NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT FOR CYCLOSPORIN A

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NTP Report on Carcinogens Listing for Cyclosporin A

Carcinogenicity

Cyclosporin A (CsA) is *known to be a human carcinogen* based on studies in humans which indicate a causal relationship between exposure to cyclosporin A and human cancer (IARC, 1990).

There are numerous case reports (IARC, 1990) describing cancer (mainly lymphoma or skin cancer) developing in organ transplant recipients, psoriasis patients, and rheumatoid arthritis patients treated with CsA for immunosuppression. Some of these patients were treated with CsA alone, whereas others were treated with other immunosuppressive agents in combination with CsA. The time between treatment initiation and tumor development ranged from as early as 1 month to 10 years. In some cases, tumors regress after discontinuation of treatment with CsA. Several cohort studies also indicate that CsA is carcinogenic in humans, inducing a tumor incidence of less than 5% in the patient population (IARC, 1990).

In grafted macaques, CsA increased the incidence of lymphomas, a neoplasm that occurs extremely infrequently in this species of monkeys. When given in combination with various other immunosuppressive regimes, CsA induced a substantial increase in the incidence of lymphomas when compared to immunosuppressive regimes excluding CsA. In mouse dietary studies, there was an increased incidence of thymic lymphoma in male mice administered 150 ppm CsA for 20 to 34 weeks, whereas the incidence of tumors of any organ was not increased in male mice administered 1, 4, or 16 ppm CsA for 78 weeks (IARC, 1990). In rats, in a study in which there was no mention of control tumor incidence, renal tumors were detected in more than 50% of streptozotocin-induced diabetic animals administered 10 mg CsA/kg bw orally for 20 weeks (Reddi et al., 1991). However, the incidence of tumors of any organ was not increased in rats administered 0.5, 2, or 8 mg CsA/kg bw orally for 95 (males) or 105 (females) weeks (IARC, 1990).

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

In initiation-promotion studies, CsA increased the incidence of lymphoid tumors in male mice either irradiated or treated with *N*-methyl-*N*-nitrosourea (MNU) (IARC, 1990), of hepatocellular carcinoma in male rats initiated with diethylnitrosamine (DEN) (Masuhara et al., 1993), and of intestinal adenocarcinoma in male rats administered MNU (IARC, 1990). Treatment with CsA also increased the incidence of cervical lymph node metastasis in Syrian golden hamsters treated with dimethylbenz[*a*]anthracene (DMBA) (Yamada et al., 1992), and metastasis of tumors to the liver in male mice inoculated via the portal vein with MCA 38 colon tumor cells (Yokoyama et al., 1994) or colon-26 tumor cells (Suzaki et al., 1995). In contrast, an increase in adenomas by CsA was not detected in male mice treated with urethan (urethane; ethyl carbamate) (IARC, 1990), in male rats initiated with 3-methylcholanthrene (Bussiere et al., 1991), or in rats treated with *N*-methyl-*N'* -nitro-*N*-nitrosoguanidine (MNNG) (IARC, 1990).

CsA is reported as negative for the induction of genetic damage (gene mutations in prokaryotes, gene mutations and chromosomal aberrations in cultured mammalian cells, chromosomal aberrations and micronuclei in rodent bone marrow cells, DNA repair in mouse testicular cells, and dominant lethal mutations in male mice) (IARC, 1990; Zwanenburg and Cordier, 1994). However, CsA was reported to induce a weak increase in sister chromatid exchanges in human lymphocytes *in vitro* and to induce unscheduled DNA synthesis and

chromosomal aberrations in the peripheral blood lymphocytes of kidney transplant patients treated with CsA and prednisolone (IARC, 1990).

The most likely explanation for the increased incidence of tumors in patients treated with CsA is immune suppression (Ryffel, 1992).

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

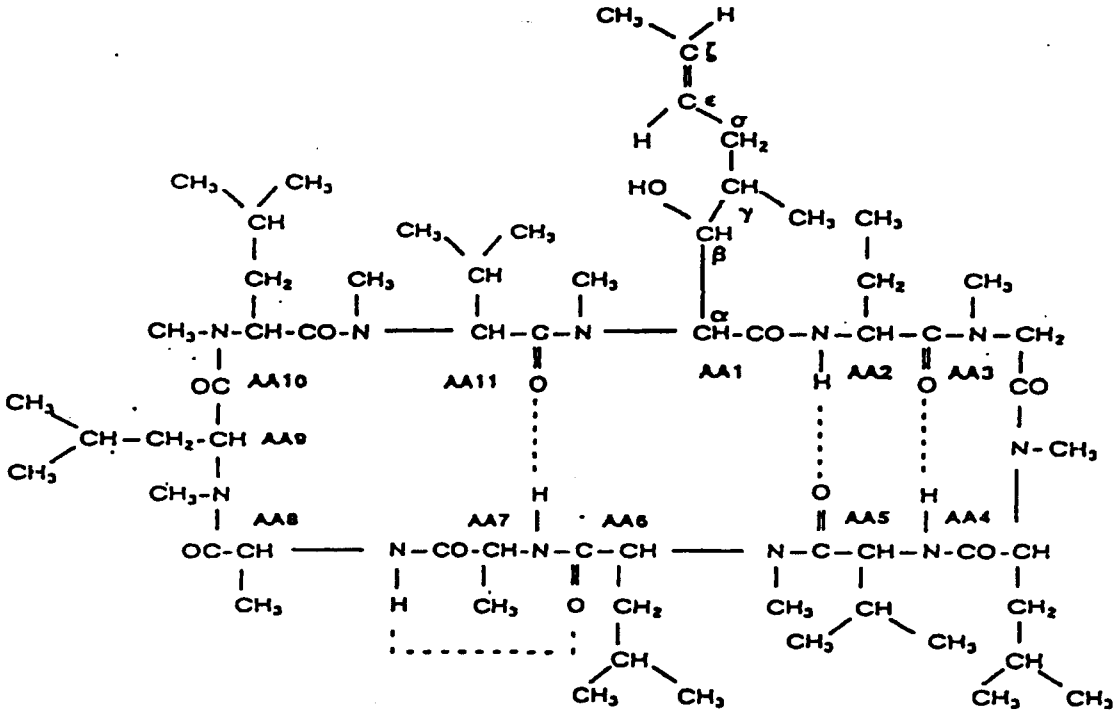
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

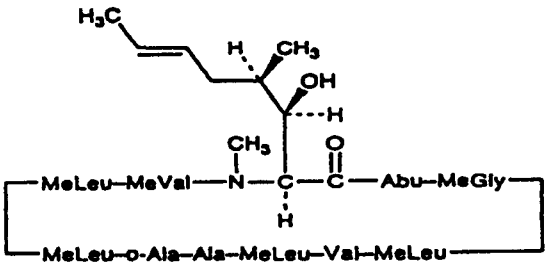
Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

Cyclosporin A
[59865-13-3]



Cyclosporin A may also be represented as:



1.1 Chemical Identification

The American term "Cyclosporin A" is used in this report because it is the name under which the drug is marketed, and the one used in clinical reference. It should be noted that IARC (1990) uses the term "ciclosporin."

Cyclosporin A ($C_{62}H_{111}N_{11}O_{12}$, mol. wt. = 1202.63) is also called:

Antibiotic S 7481F1
Ciclosporin
{*R*-[*R**,*R**-(E)]}-*L*-Cyclic- (*L*-alanyl-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl-3-hydroxy-*N*,4-dimethyl-*L*-2-amino-6-octenoyl-*L*-α-aminobutyryl-*N*-methylglycyl-*N*-methyl-*L*-leucyl-*L*-valyl-*N*-methyl-*L*-leucyl)
Cyclo{[4-(E)-but-2-enyl-*N*,4-dimethyl-*L*-threonyl]-*L*-homoalanyl-(*N*-methylglycyl) (*N*-methyl-*L*-leucyl)-*L*-valyl (*N*-methyl-*L*-leucyl)-*L*-alanyl-*D*-alanyl-(*N*-methyl-*L*-leucyl) (*N*-methyl-*L*-leucyl) (*N*-methyl-*L*-valyl)}
Cyclo{[(E)-(2*S*,3*R*,4*R*)-3-hydroxyl-4-methyl-2-(methylamino)-6-octenoyl]-*L*-2-aminobutyryl-*N*-methyglycyl-*N*-methyl-*L*-leucyl-*L*-valyl-*N*-methyl-*L*-leucyl-*L*-alanyl-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl}
Cyclosporin
Cyclosporine (USAN)
Cyclosporine A
Dyclosporin
Neoral
OL-27-400
S 7481F1
Sandimmun
Sandimmune

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	White	Budavari (1996)
Physical State	Prismatic needles	Budavari (1996)
Melting Point, °C	148-151	Budavari (1996)
Solubility:		
Water at 20 °C	Slightly soluble	Budavari (1996)
Organic Solvents	Soluble in methanol, ethanol, acetone, ether, and chloroform. Slightly soluble in saturated hydrocarbons	Budavari (1996)

1.3 Identification of Structural Analogues and Metabolites

Several structural analogues and metabolites discussed in this report may be found in Figures 6-1 and 6-2.

1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Data, and 7.0 References) and three appendixes. Appendix A describes the literature search in online databases, Appendix B provides explanatory information for Figure 5-1, and Appendix C provides an annotated bibliography for the case reports.

2.0 HUMAN EXPOSURE

2.1 Use

Cyclosporin (CsA) has been used as an immunosuppressive agent since the mid-1980s. It is used extensively in the prevention and treatment of graft-versus-host reactions in bone marrow transplantation and for the prevention of rejection of kidney, heart, and liver transplants. It has also been tested for the therapy of a large variety of other diseases in which immunological factors may have a pathogenic role, including Graves' disease, uveitis, Crohn's disease, ulcerative colitis, chronic active hepatitis, primary biliary cirrhosis, diabetes mellitus, myasthenia gravis, sarcoidosis, dermatomyositis, systemic lupus erythematosus, psoriasis, rheumatoid arthritis, and certain nephropathies (IARC, 1990; Reents, 1996). CsA is used alone or in combination with azathioprine, prednisolone, prednisone, antilymphocyte globulin, actinomycin, cyclophosphamide, methylprednisolone and/or phototherapy (e.g., PUVA, UVB) (see Appendix C [Case Reports]). CsA is administered orally or intravenously (i.v.). Oral preparations may contain corn, castor, or olive oil in ethanol; i.v. preparations contain 33% alcohol and a castor oil vehicle. In July 1995, a new microemulsion oral formula of CsA was approved by the FDA (Reents, 1996).

2.2 Production

CsA is manufactured commercially in Switzerland (IARC, 1990). Chem Sources (1996) identified two U.S. suppliers of CsA. No data on imports or exports of CsA were available.

2.3 Environmental Exposure

The primary routes of potential human exposure to CsA are i.v. and oral administrations (IARC, 1990). Patients receiving organ transplants are exposed to CsA. Potential occupational exposure may occur for workers formulating or packaging the solutions and for health care professionals administering the drug. A typical oral dose of CsA is 18 mg/kg daily, beginning 12 hours before transplantation and continuing for one to two weeks. The dosage may subsequently be reduced to 5-10 mg/kg or less. CsA may also be given by i.v. administration at one-third the oral dose (IARC, 1990). This drug is often given for several months to transplant recipients. CsA is not included in the National Occupational Exposure Survey (NIOSH, 1984) or the National Occupational Hazard Survey conducted by NIOSH (NIOSH, 1976).

2.4 Regulations

FDA regulates cyclosporin under the Food, Drug, and Cosmetic Act (FD&CA) as a prescription peptide antibiotic drug. Purities and concentrations are given for cyclosporin oral dosage forms of drugs. FDA also regulates the use of cyclosporin in ophthalmalmic ointment for dogs.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 201—PART 201—LABELING. Promulgated: 40 FR 13998 03/27/75. U.S. Code: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 358, 360, 360b, 360gg-360ss, 371, 374, 379e; 42 U.S.C. 216, 241, 262, 264. 21 CFR 201.50 ff.—Subpart B—Labeling Requirements for Prescription Drugs and/or Insulin. 21 CFR 201.57—Sec. 201.57 Specific requirements on content and format of labeling for human prescription drugs.	On human drug prescriptions, there must be a subsection of the labeling stating whether long-term studies in animals have been performed to evaluated carcinogenic potential including the species and results. Any precautionary statements on these topics shall also include practical, relevant advice on the significance of these animal findings. If there is any evidence from the human data that the drug may be carcinogenic or mutagenic or that it impairs fertility, this information will be contained in the "Warning" section.
	21 CFR 430.4—Sec. 430.4 Definitions of antibiotic substances.	Cyclosporine is a specific cyclic polypeptide consisting of 11 amino acids produced by the growth of *Cylindrocarpon lucidum Booth* or *Tolypocladium inflatum Gams*.
	21 CFR 448—PART 448—PEPTIDE ANTIBIOTIC DRUGS. Promulgated: 39 FR 19115, 05/30/74. U.S. Code: 21 U.S.C. 357. 21 CFR 448.10 ff.—Subpart A—Bulk Drugs.	This part lists the requirements for certification, standards of identity, strength, quality, and purity of peptide antibiotic drugs classified under bulk drugs and oral, injectable, ophthalmic, otic, dermatologic, and other general dosage form drugs.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 448.23—Sec. 448.23 Cyclosporine. Promulgated: 49 FR 22632, 05/31/84, as amended at 55 FR 11584, 03/29/90.	Cyclosporin content, when purified and dried, should not be < 975 μg nor > 1,020 μg on the anhydrous basis. It must also not contain > 20 ppm heavy metals and its loss of drying should not be > 3.0%.
	21 CFR 448.121 ff.—Subpart B—Oral Dosage Forms.	
	21 CFR 448.123—Sec. 448.123 Cyclosporin oral dosage forms.	
	21 CFR 448.123a—Sec. 448.123a Cyclosporin oral solution. Promulgated: 49 FR 22633, 05/31/84, as amended at 55 FR 11584, 03/29/90. Redesignated: 55 FR 19873, 05/14/90.	Cyclosporin oral solution contains cyclosporin in a suitable and harmless alcohol-vegetable oil solution. Cyclosporin oral solution shall contain not < 90 mg/mL and not > 110 mg/mL of cyclosporin. The cyclosporin used must conform to the requirements of the bulk cyclosporin except heavy metals. Labeling, sample certification, and tests and methods of assay are also included.
	21 CFR 448.123b—Sec. 448.123b Cyclosporin capsules. Promulgated: 55 FR 19873, 05/14/90; 55 FR 22014, 05/30/90.	Cyclosporin capsules contains cyclosporin in a suitable and harmless alcohol-vegetable oil solution, enclosed by a soft gelatin capsule. Cyclosporin capsules shall contain not < 90 mg/mL and not > 110 mg/mL of cyclosporin. The cyclosporin used must conform to the requirements of the bulk cyclosporin except heavy metals. Labeling, sample certification, and tests and methods of assay are also included.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 448—Subpart C—Injectable Dosage Forms.	
	21 CFR 448.223—Sec. 448.223 Cyclosporin for infusion. Promulgated: 49 FR 22633, 05/31/84, as amended at 55 FR 11584, 03/29/90.	Cyclosporin for infusion contains a solution of cyclosporin in a suitable or harmless alcohol-derivatized vegetable oil vehicle. Cyclosporin for infusion shall contain not < 90 mg/mL and not > 110 mg/mL of cyclosporin. The cyclosporin used must conform to the requirements of the bulk cyclosporin except heavy metals. It also must not contain > 42 endotoxin units/mL. Labeling, sample certification, and tests and methods of assay are also included.
	21 CFR 524—PART 524—OPHTHALMIC AND TOPICAL DOSAGE FORM NEW ANIMAL DRUGS. Promulgated: 40 FR 13873, 03/27/75. U.S. Code: 21 U.S.C. 360b.	
	21 CFR 524.575—Sec. 524.575 Cyclosporin ophthalmic ointment. Promulgated: 60 FR 48651, 09/20/95.	Cyclosporin ophthalmic ointment is used to treat chronic keratoconjunctivitis sicca in dogs. Safety of use in puppies has not been determined. Direction for use are included. Each gram of ointment contains 2 mg of cyclosporin.

