



Thalidomide: The Tragedy of Birth Defects and the Effective Treatment of Disease

James H. Kim*¹ and Anthony R. Scialli†

**ILSI Health and Environmental Sciences Institute, Washington, DC 20005*; and †*Tetra Tech Sciences, Arlington, Virginia 22201-3397*

¹To whom correspondence should be addressed at ILSI Health and Environmental Sciences Institute, 1156 15th Street, NW, Suite 200, Washington, DC 20005. Fax: (202) 659-3617. E-mail: jkim@hesiglobal.org.

Received January 28, 2011; accepted April 2, 2011

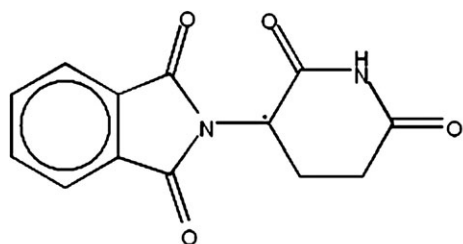
Thalidomide was a widely used drug in the late 1950s and early 1960s for the treatment of nausea in pregnant women. It became apparent in the 1960s that thalidomide treatment resulted in severe birth defects in thousands of children. Though the use of thalidomide was banned in most countries at that time, thalidomide proved to be a useful treatment for leprosy and later, multiple myeloma. In rural areas of the world that lack extensive medical surveillance initiatives, thalidomide treatment of pregnant women with leprosy has continued to cause malformations. Research on thalidomide mechanisms of action is leading to a better understanding of molecular targets. With an improved understanding of these molecular targets, safer drugs may be designed. The thalidomide tragedy marked a turning point in toxicity testing, as it prompted United States and international regulatory agencies to develop systematic toxicity testing protocols; the use of thalidomide as a tool in developmental biology led to important discoveries in the biochemical pathways of limb development. In celebration of the Society of Toxicology's 50th Anniversary, which coincides with the 50th anniversary of the withdrawal of thalidomide from the market, it is appropriate to revisit the lessons learned from the thalidomide tragedy of the 1960s.

Key Words: birth defects; teratogen; multiple myeloma; leprosy; testing.

Thalidomide was first marketed in the late 1950s as a sedative and was used in the treatment of nausea in pregnant women (Fig. 1). Within a few years of the widespread use of thalidomide in Europe, Australia, and Japan, approximately 10,000 children were born with phocomelia, leading to the ban of thalidomide in most countries in 1961. Some countries continued to provide access to thalidomide for a couple of years thereafter (Lenz, 1988). In addition to limb reduction anomalies, other effects later attributed to thalidomide included congenital heart disease, malformations of the inner and outer

ear, and ocular abnormalities (Miller and Strömmland, 1999). The thalidomide tragedy was averted in the United States because of the hold on its approval by Dr Frances Kelsey of the U.S. Food and Drug Administration, who was recognized by President John F. Kennedy as a recipient of the Gold Medal Award for Distinguished Civilian Service. Dr Kelsey's decision to hold the approval of thalidomide was not because of the birth defects, which had not yet been attributed to thalidomide, but because of her concerns about peripheral neuropathy (sometimes irreversible) in the patient and the potential effects a biologically active drug could have after treatment of pregnant women. The thalidomide tragedy also brought into sharp focus the importance of rigorous and relevant testing of pharmaceuticals prior to their introduction into the marketplace (Kelsey, 1988). Dr Kelsey was awarded an honorary membership to the Society of Toxicology in celebration of its 50th Anniversary in 2011.

Josef Warkany, one of the founders of the Teratology Society, doubted in April of 1962 that thalidomide was responsible for the epidemic of limb defects (Warkany, 1988). His reasoning was that rat experiments had not produced comparable malformations and that malformations in humans were inconsistent (i.e., some mothers who were exposed to thalidomide had normal children and some malformations occurred in children whose mothers did not knowingly take thalidomide) (Warkany, 1988). The thalidomide episode led to the adoption of requirements for the systematic testing of pharmaceutical products for developmental toxicity prior to marketing. The adoption of these requirements is sometimes considered a benefit of the thalidomide tragedy. The legacy of thalidomide extends further than the creation of detailed testing protocols. With thalidomide came, the widespread recognition that differences in sensitivity between species required consideration. As a consequence, developmental toxicity testing for pharmaceuticals is conducted in two



Note: ♦ = asymmetric carbon atom

FIG. 1. Structure of thalidomide.

species, one of which is not a rodent. It is not clear, however, that the routine use of the second species (usually rabbit) has resulted in better testing, and it has been suggested that a more thorough understanding of results in a single species may be preferable (Janer *et al.*, 2008).

