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Competing interests statement

The author declares that he has no competing financial interests.

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TIMELINE

The evolution of thalidomide and its IMiD derivatives as anticancer agents

J. Blake Bartlett, Keith Dredge and Angus G. Dalgleish

Thalidomide was originally used to treat morning sickness, but was banned in the 1960s for causing serious congenital birth defects. Remarkably, thalidomide was subsequently discovered to have anti-inflammatory and anti-angiogenic properties, and was identified as an effective treatment for multiple myeloma. A series of immunomodulatory drugs — created by chemical modification of thalidomide — have been developed to overcome the original devastating side effects. Their powerful anticancer properties mean that these drugs are now emerging from thalidomide's shadow as useful anticancer agents.

Thalidomide (α -(*N*-phthalimido)glutarimide) — a synthetic glutamic-acid derivative — was manufactured and marketed by the German pharmaceutical company Chemie Grunenthal during the mid-1950s (BOX 1; TIMELINE). It is a non-barbiturate drug with sedative and anti-emetic activity and was found to be useful because of an apparent lack of toxicity in human volunteers. These properties led to it being marketed as the safest available sedative of its time. It rapidly became popular as a drug to counter the effects of morning sickness in Europe, Australia, Asia and South America, although it did not receive Food and Drug Administration (FDA) approval in the United States because of concerns about neuropathy — tingling hands and feet after long-term administration — that were associated with its

use. It was withdrawn from the other markets in early 1961 after two clinicians — William McBride in Australia and Widukind Lenz in Germany — reported independently that thalidomide use was associated with birth defects^{1,2}. A report associating thalidomide use with neuropathies was also reported at around this time³. Unfortunately, this withdrawal was too late to prevent the birth of between 8,000 and 12,000 babies with severe developmental deformities, which include the stunted-limb development that is characteristic of 'thalidomide babies'.

In 1965, following a serendipitous discovery by Israeli dermatologist Jacob Sheskin, it was reported that thalidomide was remarkably effective at improving lesions, fever and night sweats in patients with erythema nodosum leprosum (ENL) — a potentially life-threatening inflammatory complication of lepromatous leprosy⁴. After finding thalidomide in the clinic and remembering that it was a sedative, Sheskin administered it to a patient who was having trouble sleeping and — remarkably — the next morning the patient's inflammation was significantly reduced. This discovery was investigated in a study that was coordinated by the World Health Organization in thousands of men who had ENL and showed that a vast majority had complete remission within a couple of weeks of starting thalidomide treatment⁵. This was the catalyst that eventually led to the use of thalidomide as an immunomodulatory

and anti-inflammatory drug^{6–8}. However, thalidomide was only given FDA approval for the treatment of acute ENL in 1998, after further investigations found an immunological basis for this effect⁹. Even then, its use was limited by very strict guidelines.

It is now clear that despite its teratogenicity (BOX 1), which caused the birth defects, thalidomide is useful in treating several clinical conditions for which there are few or no alternative treatment options. An early appreciation of the immunosuppressive properties of thalidomide in several animal models led to its use in various conditions that are associated with immune activation. Initial, but mainly anecdotal, reports from the early 1980s onwards indicated that thalidomide was effective in the treatment of several autoimmune disorders. However, because the use of thalidomide was necessarily restricted, large-scale studies were not undertaken until much later. Instead, the results of various small uncontrolled studies were published and these seemed to demonstrate the efficacy of thalidomide in the treatment of patients with autoimmune disorders such as rheumatoid arthritis¹⁰, cutaneous lesions of systemic lupus erythematosus and Behcet's disease^{11,12}. The immunosuppressive properties of thalidomide also led to its use in the treatment of chronic graft-versus-host disease associated with allogeneic bone-marrow transplantation^{13–15}.

As thalidomide initially seemed to show promise for the treatment of these conditions, it was quickly used in further studies in small cohorts of patients with various untreatable ailments. From these investigations, it has become apparent that thalidomide is not merely an immunosuppressant, but that it has other clinically useful properties. Each new property that has been discovered has led to thalidomide being used in different spectra of disease. As a result, thalidomide is now an option for a diverse range of clinical applications and is again a profitable drug, with sales that amount to over \$200 million per year in the United States and rising.

Mechanisms of thalidomide action

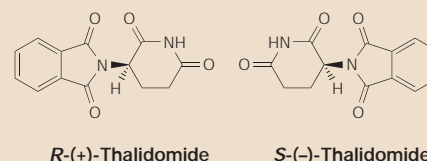
Thalidomide inhibits monocyte-derived TNF- α . The key finding that explained, at least in part, the potent anti-inflammatory activity of thalidomide came in 1991, when it was discovered that thalidomide inhibited the synthesis of tumour-necrosis factor- α (TNF- α) by activated monocytes¹⁶ — the mRNA becomes less stable. TNF- α is a pro-inflammatory cytokine that is an important regulator of the inflammatory cascade and is a useful therapeutic target in inflammatory disease, particularly if activated monocytes

have an important role in pathogenesis (FIG. 1). There is also evidence that thalidomide might inhibit TNF- α that is derived from other cellular sources that have been activated by inflammatory stimuli, such as microglia and Langerhans cells^{17,18}. The fact that thalidomide inhibits TNF- α explains its therapeutic effect in patients with ENL, as they have extremely high levels of TNF- α in their blood and in dermatological lesions. Most importantly, this finding led to the initial use of thalidomide in several, small open-label studies in which increased TNF- α production is associated with disease¹⁹, such as AIDS-related Kaposi's sarcoma and cachexia, rheumatological disease, Crohn's disease, cerebral malaria, multiple sclerosis, psoriasis, sepsis, tuberculosis and some cancers^{6,20}.