[a]The regulations in this table have been updated through the 1996 Code of Federal Regulations: 40 CFR, July 1, 1996; 21 CFR, April 1, 1996; 29 CFR, July 1, 1996.

3.0 HUMAN STUDIES

Summary: Numerous case reports (see Appendix C) described the development of cancers in patients treated with cyclosporin A (CsA) alone or CsA in combination with other immunosuppressive agents. Cohort studies evaluated the development of cancers (mostly lymphomas) in patients treated with CsA; but because the majority of these studies included patients who had also received other immunosuppressive therapies, it was not possible to attribute the development of the cancers to a single drug. There were a few studies, though, that reported an increased incidence of cancers in CsA-treated patients. Vilardell et al. (1992) reported that there was a greater incidence of lymphomas in CsA-treated patients than in patients treated conventionally (i.e., no CsA). Sheil et al. (1987; cited by IARC, 1990) reported that 2

malignancies were detected in 140 patients that received CsA alone, whereas no malignancies were detected in another 140 patients that received CsA followed by azathioprine. Montagnino et al. (1994) reported that Kaposi's sarcoma was detected in 13/820 kidney transplant recipients: Two (0.2%) were under conventional therapy and 11 (1.3%) had received CsA. However, two studies did report that the incidence of lymphoma was *lower* in patients treated with CsA alone than in patients treated with CsA in combination with other immunosuppressive therapies or in patients treated with immunosuppressive therapies besides CsA (Beveridge et al., 1984; Smith et al., 1989; cited by IARC, 1990).

3.1 Case Reports

Most case reports (see Appendix C; approximately 40 citations) described the development of cancers in organ transplant recipients, psoriasis patients, or rheumatoid arthritis patients treated with immunosuppressive therapies, most commonly including CsA alone or CsA in combination with azathioprine, prednisolone, prednisone, antilymphocyte globulin, actinomycin, cyclophosphamide, methylprednisolone and/or phototherapy (e.g., PUVA, UVB). The time between treatment initiation and development of cancer ranged from 3 months to 5 years. The most common cancers detected in these patients were lymphomas and skin cancers. In some immunosuppressed patients who developed cancer, human papillomavirus was detected.

3.2 Cohort Studies

3.2.1 CsA Alone

In a study that followed 34 organ transplant recipients of whom, 28 were treated with CsA alone and the remaining 6 were treated with a cyclophosphamide derivative and steroids in addition to CsA, the development of 3 lymphomas was reported: 2 (7.1%) in patients receiving CsA alone and 1 (17%) in a patient on the combined therapy. The maximum follow-up was 1.5 years (Calne et al., 1979; cited by IARC, 1990).

Penn (1983) stated that the Cincinnati Transplant Tumor Registry (CTTR) had a total of between 2000 and 2500 patients who had been treated with CsA as of May 1983. At least 23 patients had developed malignancies after treatment. Lymphomas appeared within 1 to 17 months after transplantation and the start of CsA therapy. Among the 23 malignancies, 17 were lymphomas; 2, nonmelanoma skin cancers; 1 each, carcinoma of the colon, naso pharynx, and kidney; and 1, neuro-ectodermal tumor of the brain (Penn, 1982, cited by Penn, 1983). Penn (1983) reported that among 1767 neoplasms arising in 1661 organ (1622 kidney) transplant recipients, who had received various immunosuppressive treatments, the percentage of lymphoma was 18% compared to 3% to 4% of all malignancies in the general population.

In the 313 patients with lymphoma, only 5% (17) had received CsA; 96% received prednisone, 90% received azathioprine, 34% received antilymphocyte globulin, 30% received local radiation of the graft, 19% received a splenectomy, 13% received actinomycin, 6% received cyclophosphamide, and 8% received other miscellaneous immunosuppressive therapies. It was concluded that the increased incidence of lymphoma could not be attributed to any single immunosuppressive agent but, rather, was "a complication of immunosuppression per se."

Within an estimated 5550 transplant patients (kidney, heart, liver, and bone marrow) worldwide as of February, 1984, 33 (0.59%) post-transplant lymphomas and 7 (0.13%) other lymphoproliferative lesions were detected. The majority of the lymphomas developed within 6 months of organ transplantation. Systematic follow-up of about two-thirds of all organ

transplant recipients in clinical studies and a post-marketing surveillance program were used. The incidence of lymphoma and lymphoproliferative disorder in renal transplant patients receiving CsA in combination with other immunosuppressive agents, such as cytostatic agents anti-lymphocyte or anti-thymocyte globulin was considerably higher (8/100 patients [8%]) than in patients on CsA alone or in combination with steroids (22/4000 patients [0.4%]) (Beveridge et al., 1984).

Sheil et al. (1987; cited by IARC, 1990) evaluated tumor incidence among renal transplant patients administered CsA chronically or CsA followed by azathioprine after 3 months. The mean follow-up period was 5 years. One malignant melanoma and 1 adenocarcinoma were detected in the remaining kidneys of 140 transplant patients treated with CsA alone. No tumors were detected in an additional 140 transplant patients treated with cyclosporin followed by azathioprine. The country where treatment occurred was not specified in the IARC review.

In another study, it was reported that 2 lymphomas were detected in 712 organ transplant recipients treated with azathioprine, none were detected in 160 organ transplant recipients treated with CsA, and 7 were detected in 132 organ transplant recipients treated with both azathioprine and CsA. The maximum follow-up period was 5 years (Smith et al., 1989; cited by IARC, 1990). The country where treatment occurred was not specified in the IARC review.

Among 2222 kidney transplant recipients, Vilardell et al. (1992) reported 66 post-transplant malignancies, with skin cancers accounting for 52% of all tumors. In 1295 patients treated with CsA, 31 cancers (2.4%) were detected after treatment. In 927 patients treated with azathioprine and prednisone, 35 cancers (3.8%) were detected after treatment. There was a greater incidence of lymphomas in CsA-treated patients than in patients treated conventionally, and these appeared rapidly after transplantation (average of 6 months, ranging from 4 to 8 months). CsA-treated patients also had an increased incidence of Kaposi's sarcoma and of lung carcinoma.

In a study conducted by Arellano and Krupp (1993), the incidence and risk of development of malignancies were evaluated in patients treated for rheumatoid arthritis with CsA. Malignancies were detected in 17 of more than 1,000 patients (4 skin cancers, 1 lymphoma, 12 solid tumors). The dose of CsA administered to these 17 patients ranged from 1.2–6 mg/kg/day. The latency time from initiation of CsA treatment to detection of cancer ranged from 8 days to 46 months. It was found that the risk of malignancy associated with CsA did not differ from the risk associated with conventional disease-modifying anti-rheumatic drugs (DMARDs).

Montagnino et al. (1994) retrospectively evaluated kidney transplant recipients, with at least 6 months follow-up, for prevalence of Kaposi's sarcoma (KS). KS was detected in 13/820 recipients (504 had been treated with CsA alone or in combination with methylprednisolone or methylprednisolone plus azathioprine). Two of the 13 patients that developed KS were under conventional therapy (azathioprine plus methylprednisolone) and 11 had received CsA (3/11 received CsA alone, 6/11 received CsA and methylprednisolone, and 2/11 received CsA, methylprednisolone, and azathioprine). The mean latency time for KS was 38.7 ± 38.3 (6-124) months after transplantation.

3.2.2 CsA, Possibly Other Immunosuppressive Therapies

Cockburn (1987) reviewed tumor incidence data for almost 20,000 patients worldwide administered CsA after kidney, heart, liver, or bone marrow transplants or for treatment of autoimmune diseases. The highest incidence of non-lymphomatous malignancies occurred in liver transplant recipients (7/550 [1.3%] vs. 65/15000 [0.43%] kidney transplant, 1/800 [0.13%] heart transplant, and 2/2300 [0.09%] bone marrow transplant recipients and 2/1200 [0.17%] patients treated with CsA for autoimmune diseases). The highest incidence of lymphoma occurred in heart transplant recipients (12/800 [1.5%] vs. 36/15000 [0.24%] kidney transplant, 5/550 [0.91%] liver transplant, and 4/2300 [0.17%] bone marrow transplant recipients, and 2/1200 [0.17%] patients treated with CsA for autoimmune diseases). Statistical analysis of these incidences was not performed. It was not clear if the patients had received other immunosuppressive therapies in addition to CsA.

In a study that followed up 4040 organ transplant recipients treated with CsA, a comparison was made between cancer incidence in this group with cancer incidence in the general population. Lymphoma risk (relative risk, 27.5; 11 cases reported), risk of skin cancers (6.8; 21 cases), and urinary-tract cancers (5.9; 10 cases) were increased. It was not clear, however, if other immunosuppressive therapies, besides CsA, were used. A 34- to 59-fold increase in lymphoma risk and a 2- to 6-fold increase in the risk of other malignancies were also reported for patients receiving conventional immunosuppressive therapy. A 2-fold, and a 2- to 4-fold increase in the overall incidence of all types of malignancies were reported for CsA and for conventional immunosuppressive therapy, respectively. The authors noted that Kaposi's sarcoma and basal cell carcinoma were the predominant forms of skin malignancies found in CsA-treated-patients. Although no differentiation could be made between CsA and conventional immunosuppressive therapy with respect to neoplasm induction, the latency period for lymphoma development was shorter in patients treated with CsA. The reversibility of Kaposi's sarcomas and lymphomas after reduction or discontinuation of CsA was also noted (Cockburn and Krupp, 1989; cited by IARC, 1990). The country where treatment occurred was not specified in the IARC review.

3.2.3 CsA in Combination with Other Immunosuppressive Therapies

The incidence of lymphomas in recipients of cardiac allografts who were treated with CsA and other immunosuppressive agents (no details given) was significantly increased (6/37) in patients less than 40 years of age with prior idiopathic cardiomyopathy (ICM). None of 54 patients with prior coronary-artery disease developed lymphomas (Anderson et al., 1978; cited by IARC, 1990).

In a follow-up study (country not specified), lymphoproliferative lesions (15 lymphomas, 2 other lesions) were reported in 8/315 renal transplant recipients, 4/129 heart transplant recipients, 3/48 liver transplant recipients, and 2/6 heart-lung transplant recipients treated with immunosuppressive therapy including CsA (dose range = 0-21 mg/kg/day). The maximum follow-up period was 4 years. Seven of the renal transplant patients who developed lesions required surgical repair of bowel perforations. In these patients tumor regression, as determined by a second laparotomy, followed a reduction or discontinuation of CsA (Starzl et al., 1984; cited by IARC, 1990).

Krupp and Monka (1990) pooled data from 11 clinical studies on the effects of CsA on patients with psoriasis. The incidence of malignancies was evaluated in patients from one of the

studies, carried out by Sandoz Pharmaceuticals (dose and duration not given). Of 842 patients, 6 (0.71%) developed malignant or pre-malignant skin lesions; a further 6 (0.71%) developed solid organ tumors of the bladder, cervix, colon, thyroid, oral mucosa, or unknown origin; and a further 5 (0.59%) developed lymphoproliferative disorders (lymphoma or lymphocytic infiltration). Almost all patients who developed skin cancer had previously been treated with other known carcinogens (psoralen photochemotherapy, UVB, or methotrexate, others not specified). In those patients with lymphoproliferative disorders, cessation of CsA treatment resulted in spontaneous regression of the lesions.

A retrospective analysis was performed by Armitage et al. (1991) to evaluate the incidence, risk, and effect of post-transplant lymphoproliferative disease (PTLD) in 439 heart and 64 lung transplant recipients who received CsA and prednisone (doses not given). Only those patients that survived more than 30 days after transplantation were included in the study. The median follow-up time for heart transplant recipients was 2.6 years and for lung transplant recipients was 1.7 years. PTLD was detected in 15 heart transplant recipients and in 5 lung transplant recipients. Epstein-Barr virus was detected in all patients.

Blohmé and Larkö (1992) compared the incidence of skin malignancy (squamous cell carcinoma, basal cell carcinoma, carcinoma in situ, and keratoacanthoma) in 3 groups of renal transplant patients treated in Scandinavia. Group 1 (98 patients) had received CsA + prednisolone, group 2 (65 patients) had received CsA + azathioprine + prednisolone, and group 3 (298 patients) had received azathioprine + prednisolone. All groups were observed for at least 5 years. There was no difference in the incidence of malignant and premalignant skin lesions among patients that received azathioprine- or cyclosporin-based therapy (group 1: 6/98 [6.1%]; group 2: 4/65 [6.2%]; group 3: 15/298 [5.0%]).