In order to address potential developmental and reproductive toxicities of pharmaceuticals, the U.S. FDA (1966) laid the foundation for the development of the segment I (fertility and general reproduction), II (teratogenicity), and III (perinatal) testing protocols in 1966. Prior to the development of the segment I, II, and III testing protocols, toxicology testing was more hypothesis driven rather than a systematic bioassay testing strategy that is in place today. The segment I, II, and III studies, or their International Conference on Harmonisation and Organisation for Economic Co-operation and Development equivalents, are performed in addition to routine short-term, subchronic, and chronic toxicity assays and have been in place with little change for over 40 years.

An examination of the PubMed literature database revealed a bimodal pattern (Fig. 2) in the number of citations containing

thalidomide as a keyword. As a direct result of the thalidomide tragedy in the early 1960s, not surprisingly, there was a steady number of publications in that decade. Because of the ban in its use, interest in thalidomide waned in the 1970s and 1980s. For reasons that will be described below, the number of thalidomide publications began increasing in the latter half of the 1990s and have increased dramatically in the last 10 years. Though its use in pregnant women was banned in 1961, thalidomide continues to be used in the treatment of leprosy because of its immunomodulatory properties. The World Health Organization still does not recommend thalidomide for the treatment of leprosy because of its use in areas of poor medical surveillance resulting in a number of thalidomide-affected children (<http://www.who.int/lep/research/thalidomide/en/>).

In 1998, thalidomide was approved by the U.S. Food and Drug Administration for the treatment of leprosy and, subsequently, for multiple myeloma. Studies are ongoing to evaluate the effectiveness of thalidomide in the treatment of other diseases. Thalidomide inhibits angiogenesis and could be used to treat human diseases that are dependent on angiogenesis. Inhibition of angiogenesis is one of the proposed mechanisms of action for thalidomide's teratogenic properties (discussed below).

As a "model" teratogen, thalidomide is also being used as a tool to evaluate the predictivity of alternative testing methods such as *in vitro* assays.

WINDOWS OF EXPOSURE AND DOSE

Toxicologists have long held dear the tenet of Paracelsus that "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy."

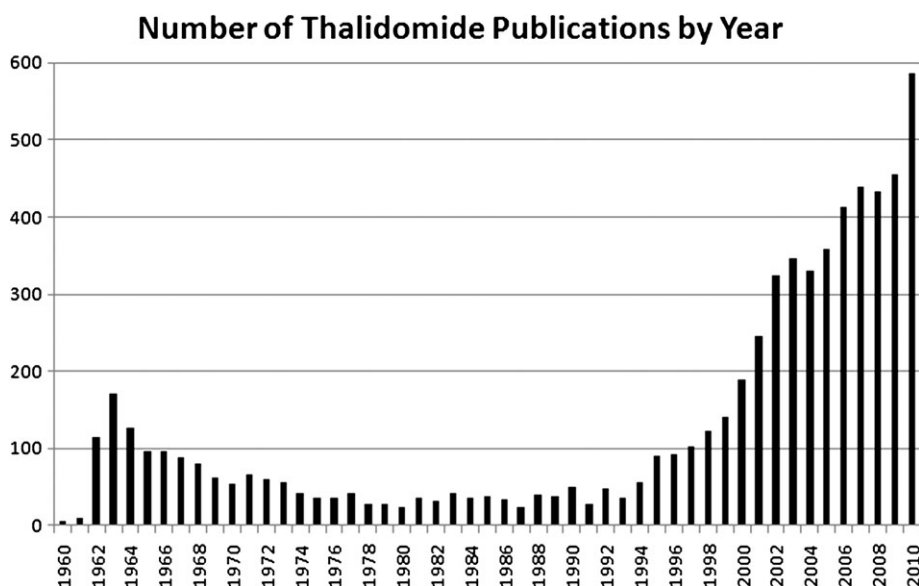


FIG. 2. Number of PubMed entries for thalidomide publications by year (searched on 12/27/2010).