Thalidomide inhibits angiogenesis. The next crucial discovery that uncovered the clinical potential of thalidomide came in 1994, when thalidomide was found to inhibit angiogenesis — the formation of new blood vessels, which is a crucial process in the growth and metastasis of solid tumours. Judah Folkman was one of the first researchers to associate angiogenesis with tumour development in the early 1970s and it was from his laboratory that the inhibitory effect of thalidomide on angiogenesis was demonstrated. He believed that the classical congenital defects that are caused by thalidomide treatment — abnormal limb development — were caused by the inhibition of blood-vessel growth in the developing fetal limb bud. Using a rabbit cornea micropocket assay, it was demonstrated that thalidomide could, in fact, inhibit basic fibroblast growth factor (bFGF)-induced angiogenesis²¹. However, despite this study, it is worth noting that the link between the teratogenic properties of thalidomide and its anti-angiogenic activity

Box 1 | The chemistry of thalidomide

Thalidomide consists of a racemic mixture of *S*(-) and *R*(+) enantiomers (isoforms) — molecules with identical chemical composition that are mirror images of one another and that can not be superimposed (see figure). In nature, compounds often exist as enantiomers, although generally only one form is physiologically useful. In the case of thalidomide, there seems to be a segregation of activities between these different forms. Of particular interest in terms of potential clinical application has been the association of the *S*(-) enantiomer with the teratogenic effects of thalidomide, which are responsible for the abnormalities that occur during embryonic development, whereas the *R*(+) isoform seems to be responsible for sedation. This indicated that purification of the *R*(+) isoform, although less effective as a TNF- α inhibitor and anti-angiogenic agent, could provide a safer drug and could have prevented the earlier tragic events. However, the rapid interconversion of the two isomers under physiological conditions, as demonstrated in humans *in vivo*, proved that purification is not a feasible option. Furthermore, although the two isoforms of thalidomide were initially shown to have different teratogenic effects in rodent models, differences were not observed in the New Zealand rabbit model that is traditionally used to measure drug toxicity.



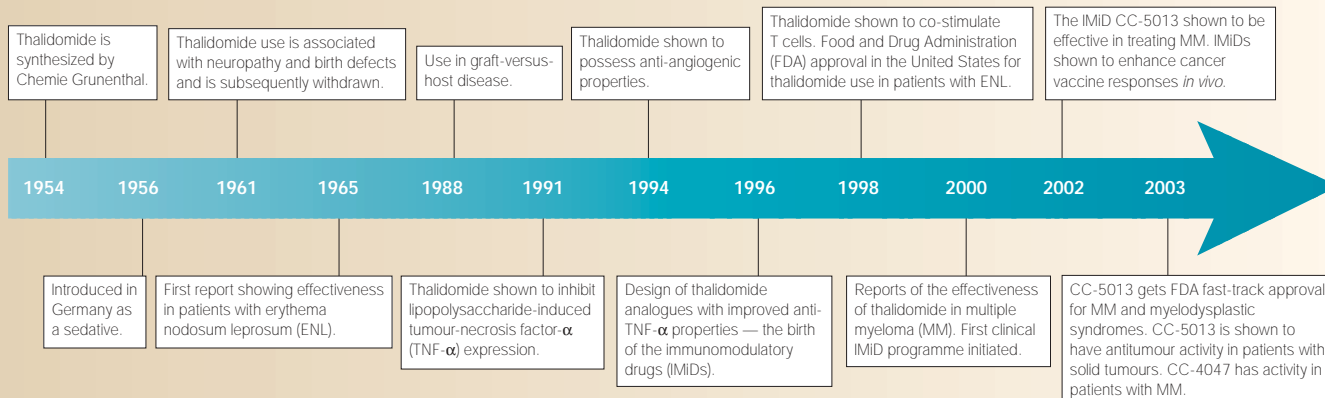
remains unproven. Other groups have more recently demonstrated that thalidomide mediates inhibitory effects on mesenchymal proliferation in the limb bud²² and induces embryonic oxidative stress²³. Irrespective of these findings, the anti-angiogenic properties of thalidomide sparked a huge interest in its use for the treatment of cancer.

T-cell co-stimulatory activity of thalidomide. Yet another activity of thalidomide was demonstrated in 1998, when it was shown that thalidomide is able to co-stimulate T cells that have been partially activated by the T-cell receptor (TCR; FIG. 2)²⁴. Co-stimulation is the crucial process by which a second signal is delivered to naive T cells, which facilitates their activation and the subsequent generation of an antigen-specific effector

response. It is mediated by interactions between members of the B7 family of proteins on antigen-presenting cells and the CD28 co-stimulatory molecule that is expressed on the surface of T cells. This interaction, in conjunction with the primary TCR-mediated signal, prevents the induction of immunological tolerance (or anergy), which would occur in the presence of the TCR alone.

The co-stimulatory activity of thalidomide is important as it could be used as an immunological adjuvant to promote an otherwise ineffective immune response. For example, it could provide an alternative approach for treating patients with cancer by enhancing their response to tumour antigens. However, it should be noted that the immunomodulatory effects of thalidomide