A retrospective analysis was performed by Melosky et al. (1992) to evaluate the incidence, risk, and effect of lymphoproliferative disorders in 478 kidney transplant recipients. Of the 478 patients, 334 received triple immunosuppressive therapy (CsA + azathioprine + prednisone) and 144 received quadruple immunosuppressive therapy (CsA + Minnesota antilymphocyte globulin + azathioprine + prednisone). Twenty-three malignancies, including carcinoma of the skin (n = 11), non-Hodgkins lymphoma (n = 5), prostatic carcinoma (n = 3), Hodgkin's lymphoma (n = 1), esophageal carcinoma (n = 1), bile duct carcinoma (n = 1), and thyroid carcinoma (n = 1), were detected in 22/478 (4.6%) patients. Lymphoproliferative disorders were detected in 2/334 (0.60%) triple-therapy patients and in 3/144 (2.1%) quadruple-therapy patients. Mean follow-up time was 26 months (0.1–63 months). The mean latency time for development of malignancy was 16.7 months (3–45 months). The overall risk of neoplasia in the patients was 3.08 times that of the general population, obtained by calculating observed-to-expected ratios for age-, sex-, and geographically matched controls.

In a follow-up study of 1620 German kidney transplant recipients (966 males and 654 females, all of whom had been treated with prednisolone and CsA), 66 were reported to have developed *de novo* malignancies (40 males [4.1%], 26 females [4.0%]). Latency time ranged from 8 to 128 months after transplantation. Of the 66 malignancies, 18 were skin cancers, 5 were lymphomas, and the remaining were solid tumors of various sites. All patients were treated with prednisolone and CsA. In addition, 20% of the males and 14% of the females received azathioprine. The risk of development of *de novo* malignancies was calculated by comparing incidences in kidney transplant recipients with incidences in patients listed in a regional cancer registry. For 2504 observed patient-years in the female population, the expected number of

malignancies was 7.3 (vs. 26 in kidney transplant recipients, a 3.5-fold increase). In 3510 patient-years observed in males, the expected number was 13.3 (vs. 40 in kidney transplant recipients, a 3-fold increase). These differences were statistically significant ($p < 0.01$, chi-square test) (Frei et al., 1993).

In a study conducted by Lewis et al. (1993; cited in Sandoz Pharmaceuticals Corporation, personal communication from M.D. Grebenau to R.W. Tennant, May 31, 1996), 84 patients were treated for plaque psoriasis with CsA for 1-72 months. Three (3.6%) developed malignancies. One patient developed cervical cell carcinoma after 3 years of therapy, but with continuation of CsA treatment, there were no recurrences. Another patient who developed 2 cutaneous cell carcinomas also developed these lesions while receiving PUVA therapy. The authors suggested that previous treatments with UV radiation and PUVA may have been responsible for the malignancies detected in patients from this study. They also noted that combined treatment with PUVA or other immunosuppressive drugs and CsA "increases the risk of cutaneous malignancy and should be avoided."

Busnach et al. (1993) reported that the incidence of cervical intraepithelial neoplasia (CIN) was increased in women that received renal allografts as compared to age-matched non-immunosuppressed healthy women (5/50 [10%] vs. 4/60 [6.6%], respectively). All the transplant recipients had received low-dose oral steroids, 27/50 (54%) also received CsA, 15/50 (30%) also received azathioprine, and 7/50 (14%) also received CsA + azathioprine. CIN incidence was not broken down by type of therapy received. HPV (human papillomavirus) prevalence was also increased among the renal allograft recipients as compared to age-matched non-immunosuppressed healthy women (10/50 [20%] vs. 7/60 [11.6%], respectively).

In a study conducted by Ritters et al. (1994), tumor incidence in kidney transplant recipients treated in Düsseldorf, Germany was evaluated. Group 1 consisted of 80 patients that received azathioprine + methylprednisolone and group 2 consisted of 466 patients that received CsA + methylprednisolone or CsA + methylprednisolone + azathioprine. In group 1, 8/80 (10%) patients developed tumors and in group 2, 22/466 (4.7%) patients developed tumors. Most malignancies (53%) were skin tumors. Malignancies in CsA-treated patients were diagnosed much earlier than those in patients who received conventional therapy. The authors were unable to confirm an increased risk of malignancy or an increased incidence of lymphoma after cyclosporin treatment as compared to other therapies.

In another study, the incidence of *de novo* tumors developing in 1887 kidney transplant recipients treated at the University of Minnesota with various immunosuppressive agents was evaluated. Patients received 1 of 4 drug regimens: azathioprine (AZA) + antilymphocyte globulin (ALG), CsA + prednisone (Pred), CsA + Pred + AZA, or CsA + Pred + AZA + ALG. The first regimen was labeled CONV and the latter 3 regimens were collectively labeled CSA. 1165 patients received the CONV regimen and 722 patients received one of the CsA regimens. All patients were stratified according to age, diabetic status, donor source, and sex. As revealed by life-table analysis, there was no difference in overall cancer incidence or in skin cancer incidence between the CONV and CSA groups (overall incidence: 124/1165 CONV [10.6%] vs. 34/722 CSA [4.7%]; skin cancer: 68/1165 CONV [5.8%] vs. 22/722 CSA [3.0%]), although the mean time to cancer occurrence for both types of cancer was significantly shorter for the CSA groups. Also, the incidence of lymphoma was increased in the CONV group, but not in the CSA groups (16/1165 CONV [1.4%] vs. 1/722 [0.1%]) (Gruber et al., 1994).

In a study conducted by Hiesse et al. (1995), the incidence of malignancies in 650 kidney transplant recipients who received conventional immunosuppressive therapy (i.e., no CsA; group 1) was compared with that in 940 kidney transplant recipients who received immunosuppressive therapy that included CsA (group 2). All patients were treated at the same hospital in Paris, France. Patients in group 1 were treated with prednisone and azathioprine. Rejections were treated with methylprednisolone boluses and polyclonal antilymphocyte or antithymocyte globulins. Patients in group 2 received either triple (CsA + azathioprine + steroids) or quadruple (CsA, polyclonal antilymphocyte globulin + steroids + azathioprine) therapy. There was a significant increase in cancer incidence (reported only as a percent) in CsA-treated patients (year 1: 0.31% [group 1] vs. 0.85% [group 2]; year 2: 0.64% [group 1] vs. 1.53% [group 2]; year 3: 1.32% [group 1] vs. 2.86% [group 2]; year 4: 1.5% [group 1] vs. 4.33% [group 2]; year 5: 3.26% [group 1] vs. 5.71% [group 2]; year 10: 6.23% [group 1] vs. 12.6% [group 2]). When cutaneous malignancies, were excluded from the data, however, the incidence of cancer was similar in groups 1 and 2. Solid tumors represented the majority of malignancies observed in both groups of patients (39.4%). Skin tumors were more common in CsA-treated patients than in conventionally-treated patients (41.8% of tumors in CsA-treated patients vs. 29.6% of tumors in conventionally-treated patients). The average follow-up time was 86.5 ± 52.4 months for group 1 and 46.7 ± 28.3 months for group 2.

Kehinde et al. (1994) retrospectively investigated the development of *de novo* neoplasia in kidney transplant recipients who received triple-drug therapy. Patients were classified as receiving triple-drug therapy if they were administered CsA, azathioprine, and prednisolone continuously for at least 1 year after transplantation. Of 492 patients, 27 (5.5%) developed *de novo* cancer. Skin tumors were the most common tumor. The mean latency time between transplantation and tumor diagnosis was 48 months (4–140). In another study in this article, 110 patients were randomized to 1 of 3 immunosuppressive regimens: high-dose CsA, high-dose CsA + oral nifedipine, or low-dose CsA + azathioprine. The 3 groups all received identical steroid treatment. Cancer was detected in 3 patients that received low-dose cyclosporin + azathioprine (2 had basal cell carcinoma of the skin, 1 had pancreatic carcinoma) and in 1 patient that received high-dose cyclosporin + nifedipine (seminoma).

4.0 MAMMALIAN CARCINOGENICITY

Full experimental details for the studies described in this section are presented in Table 4-1.

4.1 Mice

There was no increase in the incidence of tumors in OF1 male mice (age at study initiation not specified) administered 1, 4, or 16 ppm CsA in the diet for 78 weeks (Ryffel et al., 1983; cited by IARC, 1990).

The incidence of thymic lymphoma was significantly increased in male AKR mice administered 150 ppm CsA in the diet for 20–34 weeks beginning at age 6 weeks (Hattori et al., 1986; cited by IARC, 1990).

4.2 Rats

There was no increase in the incidence of tumors in male and female OFA rats (age not specified) administered 0.5, 2, or 8 mg CsA per "kilogram body weight of diet" [*sic*] (0.4, 2, or 7 μmol/kg bw) for 95 (males) or 105 (females) weeks (Hattori et al., 1986; cited by IARC, 1990).

In a study in which there was no mention of tumor incidence in control animals, renal tumors were detected in 7/13 diabetic male Wistar rats (age not specified; weight 70-100 g) administered 10 mg CsA/kg (8.3 μmol/kg) every 3 days by gavage for 20 weeks. Diabetes was induced in overnight-fasted rats by a single intraperitoneal (i.p.) injection of streptozotocin (60 mg/kg) in 0.1 M citrate buffer (Reddi et al., 1991).

Table 4-1. Mammalian Carcinogenicity of Cyclosporin A

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice - *Oral Administration*							
6-wk-old AKR mouse	30M	22M (basal diet alone)	CsA, purity not specified	150 ppm in diet	up to 34 wk	This study was a screening assay. Study did not mention which tissues were examined. Statistical analysis of tumor incidence was performed using Fisher's exact test. **Lymphatic System:** Positive (for thymic lymphoma) The incidence of thymic lymphoma was significantly increased in CsA-treated mice killed between 20 and 29 weeks (13/18 vs. 2/12 controls; p = 0.004) and between 30 and 34 weeks (9/9 vs. 3/9 controls; p = 0.005). Thymic lymphoma was first detected in CsA-treated mice at week 17 and in controls at week 23.	Hattori et al. (1986; cited by IARC, 1990)
OF1 mouse (age not specified)	50M, 50F per dose	50M, 50F (basal diet alone)	CsA, purity not specified	1, 4, or 16 ppm in diet	78 wk	Mice were killed at the end of the treatment period. All macroscopically observed lesions were examined histologically. **All Tissues:** Negative There was no increase in the incidence of tumors in CsA-treated mice as compared to controls.	Ryffel et al. (1983; cited by IARC, 1990)
Rats - *Oral Administration*							
Wistar rat (age not specified)	13M (diabetic)	4M (diabetic; vehicle alone) 5M (nondiabetic; citrate buffer + vehicle)	CsA, purity not specified	10 mg/kg (8.3 µmol/kg) every 3 days by gavage	20 wk	Diabetes was induced in overnight-fasted rats by a single i.p. injection of streptozotocin (60 mg/kg) in 0.1 M citrate buffer. Only the kidneys were examined. **Kidneys:** Renal tumors (type not specified) were detected in 7/13 CsA-treated rats. No mention was made of tumor incidence in controls. The concentrations of vitamin B6, thiamin, riboflavin, nicotinate, free inositol, and acid-soluble carnitine were significantly lower in renal tumor tissue derived from CsA-treated rats than in normal kidney tissue derived from CsA-treated rats. Concentrations of folic acid, B12, biotin, pantothenate, and biopterin did not differ significantly in tumor and non-tumor tissue derived from CsA-treated rats.	Reddi et al. (1991)
OFA rat (age not specified)	50M, 50F per dose	50M, 50F (basal diet alone)	CsA, purity not specified	0.5, 2, or 8 mg/kg "bw of diet" [*sic*] (0.4, 2, or 7 µmol/kg)	95 wk (males) 105 wk (females)	Rats were killed at the end of the treatment period. All macroscopically observed lesions were examined histologically. **All Tissues:** Negative There was no increase in the incidence of tumors in CsA-treated rats as compared to controls. IARC noted the high incidence of tumors in the controls, which may have reduced the sensitivity of the assay.	Hattori et al. (1986; cited by IARC, 1990)

Abbreviations: bw = body weight; LD = low dose; MD = mid dose; HD = high doses; s.c. = subcutaneously; i.p. = intraperitoneally

5.0 GENOTOXICITY

Studies of the genotoxic effects of CsA are summarized in Table 5-1.

Summary: Limited genotoxicity testing has been performed on CsA. It has been found to be primarily non-genotoxic in prokaryotes and mammalian cells *in vitro* [see Genetic Activity Profile, Figure 5-1(data limited to IARC, 1990)]. CsA did not induce gene mutations in *Salmonella typhimurium; hprt* mutations in Chinese hamster V79 cells; chromosomal aberrations in cultured human peripheral blood lymphocytes and Chinese hamster bone marrow; micronuclei induction in mouse and hamster bone marrow; unscheduled DNA synthesis (UDS) in mouse spermatocytes; or dominant lethal mutations in male mice. However, CsA was reported to induce a weak increase in sister chromatid exchanges (SCE) in human lymphocytes *in vitro,* and UDS and chromosomal aberrations in the peripheral blood lymphocytes of kidney transplant patients treated with CsA and prednisolone. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro.*

Information for studies reviewed in IARC Vol. 50 (1990) was often limited to qualitative data. Pertinent information on study design, doses tested, chemical purity, etc., were generally not provided.

5.1 Noneukaryotic Systems

Matter et al. (1982; cited by IARC, 1990) found that CsA from 30 to 3,000 μg/plate (0.02 to 2.5 μmol/plate) did not induce reverse mutations in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA1538 either with or without metabolic activation [HID = 3,000 μg/plate (2.5 μmol/plate)].

5.2 Mammalian Systems *In Vitro*

5.2.1 DNA Damage

Yuzawa et al. (1987; cited by IARC, 1990) reported that CsA from 0.13 to 4.0 μg/mL (0.11 to 3.3 μM) induced a weak positive increase in SCE in human peripheral blood lymphocytes tested only in the absence of exogenous metabolic activation [LED = 1.0 μg/mL (0.83 μM)].

