



## Thalidomide and birth defects

Journal:	eLS
Manuscript ID	A26052.R1
Wiley - Manuscript type:	Standard Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Fraga, Lucas; University of Aberdeen, Diamond, Alex; University of Aberdeen, Vargesson, Neil; University of Aberdeen,
Abstract:	<p>Thalidomide is a teratogenic drug that caused horrific birth defects when prescribed as an antiemetic to pregnant women in the 1960's. The most stereotypical defect is symmetrical limb malformations such as phocomelia, though ear, eye and internal organ defects are also observed. Thalidomide was consequently withdrawn from the market. However, Thalidomide has since been shown to have many beneficial anti-inflammatory and immunomodulatory effects and is therefore used in a regulated manner in the treatment against cancers and inflammatory disorders. Sadly, new cases of babies affected by thalidomide are being born in Brazil, likely due to medicine sharing. The mechanisms of how thalidomide causes a wide range of embryonic malformations are becoming clearer; thalidomide is thought to act through molecules such as cereblon and tubulin and also affect blood vessel development and cell death, resulting in teratogenesis. Fully understanding the molecular events induced by thalidomide is essential if we are to develop a safe but clinically relevant form of the drug.</p>



1

2 **Thalidomide and Birth Defects**

3 A26052

4

5

6 **Lucas Rosa Fraga\*, Alex J Diamond\*, Neil Vargesson<sup>\$</sup>**

7 School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences,

8 University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK

9

10

11

12

13 **Advanced Article**

14 \* - These authors contributed equally to this Review Article.

15 \$ - Author for correspondence: email: [n.vargesson@abdn.ac.uk](mailto:n.vargesson@abdn.ac.uk);16 [nvargesson@gmail.com](mailto:nvargesson@gmail.com). Tel: (0)1224 437374

17

18

19

## 1 **Abstract**

2 Thalidomide is a teratogenic drug that caused horrific birth defects when prescribed  
3 as an antiemetic to pregnant women in the 1960's. The most stereotypical defect is  
4 symmetrical limb malformations such as phocomelia, though ear, eye and internal  
5 organ defects are also observed. Thalidomide was consequently withdrawn from the  
6 market. However, Thalidomide has since been shown to have many beneficial anti-  
7 inflammatory and immunomodulatory effects and is therefore used in a regulated  
8 manner in the treatment against cancers and inflammatory disorders. Sadly, new  
9 cases of babies affected by thalidomide are being born in Brazil, likely due to  
10 medicine sharing. The mechanisms of how thalidomide causes a wide range of  
11 embryonic malformations are becoming clearer; thalidomide is thought to act through  
12 molecules such as cereblon and tubulin and also affect blood vessel development  
13 and cell death, resulting in teratogenesis. Fully understanding the molecular events  
14 induced by thalidomide is essential if we are to develop a safe but clinically relevant  
15 form of the drug.

16

17

## 18 **Key Words**

19 angiogenesis, cell death, Cereblon, reactive oxygen species, time sensitive window,  
20 mechanisms of teratogenesis, chicken embryo, zebrafish embryo

21

22

## 23 **Key Concepts**

- 24
- 25 • Thalidomide was used between 1957 and 1961 as a 'safe' treatment  
26 for morning sickness, but was withdrawn after it was found to cause  
severe birth defects.
  - 27 • Thalidomide has since been shown to possess anti-inflammatory,  
28 antiangiogenic and anti-proliferative properties.

- 1 • Thalidomide is now used, under strict regulations, to treat human  
2 inflammatory disorders and cancer.
- 3 • Thalidomide causes embryonic damage in a short time-sensitive  
4 window between day 20 and 36 post-fertilisation in humans.
- 5 • Thalidomide causes damage to the majority of the body tissues,  
6 amongst the most common and stereotypical damage is to the limbs.
- 7 • Effects of thalidomide can vary dependent on the species exposed,  
8 some species being more sensitive to the drug than others.
- 9 • Evidence supports blood vessels as a primary target of thalidomide.
- 10 • Possible other pathways involved in thalidomide-induced embryopathy  
11 are oxidative stress induction, cell death and binding to Cereblon.
- 12 • Cereblon acts as a target of thalidomide for treatment of multiple  
13 myeloma in adult humans.
- 14

## 1 **1 History of Thalidomide**

2  
3 Thalidomide [α-(N-phthalimido) glutaramide] was synthesised by Chemie  
4 Grunenthal, in Germany, and introduced onto the market in 1957 as a “safe”, non-  
5 addictive, over-the-counter sedative (Vargesson, 2015). The drug was marketed  
6 across 46 countries and was also sold as an effective antiemetic for pregnant  
7 women suffering morning sickness (Franks *et al.*, 2004; Vargesson, 2009;  
8 Vargesson, 2013; Vargesson, 2015).

9 Following the release of thalidomide, reports of an increase in the occurrence of  
10 severe and rare birth defects began surfacing (McCredie, 2009; Vargesson, 2013;  
11 Vargesson, 2015). The most striking defect was phocomelia of the limbs (where  
12 distal structures of the limb remain whereas proximal structures are lost or reduced),  
13 though some babies presented with amelia (no limb structures exist). A wide range  
14 of damage to the limbs could be observed as well as damage to many other body  
15 systems (see Section 3) (Lenz and Knapp, 1962; Ruffing, 1977). Damage to the  
16 ears, eyes, genitalia, heart, gastrointestinal tract and kidneys was also reported  
17 (Smithells and Newman, 1992; Vargesson, 2009; Vargesson, 2013; Vargesson,  
18 2015). The range and severity of damage to many babies across Europe confused  
19 clinicians at the time. It was not till two clinicians, McBride in Australia and Lenz in  
20 Germany, independently concluded in 1961 that the children with these birth defects  
21 were born to mothers who had consumed thalidomide (McBride, 1961; Lenz, 1962;  
22 Lenz, 1988). The drug was withdrawn from the worldwide market on 30 Nov 1961  
23 (Matthews and McCoy, 2003; Vargesson, 2013). The consumption of thalidomide  
24 during pregnancy was confirmed as the cause of birth defects since there was an  
25 almost complete loss of such defects from 1962 onwards (Lenz, 1988; Smithells and  
26 Newman, 1992; Vargesson, 2013; Vargesson, 2015). However, it is estimated that at  
27 least 10,000 children were born with deformities resulting from thalidomide exposure  
28 (Smithells and Newman, 1992; Vargesson, 2009). Thalidomide was not approved for  
29 use in America during the 1957-1962 thalidomide disaster: Dr Frances Kelsey,  
30 working for the US Food and Drug Administration (FDA), doubted its safety after  
31 reports of peripheral neuropathy in patients (Matthews and McCoy, 2003; Franks *et*  
32 *al.*, 2004; Vargesson, 2013; Vargesson, 2015). If thalidomide had been released in

1 the US, there may have been a significantly higher number of cases of birth defects,  
2 as seen in Europe, Canada, Australia and Japan.

3 Thalidomide underwent a rebirth in 1965 after studies proved its effectiveness as a  
4 treatment for erythema nodosum leprosum (ENL), a complication of leprosy  
5 (Sheskin, 1965). Following this, thalidomide was licensed in Mexico, Brazil and later  
6 in the US for the use in the treatment of ENL (Franks *et al.*, 2004) and in 2006 for  
7 treatment of multiple myeloma (MM) (Latif, 2012).

8 Programs now administer the use of thalidomide under strict guidelines where  
9 women prescribed the drug are required to use birth control and take regular  
10 pregnancy tests. When these guidelines are followed, no occurrence of thalidomide  
11 embryopathy has been reported (Uhl *et al.*, 2006). However, tragically, in Brazil,  
12 children are still being born with thalidomide embryopathy where the drug is used to  
13 effectively treat leprosy. This is likely due to a culture of sharing medicines as a  
14 result of people living so far away from hospitals, misinterpretation of the drug, and  
15 pregnant women taking it whilst suffering from leprosy (Vianna *et al.*, 2013;  
16 Vargesson, 2013). Considering the beneficial properties of thalidomide there is the  
17 possibility of increased use and a concern for a further potential increase in the  
18 frequency of thalidomide-induced birth defects. Despite research efforts the  
19 mechanisms of thalidomide-induced embryopathy are not fully understood.  
20 Continuing research is vital in the mission to synthesise a safe, clinically relevant  
21 form which is non-teratogenic, i.e. does not cause birth defects.

