Topoisomerase II Inhibitors

Topoisomerase II inhibitors are chemicals that inhibit a group of DNA enzymes called type II topoisomerases (topoisomerase IIs). Topoisomerase IIs regulate the structure of DNA and are essential in separating multiple intertwined DNA daughter strands after DNA replication and prior to mitosis. These enzymes resolve knots and tangles in the genetic material by transiently creating and resealing DNA double strand breaks. Thus, topoisomerase IIs are the target of many drugs used in cancer therapy. One category of topoisomerase II inhibitors acts by stabilizing the covalently bound form of topoisomerase II with DNA, resulting in increased topoisomerase II-cross-linked DNA strand breaks. A second category of topoisomerase II inhibitors are referred to as catalytic inhibitors. Inhibitors in this category prevent topoisomerase II from carrying out its required physiological functions, like DNA supercoil relaxation, decatenation (unlinking), and unknotting.

Exposure to topoisomerase II inhibitors may occur in patients receiving drugs for cancer therapy as well as in patients treated for multiple sclerosis.

Four individual topoisomerase II inhibitors are already listed as carcinogens under Proposition 65 (see Table 1). Topoisomerase II inhibitors (as a chemical group) passed the human and animal data screens, underwent a preliminary toxicological evaluation, and are being brought to the Carcinogen Identification Committee (CIC) for consultation. This is a compilation of the relevant studies identified during the preliminary toxicological evaluation.

Epidemiological data

Epirubicin hydrochloride (CAS No. 56390-09-1)

- Controlled clinical trials, as reviewed in FDA (1999)
    - Analysis of 7110 early breast cancer patients enrolled in controlled clinical trials and receiving epirubicin and cyclophosphamide treatment .
        - *Cumulative risk of developing acute myeloid leukemia / myelodysplastic syndromes (AML/MDS) was 0.27 percent (95% CI=0.14-0.40) at 3 years, 0.46 percent (95% CI=0.28-0.65) at 5 years and 0.55 percent (95% CI=0.33-0.78) at 8 years.*
        - *The risk of developing AML increased with increasing cumulative dose of epirubicin.*

Etoposide[1] (CAS No. 33419-42-0)

- Cohort studies
    - IARC reassessment of several cohort studies published in the 1990s in patients receiving etoposide for cancer therapy: IARC (2011)

[1] Classified by the International Agency for Research on Cancer (IARC) in Group 1. Under consideration for listing via Labor Code.

        - *Cumulative total of more than 50 cases of AML/MDS reported in these cohorts*
        - *10- to 100-fold increases in AML generally associated with etoposide treatment in these studies*
    - Cohort studies in male germ-cell tumor patients treated with etoposide-containing regimens and risk of secondary AML/MDS, as reviewed in IARC (2011, Table 2.1)
        - *Increased risk of secondary AML/MDS*
    - Cohort study of lung cancer patients treated with etoposide-containing regimens: Ratian *et al*., 1987, as reviewed in IARC (2011)
        - *Increased risk of secondary AML*

- Case-control study of AML/MDS in children with solid tumors: Le Deley *et al*. 2003, as reviewed in IARC (2011)
    - *Increased risk associated with etoposide*-containing regimens

Mitoxantrone[2] (CAS No. 70476-82-3)

- Cohort studies, as reviewed in IARC (2000a)
    - Fifty nine premenopausal women with early-stage breast cancer receiving mitoxantrone treatment: Cremin *et al*. (1996)
        - *Two patients developed AML and another developed MDS*

- Case reports
    - IARC (2000a) reviews nine case reports of acute promyelocytic leukemia, AML, or MDS in cancer and multiple sclerosis patients treated with mitoxantrone.

Teniposide[3] (CAS No. 29767-20-2)

- Cohort studies, as reviewed in IARC (2000b)
    - Sixty-two Spanish children with acute lymphoblastic leukemia (ALL) receiving teniposide treatment: Verdeguer *et al*. (1992)
        - *Three children developed AML*
    - 580 children with ALL receiving teniposide treatment. Some children also received etoposide treatment: Pui *et a*l. (1991)
        - *Twenty children developed AML. The overall cumulative risk of AML was 3.8 percent at six years.*
    - Twelve teniposide treatment trials in patients with various primary tumors that subsequently developed AML were combined for analysis Smith *et al.* (1999)
        - *The six-year actuarial risks for AML/MDS were 3.3, 0.7 and 2.2 for the low, moderate and high cumulative dose groups.*
    - Two large series of children with ALL that did not receive teniposide: Neglia *et al*. (1991); Kreissman *et al*. (1992).