Timeline | Chronology of thalidomide and IMiD development



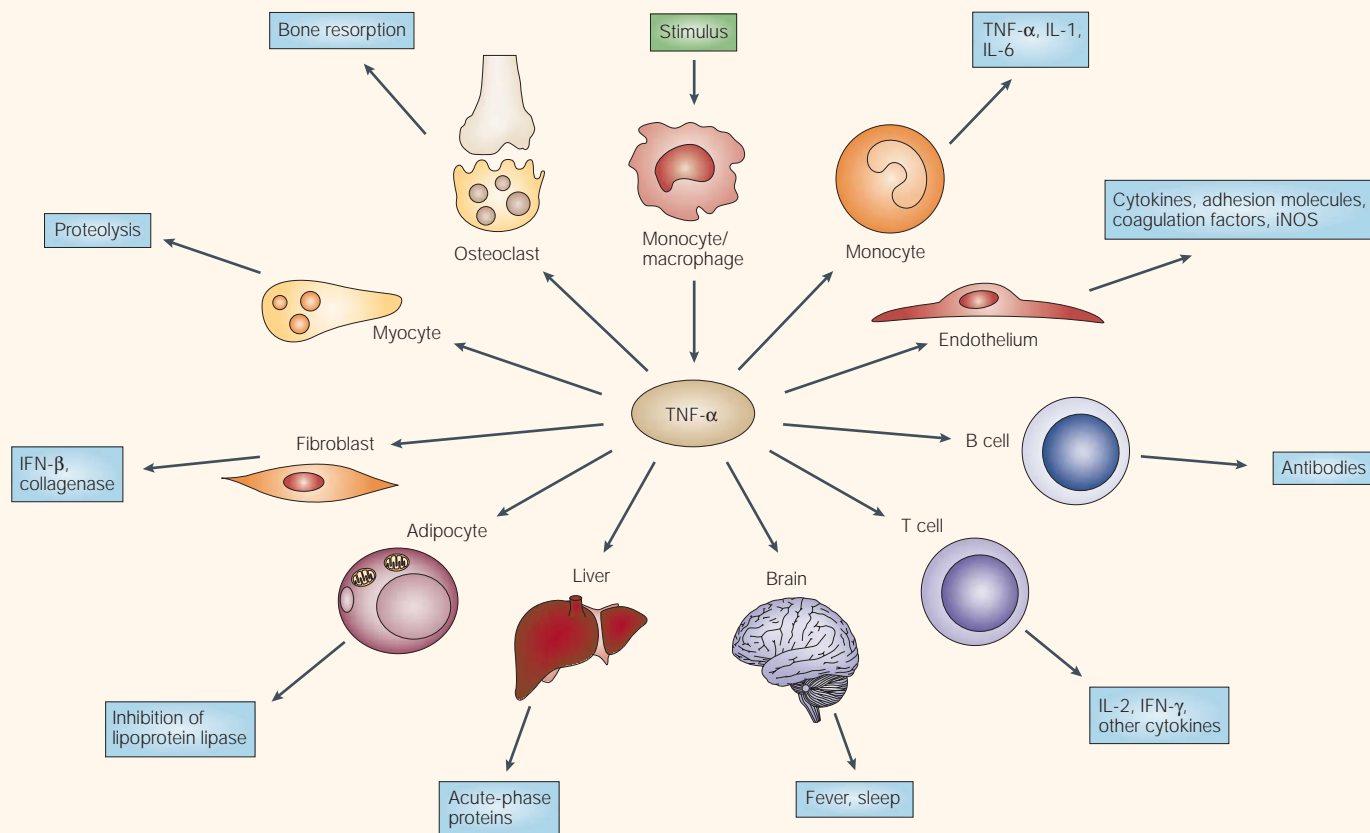


Figure 1 | **Tumour-necrosis factor- α has numerous targets.** Tumour-necrosis factor- α (TNF- α) is mainly produced by monocytes and macrophages, but is also produced by other cell types, in response to a large number of stimuli and physiological conditions. TNF receptors are expressed on most cell types, which respond to TNF- α by activating a range of transcription factors and gene products. Effects on TNF- α bioactivity, therefore, directly influence a diverse range of cell activities. IFN, interferon; IL, interleukin; iNOS, inducible nitric-oxide synthase. Figure adapted with permission from Ref. 57 © (1997) Elsevier Science Publishers.

are variable, and depend on the type of immune cell that is activated, as well as the type of stimulus that the cell receives. Therefore, the effects of thalidomide on a particular cohort of patients are likely to depend on their disease state and immunological status. For example, thalidomide-mediated inhibition of the key pro-inflammatory and regulatory cytokines TNF- α ¹⁶ and interleukin-12 (IL-12; REF. 25) during microbial stimulation of monocytes could be countered by thalidomide-mediated augmentation of the same cytokines during T-cell activation. This differential response might explain the clinically diverse effects of thalidomide, which include beneficial activity in some autoimmune conditions that are associated with increased T helper 1 (T_H1)-type cellular immunity and some cancers that are associated with lack of tumour-specific T_H1-type cellular immunity. Therefore, thalidomide can no longer be referred to simply as a TNF- α inhibitor, as T-cell co-stimulation is likely to explain the unexpected increase in TNF- α production that is observed in certain clinical settings²⁶.

Thalidomide: an anticancer agent
 The main impetus for using thalidomide to treat patients with cancer came with the discovery of its anti-angiogenic potential. This also happened to coincide with the emerging concept that treatment could be aimed at the infrastructure that supports the growth of the tumour, rather than targeting tumour cells directly. Similarities between the angiogenic process in the promotion of tumour growth and in chronic inflammation also lent further support for a possible role for thalidomide as an anti-inflammatory agent in the treatment of cancers. In particular, the anti-TNF- α effects of thalidomide were thought to be relevant, as TNF- α seems to have a role in angiogenesis by upregulating the expression of endothelial integrin, which is crucial for this process²⁷. Finally, it is well established that the increase of TNF- α in the serum of patients with cancer is often associated with advanced disease, so using thalidomide to reduce these levels might prove to be beneficial in the treatment of patients.

Thalidomide and multiple myeloma
 In the past few years, thalidomide has begun to impact on the treatment of **multiple myeloma** (MM; BOX 2). This is an incurable B-cell malignancy in which increased bone-marrow microvessel density (MVD) is associated with poor prognostic outcome, providing the rationale for treatment with thalidomide. Remarkably, an initial report published in 1999 indicated that thalidomide was an effective treatment in 30–40% of patients with advanced and refractory MM²⁸ and showed that, of the 84 patients treated, there was an overall clinical response rate of 32%. Moreover, 10% of patients had complete, or near complete, remissions. Partial remission — defined by a >50% decrease in serum or urine monoclonal protein, an established prognostic indicator — was achieved in 25% of patients. The authors of this study were unable to show an association between the clinical response to thalidomide and a decrease in bone-marrow MVD. However, very recent data showing decreased MVD only in patients who responded to thalidomide does support the theory that angiogenesis is a

therapeutic target in MM²⁹. Subsequent studies have confirmed these initial clinical findings and indicate that thalidomide treatment leads to a 25–33% response rate in patients with refractory MM, and also has significant response rates in patients at other stages of disease³⁰. More recently, thalidomide treatment in combination with the chemotherapeutic agent dexamethasone has been shown to act synergistically and induce even greater partial response rates of 60–70%, even when patients have been unresponsive to either agent alone³¹. Addition of cyclophosphamide seems to improve the response rates still further³².