(Gallo, 2008). For developmental and reproductive toxicology, this tenet needs to be modified with a consideration of the exposure period. In the early 1960s, it was recognized that the timing of exposure was as important as the dose for teratogenic effects (Lenz, 1962; Nowack, 1965). Based on the literature, Miller and Strömmland (1999) and Miller *et al.* (2009) constructed a timetable of critical exposure periods for thalidomide embryopathy. For example, the window of exposure in humans for upper limb malformations is days 24–32 postfertilization; the window for lower limb malformations is days 27–34 postfertilization (Miller and Strömmland, 1999). Other endpoints include external ear malformations (days 20–24), inner ear malformations (days 24–34), thumb hypoplasia (days 21–28), and triphalangism of the thumbs (days 32–36). The sensitive period during pregnancy for thalidomide effects in humans is approximately days 20–34 after fertilization (Miller and Strömmland, 1999; Miller *et al.*, 2009). A summary of these windows of critical exposures and limb defects is presented in Figure 3.

Miller and Strömmland (1999) also listed other findings in thalidomide-exposed children: kidney malformations, ventricular septal defects, dental malformations, autism and mental retardation, ocular anomalies, and Duane syndrome (lack of the sixth nerve and aberrant innervation of ocular muscles by the third cranial nerve) (Miller *et al.*, 2009). Autism and mental retardation, ocular anomalies, and Duane syndrome are not always accompanied by limb anomalies.

MECHANISMS OF ACTION

A diverse collection of thalidomide mechanisms of action has been proposed (Hansen and Harris, 2004; Stephens, 1988). Hansen and Harris (2004) noted over 30 hypotheses for the mechanism of thalidomide teratogenicity from 1966 to 2003,

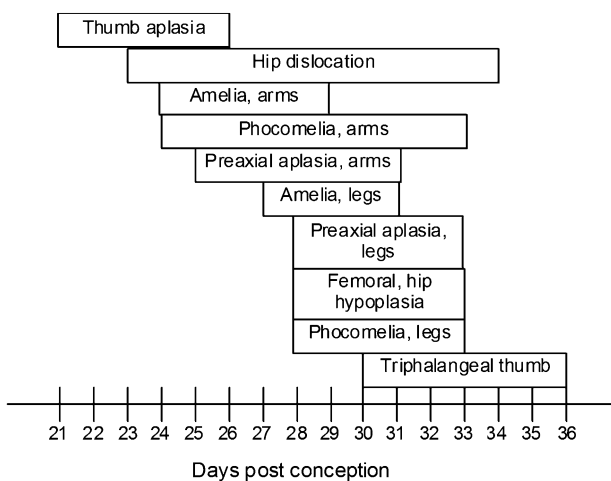


FIG. 3. Critical exposure periods for thalidomide embryopathy during human development.

including: (1) acylation of macromolecules, (2) ascorbic acid synthesis, (3) downregulation of adhesion receptors, (4) alteration of cytokine synthesis, (5) folic acid antagonism, (6) inhibition of DNA synthesis, (7) DNA oxidation, (8) interference of glutamate metabolism, and (9) mesonephros-stimulated chondrogenesis. As described below, more recent research has focused on hypotheses involving (1) oxidative stress/damage, (2) DNA intercalation, (3) inhibition of angiogenesis, and (4) cereblon (CRBN) binding.

Thalidomide has been reported to increase the production of oxygen radicals and induce oxidative stress (Hansen and Harris, 2004). For example, rabbits treated with 400 mg/kg/day thalidomide on gestational day (GD) 8–12 produced litters with phocomelia (Parman *et al.*, 1999). For DNA oxidation studies, the rabbits were treated 6 h before sacrifice with a single dose of 400 mg/kg (Parman *et al.*, 1999). Levels of DNA oxidation in maternal tissues (liver, lung, kidney, brain, and placenta) and in embryos were decreased when the rabbits were pretreated (by 15 min) with 40 mg/kg phenyl N-tert-butylnitron (PBN; a spin-trapping agent). Cotreatment of the rabbits with PBN also reduced the incidences of phocomelia (Parman *et al.*, 1999).