5.2.2 Gene Mutations

Zwanenburg et al. (1988; cited by IARC, 1990) found that CsA from 1.0 to 250 μg/mL (0.83 to 208 μM) for 24 hours did not induce mutations at the *hprt* locus in Chinese hamster V79 cells with or without metabolic activation [HID = 250 μg/mL (208 μM)].

5.2.3 Chromosomal Damage

Zwanenburg and Cordier (1994) reported that CsA tested at 5.0 to 300 μg/mL (4.2 to 249 μM) for 2 hours in the presence of S9 activation and 20 or 44 hours in the absence of S9 activation did not induce chromosomal aberrations in human peripheral blood lymphocytes [HID = 300 μg/mL (249 μM)].

5.3 Mammalian Systems *In Vivo*

5.3.1 DNA Damage

Matter et al. (1982; cited by IARC, 1990) reported that CsA at 507 mg/kg (421 μmol/kg) p.o. did not induce unscheduled DNA synthesis in the spermatocytes of male CD-1 mice.

5.3.2 Gene Mutations

Matter et al. (1982; cited by IARC, 1990) found that 100 to 1000 mg/kg (83 to 831 μmol/kg) CsA administered p.o. did not induce dominant lethal mutations in CD-1 male mice [HID = 1000 mg/kg (831 μmol/kg)].

5.3.3 Chromosomal Damage

Matter et al. (1982; cited by IARC, 1990) reported that CsA did not induce chromosome aberrations in Chinese hamster bone marrow cells tested with 1500 mg/kg (1247 μmol/kg) p.o., nor micronuclei in the bone marrow polychromatic erythrocytes of CD-1 mice or Chinese hamsters tested with 375 to 1500 mg/kg (312 to 1247μmol/kg) p.o.

5.4 Human Genotoxicity Studies

5.4.1 DNA Damage

Petitjean et al. (1986; cited by IARC, 1990) reported that CsA induced UDS in the peripheral blood lymphocytes of human kidney transplant patients. Number of patients, dose levels, and exposure regimen were not provided.

5.4.2 Chromosomal Damage

Fukuda et al. (1988; cited by IARC, 1990) reported that 17 of 25 kidney transplant patients receiving CsA orally at 5 to 14 mg/kg/day (4 to 12 μmol/kg/day) for over one year, combined with variable doses of prednisolone, had increased levels of chromosomal aberrations in their peripheral blood lymphocytes [LED = 9.0 mg/kg (7.5 μmol/kg)].

Table 5-1. Summary of Cyclosporin Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1 Noncukaryote Systems							
Salmonella typhimurium strains TA100, TA1535, TA1537, and TA1538	*his* reverse gene mutations	+/-	n.p.	30 to 3,000 µg/plate (0.02 to 2.5 µmol/plate)	negative/ negative	HID = 3,000 µg/plate (2.5 µmol/plate)	Matter et al. (1982; cited by IARC, 1990)
5.2 Mammalian Systems *In Vitro*							
5.2.1 DNA Damage							
human peripheral blood lymphocytes	sister chromatid exchanges (SCE)	-	n.p.	0.13 to 4.0 µg/mL (0.11 to 3.3 µM)	positive	CsA was only weakly positive for induction of SCE, LED = 1.0 µg/mL (0.83 µM).	Yuzawa et al. (1987; cited by IARC, 1990)
5.2.2 Gene Mutations							
Chinese hamster V79 cells	*hprt* gene mutations	+/-	n.p.	1.0 to 250 µg/mL (0.83 to 208 µM) for 24 h	negative/ negative	HID = 250 µg/mL (208 µM)	Zwanenburg et al. (1988; cited by IARC, 1990)
5.2.3 Chromosomal Damage							
human peripheral blood lymphocytes	chromosomal aberrations	+/-	n.p.	5.0 to 300 µg/mL (4.2 to 249 µM) for 2 h +S9 and 20 or 44 h -S9	negative/ negative	No dose at either fixation time induced an increase in chromosomal aberrations. HID (+/-S9) = 300 µg/mL (249 µM)	Zwanenburg and Cordier (1994)
5.3 Mammalian Systems *In Vivo*							
5.3.1 DNA Damage							
CD-1 mouse spermatocytes	unscheduled DNA synthesis (UDS)	NA	n.p.	507 mg/kg (421 µmol/kg) p.o.	negative	HID = 507 mg/kg (421 µmol/kg) .	Matter et al. (1982; cited by IARC, 1990)
5.3.2 Gene Mutations							
CD-1 male mice	dominant lethal mutations	NA	n.p.	100 to 1000 mg/kg (83 to 831 µmol/kg) p.o.	negative	HID = 1000 mg/kg (831 µmol/kg)	Matter et al. (1982; cited by IARC, 1990)
5.3.3 Chromosomal Damage							
Chinese hamsters	chromosomal aberrations in bone marrow cells	NA	n.p.	1500 mg/kg (1247 µmol/kg) p.o.	negative	HID = 1500 mg/kg (1247 µmol/kg)	Matter et al. (1982; cited by IARC, 1990)
CD-1 mice	micronuclei induction in bone marrow polychromatic erythrocytes	NA	n.p.	375 to 1500 mg/kg (312 to 1247 µmol/kg) p.o.	negative	HID = 1500 mg/kg (1247 µmol/kg)	Matter et al. (1982; cited by IARC, 1990)

Table 5-1. Summary of Cyclosporin Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
Chinese hamsters	micronuclei induction in bone marrow polychromatic erythrocytes	NA	n.p.	375 to 1500 mg/kg (312 to 1247 µmol/kg) p.o.	negative	HID = 1500 mg/kg (1247 µmol/kg)	Matter et al. (1982; cited by IARC, 1990)
5.4 Human Genotoxicity Studies							
5.4.1 DNA Damage							
human peripheral blood lymphocytes	UDS	NA	n.p.	n.g.	positive	Kidney transplant patients. Dose levels and exposure regimen not provided.	Petitjean et al. (1986; cited by IARC, 1990)
5.4.2 Chromosomal Damage							
human peripheral blood lymphocytes	chromosomal aberrations	NA	n.p.	5 to 14 mg/kg/day (4 to 12 µmol/kg) plus variable doses of prednisolone	positive	17 of 25 kidney transplant patients receiving cyclosporin A for over one year showed increased levels of chromosomal aberrations. LED = 9.0 mg/kg (7.5 µmol/kg)	Fukuda et al. (1988; cited by IARC, 1990)

Abbreviations: HID = highest ineffective dose; LED = lowest effective dose; NA = not applicable; n.g. not given; n.p. = not provided

Figure 5-1. Genetic Activity Profile of Cyclosporin A (Data Limited to IARC, 1990)

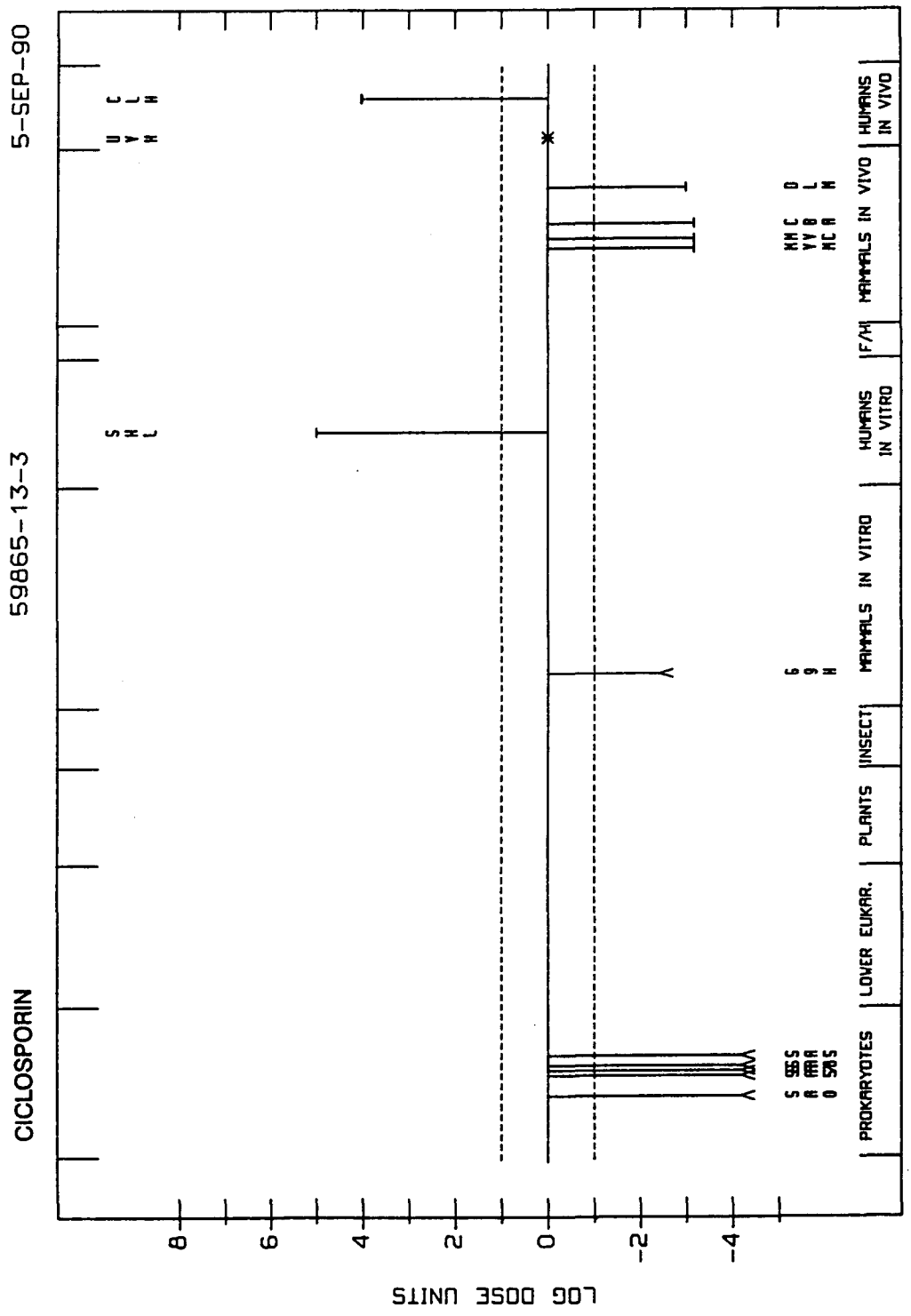
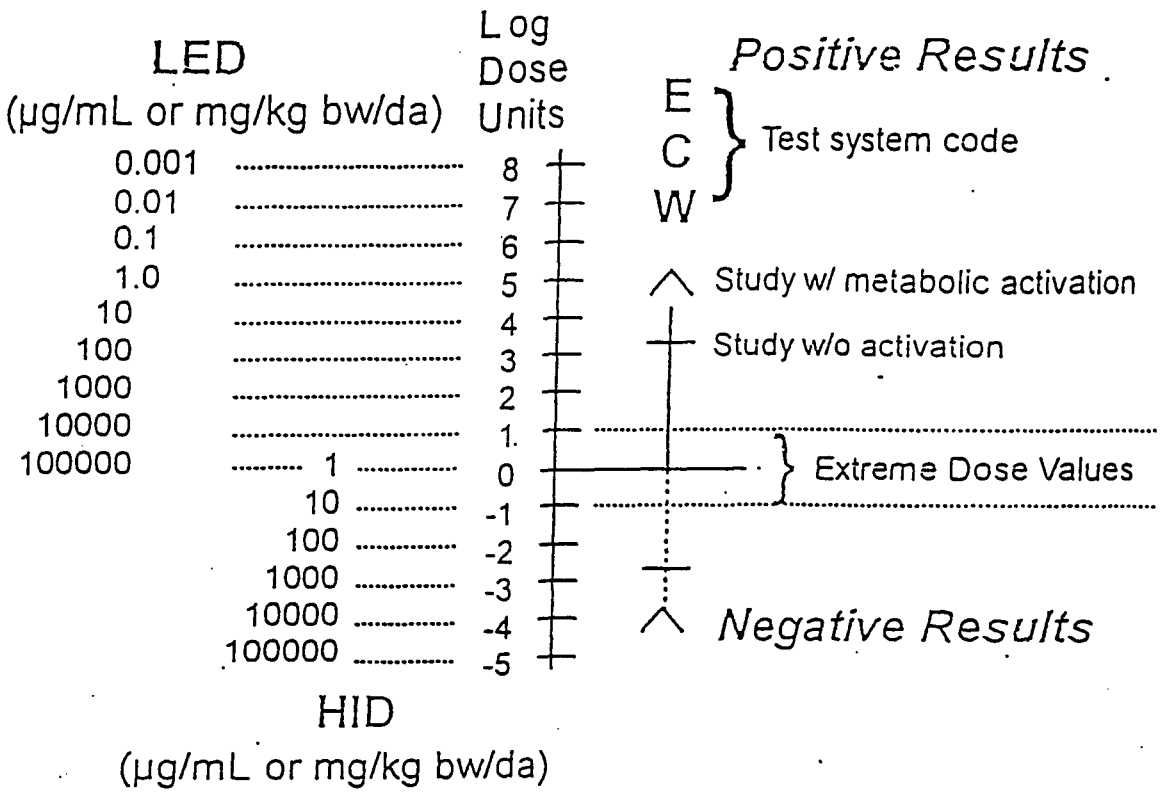


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N.E., H.F. Stack, M.R. Gross, and M.D. Waters. 1984. An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. Mutat. Res. 143:89-111.

Waters, M.D., H.F. Stack, A.L. Brady, P.H.M. Lohman, L. Haroun, and H. Vainio. 1988. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutat. Res. 205:295-312.

Waters, M.D., H.F. Stack, N.E. Garrett, and M.A. Jackson. 1991. The genetic activity profile database. Environ. Health Perspect. 96:41-45.

6.0 OTHER RELEVANT DATA

6.1 Metabolism, Absorption, Distribution, and Excretion

CsA metabolism and metabolite identification have been reviewed by IARC (1990), Wenger (1990), Akagi et al. (1991), and Christians and Sewing (1993). The nomenclature proposed at the Hawk's Cay meeting (Consensus Document, 1990) will be adopted here and is presented in Figures 6-1 and 6-2. In this nomenclature, AA (for amino acid) followed by a number designates segments of the CsA molecule. Metabolite designations are prefixed by the letters AM (for CsA metabolite).

