a). PROTACs are therefore relatively large molecules that do not conform to typical rules of small-molecule drug design.
- In contrast to thalidomide analogues, PROTACs can be prospectively designed for many liganded targets. PROTAC development remains empiric and challenging, although scientific breakthroughs and investment have spurred the development of PROTACs for many targets in oncology.
- The first clinical trial of a PROTAC, ARV-110, opened in 2019 (NCT03888612); ARV-110 targets the androgen receptor and is being tested in patients with prostate cancer.
- In many cases, the target-binding warhead is derived from existing inhibitors. In comparisons of chemically related inhibitors, PROTACs have been found to have higher target specificity, owing to the unique requirement for drug-induced stabilization of two proteins that have not evolved to interact with each other[227–229].
- Degraders can overcome point mutation-based resistance to target inhibition, for example, the BTK C481S resistance mutation to ibrutinib, using warheads that retain affinity for the mutated active site[230,231]
- Only a few of the >600 E3 ubiquitin ligases have been leveraged for targeted protein degradation to date, including the those with cereblon (CRBN), VHL, MDM2, cIAP or ß-TRCP as substrate-recognition components. Thus, extraordinary potential exists to use additional ligases to exploit crucial features, including cell type-specific activity and target selectivity[232].
- Related bifunctional molecule concepts extend to the recruitment of target proteins to effectors other than ligases, such as lysosomes and phosphatases[233,234].
- Hydrophobic tagging is a related approach in which a hydrophobic small molecule binds to a target protein, thus mimicking a partially unfolded state and inducing degradation of the target by cellular quality control machinery[198,235]. The hydrophobic-tag EZH2 degrader MS1943 (see figure part b), for example, has demonstrated activity against triple-negative breast cancer cell lines that are refractory to catalytic EZH2 inhibitors[236].



Key points

- Thalidomide analogues including lenalidomide and pomalidomide are frontline anticancer agents against multiple hematologic malignancies. The dominant mechanism of action of thalidomide analogues is via targeted protein degradation of disease-relevant proteins.
- Degrader therapeutics exhibit catalytic, event-driven pharmacology, and their biologic effects are a summation of the polypharmacology of multiple degraded neosubstrate proteins.
- Cancers acquire resistance to thalidomide analogues by circumventing target protein degradation or by rewiring pathways downstream of target protein degradation.
- Quantitative clinical proteomic assays can be used for pharmacodynamic monitoring of degrader therapeutics.
- Molecular switches gated by degrader therapeutics can be used to control anticancer cellular immunotherapies.
- Multiple classes of degrader therapeutics are expanding the draggable proteome to target non-catalytic cancer-relevant proteins including transcription factors, mRNA-splicing factors, and cyclins.