Using *in vitro* whole embryo culture techniques, rat (thalidomide-resistant Sprague-Dawley) and rabbit (thalidomide-sensitive New Zealand White) embryos were exposed to thalidomide (0, 5, 15, and 30 μ M), and changes in glutathione were assessed (Hansen *et al.*, 1999). The rabbit embryo cultures exhibited glutathione depletion (to 50% of control values) at 15 μ M, about twice the peak concentration achieved in humans on therapy, whereas rat embryo cultures did not. Glutathione depletion was also observed in the rabbit but not rat visceral yolk sacs at 15 μ M thalidomide. These experiments suggested a species-specific role for oxidative stress in thalidomide teratogenesis, though the mechanism still needs exploration.

Hansen and Harris (2004) have proposed that nuclear factor kappa-B (NF- κ B) mediates the redox regulation of limb outgrowth. Pregnant rabbits, which are thalidomide-sensitive, were treated with thalidomide (70 mg/kg bw/day) on GD 8–12 and pregnant rats, which are thalidomide insensitive, were treated with thalidomide (300 mg/kg bw/day) on GD 9–13. Limb buds were harvested on the last day of treatment, and green fluorescent protein (GFP) reporter vectors containing NF- κ B-binding promoter sites were transfected into the cells for expression experiments (Hansen *et al.*, 2002). GFP expression was decreased in thalidomide-exposed rabbit limb bud cells, whereas rat limb bud cells did not exhibit such a decrease. *In situ* hybridization of rabbit embryos (maternal rabbits treated with 400 mg/kg/day thalidomide) demonstrated decreased expression of genes (Twist, fibroblast growth factor [FGF]-8, FGF-10) involved in the proposed pathway (Hansen and Harris, 2004). Decreased GFP expression in the transfected limb bud cells and decreased expression of Twist, FGF-8, and FGF-10 could be prevented by cotreatment with the free radical spin-trapping compound PBN.

Mutant mice that are deficient in antioxidant enzymes (i.e., glucose-6-phosphate dehydrogenase) or glutathione or that have inhibition of glutathione peroxidase or reductase are more sensitive to thalidomide embryopathy than are wild-type mice (reviewed by Wells *et al.*, [2005]). In addition, *p53* knockout mice that are deficient in DNA damage responses and repair are also more sensitive to thalidomide- and radiation-induced embryopathies. Thalidomide has also been observed to induce cell death via upregulation of bone morphogenetic proteins and the *Wnt* antagonist, *Dickkopf1* (*Dkk1*), resulting in inhibition of *Wnt*/ β -catenin signaling in chicken embryos and human embryo fibroblasts (at about 5 times the maximum human therapeutic concentration) but not in mouse embryo fibroblasts (Knobloch *et al.*, 2007). Perturbation of signaling pathways via oxidative stress has been hypothesized to result in apoptosis of key progenitor cells in limb development (Knobloch and Ruther, 2008; Knobloch *et al.*, 2008).

As early as 1986, Koch and Czejka (1986) reported the intercalation by thalidomide of DNA of various specimens. Stephens *et al.* (2000) demonstrated that thalidomide binds to GC-rich promoter sites by intercalation and thereby decreases the transcription of insulin-like growth factor (IGF-1) and FGF-2. These gene products are known to stimulate transcription of α 5- and β 3-integrin subunit genes, resulting in stimulation of angiogenesis in the limb bud and proper limb growth. This theory is consistent with thalidomide exerting its teratogenic effects by inhibiting angiogenesis (Stephens *et al.*, 2000; Stephens and Fillmore, 2000). To investigate a hypothesis that thalidomide interferes with genes regulated by GC-rich promoters by blocking the binding of SP-1, Drucker *et al.* (2003) reported this same observation while studying the mechanism of thalidomide in the treatment of multiple myeloma. Inhibition of angiogenesis may be a mechanism of thalidomide action common to teratogenicity and utility against multiple myeloma (Drucker *et al.*, 2003; Stephens *et al.*, 2000).