Summary: In humans, the absorption of CsA after oral administration is incomplete, slow, and highly variable. It displays a highly variable bioavailability that is thought to be due to oxidative metabolism via cytochrome P-450 IIIA in the gut. CsA has been shown to be metabolized to over 30 metabolites in human, rat, rabbit, and dog *in vivo* and *in vitro* studies, with the four major metabolites being AM9, AM1, AM4N and AM1c. In rats, the major CsA metabolite formed is AM9, in contrast to humans and rabbits who produce AM1 as the major metabolite of CsA. CsA is preferentially metabolized via oxidation at the γ- carbons of the methylleucines at 4, 6, and 9 amino acids, the terminal methyl group of *N*-methyl-4-(2-butenyl)-4-methylthreonine, or demethylated at AA4 and 9, or cyclized at AA1.

In humans, CsA metabolism has been shown to occur at several sites in *in vitro* studies, including the g.i. tract, liver, and kidney. The liver is the main site of CsA biotransformation and subsequent elimination of metabolites is primarily via the bile. Metabolism of CsA in the g.i. tract and stomach may contribute to the high variability of CsA bioavailability in humans. Hepatic and gastrointestinal cytochrome P-450 IIIA enzymes are responsible for the biotransformation of CsA and its metabolites in humans, and it is the concentration of cytochrome P-450 IIIA that determines CsA bioavailability.

6.1.1 Metabolism and Metabolite Identification

Structures of many of the CsA metabolites are shown in the metabolic pathways depicted in Figure 6-2 (a, b, c, and d). Experimental details of the studies discussed in this section are presented in Table 6-1 (Cyclosporin A Metabolite Identification). In human, rat, rabbit, and dog *in vivo* and *in vitro* studies (Maurer et al., 1984; Christians et al., 1991a,b; Bowers et al., 1990; Hashem et al., 1988; Copeland et al., 1990a), CsA has been shown to be metabolized to over 30 metabolites, with the four major metabolites being AM9, AM1, AM4N and AM1c (see Figures 6-1 and 6-2 [a, b, and c] for structure identification) (Maurer et al., 1984; Hartman et al., 1985; Christians et al., 1991b; Christians and Sewing, 1993). In rats, the major CsA metabolite formed is AM9, in contrast to humans and rabbits who produce AM1 as the major metabolite of CsA (Venkataramanan et al., 1988; cited by Copeland et al., 1990a). CsA is preferentially metabolized via oxidation at the γ-carbons of the methylleucines at 4, 6, and 9 amino acids (AA), the terminal methyl group of MeBMT [AA1; *N*- methyl-4-(2-butenyl)-4-methylthreonine] or demethylated at AA4 and 9, or cyclized at AA1 (Christians and Sewing, 1993).

Metabolism of CsA has been shown to occur at several sites in human *in vitro* studies, including the g.i. tract, liver, and kidney (Vickers et al., 1992; Webber et al., 1992). The liver is the main site of CsA biotransformation and subsequent elimination of metabolites is primarily via the bile (Maurer et al., 1984; Maurer and Lamaire, 1986; Reents, 1996). Only 6% is excreted

via the urine, of which 0.1% is excreted as unchanged CsA (Reents, 1996). It has been suggested that metabolism of CsA in the g.i. tract and stomach may contribute to the high variability (8-60%) of CsA bioavailability in humans (Lemaire et al., 1990; cited by Vickers et al., 1995).

In humans, hepatic and gastrointestinal cytochrome P-450 IIIA enzymes are responsible for the biotransformation of CsA and its metabolites. The concentrations of functional P-450 IIIA in the gut and liver is influenced by genetic disposition, induction, and inhibition (Christians and Sewing, 1993). It is the concentration of cytochrome P-450 IIIA that determines CsA bioavailability (for review see Christians and Sewing, 1993; Wu et al., 1995).

6.1.2 Absorption, Distribution, and Excretion

The absorption, distribution, and excretion of CsA have been reviewed by IARC (1990) and Christians and Sewing (1993) and are discussed below.

6.1.2.1 Mice

C57B1 mice orally, i.p., i.v., or subcutaneously (s.c.) administered radiolabeled CsA, showed a high initial concentration of radiolabel in liver, pancreas, salivary glands, fat, and spleen tissue by whole-body autoradiography. In lymph nodes, thymus, bone marrow, and liver, relatively high levels were retained. The radiolabel was confined to the outer medulla and outer zone of the kidney. Radioactivity was not detected in fetus or central nervous system (Backman et al., 1987, 1988; cited by IARC, 1990).

CsA was detected (radioimmunoassay) in every organ investigated in BALB/c mice injected s.c. with 12.5, 50, or 200 mg (10.4, 41.6, or 166.3 μmol)/kg bw (Boland et al., 1984; cited by IARC, 1990). Brain, kidney, and liver (organs susceptible to toxicity) retained CsA or metabolites after i.p. injection of CsA (Belitsky et al., 1986; cited by IARC, 1990).

6.1.2.2 Rats

Sprague-Dawley and Wistar rats orally administered tritium-labeled CsA (10 mg [8.3 μmol]/kg bw daily in olive oil for 21 days) absorbed approximately 30% of the dose. Radiolabel was distributed widely throughout the body. The terminal elimination half-life ($t_{1/2}$) of the radiolabel was 46 hours after dosing. Elimination from the kidney and liver had a $t_{1/2}$ of 70 to 100 hours. After repeated treatments, high levels of the administered dose accumulated in the kidney, liver, blood, and lymph nodes, and in skin and adipose tissue. (Wagner et al., 1987; cited by IARC, 1990).

WAG/Rij rats administered a single oral dose of 82 mg (68.2 μmol)/kg bw resulted in levels of 80 μg/g in kidney, brain, and liver 3 and 7 hours after administration. Thereafter, slow elimination occurred, and significant concentrations (10 μg/g) were detected at 5 days after administration of the dose. Shortly after oral administration (IARC, 1990 did not specify), CsA was detected in the blood (3.5 μg/mL), and the levels remained approximately similar for about 2 days. In bile, 2% of the administered dose was eliminated unchanged and similar concentrations were eliminated via urine (Nooter et al., 1984a; cited by IARC, 1990). Ueda et al. (1983; cited by IARC, 1990) found that 2% of an oral dose of CsA administered to rats was absorbed into the lymphatic system.

6.1.2.3 Rabbits

From days 43 to 120 after repeated s.c. injections of CsA (dose not given) in rabbits, the concentrations of CsA in blood were about 0.1 μg/mL. The absorption $t_{1/2}$ was calculated as 33 days when the rabbits were injected with 20 mg (16.6 μmol) CsA/kg bw twice a week throughout days 7 to 29 (Shah et al., 1988; cited by IARC, 1990).

6.1.3 Human Transplacental Transfer

Using a dual perfusion technique of isolated human placental lobules, Nandakumaran and Eldeen (1990) found that there was a negligible transfer of CsA from maternal to fetal circulation that represented less than 5% of the maternal drug load. The authors concluded that the low placental transfer can be attributed to relatively high molecular weight of CsA. The authors also stated that the results were in agreement with those reported earlier for an *in vivo* study conducted on a pregnant woman. [The experimental details of the study were not given. However, the study conducted by Bourget et al. (1990) may be the study mentioned by Nandakumaran and Eldeen (1990)].

Bourget et al. (1990) studied the transplacental passage of CsA in a pregnant woman who received a liver transplant 2 years prior to conception. She was maintained on a combination of azathioprine, prednisone, and CsA throughout pregnancy up to delivery. On the day of delivery, transplacental passage was evaluated by obtaining plasma levels of CsA in mother, fetus, baby, and amniotic fluid. In short, the values showed that transplacental passage of CsA was minimal, with a plasma concentration ratio of 5.0 between mother and child reported. The authors stated that the placenta efficiently prevents CsA passage at the usual maintenance-dose concentration range, despite its high lipophilicity.

Subsequently, Bourget et al. (1991) studied the accumulation of CsA in the conceptus during the first trimester of pregnancy—nine months after a liver transplant. Seven venous blood samples were collected from the mother on the day of abortion, including 2 between successive administrations of CsA (first sample collected to evaluate the residual plasmatic value of the previous sample). Examination revealed a normally appearing embryo, which was subsequently dissected and the liver, bowel, and a part of the head mass were removed. The authors found a ubiquitous distribution of CsA in the conceptus, and accumulation of CsA in the liver was considerable. The authors attributed the accumulation of CsA in liver to the conceptus' inability to metabolize or excrete the drug, and stated that the levels in both trophoblastic tissue and liver should not have been so high when considering Fick's law [rate of diffusion of a species, or molar flux, in a given direction is proportional to the concentration gradient in that direction, and the rate of concentration with time is proportional to the change in concentration gradient with distance in a given direction, i.e. in one dimension (Walker, 1995)].

In contrast to the above studies, Uhing et al. (1993) stated that CsA transplacental passage in humans occurs easily. The authors cited two references in agreement with their statement: Flechner et al. (1985) and Vankatarmanan et al. (1988). Flechner et al. (1985) found that maternal blood and cord levels of CsA were equal. In addition, in amniotic fluid and the placenta, the concentration of CsA was higher than that in maternal plasma. Vankatarmanan et al. (1988) found that placenta blood levels were approximately 50% of maternal blood levels, and some active metabolites were present at equal concentrations in maternal and cord blood.

6.2 Pharmacokinetics

Summary: In blood, 33% of CsA is bound to plasma proteins and lipoproteins, 10 to 20% to leukocytes, and 58% to erthrocytes. In the plasma fraction, 8% is bound to proteins, 16% to very low density lipoproteins, 31% to low density proteins, and 46% to high density lipoproteins. The rate of first-pass metabolism is 10 to 27%, due to the metabolism of CsA in the small intestine. The metabolites found in erthrocytes are those that are preferentially found in tissue suggesting that the factors controlling CsA metabolite distribution in tissues are equal to those in blood. Approximately 95% of ^{3}H labeled CsA orally administered was recovered from feces within a period of 96 hours, and less than 0.1% of the excreted radioactivity was attributed to parent compound. Following oral administration of CsA, only 4 to 6% was eliminated by the kidney.

The pharmacokinetics of CsA have been extensively studied in humans and reviewed by Lindholm (1991; briefly), Christians and Sewing (1993), and Fahr (1993).

The absorption of CsA after oral administration is incomplete, slow, and highly variable (Lindberg et al., 1986; cited by Lindholm et al., 1991). CsA is predominantly absorbed in the small intestine following oral administration (Drewe et al., 1992; cited by Christians and Sewing, 1993) and displays a highly variable bioavailability (8 to 60%; Burckart et al., 1986a; cited by Christians and Sewing, 1993). Wu (1995) showed that in healthy volunteers, CsA was well absorbed (on average 86%) and that the major reason for poor bioavailability was oxidative metabolism via cytochrome P-450 IIIA in the gut. These studies also showed that after oral administration, approximately two-thirds of CsA metabolism occurs in the gut, with the liver responsible for only one third of CsA metabolism.

In blood, 33% of CsA is bound to plasma proteins and lipoproteins, 10 to 20% to leukocytes, and 58% to erthrocytes (Lamaire and Tillement, 1982; cited by Christians and Sewing, 1993). In the plasma fraction, 8% is bound to proteins (mainly albumin), 16% to very low density lipoproteins, 31% to low density proteins, and 46% to high density lipoproteins (Mraz et al., 1983, Rodl and Khoshsorur, 1990; cited by Christians and Sewing 1993). "The metabolites found in tissue are those that are preferentially found in erthrocytes suggesting that the factors controlling CsA metabolite distribution in tissues are equal to those in blood" (Lensmeyer et al., 1991; cited by Christians and Sewing, 1993). Per kilogram tissue, the concentrations of CsA plus its metabolites decreased in the order pancreas > spleen > liver > fat > kidney >lung > bone marrow > muscle (heart) > blood.

The rate of first-pass metabolism is 10 to 27% (Lemaire et al., 1990; cited by Christians and Sewing, 1993), due to the metabolism of CsA in the small intestine (Kolars et al., 1991, 1992, Tija et al., 1991; cited by Christians and Sewing, 1993; Webber et al., 1992). Bile is the major route of excretion of CsA in humans. Maurer and Lamaire (1986) found that approximately 95% of ^{3}H labeled CsA orally administered was recovered from feces within a period of 96 hours, and less than 0.1% of the excreted radioactivity was attributed to parent compound. CsA accounts for less than 0.1% of bile-derived material, with CsA metabolites accounting for the residual material recovered from bile (Vankataramanan et al., 1988; cited by Christians and Sewing, 1993). Following oral administration of CsA, only 4 to 6% is eliminated by the kidney. A strong correlation was observed between renal elimination of CsA metabolites and creatinine clearance. In blood of patients with normal kidney functions, metabolites with cyclization of AA1 as AM1c and AM1c9 can rarely be detected, but are excreted in considerable amounts in urine (Bleck et al., 1989; cited by Christians and Sewing, 1993).

6.3 Modes of Action

The postulated mechanisms of CsA-induced carcinogenicity in human, primate, and rodent have been reviewed by Ryffel (1992), Ryffel et al. (1992), and MacDonald et al. (1994) and are discussed below.

6.3.1 Genetic Effects

CsA (parent compound) has no DNA-binding property. See section 5.0 for discussion of CsA genotoxicity (gene mutations, DNA damage, and chromosomal damage) in prokaryotes, mammalian systems *in vivo,* and humans. CsA has no effect on basal transcription of housekeeping genes, and inhibits only induced gene transcripts (Ryffel et al., 1992;).

6.3.2 Immunosuppression

Evidence from many studies in which CsA was administered in conjunction with an initiating agent (*N*-methyl-*N*-nitrosourea [MNU], 3-methylcholanthrene [3- MC], x-radiation, streptozotocin, and virus) in rodents (mouse and rat) and monkeys supports the belief that the slight increase in the incidence of tumors is related to suppression of immune surveillance (MacDonald et al., 1994; for review see Ryffel, 1992). For example, the latency period to tumor development was reduced and/or the number of tumors observed were increased when CsA was administered in conjunction with an initiating agent (for review see Ryffel, 1992). In addition, in a study conducted on AKR mice administered a high dose CsA (150 mg/kg/day), the latency period for the appearance of thymic lymphomas was reduced (Hattori et al., 1986; cited by Ryfell, 1992, MacDonald et al., 1994).