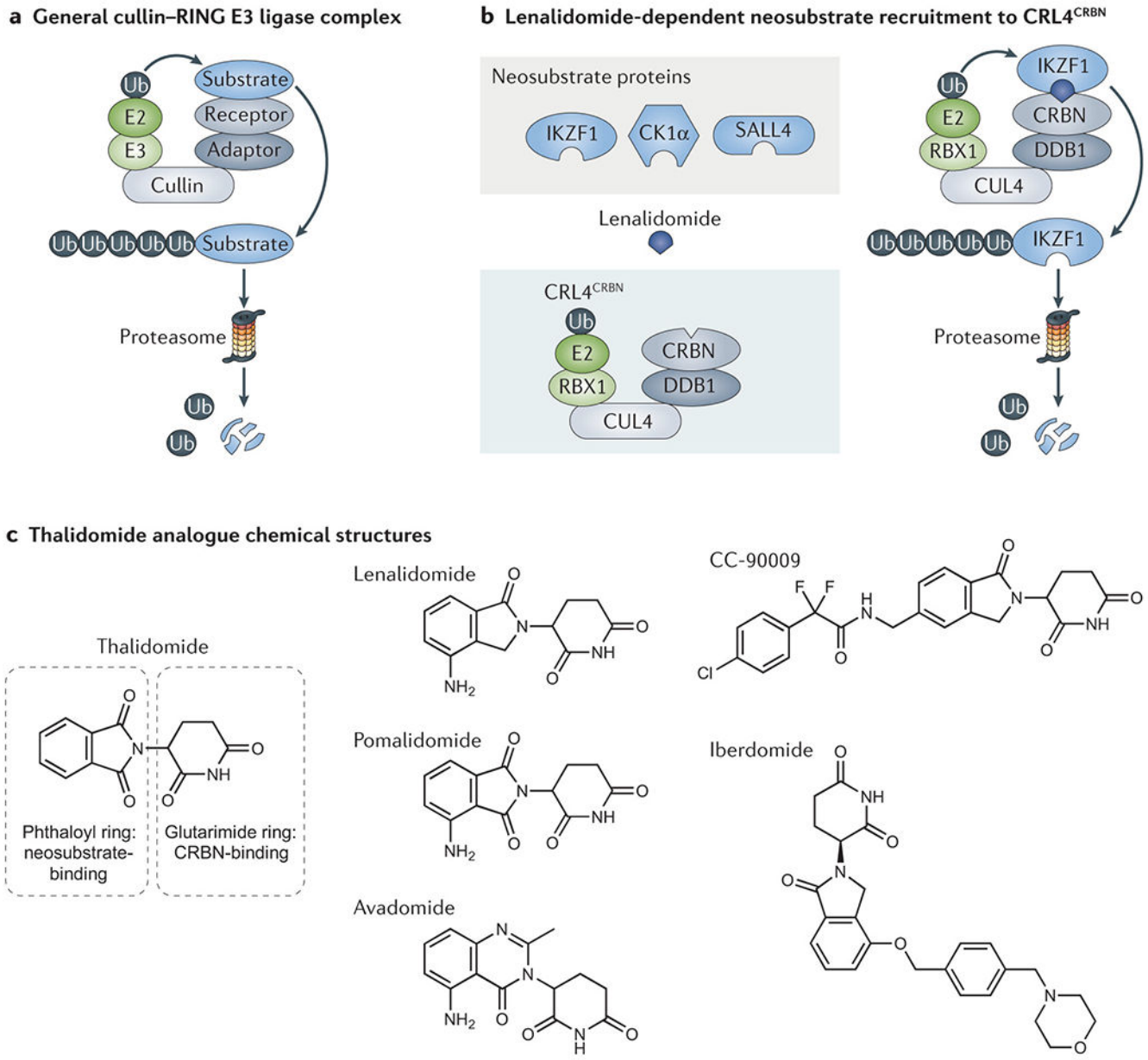


Fig. 1 |. Mechanisms of targeted protein degradation.

a | Cullin-RING ligases (CRLs) constitute the largest family of E3 ubiquitin ligases. CRL complexes mediate the selective transfer of ubiquitin (Ub) directly from an E2 enzyme to a receptor-bound substrate protein. This marking with Ub targets the substrate protein for proteasomal degradation. **b** | Lenalidomide acts as a 'molecular glue' to mediate drug-dependent recruitment of neosubstrate proteins to the cereblon (CRBN) receptor component of the cullin-RING ligase $CRL4^{CRBN}$, which results in neosubstrate ubiquitination and degradation. **c** | Thalidomide analogues all share a glutarimide ring that binds to CRBN; however, these agent all vary subtly, in the case of lenalidomide, pomalidomide, and avadomide, or more substantially, in the case of iberdomide and CC-90009, in their

neosubstrate-binding moiety. Owing to these chemical variations, different thalidomide analogues promote the degradation of overlapping but distinct sets of neosubstrate proteins.