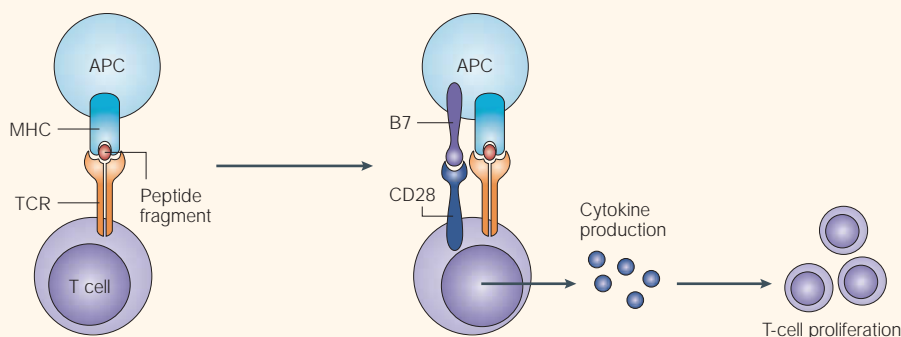
Since this discovery, thalidomide has also been evaluated in clinical trials as a treatment for various solid tumours with a varying degree of success. There are published reports of efficacy in the treatment of patients with solid tumours such as advanced renal cancer³³, metastatic prostate cancer³⁴, high-grade glioma³⁵ and metastatic melanoma³⁶. More complete overviews of thalidomide use in patients with solid tumours can be found elsewhere^{37,38}.

Development of IMiDs

Clearly, even in patients with advanced cancer, the use of thalidomide could present significant problems due to its teratogenic side effects. This requires intense patient monitoring during thalidomide administration. Therefore, it is hardly surprising that not long after the discovery of the anti-angiogenic properties of thalidomide, and given its obvious clinical benefits, attempts were made to synthesize thalidomide analogues that had fewer side effects than the parent compound.

Immunomodulatory drugs (IMiDs) are a series of compounds that were developed by using the first-generation IMiD thalidomide as the lead compound in a drug-discovery programme. The thalidomide structural backbone was used as a template by chemists to design and synthesize compounds with increased immunological and anticancer properties, but lacking the toxicity associated with the parent compound³⁹. Initially, the rationale for developing the second-generation IMiDs in the mid 1990s was to improve the inhibition of TNF- α ^{40,41} and, with this aim, a series of amino-phthaloyl-substituted thalidomide analogues were generated⁴². The 4-amino analogues — in which an amino group is added to the fourth carbon of the phthaloyl ring of thalidomide — were found to be up to 50,000 times more potent at inhibiting TNF- α than the parent compound *in vitro*. Extensive preclinical testing, involving

a Activation of naive T cells requires co-stimulation



b IMiDs overcome the requirement for co-stimulation

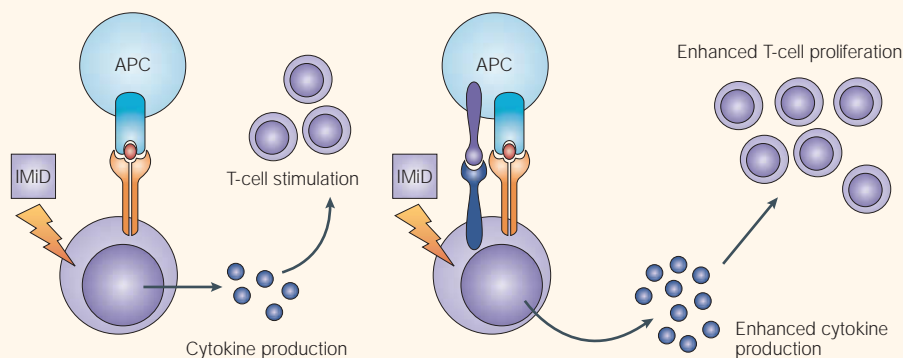


Figure 2 | **Co-stimulatory activity of thalidomide.** **a** | Antigen-presenting cells (APCs) activate T cells by presenting major histocompatibility complex (MHC)-bound peptides to the T-cell receptor (TCR). Effective T-cell activation also requires interaction between accessory molecules, such as B7 on the APC and CD28 on the T cell, which provides the secondary signals that are necessary for activation to occur and prevents T-cell anergy (non-responsiveness). **b** | Thalidomide and immunomodulatory drugs (IMiDs) seem to enhance TCR-mediated signalling both in the absence and presence of these secondary signals, thereby enhancing immune responses.

pharmacology, pharmacokinetics and toxicity, has led to the identification of CC-5013 (Revimid) and CC-4047 (Actimid) for testing in clinical trials (FIG. 3).

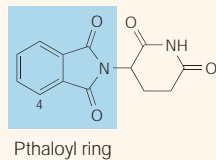
Third-generation IMiDs developed from the ongoing research programme are now in preclinical testing and will be investigated in

clinical trials if modifications to the second-generation compounds are necessary. Furthermore, as the emphasis during pre-clinical testing has changed from the anti-TNF- α activity of the IMiDs to their anti-angiogenic and immunomodulatory activities, it is possible that third-generation

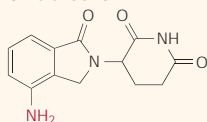
Box 2 | Multiple myeloma

Multiple myeloma (MM) is a B-cell malignancy that is incurable at present. It is characterized by the clonal proliferation of malignant cells in the bone marrow that leads to the production of a monoclonal immunoglobulin. MM accounts for approximately 1–2% of all cancers and cancer deaths, and afflicts 14,000–15,000 patients annually in the United States alone. The current median survival rate for symptomatic patients is 3–5 years. High-dose chemotherapy — typically melphalan and prednisolone — combined with transplantation of haematopoietic stem cells increases the rate of complete remission and extends event-free and overall survival. However, little progress in developing effective treatment regimens has been made over the past few decades; relapse rates are very high and there are few salvage therapies available. Thalidomide treatment was initiated in MM because this condition correlates with prominent bone-marrow vascularization, which is associated with poor prognosis. In addition, plasma levels of various pro-angiogenic molecules, such as basic fibroblast growth factor and vascular endothelial growth factor, are increased in patients with active MM. Therefore, anti-angiogenic drugs, such as thalidomide, are viable therapeutic options.

a Thalidomide



b CC-5013



CC-4047

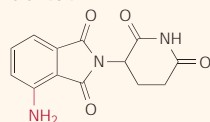


Figure 3 | Structure of thalidomide and the IMiDs CC-5013 and CC-4047. The thalidomide structure (a) was modified by adding an amino (NH₂-) group at the 4 position of the phthaloyl ring to generate the IMiDs CC-5013 and CC-4047 (b). For CC-5013, one of the carbonyls (C = O) of the 4-amino-substituted phthaloyl ring has been removed.

