Thalidomide analogs as emerging anti-cancer drugs

Keith Dredge[a], Angus G. Dalgleish[a] and J. Blake Marriott[a]

Recently, it has been demonstrated that a number of novel thalidomide analogs possess anti-cancer properties due to their T cell co-stimulatory, anti-angiogenic and/or anti-inflammatory effects. Based on such effects, a class of thalidomide analogs known as Immunomodulatory Drugs (IMiDs™) have recently entered into phase I clinical trials for the treatment of a number of cancers. The lead IMiD CC-5013 (referred to clinically as REVIMID™) is now entering phase III clinical trials for multiple myeloma and metastatic melanoma, while CC-4047 (ACTIMID™) is currently under investigation in phase I/II and II trials for multiple myeloma and prostate cancer, respectively. The other group of compounds, classified as Selective Cytokine Inhibitory Drugs (SelCIDs™), do not co-stimulate T cells, but have anti-inflammatory and anti-angiogenic properties. Moreover, a subset of SelCIDs has been found to posses direct anti-tumor activity both *in vitro* and *in vivo*. This minireview highlights the various mechanisms of action associated with these compounds and their subsequent clinical development. The enhanced efficacy and lower side-effect profiles of the analogs in comparison to thalidomide make the use of these agents very attractive as novel anti-cancer agents. *Anti-Cancer Drugs* 14: 331–335 © 2003 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2003, **14**:331–335

Keywords: anti-angiogenic, anti-inflammatory, anti-tumor, co-stimulatory, thalidomide analogs

[a]Division of Oncology, St George's Hospital Medical School, London, UK.

Sponsorship: The authors would like to thank Celgene Corp. (Warren, NJ) for their generous and continuing financial support.

Correspondence to K. Dredge, Division of Oncology, St George's Hospital Medical School, Tooting, London SW17 0RE, UK.
Tel: +44 208 7255037; fax: +44 208 7250158;
e-mail: kdredge@sghms.ac.uk

Received 18 February 2003 **Revised version accepted** 18 March 2003

Introduction

Thalidomide is an effective drug for several forms of cancer [1–3]. In particular, the drug has shown remarkable effects in patients with refractory and relapsed myeloma [4,5]. Novel compounds have been designed using thalidomide as a lead structure in the hope that this would allow optimization of immunological properties while decreasing side-effects [6–10]. Initially, this focused on improving thalidomide's anti-tumor necrosis factor (TNF)-α activity. More recently, the emphasis has changed to focus not only on anti-inflammatory properties, but also T cell co-stimulatory, anti-angiogenic and anti-cancer properties [11–15].

During the characterization of these compounds by several laboratories it has become apparent that there are at least two distinct classes of thalidomide analogs [6–9]. These have been termed SelCIDs™ (Selective Cytokine Inhibitory Drugs) and IMiDs™ (Immunomodulatory Drugs). SelCIDs are phosphodiesterase (PDE) type 4 inhibitors and are functionally linked (TNF-α inhibition) to, but are chemically distinct from thalidomide. IMiDs are chemical analogs of thalidomide which do not inhibit PDE type 4 and act via an unknown mechanism(s). Both groups of compounds are potent TNF-α inhibitors (in an inflammatory setting). However, IMiDs are T cell co-stimulators and in this context TNF-α is increased [15,16]. IMiDs have also demonstrated anti-myeloma activity which is dependent on p53 status [15]. However, a subclass of the SelCID group appears to have direct anti-tumor activity that is not due to PDE type 4 inhibition or dependent on p53 status [11]. The possible mechanisms by which IMiDs and SelCIDs exert anti-cancer effects are highlighted in Table 1.

Preclinical activity

Immunomodulatory properties

SelCID and IMiD analogs differ with respect to their effects on cytokine production following activation of monocytes/macrophages. During lipopolysaccharide (LPS) stimulation of monocytes/macrophages, it was shown that in addition to inhibiting TNF-α production, SelCIDs have a modest effect on interleukin (IL)-10 stimulation, but only weakly inhibit IL-1β and IL-12, while having no effect on IL-6 [17]. Thalidomide/IMiDs show greater inhibitory effects on IL-1β and IL-12 [18]. Interestingly, IMiDs with only minimal structural differences from the parent compound are also potent inhibitors of IL-1β in LPS-stimulated human peripheral blood mononuclear cells [17].