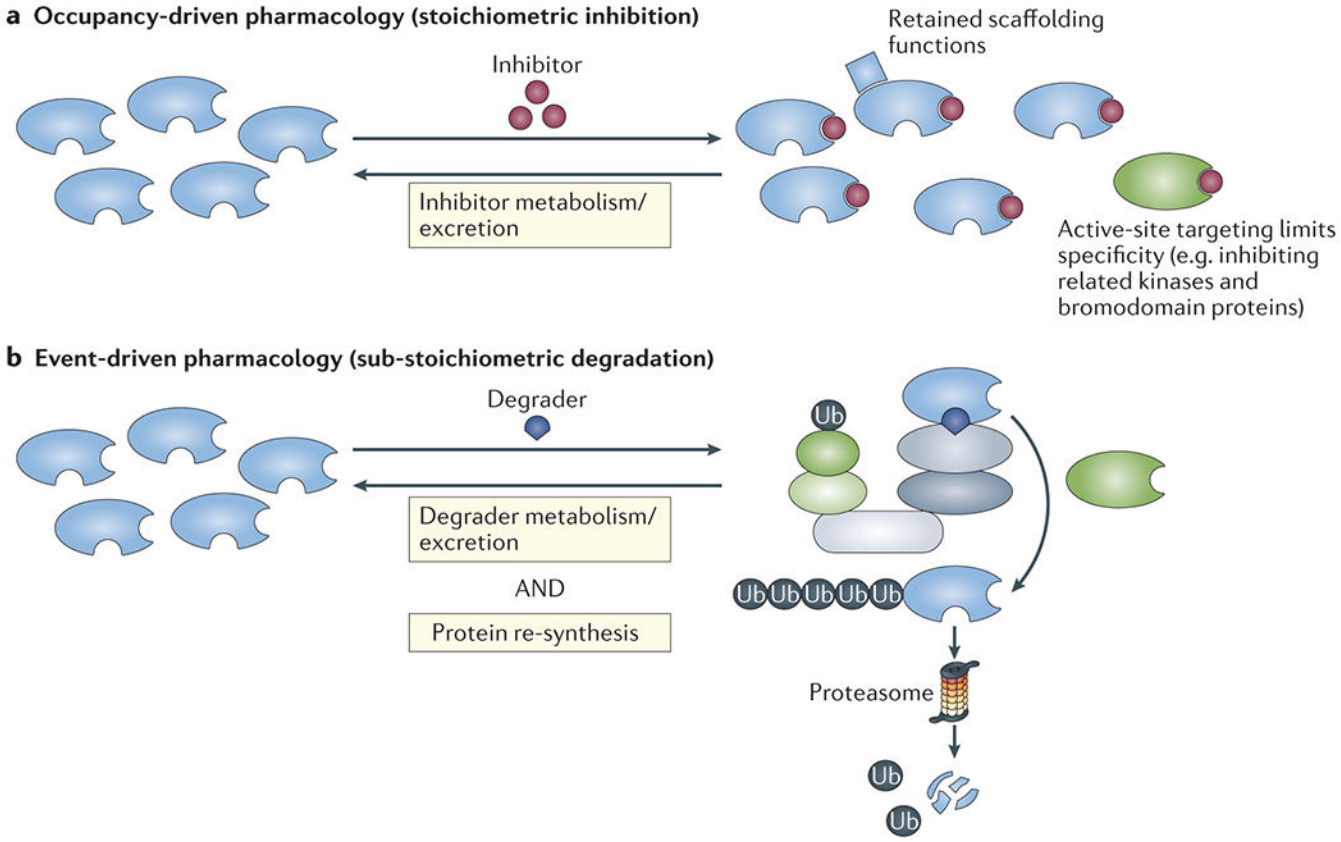


Fig. 2 |. Event-driven versus occupancy-driven pharmacology.