IMiDs could have greater anticancer activity and/or enhance immune responses. Because of the structural similarity with thalidomide, the IMiDs possess the same properties that are of potential benefit to patients with cancer — prevention of angiogenesis and co-stimulation of T cells.

IMiD functions

Angiogenesis. Recent results have confirmed that the IMiDs, in particular the clinical lead compounds CC-5013 and CC-4047, are anti-angiogenic^{43,44}. However, as with thalidomide, the mechanism(s) remains elusive. Data from *in vitro* experiments indicate that IMiDs vary in their ability to inhibit endothelial-cell proliferation. Indeed, the oral administration of CC-5013 is able to inhibit tumour growth in a mouse model of colorectal cancer despite having no effect on endothelial-cell proliferation *in vitro*. Of particular interest is the observation that CC-5013 seems to be non-teratogenic when tested in the sensitive New Zealand rabbit preclinical model, which is the only animal model in which thalidomide-associated teratogenicity can be detected.

T-cell co-stimulation. IMiDs are far more potent than thalidomide at co-stimulating T-cells that have been partially activated via the TCR⁴⁵. Furthermore, co-stimulation applies equally to CD4⁺ and CD8⁺ T cells. The potency of IMiD-induced co-stimulation seems to increase when TNF receptor 2 transport to the cell membrane is inhibited. The implications of this are unclear, although this is likely to affect T-cell homeostasis. More recently, IMiDs have been shown to trigger

the phosphorylation of CD28 and also to enhance the activity of the AP-1 transcription factor^{46,47}. However, the precise mechanism(s) that is involved in IMiD-mediated T-cell co-stimulation remains to be elucidated.

There is now clear evidence to indicate that the co-stimulatory properties of IMiD analogues *in vitro* can translate to beneficial antitumour responses *in vivo*. It has been demonstrated that CC-4047 is able to enhance a partially effective cancer vaccine and enable the generation of a long-term protective antitumour response⁴⁸. Protection seems to be mediated by the induction of protective T_H1-type cellular immunity as, although CD8⁺ cells were activated, there were more CD4⁺ cells responding to the tumour cells that comprised the vaccine. Some protection is also seen when CC-4047 is co-administered with a tumour vaccine shortly after live-tumour challenge in mice. However, a booster regimen seems to be required. This more closely mirrors the clinical situation, the aim of which is to treat an established tumour and protect against the formation of new tumours and the regrowth of residual tumour after surgical resection.

There is also emerging evidence that IMiDs can activate the innate component of the immune system. For example, CC-5013 seems to augment the cytotoxicity of natural-killer cells, leading directly to lysis of MM cells⁴⁹. Furthermore, CC-4047 seems to have a potent augmentary effect on CD28-negative $\gamma\delta$ T cells that have been stimulated with their natural bacterial antigen isopen-tenyl pyrophosphate, (J.B.B., unpublished

observations). These cells are also able to directly lyse tumour cells, augment the early expression of cytokines in response to bacterial infection and help the development of the adaptive immune response.

Direct antitumour activity of IMiDs Surprisingly, the IMiDs were found to share another important anticancer property — the ability to directly induce growth arrest and caspase-dependent apoptosis of tumour cells⁵⁰. Initial preclinical data showed that CC-5013 possesses direct anti-myeloma activity in the absence of accessory immune cells^{50,51}. Primary human MM cells derived from the bone marrow of patients resistant to chemotherapy were shown to be susceptible to IMiD-induced growth arrest. This could be overcome by the exogenous addition of the pro-inflammatory cytokine IL-6, indicating that inhibition of IL-6 is likely to be involved in the mechanism that regulates this effect. Importantly, other mechanistic details have begun to emerge, including effects on apoptotic pathways⁵². Furthermore, IMiD activity is able to potentiate the effects of TRAIL (TNF-related apoptosis-inducing ligand), dexamethasone and proteasome inhibitors that are used as anti-myeloma therapies at present. There is also strong evidence that IMiDs can interfere in interactions between myeloma cells and bone-marrow stromal cells, which seem to be crucial for MM-cell growth and survival, and prevent the upregulation of IL-6 and vascular endothelial growth factor, which is involved in angiogenesis⁵³ (FIG. 4).