D'Amato *et al.* (1994) demonstrated in a rabbit cornea micropocket assay that FGF-induced angiogenesis could be inhibited by thalidomide. Pellets containing basic fibroblast growth factor and sucralfate were implanted into the micropockets of both corneas of rabbits to stimulate angiogenesis. Thalidomide (200 mg/kg) administered by gavage on the same day as pellet implantation resulted in inhibited angiogenesis. Therapontos *et al.* (2009) reported that those thalidomide analogs that were anti-angiogenic but not anti-inflammatory could induce limb defects in chicken embryos. Thalidomide caused temporary blockage of mature blood vessels but caused complete obliteration of immature blood vessels in the chicken embryo, resulting in the hallmark limb defects (Therapontos *et al.*, 2009).

A recent publication by Ito *et al.* (2010) reported that CRBN is a thalidomide-binding protein. CRBN normally forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1) and Cullin-4A (CUL4A). DDB1 and CUL4A are important for the expression of FGF-8 and limb development. Ito *et al.* (2010) used zebrafish as a model

to evaluate CRBN function *in vivo*. Zebrafish have a CRBN ortholog (zCRBN) that is 70% identical to human CRBN. Zebrafish injected with an antisense morpholino oligonucleotide for zCRBN exhibited a phenotype (defects in fin and otic vesicle development) similar to that found in zebrafish treated with very high concentrations of thalidomide (0, 200, or 400 μ M); coinjection with zCRBN messenger RNA rescued the embryos from those antisense-induced defects. More compelling was the observation that zebrafish expressing zCRBN mutant proteins (with low thalidomide binding affinity) were resistant to thalidomide effects; in addition, normal zebrafish embryos that are thalidomide sensitive were rescued when the zCRBN mutant protein was overexpressed. These antisense experiments were repeated using the zebrafish ortholog to CUL4A, resulting in similar observations. The zebrafish knockdowns for zCRBN and zCUL4A also exhibited decreased FGF-8 expression. Chick embryos were used to confirm the findings. Thalidomide-induced limb defects were attenuated by overexpression of the mutant human CRBN protein possessing low thalidomide-binding affinity.

There is evidence to support all the hypotheses for the mechanisms of action of thalidomide limb teratogenicity: (1) oxidative stress/damage, (2) DNA intercalation, (3) inhibition of angiogenesis, and (4) CRBN binding, although the use in some experiments of very high concentrations of thalidomide raises questions about more clinically relevant exposure levels. The proposed mechanisms are not mutually exclusive. It is quite possible that several of the proposed mechanisms are working in parallel or synergistically to result in the hallmark thalidomide-associated limb anomalies.

THE CONTINUED USE OF THALIDOMIDE

Thalidomide appears to operate through anti-inflammatory and anti-angiogenesis mechanisms (Calabrese and Fleischer, 2000; Matthews and McCoy, 2003). These properties of thalidomide have been used as the basis for the treatment of leprosy (Calabrese and Fleischer, 2000; Sheskin, 1965, 1980) and multiple myeloma (Calabrese and Fleischer, 2000; Miller and Strömland, 1999). Leprosy patients with erythema nodosum leprosum exhibit vasculitic nodules and sometimes severe neuritis, conditions that cause extreme pain. Thalidomide administration to these patients is useful in suppressing these reactions.

Thalidomide has also been used to treat the wasting syndrome of advanced HIV infection (Calabrese and Fleischer, 2000; Matthews and McCoy, 2003). This syndrome is marked by increased tumor necrosis factor- α , the synthesis of which can be inhibited by thalidomide. Other diseases proposed to benefit from the anti-inflammatory properties of thalidomide include systemic lupus erythematosus, nodular prurigo, Behçet syndrome, erythema nodosum, Langerhans cell histiocytosis, and graft versus host disease (Calabrese and Fleischer, 2000; Matthews and McCoy, 2003).

As an anti-angiogenic drug, thalidomide inhibits tumor hyper-vascularity, growth, and metastasis (Calabrese and Fleischer, 2000). Because of these properties, thalidomide has been demonstrated to be useful in the treatment of multiple myeloma.