a | According to the occupancy-driven pharmacological paradigm, small-molecule inhibitors must maintain target-protein occupancy for a sustained antagonistic effect. Moreover, noncatalytic, scaffolding functions of the target protein can be retained and thus continue to contribute to oncogenesis. Furthermore, specific catalytic inhibition of a target protein at the active site, without cross-reactivity within the same protein family, is often challenging. In the figure, the blue shapes represent target proteins, with the active site at the right side and degrader-binding site at the bottom. The blue square represents a separate protein that is able to bind to the target protein regardless of inhibitor occupancy at the active site. The green shape illustrates a protein of the same family as the target that is subject off-target effects of the inhibitor, owing to active site homology, but lacks the degrader-binding site and would, therefore, be spared from off-target degrader effects. **b** | Degrader drugs deplete the target protein, and functional target inhibition persists until the protein is re-synthesized, constituting an example of event-driven pharmacology. Degraders have a selectivity advantage by mediating ligase–drug–target ternary complex formation using surface interfaces that are generally broader and less conserved than active sites.

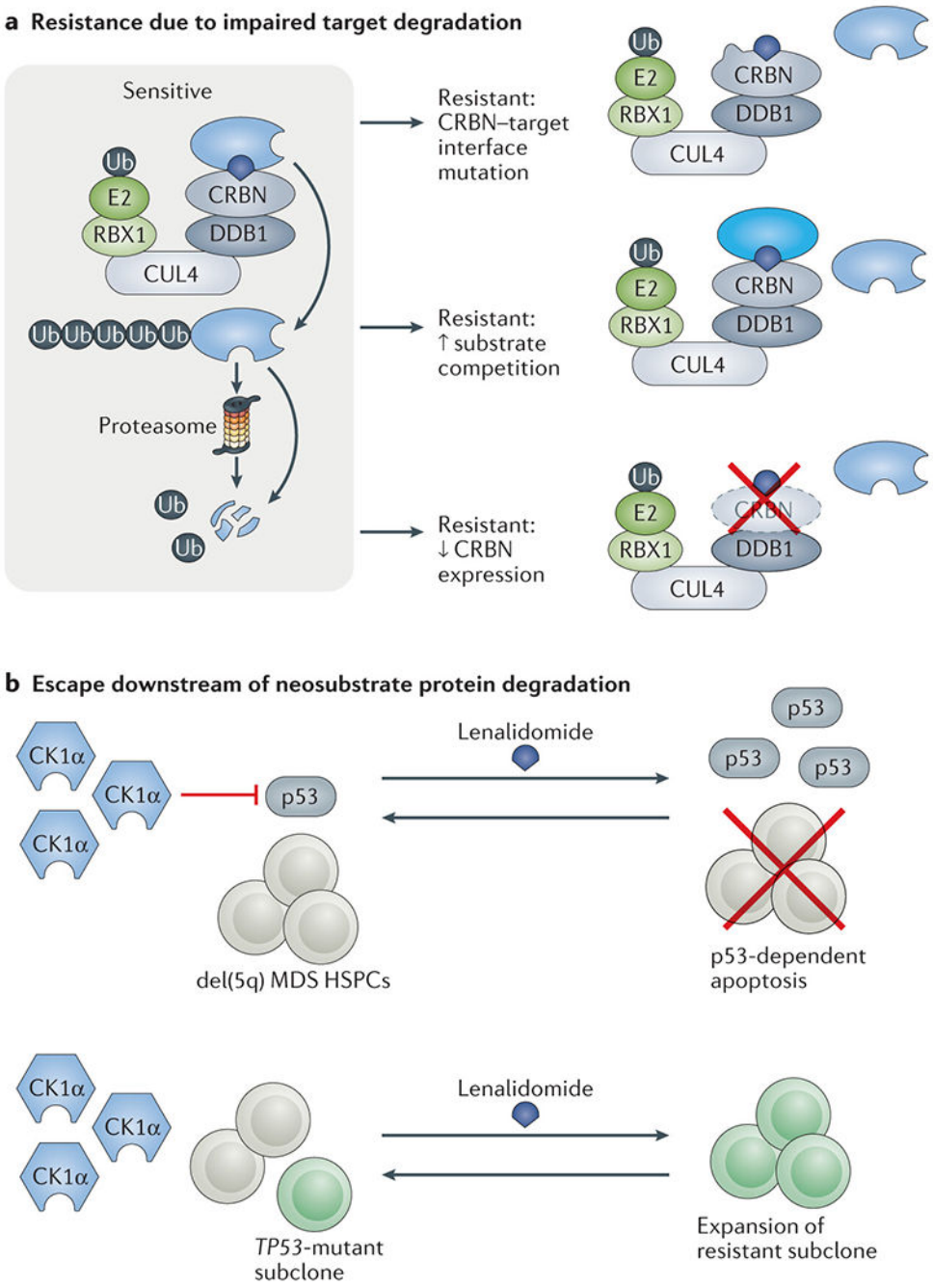


Fig. 3 |. Mechanisms of resistance to targeted protein degradation.

a | Tumour cells can escape from thalidomide analogue-induced degradation of key E3 ubiquitin ligase neosubstrate proteins via mutations affecting the cereblon (CRBN)–neosubstrate interface, reduced expression of CRBN or overexpression of competing substrates for the same ligase. **b** | Tumour cells can escape from the tumour-suppressive consequences of neosubstrate protein degradation through alterations affecting the function of downstream mediators of the antitumour effects. For example, *TP53*-mutant subclones of del(5q) myelodysplastic syndrome (MDS) haematopoietic stem and progenitor cells

(HSPCs) are resistant to p53-mediated apoptosis induce by lenalidomide-mediated degradation of CK1α.