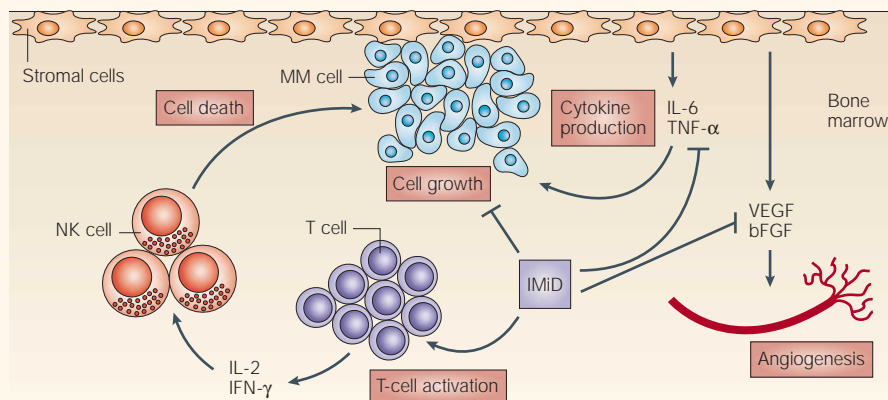


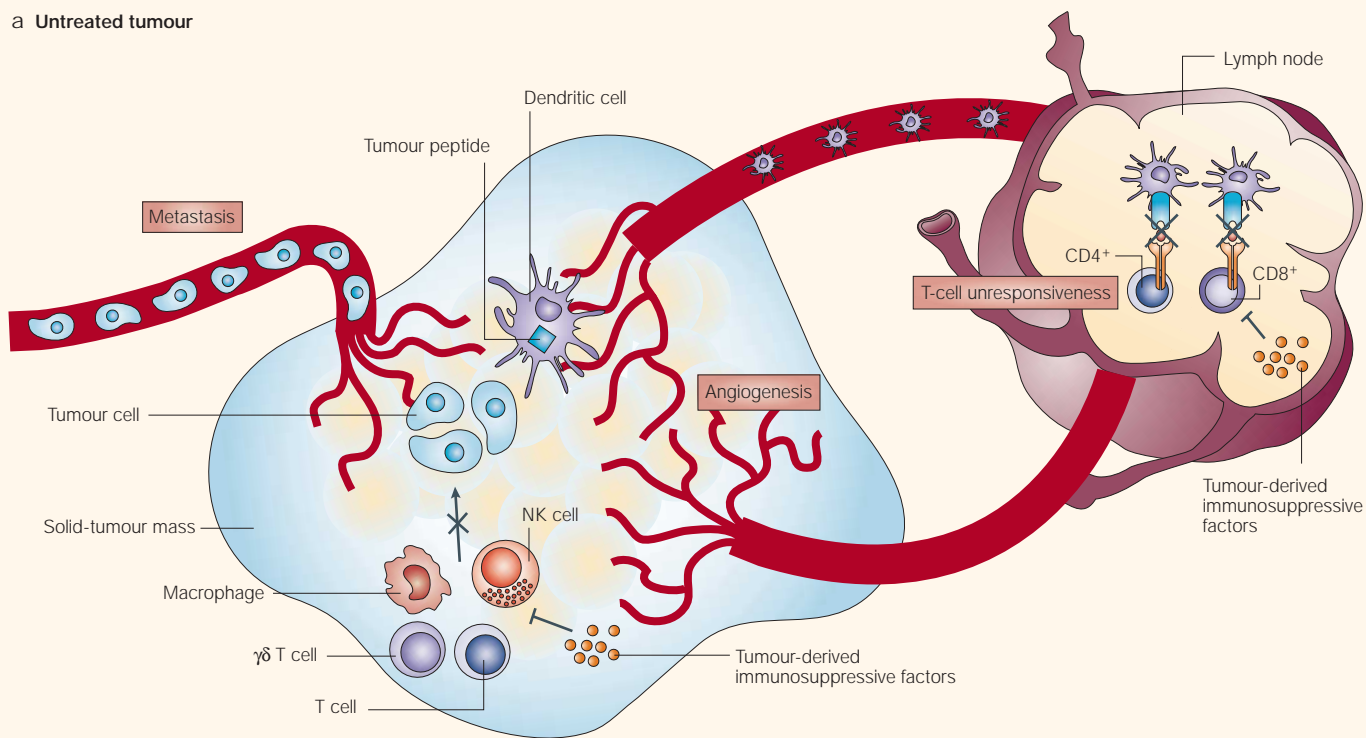
Figure 4 | Antitumour activity of IMiDs in multiple myeloma. Immunomodulatory drugs (IMiDs) induce growth arrest and/or apoptosis in multiple myeloma (MM) cells and inhibit adhesion of MM cells to bone-marrow stromal cells. Stromal-cell expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) is reduced by IMiDs, which decreases angiogenesis. Expression of interleukin-6 (IL-6) and tumour-necrosis factor- α (TNF- α) by the stromal cells is also reduced, which inhibits growth of MM cells. The IMiDs also enhance T-cell stimulation and proliferation. The activated T cells release IL-2 and interferon- γ (IFN- γ), which activate natural-killer (NK) cells (which might also be activated directly) and causes MM-cell death.

Table 1 | Current clinical studies of IMiDs

| Drug | Indication | Centre | Phase | Stage | Comments and references |
|--------------------------------|---|---|---------------------|------------------------------------|--|
| CC-5013 | Relapsed MM (<i>n</i> = 27) | Dana-Farber Cancer Institute, USA | I | Completed | First published report in MM ⁵⁴ . A dose-escalating study with 24 evaluable patients. Best responses in terms of reduction in serum M-protein in evaluated patients were >50% in 7/24 (30%), >25–50% in 10/24 (42%) and <25% in 2/24 (8%) patients. The maximum-tolerated dose was 25 mg/day. Grade 3 myelosuppression was apparent in patients treated with 50 mg/day. No somnolence or neuropathy observed. |
| CC-5013 | Metastatic malignant melanoma and other advanced solid tumours (<i>n</i> = 20) | St George's Hospital Medical School, UK | I | Completed | First published report in solid tumours ⁵⁵ . Seventeen evaluable patients. One partial response and two clear objective responses. Evidence of T-cell activation and increased serum IL-12, GM-CSF and TNF- α . No serious adverse effects were observed. |
| CC-5013 | Refractory solid tumours (<i>n</i> = 24) | Wake Forest University, USA | I | Completion due April 2005 | Still recruiting. No data reported. |
| CC-5013 | Recurrent high-grade glioma (<i>n</i> = 80) | NCI, Bethesda, USA | I | Started early 2002 | Still recruiting. No data reported. |
| CC-5013 | Refractory metastatic cancer (<i>n</i> = 30) | NCI, Bethesda, USA | I | Started early 2002 | Still recruiting. No data reported. |
| CC-5013 | Refractory solid tumours and/or lymphoma (<i>n</i> = 3–30) | NCI, Bethesda, USA | I/II | Started 2003 | Still recruiting. No data reported. |
| CC-4047 | Advanced MM (<i>n</i> = 18) | Guy's and St Thomas's, UK | I/II | Completed | Study showed anti-myeloma activity and an acceptable safety profile ⁵⁶ . CC-4047 was given in a dose-escalating regimen (1 mg/day up to 10 mg/day). All patients improved clinically. The M-protein response on trial was <25% reduction in 8/18 (44%), >25–50% in 7/18 (39%) and >50% in 3/18 (17%). The maximum-tolerated dose was 2 mg/day because of neutropaenia at the higher doses. |
| CC-5013 | Relapsed/refractory MM (<i>n</i> = 60) | Multicentre (USA) based at the Dana-Farber Institute, USA | II | Completion in early 2004 | Unpublished data presented at the 2003 American Society of Hematology meeting indicates that so far 39/46 evaluable patients (85%) with progressive disease experienced a reduction or stabilization in their M-protein levels. |
| CC-5013 | Relapsed/refractory MM (<i>n</i> = 100) | University of Arkansas, USA | II | Completion in 2004 | Still recruiting. No data reported. |
| CC-5013 | Refractory MM (<i>n</i> = 200) | Multicentre, USA | II | Completion in Dec. 2005 | Still recruiting. No data reported. |
| CC-4047 | Metastatic hormone-refractory prostate cancer (<i>n</i> = 36) | University of Colorado and Baylor College of Medicine, Texas, USA | II | Completion in mid-2005 | Still recruiting. No data reported. |
| CC-5013 | MDS with cytogenetic abnormality (<i>n</i> = 36) | NCI and Memorial Sloan-Kettering Cancer Center, USA | II | Unknown | Still recruiting. No data reported. |
| CC-5013 | MDS with 5q cytogenetic abnormality (<i>n</i> = 90) | Multicentre, USA | II | Completion in May 2004 | Still recruiting. No data reported. |
| CC-5013 | MDS (<i>n</i> = 136) | Multicentre, USA | II | Completion in Sept. 2004 | Still recruiting. No data reported. |
| CC-5013 | MDS (<i>n</i> = 25) | Multicentre, USA | II (soon Phase III) | Completed | Completed. 64% of 25 patients needed at least 50% fewer blood transfusions after CC-5013 treatment. Also, 8/8 patients with 5q- syndrome lost all sign of cells with the telltale 5q-chromosomal deletion. |
| CC-5013 | Metastatic malignant melanoma (<i>n</i> = 274) | Multicentre (USA, Europe, Australia) | III | Completion in June 2004 | Still recruiting. No data reported. |
| CC-5013 & Dex versus Dex alone | Refractory MM (<i>n</i> = 302) | Multicentre (USA, Canada) | III | Completion at end of 2005 | Still recruiting. No data reported. |
| CC-5013 & Dex versus Dex alone | Newly diagnosed MM (<i>n</i> = 500) | NCI and Southwest Oncology group, USA | III | Completion 4 years from start date | Not yet recruiting. |