## 22 **2 Biochemistry of thalidomide**

23

24 Thalidomide is a derivative of the non-essential amino acid glutamic acid (Franks *et*  
25 *al.*, 2004). The structure consists of a glutarimide ring, pthalimido ring and contains  
26 an asymmetric carbon atom (Figure 1). The presence of the chiral carbon allows  
27 thalidomide to exist in two, interchangeable states, or enantiomers (S(-)) and R(+)),  
28 within the body. One state is thought to be the causative 'teratogenic' state (S(-)),  
29 and the other the 'sedative' state (R(+)). Since the drug can switch states within the  
30 body, it is not conceivable to prescribe just the 'safe', 'sedative' version. Thalidomide  
31 can broken down in to its active state by the liver enzyme cytochrome P450 and has

1 a half-life of 6-12 hours. Thalidomide can also rapidly hydrolyse in bodily fluids  
2 (Franks *et al.*, 2004; Vargesson, 2009; Vargesson, 2013; Vargesson, 2015).

### 3 2.1 *Pharmacological Properties of Thalidomide*

4 Further research in to the mechanism of thalidomide action has revealed a wide,  
5 diverse range of functions. As well as being anti-inflammatory and  
6 immunomodulatory, thalidomide is also anti-angiogenic and has anti-proliferative  
7 activities (D'Amato *et al.*, 1994; El-Aarag *et al.*, 2014). Through these properties  
8 thalidomide has been identified as an effective treatment for a number of adult  
9 conditions. Indeed since the discovery in 1965 that thalidomide can be beneficial as  
10 an anti-inflammatory drug to treat ENL, studies have recognised its clinical purpose  
11 as treatment for multiple myeloma (MM), cancers, Behcet's disease, gastrointestinal  
12 disorders, rheumatological disorders, hereditary hemorrhagic terangiectasia (HHT),  
13 lupus, idiopathic pulmonary fibrosis, HIV and diabetic retinopathy (Franks *et al.*,  
14 2004; Vargesson, 2013; Vargesson, 2015).

#### 15 2.1.1 *Antiangiogenic actions*

16 Thalidomide has the ability to inhibit angiogenesis, the formation of new and  
17 remodelling blood vessels. This action was first reported by using rabbit and rodent  
18 cornea assays to show that thalidomide inhibits fibroblast growth factor (FGF)-  
19 induced angiogenesis (D'Amato *et al.*, 1994). In chicken embryos thalidomide  
20 inhibits nitric oxide (NO), an important molecule for endothelial cell function and  
21 protection of blood vessels (Siamwala *et al.*, 2012; Majumdar *et al.*, 2009;  
22 Tamilarasan *et al.*, 2006; see also DOI: 10.1002/9780470015902.a0003390.pub2). NO  
23 is required for normal limb development since it promotes angiogenesis and reduces  
24 oxidative stress, therefore inhibition by thalidomide leads to limb malformations.  
25 Indeed, thalidomide affected chicken and zebrafish embryos can be rescued by NO  
26 (Siamwala *et al.*, 2012). Additionally, thalidomide inhibits NO-induced endothelial cell  
27 migration as well as interfering with normal actin polymerisation patterns. This  
28 prevents cells forming tubes, thereby inhibiting angiogenesis at the cellular level  
29 (Tamilarasan *et al.*, 2006; Vargesson, 2013; Vargesson, 2015).

30 Thalidomide also induces degradation of *Tumor Necrosis Factor- $\alpha$*  (*TNF $\alpha$* ) mRNA,  
31 a pro-angiogenic cytokine, suggesting another mechanism by which thalidomide

1 inhibits angiogenesis (Moreira *et al.*, 1993). Thalidomide has been demonstrated to  
2 reduce the vascular hemorrhaging and malformations in patients suffering from HHT  
3 by inhibiting angiogenesis and through recruitment of mural cells, known to decrease  
4 endothelial cell migration and proliferation, causing early maturation of blood vessels  
5 (Lebrin *et al.*, 2010; Figure 2). In zebrafish embryos, thalidomide reduces VEGF  
6 receptor function (Yabu *et al.*, 2005; Vargesson, 2013; Vargesson, 2015). In chicken  
7 embryos, exposure of early blood vessels to thalidomide results in a breakdown of  
8 vascular formation (Tamilarasan *et al.*, 2006). Antiangiogenic analogs of thalidomide,  
9 as opposed to anti-inflammatory analogs, cause limb defects (Therapontos *et al.*,  
10 2009). The antiangiogenic actions of the drug make it a promising therapeutic agent  
11 for the treatment of tumours, since it can prevent their early vascularisation  
12 (Therapontos *et al.*, 2009).

### 13 2.1.2 Anti-proliferative actions

14 The anti-proliferative effects of thalidomide are independent of its immunomodulatory  
15 activities in hematologic malignancies. Thalidomide reduces proliferation of  
16 cancerous MM cells that are resistant to standard chemotherapy (Melcherd and List,  
17 2007). Myeloma cells are targeted by thalidomide through several mechanisms  
18 including activation of antitumor immunity and exertion of antiangiogenic effects. The  
19 treatment of MM patients with thalidomide improves their survival rate, but the exact  
20 way in which thalidomide achieves this is not fully understood. Current studies are  
21 pointing to a molecular pathway targeted by thalidomide to combat MM which  
22 involves Cereblon, Ikaros and Aiolos proteins. Cereblon is part of an E3 ubiquitin  
23 ligase complex with the proteins Damaged DNA binding protein 1 (DDB1), Cullin-4A  
24 (CUL4A), and regulator of Cullin1 (Roc1). This complex tags proteins with ubiquitin,  
25 labelling them for proteolysis, and is therefore important for the regulation of protein  
26 expression (Stewart, 2014; Ito *et al.*, 2010; Ito *et al.*, 2011). After binding to  
27 thalidomide, Cereblon protein is inactivated, resulting in the rapid ubiquitination and  
28 degradation of Ikaros and Aiolos. Both proteins are transcription factors that in  
29 normal conditions regulate T and B cell development. High degradation of Ikaros and  
30 Aiolos increase the Interleukin-2 (IL) levels and decreases TNF $\alpha$  levels (Stewart,  
31 2014) (Figure 2). In addition, a correlation exists between low amounts of Cereblon  
32 in MM cells, clinical drug resistance and poor survival outcomes (Schuster *et al.*,  
33 2014; Stewart, 2014). Thalidomide reduces expression of TNF $\alpha$ , NF- $\kappa$ B, IL -6 and -8

1 and Vascular Endothelial Growth Factor (VEGF) proteins which are related to tumour  
2 cell survival, proliferation, inhibition of apoptosis and resistance to therapy (Latif *et*  
3 *al.*, 2012).

4

5

### 6 *2.1.3 Anti-inflammatory actions*

7 Thalidomide exhibits immunomodulatory and anti-inflammatory effects through *TNF $\alpha$*   
8 mRNA degradation, Nuclear Factor-kappa-B (NF- $\kappa$ B) regulation and  
9 Cyclooxygenase-2 (COX2) inhibition (Moreira *et al.*, 1993; Vargesson, 2015).  
10 Inducing *TNF $\alpha$*  mRNA degradation suppresses the activation of interleukins and  
11 cytokines by monocytes and macrophages. ENL patients present with high levels of  
12 *TNF $\alpha$* , which reduce with thalidomide treatment (Sampaio, 1993; Vargesson, 2013).  
13 The effects of thalidomide on *TNF $\alpha$*  is beneficial when treating other autoimmune  
14 diseases which arise through an overproduction of inflammatory cytokines (Latif *et*  
15 *al.*, 2012). A key regulator of the expression of cytokines, including *TNF $\alpha$* , is  
16 transcription factor NF- $\kappa$ B. Thalidomide selectively blocks *TNF $\alpha$*  and hydrogen  
17 peroxide-induced NF- $\kappa$ B activation, interfering with *TNF $\alpha$*  expression and other  
18 inflammatory molecules such as IL-8 (Majumdar *et al.*, 2002). Cytokine COX-2,  
19 involved in both inflammatory response and cancer growth, is also suppressed by  
20 thalidomide (Melcherd and List, 2007).

21 In addition to these actions which are the basis for some of thalidomide's clinical  
22 applications the drug can also induce cell death (Knobloch *et al.*, 2007) as well as  
23 reactive oxygen species (ROS) (Parman *et al.*, 1999). The multiple and varied  
24 actions of the drug, in part, explain why it has been so difficult to determine the  
25 precise mechanism underlying thalidomide induced teratogenesis. As we will see  
26 current viewpoints favour the antiangiogenic action of the drug as a major cause of  
27 teratogenesis.