The T cell co-stimulatory activity of thalidomide was discovered in 1998 [16]. It is this activity which is responsible for differential effects on cytokine production depending on whether T cells or monocytes are activated and thalidomide analogs (IMiDs and SelCIDs) have now

0959-4973 © 2003 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000070763.25457.00

Table 1 Thalidomide analogs segregate into at least two classes

	IMiDs	SelCIDs
Anti-TNF-α		
via Toll-like receptors	yes	yes
via T cell receptors	no	yes
T cell co-stimulatory	yes	no
Anti-angiogenic	yes	yes
Direct anti-tumor	yes	yes

been segregated according to this ability. Initially, T cell co-stimulation by thalidomide was thought to involve primarily $CD8^+$ T cells (not $CD4^+$), but recently it has been shown that both cells are equally affected [19]. SelCIDs do not possess T cell co-stimulatory properties.

It has now been demonstrated that the IMiD CC-4047 can co-stimulate T cells *in vivo*. The presence of the analog during the priming phase enhanced anti-tumor responses following autologous tumor cell vaccination in a colorectal murine model. The same effect has been seen in an allogeneic model for melanoma [12]. Anti-tumor immunity was found to be mediated by the induction of the T_h1-type cytokines IFN-γ and IL-2. Further evidence that IMiD-induced T cell co-stimulation results in a T_h1-type response was observed following co-stimulation of naive T cells activated with immobilized anti-CD3 antibody. While CC-4047 alone did not induce cytokine production, stimulated splenocytes co-incubated with CC-4047 secreted increased concentrations of IFN-γ, IL-2 and granulocyte macrophage colony stimulating factor (GM-CSF), while the T_h2-type cytokines IL-4 and IL-10 were actually inhibited in comparison to stimulated controls [12].

Anti-inflammatory properties

Cyclooxygenase-2 (COX-2) is an enzyme not only involved in prostaglandin E_2 synthesis, but also thought to modulate production of angiogenic factors by colon cancer cells, thus affecting tumorigenicity. Although no clear-cut structure–activity relationships could be deduced, it has recently been shown that thalidomide analogs have the potential to be selective COX-2 inhibitors [20]. Furthermore, IMiDs have been found to inhibit LPS-mediated induction of COX-2, which should also inhibit carcinogenesis and decrease inflammation [21].

Tumor-associated macrophages can express COX-2 and we have found that a daily dosing using an IMiD does decrease the expression of COX-2 within tumors, albeit in a statistically non-significant fashion (our unpublished observations). Another inflammatory factor, TNF-α, has also been reported to be inhibited by both IMiD and SelCID analogs 1500 times more potently than thalidomide in LPS-stimulated human PBMC [8]. SelCIDs have also been shown to modestly stimulate the anti-inflammatory cytokine IL-10 in this assay [17]. All of these compounds are potent PDE4 inhibitors and this activity appears to correlate well with TNF-α inhibition (unpublished observations). Many SelCIDs have been reported to inhibit serum TNF-α levels in LPS-treated mice. Taken together, such effects could be useful in treating inflammatory conditions and/or certain cancers.