[2] Classified by IARC in Group 2B. Under consideration for listing via Labor Code.
[3] Classified by IARC in Group 2A. Under consideration for listing via Labor Code.

        - *No association between AML and cancer treatment without teniposide*

- Case-control studies, as reviewed in IARC (2000b)
    - Case-control study of secondary AML in childhood cancer patients: Hawkins *et al*. (1992)
        - *After adjusting for radiation and exposure to alkylating agents, a positive trend with dose of epipodophyllotoxin (e.g., teniposide, etoposide) was observed (e.g., p = 0.012, by tertile of dose)*.
    - Case-control study of secondary AML/MDS in Hodgkin disease patients: van Leeuwen *et al*. (1994)
        - *Treatment with teniposide did not increase the risk of AML/MDS (RR= 0.9; 95% CI= 0.12-7.0).*
        - *Treatment with teniposide and cyclophosphamide greatly increased the risk (125,000; p = 0.03), although this finding was based on only six cases and four controls.*

Animal carcinogenicity data

Epirubicin
As reviewed in FDA (1999; 2007):

- Single intravenous (i.v.) injection and one-year observation in female rats
    - *Increase in mammary fibroadenoma*
- 18-month i.v. injection study (once every three weeks for a total of 10 injections) in male rats
    - *Increase in subcutaneous fibroma*
- 24-month subcutaneous injection studies (on days 1, 2, 3, 4, 10, 11, 12, and 13 for a total of eight injections) in male and female newborn rats
    - *Increased tumor incidence (by pairwise comparison) in males*
    - *Increased tumor incidence (by pairwise comparison) in females*

Etoposide
As reviewed in IARC (2011):

- Gavage studies (six week exposure) in wild-type and heterozygous neurofibromatosis-1 gene knockout 129/Sv mice: Mahgoub *et al*. (1999)
    - *No treatment-related increase in leukemia*

Idarubicin hydrochloride (CAS No. 57852-57-0)

- "Idarubicin and related compounds have been shown to have …..carcinogenic properties when tested in experimental models (including ….female Sprague-Dawley rats)" (FDA, 2006)

Table 1. Tumors induced by topoisomerase II inhibitors listed as carcinogens under Proposition 65

Chemical (CAS Number)	Human data	Animal data	Year Listed
Actinomycin D (50-76-0)	None	Mesenchymal tumors in peritoneal cavity in rats; local sarcomas in rats and mice	1989
Amsacrine (51264-14-3)	None	Intestinal adenomas and adenocarcinomas; squamous-cell papillomas and carcinomas of the skin; mammary adenocarcinomas; all in rats	2009
Daunomycin (Daunorubicin) (20830-81-3)	None	Mammary and kidney tumors in rats and local sarcomas in mice	1988
Doxorubicin hydrochloride (Adriamycin) (25316-40-9)	None	Mammary tumors, bladder tumors, and local sarcomas in rats.	1987

Other relevant data

- Genotoxicity: See Tables 2 and 3

Table 2. Genotoxicity findings for several topoisomerase II inhibitors

Chemical	Gene mutation		Chromosomal effects		DNA breaks	UDS
	Salmonella	**Other**	**MN**	**Others**		
Epirubicin FDA (1999; 2007)	**+**	**+** (V79 Chinese hamster lung cells)	NA	**+** CA (*in vitro*; *in vivo*)	NA	NA
Etoposide IARC (2011)	**+**	NA	NA	**+** CA; SCE; aneuploidy	**+**	NA
Mitoxantrone IARC (2000a)	**+**	**+** (*Drosophila*; mouse lymphoma cells)	**+**	**+** CA (*in vitro*; *in vivo*); SCE; polyploidy	**+**	**+**
Teniposide IARC(2000b)	**+**	**+** (*Drosophila*; mouse lymphoma cells; CHO cells)	**+** (*in vitro; in vivo*)	**+** CA (*in vitro; in vivo*) SCE; aneuploidy; polyploidy (*in vitro*)	**+**	NA
Idarubicin hydrochloride Blasiak *et al*. (2002); Babudri *et al*. (1984)	**+**	**+** (V79 Chinese hamster lung cells)	**+**	NA	**+** (comet assay)	NA
Valrubicin Onrust and Lamb (1999)	**+**	NA	NA	**+** CA	NA	NA