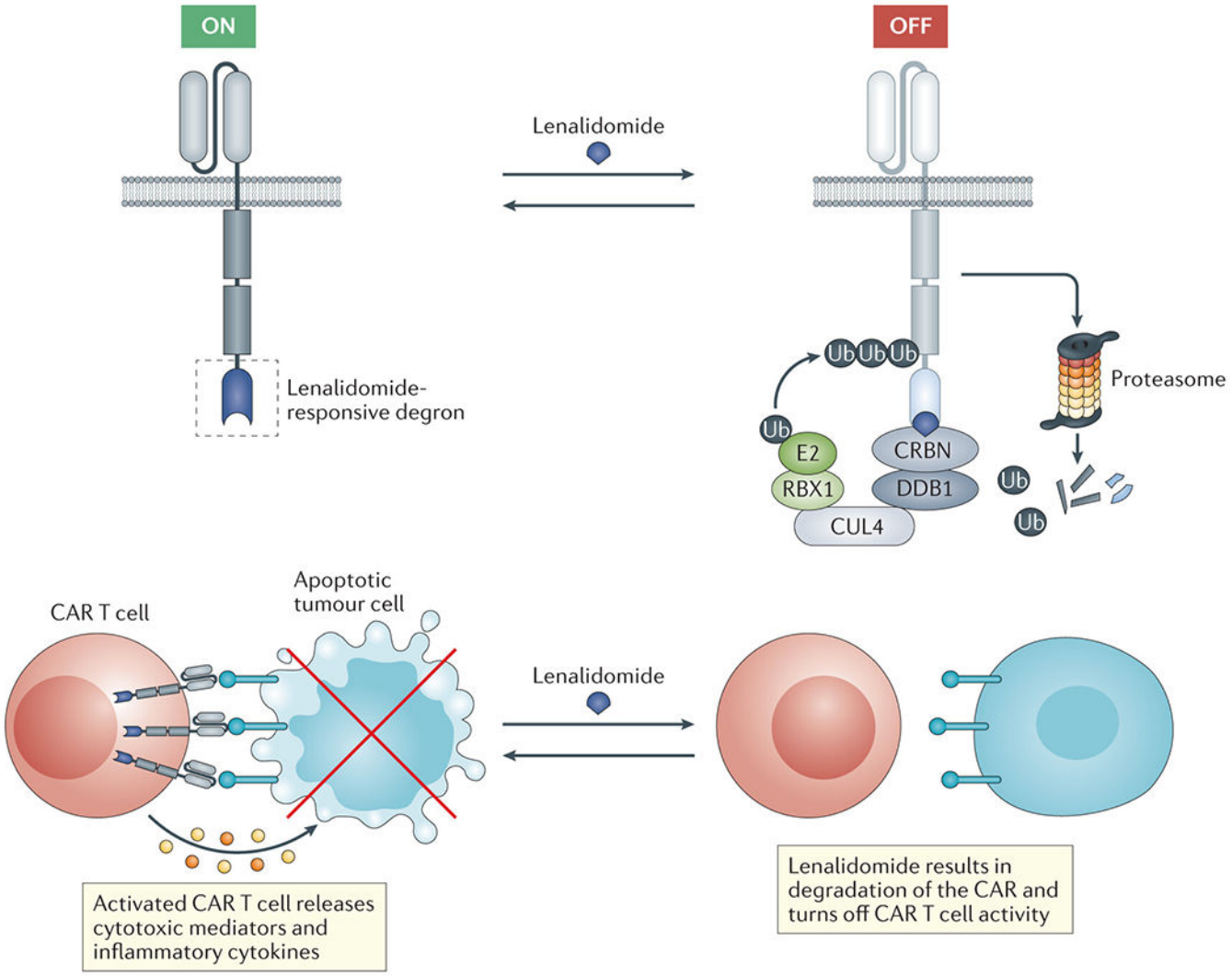


Fig. 4 |. Genetically engineered cell therapies regulated by targeted protein degraders.

The figure outlines an example of the use of lenalidomide as a molecular ‘switch’ to dynamically control the activity of chimeric antigen receptor (CAR) cells. Specifically, a lenalidomide-responsive ‘degron’ moiety (for example, a zinc finger domain derived from IKZF3 can be incorporated in the CAR construct. These degradable constructs can then be used to generate CAR T cells that can be rapidly and reversibly turned ‘off’ through lenalidomide treatment in order to tune CAR signalling or mitigate toxicities associated with T cell hyperactivation. HaloTags or dTags, which also confer the ability to control expression of the engineered protein using small-molecule degraders, could be substituted for the lenalidomide-responsive degron.

Table 1 |

Selected ongoing clinical trials of next-generation thalidomide analogues

Agent	Disease	Treatment approach	Study phase	ClinicalTrials.gov identifier
CC-90009	R/R high-risk MDS or R/R AML	Single agent	Phase I	NCT02848001
	Newly diagnosed or R/R AML	In combination with venetoclax (BCL2 inhibitor) and azacitidine (HMA) or with gilteritinib (FLT3 inhibitor)	Phase I/II	NCT04336982
Avadomide (CC-122)	Advanced-stage melanoma (naive or refractory to PD-1 inhibition)	In combination with nivolumab (anti-PD-1 antibody)	Phase II	NCT03834623
	Advanced-stage HCC (after one prior line of therapy)	In combination with nivolumab	Phase I/II	NCT02859324
	Newly diagnosed DLBCL with poor-risk factors	In combination with R-CHOP chemotherapy	Phase I	NCT03283202