In humans receiving CsA immunosuppressive treatment, the incidence of tumor findings was low when CsA was administered alone or at low doses, and increased as the dose increased or when other immunosuppressive therapy was combined with CsA treatment (Cockburn and Krupp, 1989, Wilkinson et al., 1989; cited by MacDonald et al., 1994). Furthermore, lymphomas and lymphoproliferative lesions that developed during CsA therapy were reversible upon dosage reduction (Starzl et al., 1984; cited by MacDonald et al., 1994). These findings suggest that the carcinogenic activity of CsA is dose-dependent in humans and corresponds to similar findings in mice dosed with high concentrations of CsA (see above).

6.3.3 Other Issues

No obvious relationships to mechanisms of carcinogenesis were found upon examination of mechanisms of teratogenicity (Backman et al., 1988; cited by Uhing et al., 1993; Bourget et al., 1990), nephrotoxicity (Brooks et al., 1993), hepatotoxicity (Bleck et al., 1991; cited by Christians and Sewing, 1993), and species differences in metabolism and pharmacokinetics (IARC, 1990; Wenger, 1990; Akagi et al., 1991; Lindholm et al., 1991; Fahr, 1993; Christians and Sewing, 1993).

Although no apparent relationship was found between nephrotoxicity and mechanisms of carcinogenesis, note that upon comparing pharmacokinetics of CsA in rats and humans, it was found that rats require much higher CsA doses and maintenance of much higher blood levels for induction of nephrotoxicity than humans. However, it is important to note that in no case has the induction of malignant renal tumor been associated with CsA use, even in patients manifesting drug-induced renal dysfunction" (Ryffel et al., 1992).

6.4 Structure-Activity Relationships

Rosenkranz and Klopman (1992) identified CsA substructural fragments by the CASE knowledge-based structure-activity relational expert system using several learning sets—the NTP rodent carcinogenesis bioassays, the NTP mouse bioassays, the NTP rat bioassays, and the rodent carcinogenicity database compiled by Gold and co-workers [for example see Gold et al. (1995)]. Using the NTP mouse data, CASE gave a 75% positive prediction of CsA carcinogenicity in mice for the secondary amide located between AA3 and AA2 . Using the Gold rodent database, CASE calculated a 99.3% positive prediction. The Gold biophores included the isobutyl groups in AA10, AA9, and AA4; the tertiary amide group with the N atom attached to a methylene group (strangely designated as AA3-AA1 although the residue is found only in AA3); and the group RNCHR designated as present in AA5-AA6 and AA8-AA9. CASE had no structural alerts for genotoxicity and predicted a lack thereof.

6.5 Cell Proliferation

Full experimental details for the studies described in this section are presented in Table 6-2.

Summary: Proliferative "lymphoid alterations" were detected in the gut- associated lymphoid tissue (GALT) of rats administered CsA in the diet for up to 8 weeks. In rats administered CsA in the diet for 34 weeks, "atypical epithelial proliferations of the intestinal mucosa associated with hyperplasia of gut-associated lymphoid structures" were detected. There was a significant increase in the hepatic labeling index in rats administered CsA in the diet for 2, 4, or 10 weeks and there was a significant increase in the renal labeling index in rats administered CsA subcutaneously once per day for 4, 6, or 8 days. There was an increase in proliferating cell nuclear antigen (PCNA) and an increase in the incidence of interstitial fibrosis in rats administered CsA subcutaneously for 5–35 days. In beagle dogs administered CsA in the diet for 26 weeks, skin lesions were first detected between 3 and 6 weeks. By week 7, irregular, oval, sessile, unpigmented, firm masses of the skin were detected in all CsA-treated dogs. The lesions were limited to haired skin around the eyes and to ears, lips, face, neck, chest, abdomen, genital area, and legs. The incidence and severity of lesions, however, did not correlate with blood levels of CsA.

6.5.1 Rats

Proliferative "lymphoid alterations" were detected in the gut-associated lymphoid tissue (GALT) of male Sprague-Dawley rats (age not specified) administered CsA in the diet (0.015% w/w; 150 ppm) for up to 8 weeks. These alterations were detected 2 weeks after the initiation of CsA treatment and progressed through 8 weeks of CsA treatment. Histologic examination revealed 2 types of alterations. In the first type, there were "markedly enlarged, mitotically active cortical and medullary germinal centers containing numerous tingible-body macrophages surrounded by thinned mantle zones." In the first type, there was also a proliferation of nodal vessels that frequently expanded beyond the boundaries of the nodes and infiltrated the perinodal fat. In the second type of alteration, more common in the mesenteric nodes, there were "relatively monomorphic nodules of intermediate-sized lymphocytes in the cortex and medulla. Some of these nodules contained small, ill-defined or atrophic germinal centers." There was also a significantly greater mean number of lymphoid plaques in CsA-treated rats than in controls at

2, 4, and 8 weeks. There were no abnormalities detected in the lymph nodes of control rats (Demetris et al., 1984).

The hepatic labeling index was significantly increased in 6- to 7-week-old male F344 rats administered CsA in the diet (150 ppm) for 2, 4, or 10 weeks (Masuhara et al., 1993).

No tumors were detected in male Sprague-Dawley rats (age not specified) administered CsA (110 ppm in diet) for 34 weeks, but "atypical epithelial proliferations of the intestinal mucosa associated with hyperplasia of gut-associated lymphoid structures" were detected (Perera et al., 1986; cited by IARC, 1990).

There was a significant increase in the renal labeling index in male Sprague-Dawley rats (age not specified) administered CsA (100 mg/kg body weight; 83 μmol/kg bw) subcutaneously for 4, 6, or 8 days. [^{3}H]Thymidine incorporation was measured on days 4 and 8. In the outer cortex, the mean labeling index was significantly increased in CsA-treated rats on day 8, but not on day 4, as compared to controls. In the inner cortex, the mean labeling index was significantly increased in CsA-treated rats on days 4 and 8 as compared to controls. In the inner medulla-papilla, the mean labeling index was significantly increased in CsA-treated rats on day 8, but not on day 4, as compared to controls. Focal areas of tubular atrophy and interstitial fibrosis with an increase in the overall number of interstitial cells were detected in the outer, but not inner, cortex. In the medulla, a widening of the interstitium and an increase in the number of cells in the interstitium were detected in the inner stripe of the outer medulla (Jackson et al., 1987).

There was a significant increase in the renal labeling index in male Sprague-Dawley rats (age not specified) administered CsA (100 mg/kg body weight; 83 μmol/kg body weight) subcutaneously once per day for 8 days. In the spleen, liver, and heart of these rats, there was no significant increase in the labeling index (Humes and Jackson, 1988).

There was an increase in proliferating cell nuclear antigen (PCNA) in adult male Sprague-Dawley rats administered CsA (15 mg/kg; 12 μmol/kg) subcutaneously for 5-35 days. This increase occurred early (at day 5) and was progressive, peaking at day 35. There was also an early, sustained, and significant interstitial fibrosis detected in CsA-treated rats, whereas none was detected in vehicle controls (Burdmann et al., 1994).

6.5.2 Dogs

In 2-year-old male beagle dogs administered CsA (30-55 mg/kg/day; 25-46 μmol/kg/day) in the diet for 26 weeks, skin lesions were first detected between 3 and 6 weeks. By week 7, irregular, oval, sessile, unpigmented, firm masses of the skin were detected in all CsA-treated dogs. The lesions were limited to haired skin around the eyes and to ears, lips, face, neck, chest, abdomen, genital area, and legs. The incidence and severity of lesions, however, did not correlate with blood levels of CsA. The lesions regressed spontaneously when CsA treatment was terminated; by 8 weeks post-treatment, lesions were not "clinically apparent." The incidence of skin lesions in control dogs was not mentioned. In the oral cavity of these dogs, "gingival overgrowth" was detected in 5/12 animals, but no oral papilloma-like lesions were observed. Again, no mention was made of the incidence in control dogs. Other cutaneous effects of CsA treatment included hyperkeratosis of footpads, increased growth of hair and nails, and hyperkeratinization of external surface of the prepuce of penis, which became covered by a firm dry cornified layer (Seibel et al., 1989).

6.6 Initiation/Promotion

The studies described below are presented in detail in Tables 6-3 and 6-4.

6.6.1 Mammalian Carcinogenicity

6.6.1.1 Diethylnitrosamine (DEN) and CsA

There was no increase in the incidence of hepatocellular carcinoma in 6- to 7-wk-old male F344 rats initiated with a single i.p. injection of DEN (250 mg/kg), followed 1 week later with administration of a choline-deficient diet for 7 weeks, and then followed by administration of CsA in the diet (0.015%, 150 ppm) for an additional 22, 28, or 32 weeks (controls received similar treatment except they were given basal diet). When the incidences of hepatocellular carcinoma at each measured time point were combined into a cumulative incidence, however, this cumulative incidence was significantly greater than the incidence in controls (Masuhara et al., 1993).

6.6.1.2 9,10-Dimethyl-1,2-benzanthracene (DMBA) and CsA

There was a significantly increased incidence of cervical lymph node metastasis in 10-week-old Syrian golden hamsters administered CsA (50 mg/kg/day [42 μmol/kg/day]) intravenously (i.v.) for 4 weeks. All hamsters had received 3 applications per week to the left cheek pouch of 0.3% (w/v) DMBA in acetone for 14 weeks. Hamsters were then held for 3 weeks without treatment. All hamsters developed well-differentiated squamous cell carcinomas in their treated cheek pouches. At the end of the 3-week holding period, exophytic tumors in the pouch were excised at their base and treatment with CsA was begun. The incidence of lymph node metastasis was not significantly increased when a lower dose of CsA (25 mg/kg/day [21 μmol/kg/day]) was administered (Yamada et al., 1992).

6.6.1.3 Irradiation and CsA

The incidence of lymphoid tumors was significantly increased in 6- to 7- week-old male Swiss Webster and C57B1/6J mice that were both irradiated and administered CsA (350 rad γ-radiation [single whole-body] and fed a diet containing 150 ppm CsA starting on day 10 for 35 weeks). No lymphoid tumors were detected, however, in mice that were irradiated and maintained on basal diet or in mice that were administered CsA, but were not irradiated (Hattori et al., 1988; cited by IARC, 1990).

6.6.1.4 3-Methylcholanthrene (3-MC) and CsA

No tumors were detected in 8- to 10-week-old male Sprague-Dawley rats administered CsA (2.5 or 5 mg/kg/day [2.1 or 4.2 μmol/kg/day]) subcutaneously (s.c.) for 2 days, followed immediately with a single s.c. injection of 3-MC, and then followed with s.c. administration of CsA (2.5 mg/kg/wk or 5 mg/kg twice/wk) for 2 (low dose) or 10 (high dose) more weeks. When another group of rats was administered CsA (2.5 mg/kg/day) for 2 weeks, beginning 5 days after 3-MC injection, tumors were detected (site and tumor type not specified), but the incidence was not significantly greater than that in controls. Immune function was assessed by measuring delayed-type hypersensitivity (DTH), natural killer cell (NK) activity, and production of interleukin 2 (IL-2), interferon (IFN), prostaglandin E_2 (PGE_2), and specific IgG antibody. "NK cytotoxicity was enhanced and antibody production was suppressed in rats treated with all doses

of CsA tested. IL-2 production was elevated at the two lower doses, but antibody production, DTH reactions and synthesis of IL-2 and IFN activity seen in rats treated with the lower doses of CsA may be due to the increase in IL-2 production, while enhancement of NK activity at higher doses may be due to other mechanisms" (Bussiere et al., 1991).

6.6.1.5 *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and CsA

No tumors were detected in "young" male Wistar rats that were administered 10 mg CsA/kg bw/day (8.3 μmol/kg bw/day) s.c. for 1 week. In rats that were administered CsA and MNNG, there was no increase in the incidence of tumors as compared to rats treated with MNNG alone. Refer to Table 6-3 for details of MNNG administration (Johnson et al., 1984; cited by IARC, 1990).

6.6.1.6 *N*-Methyl-*N*-nitrosourea (MNU) and CsA

There was a 4- to 8-fold increase in the incidence of thymic lymphoma in 6- to 7-week-old male Swiss Webster mice treated with MNU and CsA (single i.p. injection of MNU [12.5 or 25 mg/kg bw] followed 1 week later with 150 ppm CsA in the diet for 35 weeks) as compared to mice treated with MNU alone. No thymic lymphomas were detected, however, in mice treated with CsA alone or in untreated controls (Shinozuka et al., 1988; cited by IARC, 1990).

There was a synergistic increase in the incidence of intestinal adenocarcinoma in male Sprague-Dawley rats (age not specified) administered MNU and CsA (one week after a single i.p. injection of 25 mg MNU/kg bw, the rats were started on a diet containing 110 ppm CsA for 34 weeks). No tumors were detected, however, in rats that received CsA alone or in untreated controls, although in rats that received CsA alone, "atypical epithelial proliferations of the intestinal mucosa associated with hyperplasia of gut-associated lymphoid structures" was detected (Perera et al., 1986; cited by IARC, 1990).

6.6.1.7 Peripheral Blood Mononuclear Cells (PBMC) and CsA

The total tumor incidence was *decreased* in 7- to 9-week-old SCID mice (sex not specified) administered 50 μg CsA/g/day (42 μmol/kg/day) i.p. for up to 36 weeks as compared to controls. All mice (CsA-treated and control) had been inoculated with 60–100 x 10^6 PBMC obtained from 8 healthy human adults undergoing lymphopheresis. CsA treatment of mice was begun 1 day before PBMC inoculation (Veronese et al., 1992).