CA= chromosomal aberration; MN= micronucleus; SCE= sister chromatid exchange; UDS= unscheduled DNA synthesis; CHO= Chinese hamster ovary; NA= not available

Table 3. Genotoxicity findings for topoisomerase II inhibitors listed as carcinogens under Proposition 65

Chemical	Gene mutation		Chromosomal effects		DNA breaks	UDS
	Salmonella	Other	MN	Other		
Actinomycin D	NA	**+** (Human fibroblasts; HeLa cells; *Drosophila*)	NA	**+** CA (*In vitro*; *in vivo*)	**+**	**+**
Amsacrine	**+**	**+** (mouse lymphoma assay)	**+** (*in vitro*; *in vivo*)	**+** SCE; polyploidy	**+**	NA
Daunomycin (Daunorubicin)	**+**	**+** (V79 cells)	NA	**+** SCE (*in vivo*); CA (*in vitro*)	NA	NA
Doxorubicin hydrochloride (Adriamycin)	**+**	**+** (V79 cells)	NA	**+** CA; SCE (*in vivo*)	NA	NA

CA= chromosomal aberration; MN= micronucleus; SCE= sister chromatid exchange; UDS= unscheduled DNA synthesis; NA= not available

- Cell transformation
  - Mitoxantrone induced malignant cell transformation in C3H/10T ½ cells: Jackson *et al.* (1996)

- Mechanistic considerations: Felix (2001)
  - The epipodophyllotoxin topoisomerase II inhibitors form a complex with DNA and DNA topisomerase II, which results in decreased DNA rejoining and chromosomal breakage.
  - The metabolism of epipodophyllotoxin topoisomerase II inhibitors generates reactive oxygen species and hydroxyl radicals that can damage DNA and create abasic sites, which may further enhance position-specific DNA-topoisomerase cleavage.
  - Topoisomerase II inhibitors are associated with a distinct form of leukemia characterized by chromosomal translocations often disrupting a breakpoint cluster region in the MLL gene at chromosome band 11q23.

References[4]

Babudri N, Pani B, Tamaro M, Monti-Bragadin C, Zunino F (1984). Mutagenic and cytotoxic activity of doxorubicin and daunorubicin derivatives on prokaryotic and eukaryotic cells. *Br J Cancer* **50**:91-96.

Blasiak J, Gloc E, Wozniak K, Mlynarski W, Stolarska M, Skorki T, Majsterek I (2002). Genotoxicity of idarubicin and its modulation by vitamins C and E and amifostine. *Chem Biol Interact* **140:**1-18.

Felix CA (2001). Leukemias related to treatment with DNA topoisomerase II inhibitors. *Med Pediatr Oncol* **36**:525-535.

International Agency for Research on Cancer (IARC, 2011). *IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. A Review of Human Carcinogens. Part A. Pharmaceuticals.* Volume 100a, pp. 97-112, IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 2000a). *IARC monographs on the evaluation of carcinogenic risk of chemicals to humans; some antiviral and antineoplastic drugs, and other pharmaceutical agents.* Volume 76, Mitoxantrone, pp. 289-315, IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 2000b). *IARC monographs on the evaluation of carcinogenic risk of chemicals to humans; some antiviral and antineoplastic drugs, and other pharmaceutical agents.* Volume 76, Teniposide, pp. 259-287, IARC, Lyon, France.

Jackson MA, Stack HF, Waters MD (1996). Genetic activity profiles of anticancer drugs. *Mut Res* **355:**171-208.

Onrust SV, Lamb HM (1999). Valrubicin. *Drugs Aging* **15**:69-75.

U.S. Food and Drug Administration (FDA, 1999). Pharmacological review for New Drug Application (NDA) Number 50-807. Center for Drug Evaluation and Research, Available at: http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA/index.cfm?fuseaction=Search.Overview&DrugName=EPIRUBICIN%20HYDROCHLORIDE (Accessed, July7, 2011).

U.S. Food and Drug Administration (FDA, 2007). Approved drug label for Ellence (Epirubicin).

U.S. Food and Drug Administration (FDA, 2006). Approved drug label for Idamycin PFS (Idarubicin hydrochloride) solution. Available at: http://dailymed.nlm.nih.gov

[4] Excerpts or the complete publication have been provided to members of the Carcinogen Identification Committee, in the order in which they are discussed in this document.