## 28 **3 Thalidomide Embryopathy: What damage does thalidomide cause?**

29

### 30 *3.1 Thalidomide acts in a time sensitive window*

1 Thalidomide induces damage to the embryo in a time-sensitive window between  
2 days 20 and 36 post-fertilization (Figure 3) (Vargesson, 2009; Vargesson, 2015).  
3 The timing of damage was determined through interviews with mothers who had  
4 taken thalidomide, providing data to identify a correlation between when thalidomide  
5 was taken and the resulting malformations (Lenz and Knapp, 1962; Ruffing, 1977;  
6 Smithells and Newman, 1992). Since the symptoms of typical morning sickness  
7 coincide with a period of rapid development and embryogenesis, thalidomide was  
8 taken at a time when countless cell divisions, growth, migration, differentiation and  
9 organogenesis are occurring. Exposure to thalidomide interfered with major  
10 developmental events, triggering the defects seen in thalidomide embryopathy  
11 (Vargesson, 2013). Miscarriage results if the drug is taken before the time-sensitive  
12 window (Vargesson, 2015), however it is not known whether exposure to thalidomide  
13 after day 36 results in obvious embryonic defects. The babies identified for study and  
14 maternal interview had mainly outward, visible defects and so if damage was only  
15 obvious later in life, it was not noted. Therefore exposure to thalidomide after the  
16 time-sensitive window may not be harmless. Some reports suggest it would be rare  
17 for any embryo to be unharmed following consumption of just one tablet (Smithells  
18 and Newman, 1992). Indeed, it is estimated that one 50mg tablet is sufficient to  
19 cause birth defects in at least 20-50% of embryos exposed to thalidomide during the  
20 time-sensitive window (McBride, 1961; Lenz, 1962; Smithells and Newman, 1992;  
21 Vargesson, 2009; Vargesson, 2013; Vargesson, 2015)

### 22 3.2 *Thalidomide Embryopathy*

23 Although almost any organ can be affected by thalidomide, the type of malformations  
24 observed are dependent on the day of thalidomide intake (Table 1; Figure 3) (Lenz  
25 and Knapp, 1962; Ruffing, 1977; Smithells and Newman 1992; Vargesson, 2015).  
26 The multi-tissue damage seen is referred to as thalidomide embryopathy (Table 1)  
27 where bilateral, symmetrical limb malformation is the most stereotypical defect, but  
28 many other body systems are damaged too (Newman, 1986; Smithells and  
29 Newman, 1992). Furthermore, thalidomide embryopathy has also been termed  
30 thalidomide syndrome, as the damage seen is a collection of damage often occurring  
31 independently in other human conditions (Newman, 1986; Smithells and Newman,  
32 1992; Vargesson, 2009; Vargesson, 2013) (see also DOI:  
33 10.1002/9780470015902.a0025686).

### 1 3.2.1 *Limb Damage*

2 Phocomelia is the most striking limb malformation associated with thalidomide  
3 embryopathy, the most severe form of which being the absence of any long bones.  
4 The majority of thalidomide survivors have limb defects, ranging from amelia (no  
5 limb) to triphalangeal thumb and including radial dysplasia, and phocomelia. The  
6 majority of limb anomalies seen in thalidomide survivors are reduction events and  
7 typically bilateral in nature (Table 1). The thumb is the first bone to be affected,  
8 followed by the radius, humerus and ulna (Lenz and Knapp, 1962; McCredie, 2009;  
9 Smithells and Newman, 1992; Vargesson, 2013). Lower limb defects are less  
10 commonly seen. Shoulder and hip joints can, be weaker in thalidomide survivors and  
11 the hip and pubic bones may be missing (Vargesson, 2013).

### 12 3.2.2 *Ear and Eye Damage*

13 Ears and eyes develop around the same time as the limbs in the embryo and so are  
14 targeted during the thalidomide time-sensitive window (Figure 3). Complete absence  
15 of the eyes, small eyes and poor vision are all reported defects. Unlike limb defects,  
16 eye defects can occur unilaterally. Ear defects usually occur bilaterally and in  
17 conjunction with eye defects and facial palsies. Malformations range from absence of  
18 the ear (anotia), resulting in deafness, to elements of the outer ear remaining  
19 (microtia) (Vargesson, 2013; Vargesson, 2015).

### 20 3.2.3 *Facial and Neural Damage*

21 Facial muscles and nerves can be damaged by thalidomide and lead to facial palsy  
22 or asymmetry. A stereo-typical sign of thalidomide exposure is an enlarged facial  
23 naevus at birth, usually on the forehead, though this is no longer visible by three  
24 years of age (Vargesson, 2013). Irregular teeth, cleft palate and small noses are  
25 additional defects seen in thalidomide survivors. A second consequence of nerve  
26 damage by thalidomide during development is an increased occurrence of epilepsy  
27 and autism later in life (Smithells and Newman, 1992; Miller *et al.*, 2005).

### 28 3.2.4 *Internal Organ Damage*

29 The frequency of internal organ defects is difficult to define since they are not  
30 obviously apparent and may not present during childhood. Only the most noticeable

1 defects will have been recorded during the 1960s. The heart, kidney, gastrointestinal  
2 and urinary tracts and genitalia can all be affected by exposure to thalidomide (Lenz  
3 and Knapp, 1962; Ruffing, 1977; Smithells and Newman, 1992). Heart malformations  
4 can occur with pulmonary stenosis and patent duct arteriosus and are thought to be  
5 the main cause of miscarriages or postnatal deaths suffered after intake of  
6 thalidomide. Kidney defects include rotated, hypoplastic and ectopic kidneys. Internal  
7 and external genital defects as well as urinary tract defects are also seen. Testicular  
8 absence or malformations in males and abnormalities of the uterus in females are  
9 known defects (Lenz and Knapp, 1962; Ruffing, 1977; Smithells and Newman, 1992;  
10 Vargesson, 2013; Vargesson, 2015).

11 The true scale of the number of affected embryos and/or the range of defects caused  
12 by thalidomide may never be known since many malformed babies will have not  
13 survived to birth, or died shortly after (Smithells and Newman, 1992; Vargesson,  
14 2013; Vargesson, 2015). Furthermore, the criteria for diagnosis of thalidomide  
15 embryopathy was established in the 1960's based upon the most severely affected  
16 children (Lenz and Knapp, 1962; Ruffing, 1977; Smithells and Newman, 1992). It is  
17 possible that children born without the classical thalidomide embryopathy phenotype  
18 and therefore not considered damaged by thalidomide could have had some  
19 embryonic malformations internally and perhaps late onset disorders. Certainly  
20 analysis and follow up of affected children was done very differently in the 1960's  
21 than if the disaster had occurred today.

## 22 **4 How does Thalidomide Cause Damage To The Embryo?**

23

### 24 *4.1 Thalidomide effects are species dependent*

25 Initial studies by Grunenthal, who invented and marketed the drug, tested  
26 thalidomide on rodents, where no defects were detailed or described. Questions  
27 remain about the precise testing carried out, but Grunenthal say they carried out  
28 testing that was typical of the day. The drug was considered safe and approved for  
29 use. After thalidomide was withdrawn from the market it was actually found to act in  
30 many species including humans, primates, rabbits, marsupials, zebrafish and  
31 chickens (Stephens, 2009; Vargesson, 2013).

1 Rodents are sensitive to thalidomide but much less so than other organisms, and are  
2 affected by much higher doses (DiPaolo *et al.*, 1964; Parkhie and Webb, 1983;  
3 Vargesson, 2013). The reason for this species sensitivity difference is unclear.  
4 Thalidomide is able to inhibit angiogenesis in mice and rat aortic ring cultures, so  
5 although rodents are not insensitive to the drugs mechanisms, there may be aspects  
6 such as different rates of metabolism which offer them protection (Lu *et al.*, 2004).  
7 Indeed, incubation of thalidomide with rodent liver cytochrome enzymes results in  
8 lower angiogenic activity than if incubated with human or rabbit enzymes (Marks *et al*  
9 2002). Clearance of the drug is also much faster in mice compared to humans, so  
10 teratogenic forms may not exist for as long (Lu *et al.*, 2004; Vargesson, 2013).  
11 Differences in the length of gestation between rodents and humans could also be a  
12 factor in predisposition of sensitivity to thalidomide.