Anti-angiogenic properties

We have recently shown that both IMiDs and SelCIDs are more potent than thalidomide in terms of anti-angiogenic activity *in vitro* [13]. There is clear evidence to suggest that some SelCID analogs possess anti-angiogenic activity. CC-1069 inhibited human endothelial cell (EC) proliferation *in vitro* more than thalidomide, suggesting that it may be a more potent anti-angiogenic agent [24]. However, the potent anti-angiogenic activity *in vitro* of thalidomide analogs is independent of their anti-TNF-α properties or, in the case of SelCIDs, their PDE type 4 inhibitory effects [13]. Inhibition of angiogenic sprouting in the rat aorta assay and inhibition of formation of tubules in a human angiogenesis system was observed following co-incubation with all analogs tested. The most potent analog, CC-5013, blocked the migration of EC in a wound-healing assay, but did not inhibit EC proliferation. Daily i.p. administration of CC-5013 slowed tumor growth in a mouse model of colorectal cancer [13]. A different IMiD has also been found to inhibit angiogenesis and growth of B cell neoplasias in mice [22]. More recently, IMiDs were found to be significantly more potent than thalidomide *in vivo* in suppressing tumor growth as well as mediating anti-angiogenic effects in a mouse model for B cell malignancies [23].

Direct anti-tumor properties

It has recently become evident that IMiD analogs possess direct anti-myeloma activity in the absence of accessory cells [3,25]. Initial results suggest that IMiDs are far more effective than thalidomide or SelCIDs. A range of myeloma cell lines and chemoresistant primary human myeloma cells derived from patient bone marrow were shown to be susceptible to IMiD-induced G_1 growth arrest. This anti-myeloma effect could be overcome by the addition of exogenous IL-6, suggesting that inhibition of IL-6 may be the mechanism for this effect. The induction of either myeloma cell cycle arrest or apoptosis by IMiDs was dependent on p53 status since the presence of wild-type p53 facilitated G_1/S transition and subsequent apoptosis. Conversely, in cells with mutant p53, including patient cells, IMiDs induce G_1 arrest, but not apoptosis [25]. Therefore, it is likely that the release of IMiD/thalidomide-induced G_1 arrest may

lead to the re-growth of myeloma cells on discontinuation of treatment.

A follow-up study investigated the effect of IMiDs on apoptotic pathways in myeloma cells [15]. The study demonstrated multiple effects of IMiDs including inhibition of NF-κB and enhanced sensitivity to Fas-induced apoptosis. IMiD activity was able to potentiate TRAIL, dexamethasone and proteosome inhibitor anti-myeloma therapy. Furthermore, Gupta *et al.* also showed strong evidence that IMiDs can interfere with myeloma cell–bone marrow stromal cell interactions, and prevent the up-regulation of IL-6 and vascular endothelial growth factor [26].

More recently, a novel subgroup of SelCIDs analogs has been identified with direct anti-tumor effects on solid tumor cell lines including melanoma, prostate, colorectal and pancreatic [11], and myeloma cells (manuscript in preparation). In contrast to the effects of IMiDs in multiple myeloma, growth arrest was achieved via the early induction of G_2/M cell cycle arrest, which led to caspase-3-mediated apoptosis. This activity was shown to be independent of known PDE type 4 activity, implying that it is a novel activity. Furthermore, it was also independent of the p53 status of the tumor cell.

This has implications in the treatment of advanced chemoresistant cancers and should enable apoptosis of the tumor cells. Apoptosis was also associated with altered expression of the bcl family proteins, in particular, increased bax:bcl2 and bak:bcl2 ratios were apparent [11]. These results suggest that such compounds are distinct from previously characterized thalidomide analogs, since IMiDs induce G_1 arrest in myeloma cells which leads only to apoptosis in wild-type p53 cells [25].

Finally, a structure–activity relationship study found enhanced anti-metastatic action of thalidomide analogs known as 2-phthalimidinoglutaric acid (PGA) analogs over thalidomide itself in the B16 murine melanoma model [27]. The increased stability of the PGA series of analogs may provide additional help in the identification of the cellular targets, and hence the mechanism of the anti-cancer action of thalidomide and its analogs.

Clinical trials

Thalidomide analogs are beginning to emerge into clinical trials in conditions where there is little other therapeutic option. In this regard, the National Cancer Institute is supporting both the research and clinical development of IMiD and SelCID analogs. Table 2 shows examples of ongoing clinical trials of thalidomide analogs.