The FDA has mandated a strict surveillance program to prevent the availability of thalidomide to pregnant women. The Celgene Corporation (the maker of thalidomide) has developed a program called System for Thalidomide Education and Prescribing Safety (STEPS) to safeguard against potential exposure of pregnant women (Public Affairs Committee [PAC], 2000). The STEPS program requires that physicians prescribing thalidomide in the United States be registered with the program and that both male and female patients adhere to mandatory contraceptive plans. STEPS is a three-step program: (1) physicians must educate patients on the potential benefits and side-effects of thalidomide, (2) patients must receive contraceptive counseling and regular pregnancy testing, and (3) patients must provide informed consent and continued participation in mandatory surveys. Prescribers of thalidomide that do not comply with any of these steps will not have prescriptions honored at registered pharmacies (PAC, 2000). The STEPS program also has the following strict requirements to decrease the possibility of exposure to pregnant women: (1) only a 4-week supply can be prescribed with no automatic refills and (2) female patients must use two forms of birth control. The STEPS program has so far been successful in managing the risks of thalidomide teratogenicity in the United States (Uhl *et al.*, 2006). However, the availability of thalidomide has not been restricted in other areas of the world for the treatment of leprosy; access to thalidomide has likely resulted in cases of thalidomide-associated phocomelia on many continents but has been particularly well documented in South America (Castilla *et al.*, 1996; Schuler-Faccini *et al.*, 2007).

A recent publication by Johnson *et al.* (2011) investigated the association of thalidomide-associated peripheral neuropathy with single nucleotide polymorphisms (SNP) in 1495 multiple myeloma patients. This study reported that the risk of developing peripheral neuropathy was mediated by polymorphisms in genes involved in repair and inflammation of the peripheral nervous system. Though the mechanism of thalidomide-associated peripheral neuropathy is unclear, this report illustrates the continued interest in understanding thalidomide's effects.

Lenalidomide is a thalidomide analog currently used in the treatment of multiple myeloma. An evaluation of lenalidomide developmental toxicity in rabbits concluded that embryo-fetal effects were only manifested at maternally toxic doses (Christian *et al.*, 2007). This finding contrasts with thalidomide studies, in which teratogenesis is observed at nonmaternally toxic doses. Lenalidomide possesses antineoplastic activities that are important for its therapeutic use but does not appear to have the developmental toxicity associated with thalidomide. However, more research is needed to understand the mechanistic differences between the two compounds.

CURRENT AND FUTURE CHALLENGES

As a result of the thalidomide tragedy of the 1960s, the following lessons were learned:

Pharmaceutical products should be systematically tested for developmental effects prior to marketing.

There are differences in species sensitivity and manifestations of developmental toxicity.

Use of a second species or more thoroughgoing interpretation of results in a single species (taking pharmacokinetics into consideration) are important considerations in drug testing.

Because thalidomide is useful in the treatment of serious diseases, it is likely that this product will continue to be used in therapeutics until safer alternatives become available. Prevention of inadvertent exposure of pregnant women to this drug is a continuing challenge, particularly in parts of the world where access to the drug is less restricted than in the United States.

The development of thalidomide analogs that retain the therapeutic benefits of the drug without its teratogenic liability is a second challenge. The goal of a safe thalidomide analog may be elusive if the therapeutic mechanisms of action and the teratogenic mechanisms of action are closely related or even identical.

Research into mechanisms of action remains a priority for a better understanding of whether and how safer alternatives can be developed. Toward this end, significant advances over the last two decades in molecular techniques and alternative test species have provided unique methodologies to test hypotheses on thalidomide's mechanisms of action. Technologies such as the *in vitro* whole embryo culture test and zebrafish have been used to gain insight into mechanisms of action. The recent publication by Ito *et al.* (2010) exemplifies the use of advanced molecular techniques and an alternative test species (zebrafish) for facile phenotypic evaluations to understand mechanisms. Advances in gene expression analysis (pathway analysis, SNP) and omics technologies within the last 10–15 years now allow a rapid collection of information to further our understanding of embryonic development and perturbation of key developmental pathways. Hopefully, technological advances will also provide opportunities to investigate the less frequently encountered endpoints associated with thalidomide (e.g., autism, mental retardation, ocular anomalies, Duane syndrome) on which literature is limited.

