Thalidomide (Figure 1) was derived from alpha-phnthaloylisoglutamine, a derivative of glutamic acid® by Chemie Grünenthal GmbH, a West German Company in 1954. Thalidomide has a low level of toxicity and no LD<sub>50</sub> could be established. Indeed, high doses of thalidomide did not cause respiratory or cardiac failure, suggesting that accidental death or suicide with this compound was highly unlikely. As a matter of fact, 17 patients, including small children and one suicide attempt, survived ingestion of excessive amounts of thalidomide. In 1957, thalidomide was approved for commercial use in West Germany as a sedative and sold under the brand name Kevadon. About this time, the anti-emetic (anti-nausea) activity was discovered and the drug was prescribed to counteract morning sickness in pregnant women. Unfortunately, the teratological effects of thalidomide were not revealed through studies in rodents and approximately 12,000 children were born deformed before thalidomide was banned for clinical use in March 1962 by the Canadian Food and Drug Directorate®. In 1998, thalidomide was approved to treat erythema nodosum leprosum, a painful inflammatory dermatologic reaction of leprosy. In 2006, the FDA approved thalidomide under the brand name Thalomid (Celgene Corp) for treatment of newly diagnosed multiple myeloma. As a result of these approvals, the interest in thalidomide as a chemotherapeutic agent for other cancers, including prostate cancer (Figure 2), has emerged.

Initial clinical studies on the efficacy of thalidomide in prostate cancer therapy were reported and reviewed by Brennen et al.®. To briefly summarize, two clinical trials were completed and four clinical trials were recruiting at that time. The two clinical trials were in the setting of androgen independent or castration resistant prostate cancer (AIPC or CRPC) and Dr William Figg was the principle investigator for both investigations. Figg et al. reported in 2001® that 27% of men with AIPC responded to thalidomide monotherapy with a 40% reduction in prostate specific antigen (PSA) levels. In another study, Figg et al. reported that patients treated with a combination of thalidomide and docetaxel had increased survival compared to patients treated with each drug as a monotherapy. The main adverse effects were peripheral neuropathy and deep vein thrombosis (DVT). These observations led to more basic, translational and clinical investigation on the benefit of thalidomide for men with AIPC.

There are several recently developed therapeutic agents for CRPC®. They are:

- Abiraterone (Johnson and Johnson), a dual inhibitor of CYP17A1 and the androgen receptor (AR), MDV3100 (Meditron), a new anti-androgen
- Sipuleucel-T also known as D9901 or Provence® (Dendreon), a personalized cell-based vaccine.
- Dasatinib (Sprycel) and Sunitinib (Sutent), broad spectrum kinase inhibitors that target BCRABL, SRC, Ephrins, and GFR among others
- Atrasentan or Xinlay® and ZD4054 (both by Astrazeneca), endothelin antagonists which target patient pain
- Custirsen (Oncogenex Pharm, Inc.), an antisense oligonucleotide-based therapy designed to inhibit clusterin expression thereby decreasing a cells’ adaptive response to stress and lowering the apoptotic threshold
- Larotaxel (XRP9881) and Cabazitaxel (XRP6258), novel taxanes (Sanofi-Aventis) that are transported poorly by the multidrug resistance efflux pump, Pgp
- Aflibercept (Regeneron/Sanofi-Aventis) and Avastin or Bevacizumab (Genetech/Roche), VEGF-A antagonists that prevent the ligand from binding to its receptor thereby preventing new blood vessel development

And also in this wave of anti-angiogenesis compounds is Thalidomide and its analogue, Lenalidomide or Revlimid (Celgene).

Herein we describe in brief a selected few recent basic, translational and clinical studies. We will focus on key data regarding the mechanism of action, in vitro and animal studies, as well as clinical trials with thalidomide and its analogues. This is not meant to be a detailed review, but a synopsis of the progress being made converting Thalidomide’s story from one of tragedy to one of triumph.

**Mechanism of action**

Currently much more is known about the mechanisms of action of Thalidomide. In 2004, we summarized...
in prostate cancer therapy

key mechanisms of thalidomide and the readers can refer to Brennen et al. for more information. In this section, we will focus on one earlier report and a more recent paper.