Table 2 Examples of thalidomide analogs in clinical development

Drug	Condition	Phase
CC-5013 (REVIMID™)	glioma	I
	refractory metastatic disease	I
	solid tumors	I
	lymphoma	I/II
	multiple myeloma	II/III
CC-4047 (ACTIMID™)	refractory and relapsed multiple myeloma	I/II
	prostrate cancer	II
CC-1088	myelodysplastic syndromes	II
CC-8490	recurrent high-grade gliomas	I/II

Complied from the National Cancer Institute (www.nci.nih.gov), the National Institute of Health (www.clinicaltrials.gov) and Celgene Corp. (www.celgene.com).

In initial studies, CC-5013 has been reported in two phase I dose-escalating clinical trials for refractory and relapsed multiple myeloma [28,29]. In the former study, 12 of 19 patients showed at least a 25% paraprotein reduction, while in the latter study, 20% of patients showed more than 50% paraprotein reduction with a concomitant bone marrow response. More recently, 79% of the 24 patients receiving CC-5013 were reported to experience stable disease or other clinical improvement without many of the side-effects observed with thalidomide [Source: Clinical Updates from the Angiogenesis Foundation, 2002]. Another IMiD CC-4047 has recently been found to possess an acceptable safety profile in a phase I trial for relapsed/refractory multiple myeloma [30]. From the cohort of 18 patients all showed signs of clinical improvement as demonstrated by the M-protein response: less than 25% in eight of 18 (44%), 25–50% in seven of 18 (39%) and greater than 50% in three of 18 (17%). On a named patient basis, eight of 18 developed greater than 50% M-protein response, including one complete response and two near complete responses. The authors concluded that CC-4047 has an acceptable toxicity profile with anti-tumor activity, and should be evaluated in future phase II studies in hematological and solid tumor malignancies.

A phase I trial of solid tumors (mainly stage IV advanced melanoma) also revealed CC-5013 to be safe and lead to clear objective responses, such as reduction of s.c. lesions, in seven of 20 patients. Furthermore, evidence of T cell activation (increased CD45RO expression on $CD4^+$ and $CD8^+$ cells and increased shedding of IL-2 receptor), and increases in serum cytokines such as TNF-α, IL-12 and GM-CSF, suggested that T cells and monocytes/macrophages were being activated following CC-5013 treatment [31]. Other trials using CC-5013 are currently recruiting for patients with glioma, leukemia, lymphoma, myleodysplastic syndrome and solid neoplasms [Source: www.clinicaltrials.gov].

There have been no published reports of SelCIDs in clinical trials for cancer indications. However, the lead SelCID CC-1088 is being studied as a potential

treatment for myelodysplastic syndromes in a phase II trial. A second SelCID, CC-7085, was well tolerated in a phase I trial and a third, more potent SelCID is expected to begin clinical trials in 2003. Another compound, CC-8490, found to cause marked inhibition of glioma growth in preclinical *in vivo* models, was safe and well tolerated in healthy human volunteers [Source: www.celgene.com].

Conclusions

The preclinical data on IMiDs and SelCIDs has demonstrated that both classes of thalidomide analogs show potential as anti-cancer agents. Immunomodulatory, anti-angiogenic and anti-inflammatory activities of these analogs provide novel mechanistic avenues for cancer treatment. For example, IMiDs which possess T cell co-stimulatory activity may be used in conjunction with cancer vaccines to boost immune responses against tumors. The anti-angiogenic effects of thalidomide analogs have resulted in reduced tumor growth in preclinical models and were hypothesized to be a reason for clinical efficacy in some cancers. Inhibition of gastrointestinal tumors by anti-inflammatory agents suggests that IMiDs and especially SelCIDs may slow or prevent tumor growth by interfering with inflammatory pathways.

The ability of an analog from within the SelCID group to directly inhibit tumor growth *in vitro* and *in vivo* suggests that this analog could provide effective treatment as a single agent for susceptible tumors. Elucidation of the mechanism of action for each analog in a particular setting should provide new avenues of treatment for a number of cancers.

The reduced side-effect profile of these analogs has ensured their transition into clinical trials. Encouraging early reports indicate that clinical responses have been observed in some patients treated for refractory and relapsed multiple myeloma and late-stage metastatic melanoma. Phase II trials of CC-4047 in prostate cancer and phase III of CC-5013 for multiple myeloma and metastatic melanoma should further provide evidence that thalidomide analogs are emerging anti-cancer drugs.