6.6.1.8 Tumor-Cell Inoculation and CsA

Metastasis of tumors to the liver was evaluated in 5- to 6-week-old male C57B1/6J mice administered CsA (or vehicle) by gavage in combination with inoculation of MCA 38 colon tumor cells (derived from mice bearing murine colon adenocarcinoma) into the portal vein. Group A was administered 30 mg CsA/kg bw (25 μmol/kg bw) on the seventh, eighth, and ninth days post tumor inoculation. Group B was administered 15 mg CsA/kg bw (12.5 μmol/kg bw) 30 minutes before tumor inoculation and 2 times more at 24 hour intervals. Group C was administered 30 mg CsA/kg bw (25 μmol/kg bw) in the same manner as group B. At 2 and 3 weeks post tumor inoculation, the abdomen of each mouse was opened and explored for evidence of hepatic metastasis. Mice in all groups were killed 4 weeks post tumor inoculation. At 2 weeks post inoculation, the mean number of MCA 38 tumors in the liver and their mean diameter

were significantly increased in group B, but not in groups A and C, as compared to vehicle controls. At 3 weeks post inoculation, the mean number of MCA 38 tumors in the liver and their mean diameter were significantly increased in groups B and C, but not in group A, as compared to vehicle controls. At 4 weeks post inoculation, the mean number of liver tumors and their mean diameter were significantly increased in group C, but not in groups A and B, as compared to vehicle controls (Yokoyama et al., 1994).

The mean number of hepatic metastases was significantly increased in 6- wk-old male CDF1 mice administered CsA (10, 20, or 50 mg/kg/day [8, 17, or 42 μmol/kg/day]) s.c. for 22 days as compared to vehicle controls. One day following initiation of treatment, all mice (CsA-treated and control) had been inoculated in the portal vein, through the superior mesenteric vein, with colon-26 tumor cells (derived from a mouse colon adenocarcinoma cell line). All CsA-treated and control mice developed hepatic metastases following this procedure. After the 22-day treatment period, peritoneal metastases were detected in 4/10 low-dose, 5/6 mid-dose, and 2/2 high-dose mice, but in none of the control mice. Pulmonary metastases were only detected in 1/6 mid-dose and 2/2 high-dose mice (Suzaki et al., 1995).

6.6.1.9 Urethan and CsA

There was no significant difference between the number of adenomas detected in 6- to 7-week-old male Swiss Webster mice that received urethan (urethane; ethyl carbamate) and CsA (single i.p. injection of 1 g [0.83 μmol]/kg bw followed 1 week later with 150 ppm CsA in the diet for 22 weeks) and mice that received urethan alone (single i.p. injection of 1 g/kg bw). Although this study also included a group administered CsA alone, no comparison was made between this group and an untreated control group (Shinozuka et al., 1988; cited by IARC, 1990).

6.6.1.10 Multiple Treatments

The mean number of hepatic metastatic colonies was significantly increased in 6-week-old male CDF1 mice administered CsA (10 mg/kg/day [8 μmol/kg/day]), in olive oil, s.c. for 22 days (it was not clear, however, whether the comparison was made with saline controls or olive oil controls). One day following initiation of treatment, all mice had undergone laparotomy by an upper median incision: The duodenal loop was exposed and a 0.5-mL aliquot of colon-26 tumor cells (5×10^3 cells) (from a mouse colon adenocarcinoma cell line) was inoculated into the portal vein through the superior mesenteric vein. In mice treated with CsA and 5-fluorouracil (15 mg/kg/day every other day) for 22 days, the mean number of hepatic metastatic colonies was not significantly different from the number in mice treated with CsA alone. The incidence of hepatic metastasis was not specified (Suzaki et al., 1995).

The incidence of B-cell lymphoma was increased in macaques (species, age, and sex not specified) administered 25 mg CsA/kg bw/day (21 μmol/kg bw/day) intramuscularly for 2 weeks followed by 17 mg CsA/kg bw (14 μmol/kg bw) every day or every other day continuously (length of exposure not specified), as compared to macaques that received other immunosuppressive drugs and/or radiation (see Table 6-3 for details). The incidence of B-cell lymphoma was also increased in macaques that were administered CsA concurrently with other immunosuppressive drugs (Bieber et al., 1982; cited by IARC, 1990).

6.6.2 Cell Proliferation

6.6.2.1 Tumor-Cell Inoculation and CsA

There was a significant increase in the hepatic labeling index in 5- to 6-week-old male C57B1/6J mice administered CsA 7 days after being inoculated in the portal vein with colon tumor cells derived from mice bearing murine colon adenocarcinoma. CsA (15 or 30 mg/kg body weight; 12.5 or 25 μmol/kg body weight) was administered by gavage 30 minutes before tumor inoculation and two more times at 24-hour intervals. When CsA (30 mg/kg body weight; 25 μmol/kg body weight) was administered on the seventh, eighth, and ninth days following tumor inoculation, there was no significant increase in the labeling index (Yokoyama et al., 1994).

6.6.2.2 Topical Steroids and CsA

In a female patient treated for psoriasis with CsA for 12 weeks (2.5 mg/kg [2.1 μmol/kg] 3 times per week every 12 hours for weeks 1–10 followed by 7 mg/kg 3 times per week every 12 hours for weeks 11–12), 14 verrucous papules were detected at week 12, both within and next to psoriatic plaques, as well as on uninvolved skin on extremities and trunk. Histologic examination revealed hyperkeratosis, mold hypergranulosis, and acanthosis, consistent with benign keratoses. Koilocytosis was absent, and pilar structures were not involved. Human papillomavirus DNA was not detected. The authors concluded that "CsA appears to have contributed to the induction of these benign keratoses in our patient, but the mechanism is unknown." The patient had a 15-year history of psoriasis. She had previously been treated with topical steroids, but had never been treated with tars, UV light, or systemic medications (Ross et al., 1992).

Table 6-1. Cyclosporin A Metabolite Identification

Name[a] (No.)[b]	In Vivo	In Vitro	Reaction/Enzymes	Comments
Parent Compound				
Cyclosporin A; CsA; Cs	Human liver allograft recipient (Lucey et al., 1990).	Human liver and kidney slices (Vickers et al., 1995). Human gastrointestinal and liver microsomes obtained from kidney transplant donors (Webber et al., 1992). Human liver microsomes (Henricsson et al., 1990; Christians et al., 1991b).	In the liver of humans and animals, CsA is extensively metabolized by cytochrome P-450 (Maurer et al., 1984, Maurer, 1985, and Ryfell et al., 1988, cited by Copeland et al., 1990a; Henricsson et al., 1990). In vivo, CsA is metabolized by hepatic cytochrome P-450 IIIA to more than 21 metabolites, with the 3 major ones being II, M-17, and M-21 (Ryfell et al., 1988; Yascoff et al., 1991, cited by Pham-Huy et al., 1995; Kronbach et al., 1988, cited by Vickers et al., 1992; Lucey et al., 1990; Kolars et al., 1992; Webber et al., 1992)	CsA metabolism has been shown to occur at several sites: Gastrointestinal tract (site of absorption) and liver and kidney (sites of excretion) (Vickers et al., 1992). The liver is the main site of CsA biotransformation and subsequent extensive elimination of metabolites is via the bile (Maurer et al., 1984, Maurer and Lamaire, 1986, and Copeland et al., 1990a, cited by Vickers et al., 1995). The rate of total CsA metabolism by human kidney slices represented about 42% the rate in liver slices. CsA metabolism not detected in human kidney microsome incubations. Liver slice cultures revealed that the rat metabolized CsA about 81% the rate of human liver slices, and the dog is about 2.8 times faster. The metabolism of CsA by human intestinal colonic mucosal slices suggested that intestinal metabolism of CsA contributes to the first-pass effect of this compound. Human colonic mucosal slices metabolized CsA at a faster rate than human liver slices (2.41 ± 0.40 nmol/h/g vs. 1.08 ± 0.3 nmol/h/g) (Vickers et al., 1992). Similar findings have been reported by Webber et al. (1992).

Table 6-1. Cyclosporin A Metabolite Identification (Continued)

Name[a] (No.)[b]	In Vivo	In Vitro	Reaction/Enzymes	Comments
Cyclosporin A; CsA; Cs			CsA induced rat P-450 4A2 in kidney microsomes (Yoshimura et al., 1989, cited by Pham-Huy et al., 1995; Nakamura et al., 1994). Metabolism is initiated by *N*-demethylation at residue 4 or 9 or by hydroxylation at residue 1 or 9 (Hartman et al., 1985, Maurer et al., 1984, Gmur et al., 1988, and Yee et al., 1988, cited by Meier et al., 1990). Further metabolism of the primary metabolites via hydroxylation or *N*-demethylation occurred, but yielded lower levels of secondary metabolites (Gmur et al., 1988, and Yee et al., 1988, cited by Meier et al., 1990).	Four major metabolites formed from CsA: AM1, AM9, AM4N, and AM1c (Christians et al., 1991b). CsA is preferentially metabolized via oxidation at the γ-carbons of the methylleucines at amino acids (AA) 4, 6, and 9, the terminal methyl group of MeBMT, or cyclized at AA1 (Christians and Sewing, 1993).

Table 6-1. Cyclosporin A Metabolite Identification (Continued)

Name[a] (No.)[b]	In Vivo	In Vitro	Reaction/Enzymes	Comments
Phase I Metabolites				
AM1; M17; H370 (I)	Human bile following CsA administration (Copeland et al., 1990a). Human urine (renal transplant recipients) (Copeland et al., 1990b; Brooks et al., 1993). Human blood and urine (Meier et al., 1990; Schwinghammer et al., 1991). Human bile and urine from a liver grafted patient (Christians et al., 1988). Phenobarbital-induced male rat hepatic microsomes (Serino et al., 1993).	Human liver, colonic mucosal, and kidney slices (Vickers et al., 1992 & 1994). Rabbit renal or hepatic microsomes (Pham-Huy et al., 1995). Human gastrointestinal and liver microsomes obtained from kidney transplant donors (Webber et al., 1992). Human liver microsomes (Back et al., 1989 and Tjia et al., 1989, cited by Webber et al., 1992). Rat microsomes prepared from liver and jejunal enterocytes (Kolars et al., 1992).	Monohydroxylated CsA to form AM1 (Copeland et al., 1990a). Cytochrome P-450 IIIA (Webber et al., 1992; Vickers et al., 1994).	AM1, AM9, and AM4N are the primary CsA metabolites, with the remaining arising from further metabolism of these primary metabolites, although direct proof is lacking (Maurer et al., 1984; cited by Copeland et al., 1990a). AM1 is the major metabolite in humans (Venkataramanan et al., 1988, cited by Copeland et al., 1990a) and rabbit (Copeland et al., unpublished; cited by Copeland et al., 1990a), in contrast to rats who metabolize CsA primarily to AM9 (Ryfell et al., 1986; cited by Copeland et al., 1990a). Duodenal protein converted 15% of CsA to AM9 and AM1 after a 2-h incubation (Webber et al., 1992). It has been suggested that metabolism in the stomach may account for the variations in CsA bioavailability (Ueda et al., 1984, and Wadhwa et al., 1987; cited by Webber et al., 1992). In addition, the findings of Webber et al. (1992) suggest that the variations in g.i. tract enzyme profiles may also affect the bioavailability of CsA.
AM11D; H355 (II)	Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver microsomes (Christians et al., 1991b).	AM1 further hydroxylated at the δ-C of AA1 to form AM11D (Christians et al., 1991b).	H355 detected by HPLC and eluted at 35.5 min. (Christians et al., 1988).

Table 6-1. Cyclosporin A Metabolite Identification (Continued)

Name[a] (No.)[b]	In Vivo	In Vitro	Reaction/Enzymes	Comments
AM1c; M18; H400 (III)	Rabbit bile and urine after i.v. injection of 1.0 mg/kg AM1 derived from human bile (Copeland et al., 1990a). Human urine (renal transplant recipients) (Copeland et al., 1990b; Brooks et al., 1993). Human whole blood (Schwinghammer et al., 1991). Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver slices (Vickers et al., 1992). Human liver microsomes (Christians et al., 1991b).	AM1 or CsA cyclized to AM1c (Christians et al., 1991b).	
AM19; M8; H250 (IV)	Rabbit bile and urine after i.v. injection of 1.0 mg/kg AM1 derived from human bile (Copeland et al., 1990a). Human urine (renal transplant recipients) (Copeland et al., 1990b; Brooks et al., 1993). Human bile and urine from a liver grafted patient (Christians et al., 1988). Rat bile (Wagner et al., 1987; cited by Christians and Sewing, 1993).	Human liver and kidney slices (Vickers et al., 1992 & 1994). Human liver microsomes (Christians et al., 1991b).	AM1 hydroxylated at AA9 to form AM19 or hydroxylated at AA1 of AM9 to form AM19 (Copeland et al., 1990a; Christians et al., 1991b).	Major metabolite found in rat bile (Wagner et al., 1987; cited by Christians and Sewing, 1993).

Table 6-1. Cyclosporin A Metabolite Identification (Continued)

Name[a] (No.)[b]	In Vivo	In Vitro	Reaction/Enzymes	Comments
AM1AL; H410/420 (V)	Human bile (Hashem et al., 1988). Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver microsomes (Christians et al., 1991b).	AM1 oxidized to AM1AL (Christians et al., 1991b).	
AM1A; M203-208; H350 (VII)	Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver microsomes (Christians et al., 1991b).	AM1AL carboxylated to form AM1A (Christians et al., 1991b).	
AM9; M1; H390 (XII)	Human urine (renal transplant recipients) (Copeland et al., 1990b; Brooks et al., 1993). Human blood and urine (Meier et al., 1990; Schwinghammer et al., 1991). Rat jejunum (Kolars et al., 1992). Human bile and urine from a liver grafted patient (Christians et al., 1988). Rat blood, urine, and tissues (Wagner et al., 1987; cited by Christians and Sewing, 1993). Phenobarbital-induced male rat hepatic microsomes (Serino et al., 1993).	Human liver and kidney slices (Vickers et al., 1992 and 1994). Human hepatocytes (Pichard et al., 1992). Rat microsomes prepared from liver and jejunal enterocytes (Kolars et al., 1992). Rabbit renal or hepatic microsomes (Serino et al., 1993; Pham-Huy et al., 1995). Human gastrointestinal and liver microsomes obtained from kidney transplant donors (Webber et al., 1992).	Monohydroxylated CsA to form AM9 (Copeland et al., 1990a). Cytochrome P-450 III gene family: P-450hA7 in humans, P-450 3C in rabbit, and P-450 pcn in rats (Webber et al., 1992; Pichard et al., 1992; Serino et al., 1993; Vickers et al., 1994).	AM9 was the major metabolite found in rats (Ryfell et al., 1986, cited by Copeland et al., 1990a). The metabolism of I by enterocytes *in vivo* is substantial and likely contributes to first pass metabolism following oral administration of I (Kolars et al., 1992).