13 Among the mammals, primates are considered the best model to study thalidomide  
14 embryopathy giving phenotypes that most similarly reflect those seen in humans  
15 (Ema *et al.*, 2010; Vargesson, 2013; Vargesson, 2015). However primates present  
16 ethical and practical challenges including low offspring numbers, long gestation  
17 times and are costly to work with. Studies in non-human primates have shown  
18 characteristic limb reduction malformations, ranging from amelia to phocomelia, and  
19 defects in the tail and genitalia (Ema *et al.*, 2010). Rabbit model studies identified a  
20 range of defects similar to those found in humans, including limb and internal organ  
21 defects (Fratta, 1965). Rabbits are therefore one of the most reliable models used to  
22 demonstrate the teratogenic effects of thalidomide. Regarding non-mammalian  
23 models, thalidomide is toxic to *Xenopus* and exposure causes teratogenic effects  
24 (Fort *et al.*, 2000). Chicken and zebrafish embryos are excellent for studying  
25 thalidomide embryopathy since they develop rapidly and provide easy access to  
26 follow development (Stephens, 2009; Vargesson, 2009; Vargesson, 2013). Since  
27 these models are perfect for drug screening studies, the effect of thalidomide upon  
28 their development is well established making these animal models excellent for the  
29 study of thalidomide teratogenicity. In the chicken embryo, thalidomide causes limb  
30 and eye defects (Knobloch *et al.*, 2007; Stephens, 2009; Therapontos *et al.*, 2009;  
31 Ito *et al.*, 2010; Mahony *et al.*, 2013; Siamwala *et al.*, 2012). In Zebrafish, embryonic  
32 fins and eyes are affected (Ito *et al.*, 2010, Mahony *et al.*, 2013; Yabu *et al.*, 2005). In  
33 humans thalidomide affects the development of embryos in a time-sensitive manner.

1 This is also true for other animals, so embryos will be most sensitive to thalidomide  
2 during a particular window of development (Stephens, 2009; Therapontos *et al.*,  
3 2009; Ito *et al.*, 2010; Mahony *et al.*, 2013). Thalidomide also exhibits intra-species  
4 specificity; of eight dizygotic twin pairs examined during the 1960s thalidomide  
5 disaster in Brazil, only four pairs were born with the same malformations (Schmidt  
6 and Salzano, 1980). Drug distribution, metabolism and the genetic background of  
7 each species, strain or individual must be taken in account.

8

#### 9 *4.2 Morphological and Molecular Actions of Thalidomide Teratogenicity*

10 More than 30 theories attempting to explain the mechanisms of thalidomide  
11 teratogenesis have been postulated since the 1960s, though most cannot be backed  
12 up with *in-vivo* evidence (Vargesson, 2009; Vargesson, 2015). These theories  
13 include actions on DNA, bone cells, integrins and many others. Explanations need to  
14 address the range of defects seen in thalidomide embryopathy and how the time-  
15 sensitive window of exposure affects all tissues. Three of the most widely accepted  
16 theories are (i) the antiangiogenic action of the drug; (ii) the drugs ability to induce  
17 reactive oxygen species (ROS) and cell death; (iii) thalidomide binding to Cereblon.

##### 18 *4.2.1 Blood Vessels as Targets of Thalidomide*

19 Blood vessels supply oxygen and nutrients to growing tissues so are essential for  
20 embryonic development. It is established that loss or disruption of blood vessels  
21 during embryogenesis can lead to death or embryonic malformations (Vargesson,  
22 2003; Vargesson, 2013). It was postulated that limb defects might be caused by the  
23 antiangiogenic effect of thalidomide (D'Amato *et al.*, 1994). Indeed damage to  
24 vessels can cause limb defects in chicken embryos (Vargesson and Laufer, 2001;  
25 Vargesson, 2003; Vargesson, 2009). Studies in chicken embryos have further  
26 demonstrated that thalidomide affects angiogenesis even before the expression of  
27 some signalling molecules essential for limb development, such as FGFs  
28 (Therapontos *et al.*, 2009).

29 Thalidomide can be broken down in to various by-products, and a large number of  
30 structural analogs of thalidomide can be synthesised. This is invaluable to help  
31 understand drug function and actions and also determine which characteristic of the

1 drug is the cause of teratogenesis. The production of CPS49, an antiangiogenic  
2 analog, has shed light on the method of teratogenesis. Blood vessels are destroyed  
3 within one hour of exposure to CPS49 in an E2.5 chicken embryo, with phocomelia  
4 presenting 7 days later (Therapontos *et al.*, 2009; Vargesson, 2009). Cell death is  
5 observed after application of CPS49, as well as loss of *Fgf8* and *Sonic Hedgehog*  
6 (*Shh*) expression, both key regulators of limb development and outgrowth.  
7 Thalidomide has also been shown to induce cell death and cause the loss of limb  
8 signalling events in chicken embryos (Knobloch *et al.*, 2007). Studies indicate that  
9 CPS49 destroyed newly forming vessels without a smooth muscle coat. Smooth  
10 muscle protects vessels and prevents angiogenesis. In-vitro studies demonstrated  
11 that smooth muscle negative vessels undergoing angiogenesis were destroyed but  
12 mature, smooth muscle positive vessels were unharmed (Therapontos *et al.*, 2009).  
13 CPS49 also disrupts blood vessels in zebrafish embryos and both CPS49 and  
14 thalidomide inhibit the actin cytoskeleton of vascular cells in-vitro (Therapontos *et al.*,  
15 2009; Tamilarasan *et al.*, 2006; Lebrin *et al.*, 2010).

#### 16 4.2.2 Reactive Oxygen Species (ROS) and Cell Death

17 The production of ROS in embryos causes oxidative stress, cell death and is  
18 upregulated in presence of thalidomide (Vargesson, 2013; Vargesson, 2015).  
19 Oxidative stress is required for cell-death-dependent thalidomide embryopathy;  
20 therefore this model could explain damage to limbs and other tissue. If thalidomide  
21 increases production of ROS, this will lead to cell death in affected tissues, causing  
22 defects. The function of redox-sensitive NF- $\kappa$ B is also affected by oxidative stress.  
23 NF- $\kappa$ B is a transcription factor important for limb development, and thalidomide  
24 diminishes its ability to bind to DNA promoter targets. This alters expression of *Fgf8*,  
25 *Fgf10* and Bone Morphogenetic Proteins (BMP) (Hansen and Harris, 2004),  
26 important genes in the process of limb development. Indeed, it has been shown that  
27 thalidomide exposure results in upregulation of *Bmp-4*, *-5* and *-7* expression in  
28 chicken embryos (Knobloch *et al.*, 2007). However, just how thalidomide induces  
29 ROS and/or cell death in a time-sensitive and tissue specific manner is unclear,  
30 though it could be a secondary effect to the loss of blood vessels. Considering that  
31 oxidative stress is a physiological process and occurs during embryogenesis, how it  
32 causes tissue specific damage is unknown. It is understood that NF- $\kappa$ B can  
33 negatively regulate BMP signalling which could explain, in part, why limbs are

1 affected by thalidomide through oxidative stress. How the other tissues are affected  
2 and how the range of damage is caused remains unclear.

### 3 *4.2.3 Cereblon and E3 Ubiquitin-ligase Complex*

4 Thalidomide is proposed to initiate teratogenesis by binding Cereblon, preventing  
5 establishment of the E3 ubiquitination complex and consequently causing mis-  
6 regulation of developmental signalling molecules (Ito *et al.*, 2010; Ito *et al.*, 2011;  
7 Stewart *et al.*, 2014; Vargesson, 2015).

8 In adult humans the Cereblon (*CRBN*) gene, conserved in species including plants  
9 and invertebrates (Higgins *et al.*, 2004), is expressed in several tissues such as the  
10 testis, spleen, liver, pancreas, lung and skeletal muscle (Xin *et al.*, 2008). Cereblon  
11 was identified as a primary binding target of thalidomide (Ito *et al.*, 2010), supported  
12 by results showing mutations preventing the binding between Cereblon and  
13 thalidomide suppressed limb loss in chicken embryos (Ito *et al.*, 2010). In addition,  
14 through inhibiting the translation of *Cereblon* mRNA, in zebrafish embryos, some  
15 phenotypes were found that appeared similar to those seen in thalidomide treated  
16 embryos, though not with the range or severity of damage seen in human  
17 thalidomide embryopathy (Ito *et al.*, 2010). Furthermore, Cereblon loss-of-function  
18 mice appear normal and unharmed (Lee *et al.*, 2013). Data suggests a participation  
19 of Cereblon in thalidomide embryopathy; however how thalidomide binding to  
20 Cereblon causes the damage, the range of damage and in a time sensitive manner  
21 is unclear, as is the precise role/function of Cereblon in normal embryonic  
22 development.