Data compiled from www.clinicaltrials.gov and www.celgene.com. Dex, dexamethasone; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-12, interleukin-12; IMiDs, immunomodulatory drugs; MDS, myelodysplastic syndromes; MM, multiple myeloma; M-protein, monoclonal protein; NCI, National Cancer Institute; TNF- α , tumour-necrosis factor- α .

a Untreated tumour



b IMiD-treated tumour

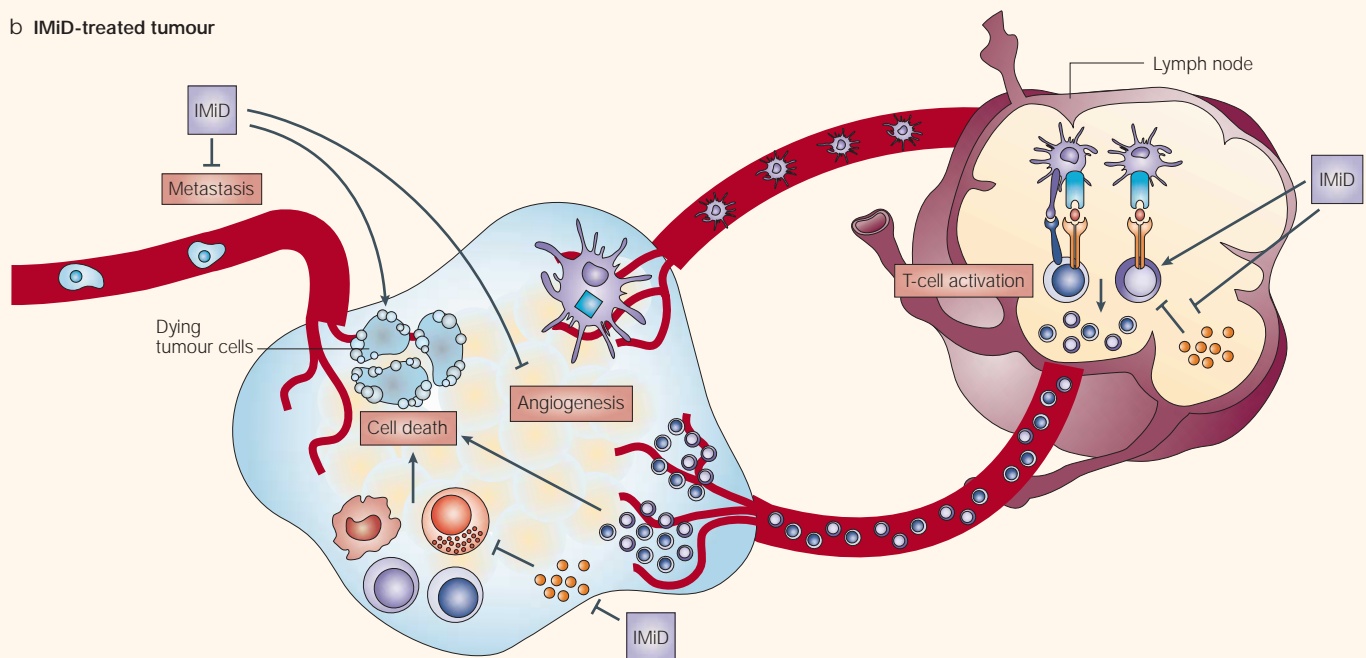


Figure 5 | **The potential mechanisms of IMiD-mediated antitumour activity.** **a** | A large untreated solid tumour contains an established blood supply — angiogenesis within tumours can also contribute to metastasis, depending on the nature of the tumour. Antigen-presenting cells, such as dendritic cells, ingest and process tumour antigens. However, immunosuppressive factors that are produced by tumour cells prevent subsequent priming and activation of CD4⁺ and CD8⁺ T cells in the lymph nodes, leading to immunological tolerance/anergy. In addition, cells of the innate immune system, such as macrophages, $\gamma\delta$ T cells and natural-killer (NK) cells, are also suppressed by these factors and are ineffective at killing tumour cells. **b** | Immunomodulatory drugs (IMiDs) directly kill certain types of tumour cells or induce cell-cycle arrest. They also possess potent anti-angiogenic activity *in vitro* and this is likely to contribute to their antitumour effects *in vivo*. IMiDs help to minimize metastasis by reducing the expression of pro-angiogenic cytokines, such as vascular endothelial growth factor, decreasing blood-vessel density and affecting cell-adhesion molecules. Finally, IMiDs co-stimulate T cells and enhance antitumour immunity, which is mediated by T-helper-1-type cytokines, such as interferon- γ and interleukin-2. T-cell co-stimulation might overcome T-cell unresponsiveness and block tumour-cell-induced immunosuppressive factors, allowing tumour-specific immune cells to destroy the tumour cells. IMiDs also co-stimulate $\gamma\delta$ T cells and enhance other innate immune cells such as NK cells, which can enhance tumour-cell death.