	Treatment-naive or R/R CLL or SLL	Single-agent, or in combination with ibrutinib (BTK inhibitor) or obinutuzumab (anti-CD20 antibody)	Phase I/II	NCT02406742
	R/R DLBCL or R/R FL (naive or refractory to lenalidomide)	In combination with obinutuzumab	Phase Ib	NCT02417285
	R/R DLBCL or R/R FL (naive or refractory to lenalidomide)	In combination with rituximab (anti-CD20 antibody) and/or either onatasertib (CC-223; mTOR inhibitor) or spebrutinib (CC-292; BTK inhibitor)	Phase Ib	NCT02031419
	R/R B cell malignancies	In combination with JCAR017 (autologous anti-CD19 4-1BB CAR T cell product)	Phase I/II	NCT03310619
	R/R advanced-stage solid tumours, NHL or MM	Single agent	Phase I	NCT01421524
	Advanced-stage solid tumours or NHL	Single agent	Phase I	NCT02509039
Iberdomide (CC-220)	R/R MM	Single agent, or in combination with daratumumab (anti-CD38 antibody), bortezomib (PI), carfilzomib (PI) and/or dexamethasone (corticosteroid)	Phase Ib/IIa	NCT02773030
	R/R MM	In combination with low-dose cyclophosphamide (chemotherapy) and dexamethasone	Phase II	NCT04392037
	Newly diagnosed MM	Single-agent maintenance after ASCT	Phase II	NCT04564703
	R/R lymphomas	Single agent, or in combination with rituximab or obinutuzumab	Phase I/II	NCT04464798
	R/R B cell malignancies	In combination with JCAR017 (autologous anti-CD19 4-1BB CAR T cell product)	Phase I/II	NCT03310619
CC-92480	R/R MM	Single agent, in combination with dexamethasone	Phase I	NCT03374085
	Newly diagnosed or R/R MM	In combination with dexamethasone plus either daratumumab, bortezomib or carfilzomib	Phase I/II	NCT03989414