23 Thalidomide binding to Cereblon has been shown to mediate thalidomide's beneficial  
24 anti-inflammatory and anti-myeloma actions in adult and diseased tissues (Figure 2).  
25 The downstream targets of Cereblon-Thalidomide binding relating to teratogenesis,  
26 however, are not known.

### 27 *4.2.3 Tubulin*

28 Through the use of an antiangiogenic thalidomide analog, 5HPP-33, biochemical and  
29 computational assays have shown the affinity of 5HPP-33 to bind tubulin. In addition,  
30 5HPP-33 causes depolymerisation of microtubules and affects rebuilding of mitotic  
31 spindles, interfering with the alignment of chromosomes at metaphase (Rashid *et al.*,

1 2015). Changes in actin and microtubule cytoskeleton cause actin stress fibre and  
2 microtubule depolymerisation, altering cell migration and proliferation. Thalidomide  
3 exposure to human umbilical vein endothelial cells (HUVECs) results in a disruption  
4 of actin cytoskeleton (Tamilarasan *et al.*, 2006), and CPS49 affects migration and  
5 cytoskeletal organisation of endothelial cells (Therapontos *et al.*, 2009).

6 These studies provide evidence that tubulin may be a target of thalidomide  
7 preventing angiogenesis, leading to cell death of tissues causing thalidomide  
8 teratogenesis.

9

#### 10 4.2.4 Soluble Guanylyl Cyclase and Nitric Oxide

11 Thalidomide has also been shown to potentially interact with soluble guanylyl  
12 cyclase (sGC). sGC stimulation by NO leads to production of cyclic guanosine  
13 monophosphate (cGMP) which is involved in several cellular processes, including  
14 apoptosis, vasodilation and blood flow increase through the control of vascular  
15 smooth muscle (Majumder *et al.*, 2009, Siamwala *et al.*, 2012). Experiments in  
16 HUVEC cultures showed that thalidomide exposure reduced cGMP levels, causing  
17 failure of angiogenesis. This phenotype can be reversed by inducing an increase in  
18 sGMP levels (Majumder *et al.*, 2009).

19 Moreover, thalidomide has been shown to exert effects through alterations in NO-  
20 mediated endothelial cell migration and apoptosis (Tamilarasan *et al.*, 2006,  
21 Siamwala *et al.*, 2012). Assays in chicken embryos show increasing NO may rescue  
22 thalidomide teratogenicity (Tamilarasan *et al.*, 2006, Majumder *et al.*, 2009,  
23 Siamwala *et al.*, 2012).

#### 24 4.2.5 Genetic Studies

25 Many other gene expression patterns have been shown to be altered following  
26 thalidomide exposure in chicken, zebrafish and non-human primate studies  
27 including, for example, *Shh*, *Fgf8* and *Integrins* (Vargesson, 2009; Vargesson, 2015).  
28 How these fit into the molecular pathway/s altered by thalidomide is unclear.  
29 Furthermore, studies looking at differential gene expression after direct thalidomide  
30 exposure have been carried out using microarray techniques in monkey embryos

1 and in human and mouse embryonic stem cells. Expression levels of around 2000  
2 genes were found to be altered following thalidomide exposure including those  
3 involved in cell differentiation, development, metabolism, cytoskeleton organization,  
4 limb and heart development and the immune response (Gao *et al.*, 2014; Gao *et al.*,  
5 2015; Ema *et al.*, 2010, Meganathan *et al.*, 2012). Some of these changes may be  
6 primary, secondary or even tertiary. Indeed, the precise molecular pathway/s  
7 influenced by thalidomide remain to be fully determined. The possibility that there  
8 may be more than one direct molecular target and pathway affected by thalidomide  
9 is plausible.

10 A genomic study, carried out in human thalidomide affected patients, aimed to  
11 assess if a potential genetic susceptibility to thalidomide embryopathy exists by  
12 analysing the endothelial Nitric Oxide Synthase gene in thalidomide survivors and  
13 non-thalidomide affected individuals. It was observed that alleles relating to a  
14 reduced production of NO are found more frequently in thalidomide subjects. This  
15 not only reinforces the involvement of NO in thalidomide embryopathy but also the  
16 role for angiogenesis in thalidomide teratogenesis (Vianna *et al.*, 2013).

## 17 **5 Conclusion**

18

19 Despite numerous studies and recent advances in our understanding, the  
20 mechanisms that result in thalidomide embryopathy are still not completely known.  
21 Actions upon blood vessels, induction of cell death and involvement of several gene  
22 targets including Cereblon and tubulin are all involved. Just how thalidomide  
23 exposure causes changes in molecular pathways and any interrelation among these  
24 pathways is unclear. Indeed multiple pathways may be affected to cause the  
25 different tissue specific damage. Currently blood vessels as a primary target tissue of  
26 thalidomide, which locally induces ROS and cell death in affected tissues, is a  
27 strongly favoured teratogenic mechanism of action of thalidomide (Vargesson, 2013;  
28 Vargesson, 2015; Figure 4).

29 Thalidomide was used to treat a range of conditions, including morning sickness,  
30 which typically occurs between week 4 and week 12 (although timing and severity  
31 can vary between women). Between weeks 4 and 9 major events in embryology

1 occur along with major cell signalling events, massive cell migration and tissue  
2 morphogenetic events. As we have outlined, angiogenesis and vascularisation is an  
3 essential step in tissue formation, outgrowth and maintenance. Smooth muscle  
4 negative vessels undergo rapid angiogenic changes and migration. Disruption of  
5 vessels or loss of vessels in forming tissues could result in cell death and localised  
6 ROS activity tissue loss with interrupted signalling devastating rapidly growing  
7 tissues and causing malformations. For example, phocomelia in the limbs could  
8 occur as vessels are prevented from vascularising the limb, which then starts to  
9 undergo cell death, loss of gene expression or gene misexpression. As the activity of  
10 the drug wears off, the remaining cells can be vascularised and undergo proliferation  
11 and the developing limb gene signalling pathways recover but as too few cells  
12 remain, only distal structures develop (Therapontos *et al.*, 2009; Vargesson, 2009;  
13 Vargesson, 2015). Appearance of secondary cell types and their development into  
14 tissues, for example nerves, muscles and bones, will then be altered as the limb  
15 tissue is malformed or even missing (Vargesson, 2013; Vargesson, 2015).

16 By around week 9 the major tissues are formed and vasculature is also maturing  
17 through recruitment of smooth muscle, with reduced angiogenesis. Exposure to  
18 thalidomide does not appear to result in outwardly visible malformations after this  
19 stage. However, the fact that thalidomide acts in an antiangiogenic manner both in  
20 the early embryo and the adult suggests late embryonic exposure could damage  
21 physiological function of the internal organs as they mature and enlarge, since tissue  
22 expansion requires angiogenesis. The framework of thalidomide embryopathy as  
23 described above (Figure 4; and in further detail in Vargesson, 2015) is a good  
24 explanation for thalidomide-induced damage to the tissues. It can explain the range  
25 of damage and time sensitive nature of the induced damage. Malformations occur  
26 dependent upon the maturity of blood vessels and whether they are undergoing  
27 angiogenesis, and the chance of defects presenting is reduced as tissues and  
28 vessels mature (Therapontos *et al.*, 2009; Vargesson and Laufer, 2001; Vargesson,  
29 2013; Vargesson, 2015).

30 Challenges do remain, and these include to understand which molecular pathway, or  
31 multiple pathways, are affected by thalidomide to cause teratogenesis. We know  
32 several molecular targets of thalidomide, Cereblon and tubulin, and know many other  
33 gene profiles can be changed following thalidomide exposure. However, just how

1 thalidomide binding to these targets results in embryopathy is unclear.  
2 Understanding the molecular pathways and elucidating any other candidate targets  
3 may shed light on novel roles for genes and help to understand how birth defects  
4 can be prevented. In addition, determining if a form or analog of thalidomide can be  
5 produced with the clinical benefits (for example, an analog that will still treat leprosy)  
6 but without the side effect of birth defects, is a significant and essential challenge  
7 especially given the new generation of thalidomide affected children in Brazil (Beedie  
8 *et al.*, *In Press*; Vargesson, 2015). Structural variants of thalidomide, for example,  
9 Lenalidomide and Pomalidomide, function slightly differently and are used clinically  
10 to treat inflammatory diseases and cancer, though with some species-specific  
11 teratogenic side-effects (Vargesson, 2013; Vargesson, 2015). Can a form of the drug  
12 be made or found that retains clinical relevance but without the drugs side-effects?