References

1 Thomas DA, Kantarjian HM. Current role of thalidomide in cancer treatment. *Curr Opin Oncol* 2000; **12**:564–573.
2 Gasparini G, Morabito A, Magnani E, Gattuso D, Capaccetti B, Alberti AM. Thalidomide: an old sedative-hypnotic with anticancer activity? *Curr Opin Invest Drugs* 2001; **2**:1302–1308.
3 Raje N, Anderson KC. Thalidomide and immunomodulatory drugs as cancer therapy. *Curr Opin Oncol* 2002; **14**:635–640.
4 Singhal S, Mehta J, Desikan R, Ayers D, Roberson P, Eddlemon P, *et al.* Antitumor activity of thalidomide in refractory multiple myeloma. *N Eng J Med* 1999; **341**:1565–1571.
5 Richardson P, Hideshima T, Anderson K. Thalidomide in multiple myeloma. *Biomed Pharmacother* 2002; **56**:115–128.
6 Muller GW, Corral LG, Shire MG, Wang H, Moreira A, *et al.* Structural modifications of thalidomide produce analogs with enhanced tumor necrosis factor inhibitory activity. *J Med Chem* 1996; **39**:3238–3240.
7 Corral LG, Muller GW, Moreira AL, Chen Y, Wu M, Stirling D, *et al.* Selection of novel analogs of thalidomide with enhanced tumor necrosis factor alpha inhibitory activity. *Mol Med* 1996; **2**:506–515.
8 Muller GW, Shire MG, Wong LM, Corral LG, Patterson RT, Stirling DI. Thalidomide analogs and PDE4 inhibition, *Bioorg Med Chem Lett* 1998; **8**:2669–2674.
9 Muller GW, Chen R, Huang SY, Corral LG, Wong LM, Patterson RT, *et al.* Amino-substituted thalidomide analogs: potent inhibitors of TNF-alpha production. *Bioorg Med Chem Lett* 1999; **9**:1625–1630.
10 Marriott JB, Westby M, Cookson S, Guckian M, Goodbourn S, Muller G, *et al.* CC-3052: a water soluble analog of thalidomide and potent inhibitor of activation-induced TNF-α production. *J Immunol* 1998; **161**:4236–4243.
11 Marriott JB, Clarke IA, Czajka A, Dredge K, Childs K, Man HW, *et al.* A novel subclass of thalidomide analogue with anti-solid tumor activity in which caspase dependent apoptosis is associated with altered expression of bcl-2 family proteins. *Cancer Res* 2003; **63**:593–599.
12 Dredge K, Marriott JB, Todryk SM, Muller GW, Chen R, Stirling DI, *et al.* Protective antitumor immunity induced by a co-stimulatory thalidomide analog in conjunction with whole cell vaccination is mediated by increased T_h1-type immunity. *J Immunol* 2002; **168**:4914–4919.
13 Dredge K, Marriott JB, Macdonald CD, Man HW, Chen R, Muller GW, *et al.* Novel thalidomide analogs display anti-angiogenic activity independently of immunomodulatory effects. *Br J Cancer* 2002; **87**:1166–1172.
14 Raje N, Anderson KC. Thalidomide and Immunomodulatory drugs as cancer therapy. *Curr Opin Oncol* 2002; **14**:635–640.
15 Mitsiades N, Mitsiades CS, Poulaki V, Chauhan D, Richardson PG, Hideshima T, *et al.* Apoptotic signalling induced by immunomodulatory thalidomide analogs in human multiple myeloma cells: therapeutic implications. *Blood* 2002; **99**:4525–4230.
16 Haslett PA, Corral LG, Albert M, Kaplan G. Thalidomide co-stimulates primary human T lymphocytes, preferentially inducing proliferation, cytokine production, and cytotoxic responses in the $CD8^+$ subset. *J Exp Med* 1998; **187**:1885–1892.