Thalidomide has been studied extensively for its anti-angiogenic properties. Angiogenesis is a complicated process involving cross-talk between tumour cells, tumour associated host cells, perivascular cells, endothelial cells and tumour-associated macrophages (TAMs). TAMs are responsible for secreting key growth factors (granulocyte-macrophage colony-stimulating factor (GM-CSF) and cytokines (TNF-α in particular) that can regulate degradation of the extracellular matrix, endothelial cell migration, and endothelial cell proliferation. They can initiate the production of certain plasminogen activator (PA) inhibitors (PAIs) that inhibit angiogenesis by destroying Urokinase-type (uPA). The increased production of this inhibitor appears to block matrix degradation mediated by uPA secreted by colon cancer cell lines as well as suppress tumour growth in mouse models of prostate cancer.

Joseph also demonstrated that Thalidomide comprises TAM function by reducing their ability to produce both GM-CSF and TNF-α. Since these cytokines are reduced by Thalidomide, PAI-2 (plasminogen activator inhibitor type 2) was reduced leading one to speculate on an alternative mechanisms for action, most likely by blocking angiogenesis. This is one of the pivotal investigations leading to the biosynthesis of Thalidomide analogues for maximum anti-cancer activity against PCa.

N-substitution (CPS11) and tetrafluorination (CPS49) are both Thalidomide analogues that have been proven cytotoxic to both cancer and endothelial cells. In vitro studies performed using lung cancer cells (H157) demonstrated that CPS49 induced cell death by 5-fold. Kinase activation was examined to determine the most likely pathway affected by analog therapy. Akt, ERK, and p38 are kinases and members of the MAPK (mitogen-activated protein kinase) superfamily were examined. Akt, which is responsible for enhanced cell survival when activated, did not show signs of inactivation during CPS49 treatment. ERK, a MAPK that promotes cell survival and proliferation, activity increased with CPS49 treatment. P38α, a member of the MAPK superfamily that promotes cellular necrosis and/or apoptosis, activity was increased by CPS49. To confirm p38α phosphorylation, the authors analyzed two sub-

Figure 1. The two enantiomers of thalidomide: (S)-thalidomide (left) and (R)-thalidomide (right)

strates downstream from p38, HSP27 and ATF2. The results from this experiment proved that CPS49 rapidly and transiently activates both p38 and ERK but does not inhibit Akt. Apparently, the balance was shifted in favor of cell death, p38 activation, over survival through these treatments. These results were similar for (2) lung cancer cell lines and (1) prostate cancer cell line. Next, the authors determined if the mechanism of action of CPS49 identified in lung and prostate cancer cells, is similar in endothelial cells. Treatment of human umbilical vein endothelial cells (HUVEC) indicated that CPS49 increased p38 activation without activation of ERK and Akt. The phosphorylation of these prosurvival molecules actually decreased.

Animal studies

Above, we summarized in vitro data that one mechanism of Thalidomide action against PCa may be to reduce macrophage function in vitro by compromising their ability to secrete TNF-α and GM-CSF. Animal studies revealed that thalidomide reduced the number of TAMs, tumour microvessel density and tumour size in the Mat-Lu rat prostate cancer model in vivo. Warfel et al. showed that CPS49 activates MAPK p38, thereby promoting stress induced cell death in the castrate resistant, androgen receptor negative PC3 xenograft model. Akt and Erk activities during CPS49 treatment were not reported for the xenograft study as they were in vitro. Additionally, the effect of this analog on microvessel density and tumour hypoxia were not reported. This report suggests, however, that p38 activation could serve as a biomarker for CPS49 administration or compounds that activate p38 may be useful as chemotherapeutics by inducing stress activated cell death.
Recent clinical trials