Clinical development of the IMiDs

The clinical development of the IMiDs has been rather spectacular considering clinical trials only began in 2000. The rapid emergence of the IMiDs from the shadow of thalidomide is indicative of the potential for these compounds to treat conditions — in particular some cancers — for which there are few alternative therapeutic options. CC-5013 is the lead IMiD being tested and CC-4047 is also in clinical development. These two compounds vary in the extent of their co-stimulatory activity *in vitro*; CC-4047 is the more potent T-cell co-stimulator, although they seem to have similar anti-angiogenic activity. Variations in other factors, such as pharmacokinetics and drug stability in plasma, mean that CC-5013 and CC-4047 have different activity profiles and these are likely to suit different disease types.

The first completed clinical trial of CC-5013 in relapsed and refractory MM patients was published in 2002 (REF. 54; TABLE 1). No significant side effects such as those typified by thalidomide treatment — somnolence, constipation or neuropathy — were seen in any patient. Interestingly, five of seven patients who progressed during CC-5013 monotherapy then responded after CC-5013 plus dexamethasone, indicating that combination therapy could be effective. The initial trial data indicated that further investigation of this compound was warranted and, in October 2001, CC-5013 was granted orphan-drug status by the FDA. By mid-2002, CC-5013 had entered Phase II studies in other haematological cancers and, by early 2003, CC-5013 had entered Phase III clinical trials for metastatic malignant melanoma and MM. In February 2003, based on initial reports of efficacy, the FDA granted fast-track status to CC-5013 for the treatment of relapsed or refractory MM. Fast-track status is designated to compounds that might provide a significant improvement in the safety or effectiveness of the treatment for a serious or life-threatening disease. The use of CC-5013 is now gathering momentum, with several other CC-5013 studies now recruiting for patients with glioma, leukaemia, lymphoma and solid neoplasms (TABLE 1). The first published study of CC-5013 in patients with solid tumours indicates that it also has clinical activity in patients with advanced and heavily pre-treated metastatic malignant melanoma and other solid cancers⁵⁵. The primary objective of this Phase I study was to assess the safety and tolerability of CC-5013 and, in this regard, there were no serious adverse effects attributed to treatment. Also, analyses of serum cytokines and peripheral-blood cell-surface

markers showed conclusive evidence for immune activation in all of the patients who were tested.

In April 2003, fast-track status was also granted for its use in the **myelodysplastic syndromes** (MDS). These are a spectrum of malignant disorders of blood-cell production that can eventually lead to acute leukaemia, which affect over 250,000 people worldwide. At present, there is no FDA-approved agent for the treatment of MDS. However, it has become evident that CC-5013 is highly effective at restoring red-blood-cell production in this group of patients. In fact, CC-5013 is the first therapy that improves red-blood-cell production in patients with MDS-related anaemia, so they no longer have to rely on blood transfusions. Even more remarkable is the effect of CC-5013 on patients with 5q- syndrome (a type of MDS), in which treatment seems to rid the patient of the effects of the 5q-chromosomal deletion that defines the condition.

The IMiD CC-4047 has also been used to treat patients with relapsed and refractory MM, where it has been shown to have an acceptable safety profile in a Phase I/II trial⁵⁶. This study highlighted that CC-4047 also has antitumour activity and an acceptable toxicity profile and should be evaluated in future Phase II studies in haematological and solid-tumour malignancies. In the first quarter of 2003, a Phase II study in metastatic hormone-refractory prostate cancer was started. The aim of this study is to evaluate the safety and preliminary efficacy of CC-4047 for treatment of this cancer.

Future directions

Thalidomide is now recognized as a clinically effective drug because of its anti-angiogenic and anti-inflammatory properties. This has provided the rationale for developing structural IMiD analogues with increased potency. The development of these compounds is rather unusual because at the same time as they are entering Phase III clinical trials, much effort is being undertaken to define the cellular targets and determine how they actually work, thereby reversing the natural drug-discovery process. The IMiDs represent a class of compound that is anti-angiogenic, has direct antitumour effects and is both anti-inflammatory (during monocyte/macrophage activation) and T-cell co-stimulatory (during partial T-cell activation; FIG. 5). The IMiDs — in particular the two lead compounds CC-5013 and CC-4047 — have entered the clinic for the treatment of various cancers, including MM, MDS and malignant melanoma.

Initial clinical data indicate that IMiDs are effective in the treatment of advanced cancers and in patients who have received extensive previous treatment that has been unsuccessful.

These compounds entered the cancer field as anti-angiogenic drugs. However, single-agent anti-angiogenic therapy has so far proved disappointing and it is now widely believed that, to be effective, anti-angiogenic treatment — especially in the context of cancer — will regulate chronic, life-long therapy to maintain disease control. It is now known that thalidomide and its IMiD derivatives also possess direct antitumour and co-stimulatory activity; this combination perhaps explains their efficacy as single agents. However, judicious combination therapy with IMiDs and cytotoxic agents or other anticancer agents could lead to additive or synergistic interactions and also reduce the possibility of chemical resistance. Further investigation of the use of these compounds in combination with existing therapies, such as the chemotherapeutic agent dexamethasone (now underway in two key Phase III trials) is also beginning to gain momentum and early data are encouraging.

A possible role in enhancing protective antitumour immunity might produce an adjuvant effect in the context of a vaccination regimen. This is supported by pre-clinical *in vivo* data, but has yet to be explored in patients. Also, the ability of IMiDs to activate innate immune responses might be crucial to the generation of effective adaptive anti-tumour responses *in vivo*, although this area remains relatively unexplored.

Although there is only limited data concerning clinical efficacy of the IMiDs in the literature, results are starting to emerge that support their continued clinical development. Both preclinical and initial clinical studies are encouraging, but there is still much to learn about the mechanisms of action of these compounds and the cellular targets that characterize their activities. Even so, the IMiDs clearly represent an exciting new generation of anticancer drugs. Definitive evidence of clinical efficacy in Phase III studies is eagerly awaited and will hopefully be available by the end of 2004.

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Competing interests statement

The authors declare **competing financial interests**: see web version for details.

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