17 Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ, *et al.* Differential cytokine modulation and T cell activation by two distinct classes of Thalidomide analogs that are potent inhibitors of TNF-α. *J Immunol* 1999; **163**:380–386.
18 Moller DR, Wysocka M, Greenlee BM, Ma X, Wahl L, Flockhart DA, *et al.* Inhibition of IL-12 production by thalidomide. *J Immunol* 1997; **159**:5157–5161.
19 Marriott JB, Clarke IA, Dredge K, Muller G, Stirling D, Dalgleish AG. Thalidomide and its analogs have distinct and opposing effects on TNF-α and TNFR2 during co-stimulation of both $CD4^+$ and $CD8^+$ cells. *Clin Exp Immunol* 2002; **130**:75–84.
20 Noguchi T, Shimazawa R, Nagasawa K, Hashimoto Y. Thalidomide and its analogues as cyclooxygenase inhibitors. *Bio Med Chem Lett* 2002; **12**:1043–1046.
21 Fujita J, Mestre JR, Zeldis JB, Subbaramaiah K, Dannenberg AJ. Thalidomide and its analogues inhibit lipopolysaccharide-mediated induction of cyclooxygenase-2. *Clin Can Res* 2001; **7**:3349–3355.
22 Lentzsch S, Rogers MS, LeBlanc R, Birsner AE, Shah JH, Treston AM, *et al.* *S*-3-Amino-phthalimido-glutarimide inhibits angiogenesis and growth of B-cell neoplasias in mice. *Cancer Res* 2002; **62**:2300–2305.
23 Lentzsch S, LeBlanc R, Podar K, Davies F, Lin B, Hideshima T, *et al.* Immunomodulatory analogs of thalidomide inhibit growth of Hs Sultan cells and angiogenesis *in vivo*. *Leukemia* 2003; **17**:41–44.
24 Moreira AL, Friedlander DR, Shif B, Kaplan G, Zagzag D. Thalidomide and a thalidomide analogue inhibit endothelial cell proliferation *in vitro*. *J Neuro-Oncol* 1999; **43**:109–114.
25 Hideshima T, Chauhan D, Shima Y, Raje N, Davies FE, Tai YT, *et al.* Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. *Blood* 2002; **96**:2943–2950.
26 Gupta D, Treon SP, Shima Y, Hideshima T, Podar K, Tai YT, *et al.* Adherence of multiple myeloma cells to bone marrow stromal cells upregulates vascular endothelial growth factor secretion: therapeutic applications. *Leukemia* 2001; **15**:1950–1961.
27 Shah JH, Swartz GM, Papathanassiu AE, Treston AM, Fogler WE, Madsen JW, *et al.* Synthesis and enantiomeric separation of 2-phthalimidino-glutaric acid analogues: potent inhibitors of tumor metastasis. *J Med Chem* 2002; **42**:3014–3017.
28 Richardson PG, Schlossman RL, Weller E, Hideshima T, Lentzsch S, Davies F, *et al.* Immunomodulatory drug CC-5013 overcomes drug resistance and

is well tolerated in patients with relapsed multiple myeloma (MM). *Blood* 2002; **100**:3062–3067.

29 Zangari M, Tricot G, Zeldis J, Eddlemon P, Saghafifar F, Barlogie B. Results of phase I study of CC-5013 for the treatment of multiple myeloma (MM) patients who relapse after high dose chemotherapy (HDCT). *Blood* 2001; **98**:775a.

30 Schey SA, Jones RW, Raj K, Streetley M. A phase I study of an immunomodulatory drug (CC-4047), a structural analogue of thalidomide, in relapsed/refractory multiple myeloma. In: *Proc 31st Annu Meet Int Soc Exp Haematol* 2002.

31 Marriott JB, Clarke IA, Dredge K, Pandha H, Kristaleit H, Polychronis A, *et al.* Thalidomide analogue CDC-501 is safe and well tolerated by patients with end stage cancer and shows evidence of clinical responses and extensive immune activation. *Br J Cancer* 2002; **86**:S26–S26.