The microenvironment surrounding a prostate tumour contains several factors necessary for metastatic progression\(^8\). Therefore, disrupting communication between a tumour mass and the microenvironment may be another viable therapeutic option. Efstathiou et al.\(^9\) tested the hypothesis that thalidomide alters the tumour microenvironment in a manner that reduces prostate cancer aggressiveness. Fifteen men with PCa were treated with escalating doses of thalidomide (up to 600 mg) for 12 weeks, increasing at a rate of 200 mg/week, followed by radical prostatectomy. Tissue microarrays were constructed from prostatectomy samples and the blood level of several cytokines germane to PCa metastatic progression were evaluated. The analyses of tissue microarrays indicated a reduction in two principal markers of PCa angiogenesis, VEGF and Interleukin-6. bFGF expression was elevated in the thalidomide treated arm, but was confined to the nucleus and not the cytoplasm. Microvessel density was reduced in treatment group, as indicated by a decline in CD31+ endothelial cells. In addition, men treated with thalidomide had increased expression of E-cadherin, reduced expression of metalloproteinase 9 and 11, as well as attenuated Hedgehog signaling. This suggests that aggressive PCa cells were dying and more indolent, better differentiated cells were remaining as thalidomide modulated the tumour surrounding microenvironment. Interestingly, TNF-\(\alpha\) plasma levels increased in the treatment group. This is indeed unexpected, since thalidomide is known to reduce TNF-\(\alpha\) secretion by macrophages (stated and referenced above). Although the authors of this study, did not comment on this observation, it is possible the TNF-\(\alpha\) was secreted by other cell types to overcompensate for the defunct macrophage. At this time, it is just speculation and warrants testing experimentally.

In an effort to improve the clinical usefulness of thalidomide in the CRPC setting, analogues have been synthesized and tested. Few agents have made it beyond preclinical evaluation; however, one analog that has moved forward is Lenalidomide\(^10\). A recent phase I study demonstrated that this compound is well tolerated at 35 mg/day using an intermittent dosing schedule and shows promising therapeutic activity in a select group of refractory solid tumours, most notably, PCa. This trial consisted of 45 patients, 35 of whom had prostate cancer. The two most common treatment-related adverse events for continuous dosing were 1) pruritus and 2) fatigue, rash, diarrhea, nausea and neuropathy. The two most common treatments related adverse events for intermittent dose were 1) Fatigue and 2) Nausea. Despite these side effects, lenalidomide therapy halted disease progression in 12 patients, 9 of which were PCa patients. This observation suggest that CRPC cells, in particular, are sensitive to lenalidomide and a phase II is warranted to define its efficacy in men with hormonally refractory PCa. Currently, there are 8 phase II trials for PCa using Lenalidomide as a single agent or in combination with other drugs. Five of these trials are focus on CRPC (http://clinicaltrials.gov/ct2/results?term=lenalidomide+and+prostate+cancer).

Closing comments

Dr Kevin Anderson, my microbiology professor at Mississippi State University, once told me that a pathogen is a microbe out of place or misplaced (personal communication). Thalidomide has made a significant recovery, from a drug of monstrous effects to one of magnificent clinical benefit in the right setting. It also appears that the severity of a drug’s side effects and its clinical efficacy is dependent on its context. In other words, prescribing thalidomide to pregnant women obviously indicates that this drug was out of place, however prescribing to patients with ENL and refractory multiple myeloma provides significant benefit and now thalidomide has found its place.

For men with CRPC, thalidomide and its analogues are demonstrating significant promise. As scientists learn more about its complex mechanisms of action and develop better analogues, the clinical usefulness of thalidomide and derivatives in these men could very well improve. Ongoing clinical trials are testing the thalidomide analog Lenalidomide in men with PCa. One major oversight regarding completed trials is that the
patient demographics did not indicate race. All clinical studies summarized in this article, failed to include racial background in tables presenting patient characteristics. Considering that African-American men have the highest incidence of and mortality from PCa, it is important that all clinical studies on PCa clearly state the number of African-Americans included or state that no African Americans were recruited or retained. This information will be useful for retrospective studies to determine the biology behind the racial disparity of prostate cancer in African-Americans. This is exactly how BiDil was discovered; the first race-specific drug approved by the FDA for congestive heart failure in African-American. It may be that thalidomide or one of its analogues may be more effective on African-Americans, compared to the general population, since the level of nitric oxide, an angiogenic agent is lower in African-Americans.

Summary

Thalidomide and its derivatives generally are well tolerated by patients and have somewhat selective diseases for which they are useful. While future strategies aim to restrict the racemization and enhance efficacy, others point to creation of analogues having lightly different biological efficacy through different mechanisms. Some analogues appear to modulate the immune system better while others tend to block angiogenesis. The molecular mechanisms of thalidomide and its derivatives will also provide additional targets for rationale drug design and development.

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References


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