13 Great strides in our understanding of thalidomide-induced embryopathy have been  
14 made in the recent few years. Thalidomide's use for treating inflammatory disorders  
15 in adult humans has increased interest in the drug and other uses for it. In addition,  
16 following the birth of recent thalidomide survivors in Brazil interest in determining the  
17 teratogenic mechanisms of the drug has also increased. Hopefully it will only be a  
18 matter of time before all the mechanisms this drug uses are finally determined and a  
19 safe form can be developed.

20

21

## 22 **Acknowledgements**

23 Apologies to the many papers we were unable to cite, due to space constraints. We  
24 thank Lynda Erskine, Shaunna Beedie and Chris Mahony for helpful discussions.  
25 Lucas Rosa Fraga is funded by a PhD scholarship from the Science without Borders  
26 program - CNPq Brazil - INAGEMP/ Grant CNPq 573993/2008-4. Alex J. Diamond is  
27 funded by a BBSRC DTP PhD Scholarship.

28

## References

- 1  
2  
3 Beedie S, Peer CJ, Pisle S *et al.* Anti-cancer properties of a novel class of  
4 tetrafluorinated thalidomide analogs. *Molecular Cancer Therapeutics In Press*  
5  
6 D'Amato RJ, Loughnan M, Flynn E and Folkman J (1994) Thalidomide is an inhibitor  
7 of angiogenesis. *Proc Natl Acad Sci U S A* **91**(9): 4082-4085.  
8  
9 DiPaolo JA, Gatzek H and Pickren J (1964) Malformations Induced in the Mouse by  
10 Thalidomide. *Anat Rec* **149**: 149-155.  
11  
12 El-Aarag, BYA *et al.* (2014) In vitro anti-proliferative and antiangiogenic activities of  
13 thalidomide dithiocarbamate analogs. *Int Immunopharmacol* **21**(2): 283.  
14  
15 Ema M, Ise R, Kato H *et al.* (2010) Fetal malformations and early embryonic gene  
16 expression response in cynomolgus monkeys maternally exposed to thalidomide.  
17 *Reprod Toxicol* **29**(1): 49-56.  
18  
19 Fort DJ, Stover EL, Bantle J and Finch RA (2000) Evaluation of the developmental  
20 toxicity of thalidomide using frog embryo teratogenesis assay-xenopus (FETAX):  
21 biotransformation and detoxification. *Teratog Carcinog Mutagen* **20**(1): 35-47.  
  
22 Franks ME, Macpherson GR and Figg WD (2004) Thalidomide. *Lancet* **363**(9423):  
23 1802-1811.  
24  
25 Fratta ID, Sigg EB and Maiorana K (1965) Teratogenic Effects of Thalidomide in  
26 Rabbits, Rats, Hamsters, and Mice. *Toxicol Applied Pharmacol* **7**: 268.  
  
27 Gao X, Yourick JJ and Sprando RL (2014) Transcriptomic characterization of  
28 C57BL/6 mouse embryonic stem cell differentiation and its modulation by  
29 developmental toxicants. *PLoS One* **9**(9):e108510.  
30  
31 Gao X, Sprando RL and Yourick JJ (2015) Thalidomide induced early gene  
32 expression perturbations indicative of human embryopathy in mouse embryonic stem  
33 cells. *Toxicol Appl Pharmacol* **287**(1): 43-51.  
  
34 Hansen JM and Harris C (2004) A novel hypothesis for thalidomide-induced limb  
35 teratogenesis: redox misregulation of the NF-kappaB pathway. *Antioxid Redox*  
36 *Signal* **6**(1): 1.  
37  
38 Higgins JJ, Pucilowska J, Lombardi RQ and Rooney JP (2004) A mutation in a  
39 novel ATP-dependent Lon protease gene in a kindred with mild mental retardation.  
40 *Neurology* **63**(10): 1927.  
41  
42 Ito T, Ando H and Handa H (2011) Teratogenic effects of thalidomide: molecular  
43 mechanisms. *Cell Mol Life Sci* **68**(9): 1569.  
44  
45 Ito T, Ando H, Suzuki T *et al.* (2010) Identification of a primary target of thalidomide  
46 teratogenicity. *Science* **327**(5971): 1345-1350.

- 1  
2 Knobloch J, Shaughnessy Jr, JD and Ruther U (2007) Thalidomide induces limb  
3 deformities by perturbing the Bmp/Dkk1/Wnt signaling pathway. *FASEB J* **21**(7):  
4 1410.  
5  
6 Latif T, Chauhan N, Khan R, Moran A and Usmani SZ (2012) Thalidomide and its  
7 analogs in the treatment of Multiple Myeloma. *Exp Hematol Oncol* **1**(1): 27.  
8  
9 Lebrin, F., Srun, S., Raymond, K *et al.* (2010) Thalidomide stimulates vessel  
10 maturation and reduces epistaxis in individuals with hereditary hemorrhagic  
11 telangiectasia. *Nature Medicine* **16**: 420-428  
12  
13 Lee KM, Yang SJ, Kim YD *et al.* (2013) Disruption of the cereblon gene enhances  
14 hepatic AMPK activity and prevents high-fat diet-induced obesity and insulin  
15 resistance in mice. *Diabetes* **62**:1855-1864.  
16  
17 Lenz W and Knapp K (1962) Foetal malformations due to thalidomide. *German Med.*  
18 *Monthly* **7**: 253-258.  
  
19 Lenz W (1988) A short history of thalidomide embryopathy. *Teratology* **38**(3): 203-  
20 215.  
21  
22 Lenz W, Pfeiffer RA, Kosenow K and Hayman DJ (1962) Thalidomide and  
23 Congenital Abnormalities. *Lancet* **279**(7219): 45.  
24  
25 Lu J, Helsby N, Palmer BD *et al.* (2004) Metabolism of thalidomide in liver  
26 microsomes of mice, rabbits, and humans. *J Pharmacol Exp Ther* **310**(2): 571.  
27  
28 Mahony C, Erskine L, Niven J *et al.* (2013) Pomalidomide is nonteratogenic in  
29 chicken and zebrafish embryos and nonneurotoxic in vitro. *Proc Natl Acad Sci U S A*  
30 **110**(31): 12703.  
31  
32 Majumdar S, Lamothe B and Aggarwal BB (2002) Thalidomide suppresses NF-  
33 kappa B activation induced by TNF and H<sub>2</sub>O<sub>2</sub>, but not that activated by ceramide,  
34 lipopolysaccharides, or phorbol ester. *J Immunol* **168**(6): 2644-2651.  
  
35 Majumder S, Rajaram M, Muley A *et al.* (2009) Thalidomide attenuates nitric oxide-  
36 driven angiogenesis by interacting with soluble guanylyl cyclase. *Br J Pharmacol*  
37 **158**(7): 1720.  
38  
39 Marks MG, Shi J, Fry MO *et al.* (2002) Effects of putative hydroxylated thalidomide  
40 metabolites on blood vessel density in the chorioallantoic membrane (CAM) assay  
41 and on tumor and endothelial cell proliferation. *Biol Pharm Bull* **25**(5): 597-604.  
  