REFERENCES

- D'Amato, R. J., Loughnan, M. S., Flynn, E., and Folkman, J. (1994). Thalidomide is an inhibitor of angiogenesis. *Proc. Nat. Acad. Sci. U. S. A.* **91**, 4082–4085.
- Calabrese, L., and Fleischer, A. B. (2000). Thalidomide: current and potential clinical applications. *Am. J. Med.* **108**, 487–495.
- Castilla, E. E., Ashton-Prolla, P., Barreda-Mejia, E., Brunoni, D., Cavalcanti, D. P., Correa-Neto, J., Delgadillo, J. L., Dutra, M. G., Felix, T., Giraldo, A., *et al.* (1996). Thalidomide, a current teratogen in South America. *Teratology*. **54**, 273–277.

- Christian, M. S., Laskin, O. L., Sharper, V., Hoberman, A., Stirling, D. I., and Latriano, L. (2007). Evaluation of the developmental toxicity of lenalidomide in rabbits. *Birth Defects Res. B Dev. Reprod. Toxicol.* **80**, 188–207.
- Drucker, L., Uziel, O., Tohami, T., Shapiro, H., Radnay, J., Yarkoni, S., Lahav, M., and Lishner, M. (2003). Thalidomide down-regulates transcript levels of GC-rich promoter genes in multiple myeloma. *Mol. Pharmacol.* **64**, 415–420.
- Gallo, M. A. (2008). History and scope of toxicology. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, (7th ed.) (C. D. Klaassen, Ed.), pp. 3–10. McGraw-Hill, New York, NY.
- Hansen, J. M., Carney, E. W., and Harris, C. (1999). Differential alteration by thalidomide of the glutathione content of rat vs. rabbit conceptuses in vitro. *Reprod. Toxicol.* **13**, 547–554.
- Hansen, J. M., and Harris, C. (2004). A novel hypothesis for thalidomide-induced limb teratogenesis: redox misregulation of the NF-kappaB pathway. *Antiox. Redox. Sig.* **6**, 1–14.
- Hansen, J. M., Gong, S. G., Philbert, M., and Harris, C. (2002). Misregulation of gene expression in the redox-sensitive NF-kappaB-dependent limb outgrowth pathway by thalidomide. *Dev. Dyn.* **225**, 186–194.
- Ito, T., Ando, H., Suzuki, T., Ogura, T., Hotta, K., Imamura, Y., Yamaguchi, Y., and Handa, H. (2010). Identification of a primary target of thalidomide teratogenicity. *Science*. **327**, 1345–1350.
- Janer, G., Slob, W., Hakkert, B. C., Vermeire, T., and Piersma, A. H. (2008). A retrospective analysis of developmental toxicity studies in rat and rabbit: what is the added value of the rabbit as an additional test species? *Regul. Toxicol. Pharmacol.* **50**, 206–217.
- Johnson, D. C., Corthals, S. L., Walker, B. A., Ross, F. M., Gregory, W. M., Dickens, N. J., Lokhorst, H. M., Goldschmidt, H., Davies, F. E., Durie, B. G. M., et al. (2011). Genetic factors underlying the risk of thalidomide-related neuropathy in patients with multiple myeloma. *J. Clin. Oncol.* **29**, 797–804.
- Kelsey, F. O. (1988). Thalidomide update: regulatory aspects. *Teratology*. **38**, 221–226.
- Knobloch, J., and Ruther, U. (2008). Shedding light on an old mystery: thalidomide suppresses survival pathways to induce limb defects. *Cell Cycle*. **7**, 1121–1127.
- Knobloch, J., Schmitz, I., Gotz, K., Schulze-Osthoff, K., and Ruther, U. (2008). Thalidomide induces limb anomalies by PTEN stabilization, Akt suppression, and stimulation of caspase-dependent cell death. *Mol. Cell. Biol.* **28**, 529–538.
- Knobloch, J., Shaughnessy, J. D., Jr., and Ruther, U. (2007). Thalidomide induces limb deformities by perturbing the Bmp/Dkk1/Wnt signaling pathway. *FASEB. J.* **21**, 1410–1421.