Trials involving patients with cancer and noted as being ‘not yet recruiting’, ‘active, not yet recruiting’, ‘recruiting’ or ‘completed’ with no results posted were retrieved from the ClinicalTrials.gov database. AML, acute myeloid leukaemia; ASCT, autologous haematopoietic stem cell transplantation; CLL, chronic lymphocytic leukaemia; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; HCC, hepatocellular carcinoma; HMA, hypomethylating agent; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; PI, proteasome inhibitor; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; R/R, relapsed and/or refractory; SLL, small lymphocytic leukaemia.

Table 2 |

FDA approved anticancer indications of thalidomide analogues

Agent	Disease	Treatment approach	Approval year	Studies used for approval
Thalidomide	Newly Diagnosed Multiple Myeloma (NDMM)	In combination with dexamethasone	2006	PMID: 16365178
	NDMM (transplant eligible)	In combination with daratumumab, bortezomib and dexamethasone	2019	PMID: 31171419
Lenalidomide	Del(5q) MDS, low or intermediate risk	Single agent	2005	PMID: 17021321
	R/R MM	In combination with dexamethasone	2006	PMID: 18032763 PMID: 18032762
	R/R MM	In combination with carfilzomib and dexamethaonse	2018	PMID: 25482145
	R/R MM	In combination with ixazomib and dexamethasone	2015	PMID: 27119237
	R/R MM	In combination with daratumumab and dexamethasone	2016	PMID: 27705267
	R/R MM	In combination with elotuzumab and dexamethasone	2017	PMID: 26035255
	NDMM	In combination with dexamethasone	2015	PMID: 25184863
	NDMM (transplant ineligible)	In combination with daratumumab and dexamethasone	2019	PMID: 31141632
	MM maintenance after ASCT	Single agent	2017	PMID: 22571201 PMID: 22571202
	MCL relapsed after two or more prior lines, one of which was with bortezomib	Single agent	2013	PMID: 24002500
	R/R FL	In combination with rituximab	2019	PMID: 30897038 NCT01996865
	R/R MZL	In combination with rituximab	2019	PMID: 30897038 NCT01996865
Pomalidomide	MM after two prior lines	In combination with dexamethasone	2013	PMID: 24421329 PMID: 24007748
	MM after two prior lines	In combination with daratumumab and dexamethasone	2017	PMID: 28637662
	MM after two prior lines	In combination with isatuximab and dexamethasone	2020	PMID: 31735560
	Kaposi Sarcoma after failure of HAART in HIV+ patients or in non-HIV patients	Single agent	2020	PMID: 27863194

MM, multiple myeloma; MDS, myelodysplastic syndrome; ASCT, autologous stem cell transplant; MCL, mantle cell lymphoma; FL, follicular lymphoma; MZL, marginal zone lymphoma; HAART, highly active anti-retroviral therapy