42 Matthews SJ and McCoy C (2003) Thalidomide: a review of approved and  
43 investigational uses. *Clin Ther* **25**(2): 342-395.  
44  
45 McBride WG (1961) Thalidomide and Congenital Abnormalities. *Lancet* **278**(7216):  
46 1358.  
47

- 1 McCredie J (2009) History, heresy and radiology in scientific discovery. *J Med*  
2 *Imaging Radiat Oncol* **53**(5): 433-441.
- 3 Meganathan K, Jagtap S, Wagh V *et al.* (2012) Identification of thalidomide-specific  
4 transcriptomics and proteomics signatures during differentiation of human embryonic  
5 stem cells. *PloS One* **7**(8): e44228.
- 6 Melchert M and List A (2007) The thalidomide saga. *Int J Biochem Cell Biol* **39**(7-8):  
7 1489-1499.
- 8 Miller MT, Stromland K, Ventura L *et al.* (2005) Autism associated with conditions  
9 characterized by developmental errors in early embryogenesis: a mini review. *Int J*  
10 *Dev Neurosci* **23**(2-3): 201.
- 11  
12 Moreira AL, Sampaio EP, Zmuidzinis A *et al.* (1993) Thalidomide exerts its inhibitory  
13 action on tumor necrosis factor alpha by enhancing mRNA degradation. *J Exp Med*  
14 **177**(6): 1675-1680.
- 15  
16 Newman CG (1986) The thalidomide syndrome: risks of exposure and spectrum of  
17 malformations. *Clinics in perinatology* **13**(3): 555.
- 18 Parkhie M and Webb M (1983) Embryotoxicity and teratogenicity of thalidomide in  
19 rats. *Teratology* **27**(3): 327.
- 20  
21 Parman T, Wiley MJ and Wells PG (1999) Free radical-mediated oxidative DNA  
22 damage in the mechanism of thalidomide teratogenesis. *Nature Medicine* **5**:582-585.
- 23  
24 Rashid A, Kuppa A, Kunwar A and Panda D (2015) Thalidomide (5HPP-33)  
25 suppresses microtubule dynamics and depolymerizes the microtubule network by  
26 binding at the vinblastine binding site on tubulin. *Biochemistry* **54**(12): 2149-2159.
- 27 Ruffing L (1977) Evaluation of thalidomide children. *Birth Defects Orig Artic Ser*  
28 **13**(1): 287-300.
- 29 Sampaio EP, Kaplan G, Miranda A *et al.* (1993) The influence of thalidomide on the  
30 clinical and immunologic manifestation of erythema nodosum leprosum. *J Infect Dis*  
31 **168**(2): 408-414.
- 32 Schmidt M and Salzano FM (1980) Dissimilar effects of thalidomide in dizygotic  
33 twins. *Acta Genet Med Gemellol (Roma)* **29**(4): 295-297.
- 34 Schuster SR, Kortuem KM, Zhu YX *et al.* (2014) The clinical significance of cereblon  
35 expression in multiple myeloma. *Leuk Res* **38**(1): 23.
- 36  
37 Sheskin J (1965) Thalidomide in the treatment of lepra reactions. *Clin Pharmacol*  
38 *Ther* **6**: 303-306.
- 39  
40 Siamwala JH, Veeriah V, Priya MK *et al.* (2012) Nitric oxide rescues thalidomide  
41 mediated teratogenicity. *Sci Rep* **2**: 679.

- 1  
2 Smithells RW and Newman CG (1992) Recognition of thalidomide defects. *J Med*  
3 *Gen* **29**(10): 716.  
4  
5  
6 Stephens TD (2009) The effect of thalidomide in chicken embryos. *Birth Defects Res*  
7 *A Clin Mol Teratol* **85**(8): 725.  
8  
9 Stewart AK (2014) Medicine. How thalidomide works against cancer. *Science*  
10 **343**(6168): 256.  
11  
12 Tamilarasan KP, Kolluru GK, Rajaram M *et al.* (2006) Thalidomide attenuates nitric  
13 oxide mediated angiogenesis by blocking migration of endothelial cells. *BMC Cell*  
14 *Biol* **7**: 17.  
15  
16 Therapontos C, Erskine L, Gardner ER *et al.* (2009) Thalidomide induces limb  
17 defects by preventing angiogenic outgrowth during early limb formation. *Proc Natl*  
18 *Acad Sci U S A* **106**(21): 85738.  
19  
20 Uhl K, Cox E, Rogan R *et al.* (2006) Thalidomide use in the US : experience with  
21 pregnancy testing in the S.T.E.P.S. programme. *Drug Saf* **29**(4): 321-329.  
22  
23 Vargesson N (2003) Vascularisation of the developing chick limb bud: role of the  
24 TGFbeta signalling pathway. *J. Anat* **202**:93-103.  
25  
26  
27  
28  
29 Vargesson N (2009) Thalidomide-induced limb defects: resolving a 50-year-old  
30 puzzle. *Bioessays* **31**(12): 1327-1336.  
31  
32 Vargesson N (2013) Thalidomide Embryopathy: An Enigmatic Challenge. *ISRN*  
33 *Developmental Biology* **Article ID 241016**: 18 pages.  
34 2013. <http://dx.doi.org/2010.1155/2013/241016>.  
35  
36 Vargesson N (2015) Thalidomide-induced teratogenesis: History and mechanisms.  
37 *Birth Defects Research Part C: Embryo Today: Reviews* **105**: 140–156  
38  
39 Vargesson N and Laufer E (2001) Smad7 misexpression during embryonic  
40 angiogenesis causes vascular dilation and malformations independently of vascular  
41 smooth muscle cell function. *Dev Biol* **240**(2): 499-516.  
42  
43 Vianna FS, Schuler-Faccini L, Leite JC *et al.* (2013) Recognition of the phenotype of  
44 thalidomide embryopathy in countries endemic for leprosy: new cases and review of  
45 the main dysmorphological findings. *Clin Dysmorphol* **22**(2): 59.  
46  
47 Xin W, Xiaohua N, Peilin C *et al.* (2008) Primary function analysis of human mental  
48 retardation related gene CRBN. *Mol Biol Rep* **35**(2): 251.

1 Yabu T, Tomimoto H, Taguchi Y *et al.* (2005) Thalidomide-induced antiangiogenic  
2 action is mediated by ceramide through depletion of VEGF receptors, and is  
3 antagonized by sphingosine-1-phosphate. *Blood* **106**(1): 125-134.

4

5

6

7

#### 8 **Further Reading**

9 Magazanik M (2015) *Silent Shock: The Men Behind the Thalidomide Scandal and an*  
10 *Australian Family's Long Road to Justice*. Melbourne: Text Publishing.

11

12 Stephens TD, Bunde CJJ and Fillmore BJ (2000) Mechanisms of action in  
13 thalidomide teratogenesis. *Biochemical Pharmacology* **59**(12):1489-1499.

14

15 Vargesson N (2011) *Thalidomide*. Reproductive and Developmental Toxicology;  
16 Editor: R Gupta; pp395-403. Amsterdam: Academic Press Elsevier.

17

18 Zuniga A, Zeller R and Probst S (2012) The molecular basis of human congenital  
19 limb malformations. *Wiley Interdisciplinary Reviews: Developmental Biology*  
20 **1**(6):803-822.

21

22

- 1 **List of Abbreviations**
- 2
- 3 BMP – bone morphogenetic protein
- 4 cGMP – cyclic guanosine monophosphate
- 5 COX2 – cyclooxygenase 2
- 6 CUL4A – cullin 4A
- 7 DDB1 – DNA damage-binding protein 1
- 8 ENL – erythema nodosum leprosum
- 9 FDA – US Food and Drug Administration
- 10 FGF – fibroblast growth factor
- 11 HHT – hereditary hemorrhagic telangiectasia
- 12 HUVEC – human umbilical vein endothelial cells
- 13 IL – interleukin
- 14 MM – multiple myeloma
- 15 NF- $\kappa$ B – nuclear factor - kappa beta
- 16 NO – nitric oxide
- 17 Roc1 – Regulator of cullin1
- 18 ROS – reactive oxygen species
- 19 sGC - soluble guanylyl cyclase
- 20 Shh – sonic hedgehog
- 21 TNF $\alpha$  – tumour necrosis factor alpha
- 22 VEGF – vascular endothelial growth factor
- 23
- 24
- 25
- 26
- 27

**Table 1:** A list of common defects seen in TE, the specific defects seen in each organ, and an explanation of each. Also listed are the time points at which thalidomide is taken that can result in each defect.