- Koch, H. P., and Czejka, M. J. (1986). Evidence for the intercalation of thalidomide into DNA: clue to the molecular mechanism of thalidomide teratogenicity? *Zeitschrift für Naturforschung*. **41**, 1057–1061.
- Lenz, W. (1962). [How can the physician prevent dangers for the offspring?] *Die Medizinische Welt*. **48**, 2554–2558.
- Lenz, W. (1988). A short history of thalidomide embryopathy. *Teratology* **38**, 203–215.
- Matthews, S. J., and McCoy, C. (2003). Thalidomide: a review of approved and investigational uses. *Clin. Therap.* **25**, 342–395.
- Miller, M. T., and Strömmland, K. (1999). Teratogen update: thalidomide: a review, with a focus on ocular findings and new potential uses. *Teratology*. **60**, 306–321.
- Miller, M. T., Ventura, L., and Stromland, K. (2009). Thalidomide and misoprostol: ophthalmologic manifestations and associations both expected and unexpected. *Birth Defects Res. A Clin. Mol. Teratol.* **85**, 667–676.
- Nowack, E. (1965). [The sensitive phase in thalidomide embryopathy]. *Humangenetik*. **1**, 516–536.
- Parman, T., Wiley, M. J., and Wells, P. G. (1999). Free radical-mediated oxidative DNA damage in the mechanism of thalidomide teratogenicity. *Nat. Med.* **5**, 582–585.
- Public Affairs Committee (PAC) (2000). Teratology Society Public Affairs Committee position paper: thalidomide. *Teratology*. **62**, 172–173.
- Schuler-Faccini, L., Soares, R. C., de Sousa, A. C., Maximino, C., Luna, E., Schwartz, I. V., Waldman, C., and Castilla, E. E. (2007). New cases of thalidomide embryopathy in Brazil. *Birth Defects Res. A Clin. Mol. Teratol.* **79**, 671–672.
- Sheskin, J. (1965). Thalidomide in the treatment of lepra reactions. *Clin. Pharmacol. Therap.* **6**, 303–306.
- Sheskin, J. (1980). The treatment of lepra reaction in lepromatous leprosy. Fifteen years' experience with thalidomide. *Int. J. Dermatol.* **19**, 318–322.
- Stephens, T. D. (1988). Proposed mechanisms of action in thalidomide embryopathy. *Teratology*. **38**, 229–239.
- Stephens, T. D., Bunde, C. J., and Fillmore, B. J. (2000). Mechanism of action in thalidomide teratogenesis. *Biochem. Pharmacol.* **59**, 1489–1499.
- Stephens, T. D., and Fillmore, B. J. (2000). Hypothesis: thalidomide embryopathy-proposed mechanism of action. *Teratology*. **61**, 189–195.
- Therapontos, C., Erskine, L., Gardner, E. R., Figg, W. D., and Vargesson, N. (2009). Thalidomide induces limb defects by preventing angiogenic outgrowth during early limb formation. *Proc. Nat. Acad. Sci. U. S. A.* **106**, 8573–8578.
- Uhl, K., Cox, E., Rogan, R., Zeldis, J. B., Hixon, D., Furlong, L. A., Singer, S., Holliman, T., Beyer, J., and Woolever, W. (2006). Thalidomide use in the US: experience with pregnancy testing in the S.T.E.P.S. programme. *Drug Saf.* **29**, 321–329.
- U.S. FDA (1966). *Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use*. U.S. FDA, Rockville, MD.
- Warkany, J. (1988). Why I doubted that thalidomide was the cause of the epidemic of limb defects of 1959 to 1961. *Teratology*. **38**, 217–219.
- Wells, P. G., Bhuller, Y., Chen, C. S., Jeng, W., Kasapinovic, S., Kennedy, J. C., Kim, P. M., Laposa, R. R., McCallum, G. P., Nicol, C. J., et al. (2005). Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. *Toxicol. Appl. Pharmacol.* **207**(Suppl. 1), S354–S366.