<b>Region Affected</b>	<b>Specific Defects Seen</b>	<b>Additional</b>	<b>Time Point of Exposure (Days)</b>	
<b>Limb</b>	Reduced hand/footplate	Digit effects	Thumb aplasia: 21-26 Triphalangeal thumb: 31-36	
	Amelia	Complete absence of limb	Upper limb: 24-29 Lower limb: 27-31	
	Phocomelia	Limb long bones are shortened or absent	Upper limb: 24-33 Lower limb: 28-33	
<b>Limb Girdles</b>	Sharpened shoulder	Acromioclavicular joint is more prominent		
	Hip joint / Pubic bone	Hypoplasia	Hip dislocation: 23-34 Femoral hip hypoplasia: 28-33 Hip dysplasia: 20-24	
<b>Eye</b>		Complete absence		
	Cataracts		20-24	
	Microphthalmia	Congenital small eye	24-30	
	Anophthalmos	Absence of eyeball		
	Poor vision			
	Aberrant lacrimation		20-26	
	Colobomas	Derformity of iris and retina	24-26	
<b>Ear</b>	Abnormalities in eye movement			
	Anotia	Complete absence of outer ear, results in deafness	20-24 Inner ear defects: 24-33	
	Microtia	Part of the outer ear remains	24-33	
<b>Internal organs</b>	Heart	Ventricular and atrial septum defects	22-31	
		Pulmonary stenosis		
		Patent ductus arteriosus		
	Lung	Lung malformation	29-32	
	Kidney	Horseshoe, hypoplastic, rotated and ectopic malformations	Ectopic kidney: 24-29	
	Intestines	Duodenum		Duodenal atresia: 20-33 Duodenal stenosis: 27-34
		Anal atresia		Anal atresia: 27-29 Rectal stenosis: 35-36
		Gall bladder atresia		28-29
		Pyloric stenosis		26-33
	Urinary tract	Bladder atresia		28-29
	Genitals	In males: absence of testes or testicular abnormalities. Hypospadias		Testicular agenesis: 31-33
		In females: malformations of uterus and reproductive tract		35-39 and 49-50
<b>Nerves and CNS</b>	Facial palsies	Ear defects are associated with cranial nerve palsies	Facial palsy: 20-26 Cranial palsy: 21-23	

Adapted from Kim et al. 2011; Newman, 1986; Smithells and Newman, 1992; Ruffing, 1977; Vargesson, 2009; Vargesson, 2015.

1 **Figure Legends**

2

3 **Figure 1: Structures of thalidomide and its analogs** Thalidomide enantiomers R  
4 (+) and S(-) can interchange at physiological pH (asterisk indicates chiral centre).

5 **Figure 2: Therapeutic mechanisms of thalidomide in adults.** Illustrated are the  
6 pathways through which thalidomide is thought to act in the treatment of HHT and  
7 Multiple Myeloma. (Adapted from Stewart, 2014; Lebrin *et al.*, 2010)

8 **Figure 3: Thalidomide time-sensitive window.** Chart indicates the period (days  
9 and weeks post-fertilisation) in which the most common defects occur. See also  
10 Table 1. (Adapted from Vargesson, 2015; Miller *et al.*, 2005).

11 **Figure 4: Thalidomide and embryonic teratogenesis.** Thalidomide has been  
12 shown to induce loss of blood vessels, increased cell death and reactive oxygen  
13 species resulting in embryonic damage. Thalidomide may cause teratogenesis  
14 through interaction with targets such as Cereblon, tubulin and/or sGC, interrupting  
15 blood vessel development and resulting in localised reactive oxygen species and cell  
16 death induction.

17

18

19

20

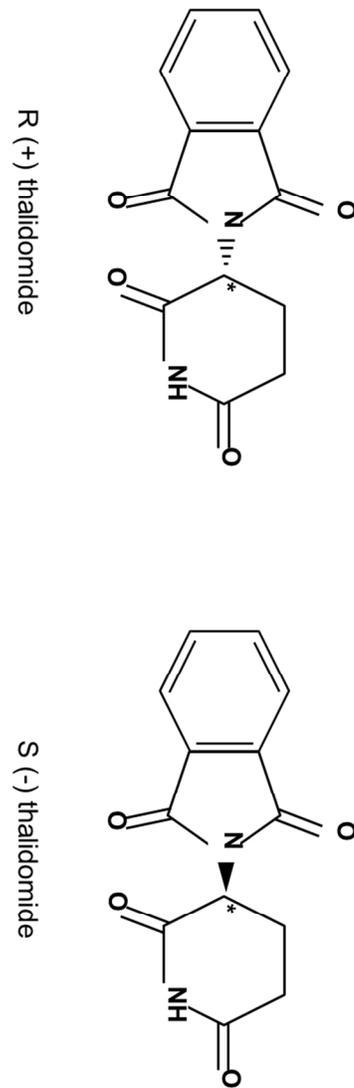
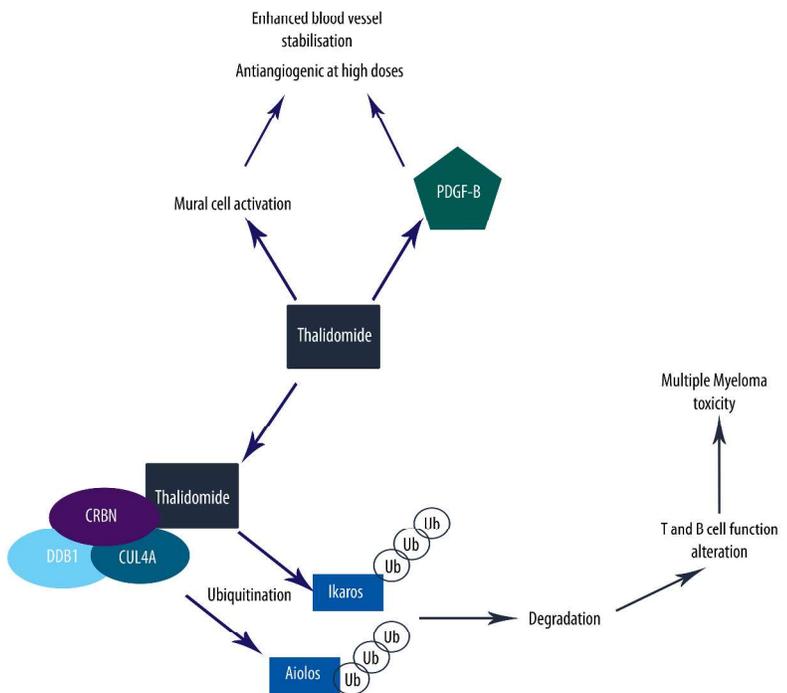


Figure 1  
266x382mm (96 x 96 DPI)

### Therapeutic Mechanisms of Thalidomide in the Adult



Thalidomide in Hereditary Hemorrhagic Telangiectasia (HHT)

Thalidomide in Multiple Myeloma

444x366mm (300 x 300 DPI)

Only

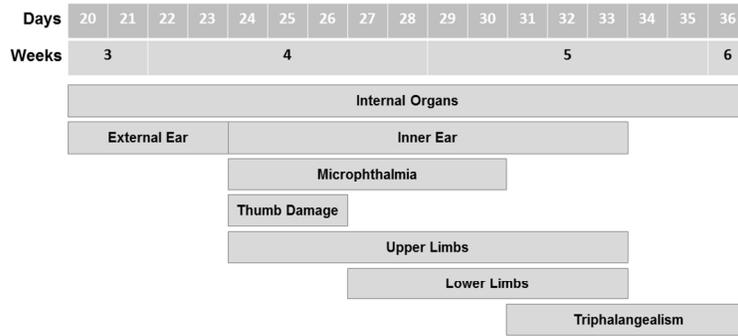
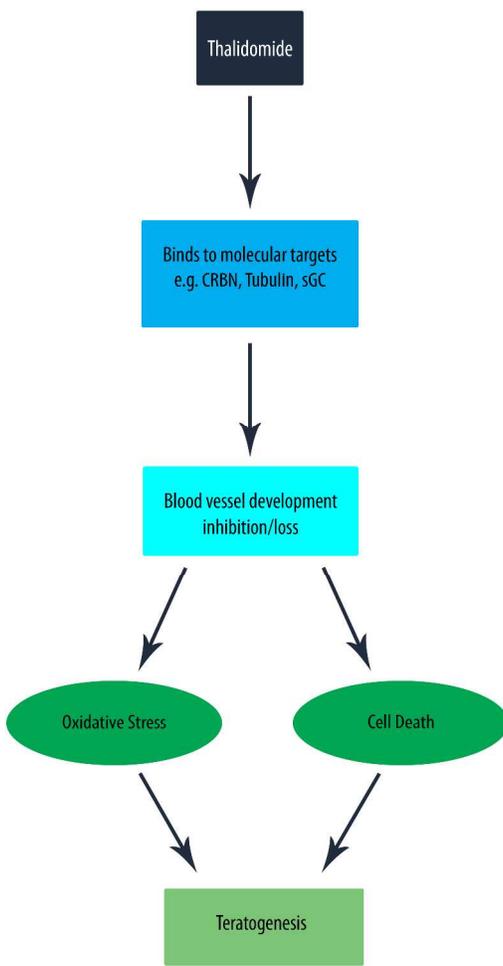


Figure 3  
382x266mm (96 x 96 DPI)

Preview Only

### Thalidomide and Embryonic Teratogenesis



277x466mm (300 x 300 DPI)