Table 6-1. Cyclosporin A Metabolite Identification (Continued)

Name[a] (No.)[b]	In Vivo	In Vitro	Reaction/Enzymes	Comments
AM1Ac; H310 (XVII)	Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver microsomes (Christians et al., 1991b).	AM1c carboxylated and cyclized at AA1 forming AM1Ac (Christians et al., 1991b).	
AM1A4N (IX)		Human liver microsomes (Christians et al., 1991b).	AM1A *N*-demethylated at AA4 forming AM1A4N (Christians et al., 1991b).	
AM1c9; M26; H270 (XVIII)	Rabbit bile and urine after i.v. injection of 1.0 mg/kg AM1 derived from human bile (Copeland et al., 1990a). Human urine (renal transplant recipients) (Copeland et al., 1990b; Brooks et al., 1993). Human bile and urine from a liver grafted patient (Christians et al., 1988).		Dihydroxylated AM1c to form AM1c9 (Copeland et al., 1990a).	
AM4N; M21; H430 (XXII)	Human urine (renal transplant recipients) (Copeland et al., 1990b). Human whole blood (Schwinghammer et al., 1991). Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver and kidney slices (Vickers et al., 1992 &1994). Rabbit renal or hepatic microsomes (Pham-Huy et al., 1995). Human liver microsomes (Back et al., 1989, and Tjia et al., 1989; cited by Webber et al., 1992). Rat microsomes prepared from liver and jejunal enterocytes (Kolars et al., 1992).	*N*-Demethylated I to form AM4N (Copeland et al., 1990a). Cytochrome P-450 IIIA (Vickers et al., 1994).	Renal microsomes of rabbits treated with rifampicin or erythromycin or untreated (control) metabolized CsA 15-, 37-, and 30-fold lower, respectively, than hepatic microsomes of rabbits treated identically (Pham-Huy et al., 1995).

Table 6-1. Cyclosporin A Metabolite Identification (Continued)

Name[a] (No.)[b]	In Vivo	In Vitro	Reaction/Enzymes	Comments
AM1c4N9 (XX)		Human liver microsomes (Christians et al., 1991b).	AM1c9 *N*-demethylated at AA4 (Christians et al., 1991b).	
AM4N9; M13; H320 (XXIII)	Human urine (renal transplant recipients) (Copeland et al., 1990b). Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver slices (Vickers et al., 1992).	AM4N was hydroxylated to form AM4N9 (Copeland et al., 1990a; Christians et al., 1991b).	
AM49 (XXIV)		Human liver microsomes (Christians et al., 1991b).	Dihydroxylated CsA to form AM49 (Copeland et al., 1990a).	
AM69; M16; H340 (XXV)	Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver microsomes (Christians et al., 1991b).	Dihydroxylated CsA forming AM69 (Copeland et al., 1990a).	
AM14N; M25; H300 (XXVI)	Human bile and urine from a liver grafted patient (Christians et al., 1988).		CsA monohydroxylated and *N*-demethylated to form AM14N (Copeland et al., 1990a).	
AM4N69 (XXVIII)			CsA is dihydroxylated and *N*-demethylated to form AM4N69 (Copeland et al., 1990a).	
AM1A9		Human liver microsomes (Christians et al., 1991b).	Carboxylated derivative of AMI9 (Christians et al., 1991b).	
AM14N9; M9; H255	Human bile and urine from a liver grafted patient (Christians et al., 1988).	Human liver microsomes (Christians et al., 1991b).	*N*-demethylated derivative of AM19 (Christians et al., 1991b).	
Dihydro-CSA-M17	Human blood and urine (Meier et al., 1990).		Oxidized (Meier, 1990)	A novel metabolite of CsA. Amino acid 1 oxidized at the terminal η-carbon and the double bond reduced (Meier et al., 1990).
AM99N	Human transplant patients (Bowers et al., 1990).		CsA demethylated and hydroxylated (Bowers et al., 1990)	

Table 6-1. Cyclosporin A Metabolite Identification (Continued)

Name[a] (No.)[b]	In Vivo	In Vitro	Reaction/Enzymes	Comments
Phase II Metabolites				
AM1c-GLC; H235 (XIX)	Human liver grafted patients (Christians et al., 1991a). Human bile and urine from a liver grafted patient (Christians et al., 1988).		AM1c glucuronylated to AM1c-GLC (Christians et al., 1991a).	
CsA-SO_4	Human bile and plasma (Henricsson, 1990; Johansson et al., 1990).		CsA conjugated with sulfuric acid (Johansson et al., 1990).	The hydroxyl group at the MeBMT amino acid is the only position that is available for conjugation of CsA. In plasma, the concentration of the conjugate CsA was estimated to exceed that of CsA by a factor of 50 (Johansson et al., 1990).
α, β Unsaturated carboxylic acid	Human and rabbit bile (Hartman et al., 1985).		*N*-Methyl goup of CsA 9-C AA1 oxidized to form α,β unsaturated carboxylic acid (Hartman et al., 1985).	Primary biliary metabolite of CsA (Hartman et al., 1985).

[a]The Hawk's Cay nomenclature (Consensus Document, 1990) is used for this review.
[b]Roman numeral corresponds to metabolite identification found in Figure 6-1 or 6-2 (a, b, c).

Table 6-2. Cell Proliferation Induced By Cyclosporin A

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Oral Administration*							
Sprague-Dawley rat (age not specified)	3M (killed at 2 wk) 3M (killed at 4 wk) 10M (killed at 8 wk)	*basal diet alone*: 3M (killed at 2 wk) 3M (killed at 4 wk) 10M (killed at 8 wk)	CsA, purity not specified	0.015% (w/w) in diet (150 ppm)	up to 8 wk	At necropsy, particular attention was paid to thymus, lymph nodes, spleen, and gut- associated lymphoid tissue (GALT). The entire gastrointestinal tract was also isolated. **Lymphatic System:** Positive (for proliferative activity in GALT) Proliferative lymphoid alterations were detected in the GALT beginning 2 weeks after the initiation of CsA treatment and progressing through 8 weeks of CsA treatment. Histologic examination revealed 2 types of alterations. In the first type, there were "markedly enlarged, mitotically active cortical and medullary germinal centers containing numerous tingible-body macrophages surrounded by thinned mantle zones." In the first type, there was also a proliferation of nodal vessels that frequently expanded beyond the boundaries of the nodes and infiltrated the perinodal fat. In the second type of alteration, more common in the mesenteric nodes, there were "relatively monomorphic nodules of intermediate-sized lymphocytes in the cortex and medulla. Some of these nodules contained small, ill-defined or atrophic germinal centers." There were no abnormalities detected in the lymph nodes of control rats. There was a significantly greater mean number of intestinal plaques in CsA-treated rats than in controls at 2, 4, and 8 weeks (4.7 [$p < 0.01$; Student's *t*-test], 4.4 [$p < 0.05$], and 5.7 [$p < 0.001$] vs. 3.9, 3.9, and 3.8 in controls at 2, 4, and 8 weeks, respectively). **Other Tissues:** Negative No gross abnormalities were detected in other organs.	Demetris et al. (1984)

Table 6-2. Cell Proliferation Induced By Cyclosporin A (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
6- to 7-wk- old F344 rat	6M (killed after wk2) 7M (killed after wk 4) 6M (killed after wk 10)	*basal diet alone*: 6M (killed after wk 2) 7M (killed after wk 4) 6M (killed after wk 10)	CsA, purity not specified	150 ppm in diet	2, 4, or 10 wk	One hour before being killed, rats were injected intraperitoneally with BrdU (50 mg/kg body weight). **Liver:** Positive (for proliferative activity, as indicated by labeling index) The hepatic labeling index was significantly increased in CsA-treated rats at all measured time points (wk 2: 10.0 ± 1.3 vs. 2.2 ± 0.3 in controls [$p < 0.01$, Fisher's exact test]; wk 4: 7.8 ± 1.4 vs. 4.1 ± 0.7 in controls [$p < 0.01$, Fisher's exact test]; wk 10: 2.7 ± 0.7 vs. 0.6 ± 0.1 in controls [$p < 0.05$, Fisher's exact test]).	Masuhara et al. (1993)
Sprague- Dawley rat (age not specified)	10-12M	10-12M (basal diet alone)	CsA, purity not specified	110 ppm in diet	34 wk	Rats that were killed both during the course of the treatment period and at the end of the treatment period were necropsied. Thymus gland, mesenteric lymph nodes, intestinal lymphoid plaques, spleen, lungs, kidneys, and liver were examined histologically. **Intestinal Tract:** No tumors were detected in CsA-treated rats or in untreated controls. In rats that received CsA, however, "atypical epithelial proliferations of the intestinal mucosa associated with hyperplasia of gut-associated lymphoid structures" were detected. IARC noted the small number of rats used.	Perera et al. (1986; cited by IARC, 1990)

Table 6-2. Cell Proliferation Induced By Cyclosporin A (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Subcutaneous Injection*							
Sprague- Dawley rat (age not specified)	M (number not specified)	M (10% ethanol in sesame oil alone)	CsA, purity not specified	100 mg/kg bw/day (83 mol/kg bw/day)	4, 6, 8, or 10 days	One day after the last injection and 1 hour before being killed, rats were injected with 250 μCi of methyl ^{3}H-thymidine. The amount of radioactivity incorporated in DNA was measured in kidneys. Student's *t*-test was used for statistical analysis. **Kidneys:** Positive (for proliferative activity as indicated by labeling index and observation of cellular proliferation) *Labeling Index*: [^{3}H]Thymidine incorporation was measured on days 4 and 8. In the outer cortex, the mean labeling index was significantly increased in CsA-treated rats on day 8, but not on day 4, as compared to controls (60.4 ± 11.1 vs. 20.3 ± 2.1 for controls; $p < 0.01$). In the inner cortex, the mean labeling index was significantly increased in CsA-treated rats on days 4 and 8 as compared to controls (day 4: 40.4 ± 9.1 vs. 17.2 ± 2.7 in controls, $p < 0.05$; day 8: 82.7 ± 14.9 vs. 11.1 ± 0.8 in controls, $p < 0.02$). In the inner medulla-papilla, the mean labeling index was significantly increased in CsA-treated rats on day 8, but not on day 4, as compared to controls (89.4 ± 15.5 vs. 7.8 ± 2.1 in controls; $p < 0.01$). *Morphology*: Focal areas of tubular atrophy and interstitial fibrosis with an increase in the overall number of interstitial cells were detected in the outer, but not inner, cortex. In the medulla, a widening of the interstitium and an increase in the number of cells in the interstitium were detected in the inner stripe of the outer medulla.	Jackson et al. (1987)

Table 6-2. Cell Proliferation Induced By Cyclosporin A (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Sprague- Dawley rat (age not specified)	6M	6M (vehicle alone)	CsA, purity not specified	100 mg/kg bw/day (83 μmol/kg bw/day) in 10% ethanol in sesame seed oil	8 days	One day after the last injection and 1 hour before being killed, rats were injected with 250 μCi of methyl [^{3}H]thymidine. The amount of radioactivity incorporated in DNA was measured in kidneys, spleen, liver, and heart. The statistical test used to compare labeling indices was not specified. **Kidneys:** Positive (for proliferative activity as indicated by labeling index) [^{3}H]Thymidine incorporation into renal DNA was significantly increased in CsA-treated rats (~ 12000 dpm/mg DNA vs. ~ 5000 dpm/mg DNA in controls). **Other Organs:** Negative The labeling indices in the spleen, liver, and heart of CsA-treated rats were increased as compared to controls, but the increase was not statistically significant.	Humes and Jackson (1988)
adult Sprague-Dawley rat	6M per treatment period	6M per treatment period (vehicle alone)	CsA, purity not specified	15 mg/kg/day (12 μmol/kg/day) in olive oil	5, 10, 26, or 35 days	All rats were kept on a low-salt diet 1 week prior to treatment. It was not clear if this diet was continued during the treatment period. Only the kidneys were examined. Comparisons between control and CsA-treated rats were made using Student's *t*-test or Mann-Whitney test, "as appropriate." **Kidneys:** Positive (for proliferative activity, as indicated by PCNA and presence of interstitial fibrosis) CsA "induced an early increase in proliferating cell nuclear antigen (PCNA) at day 5 (3.2 ± 0.8 vs. 0.8 ± 0.2 in controls [$p < 0.03$]) which was progressive (5.7 ± 1.3 at day 10) and peaked at day 35 (7.9 ± 1.4 vs. 0.5 ± 0.1 in controls [$p < 0.001$]). There was also an early, sustained, and significant interstitial fibrosis in CsA-treated rats, whereas vehicle animals showed none of these abnormalities."	Burdmann et al. (1994)

Table 6-2. Cell Proliferation Induced By Cyclosporin A (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Dogs - *Oral Administration*							
2-yr-old beagle dog	12M	2M (olive oil alone)	CsA, purity not specified	*wk 1–18*: 30 mg/kg/day (25 µmol/kg/day) *wk 19–22*: 30–55 mg/kg/day (25–46 µmol/kg/day) *wk 23–26*: 30 mg/kg/day (25 µmol/kg/day) administered in gelatin capsules placed in soft dog food.	26 wk	Dogs were observed for skin lesions every other week. Biopsies of skin lesions and unaffected skin were performed at week 18 and 26. Biopsies of remnants of skin lesions were performed 4 weeks following cessation of CsA treatment. **Skin:** Skin lesions were first detected in cyclosporin-treated dogs between 3 and 6 weeks. By week 7, irregular, oval, sessile, unpigmented, firm masses of the skin were detected in all CsA-treated dogs. The lesions were limited to haired skin around the eyes and to ears, lips, face, neck, chest, abdomen, genital area, and legs. The incidence and severity of lesions did not correlate with blood levels of CsA. The lesions regressed spontaneously when CsA treatment was terminated. By 8 weeks post-treatment, lesions were not "clinically apparent." The incidence of skin lesions in control dogs was not mentioned. **Oral Cavity:** "Gingival overgrowth" was detected in 5/12 CsA-treated dogs, but no oral papilloma-like lesions were observed. Control dogs were not mentioned. **Other:** Other cutaneous effects of CsA treatment included hyperkeratosis of footpads, increased growth of hair and nails, and hyperkeratinization of external surface of the prepuce of penis, which became covered by a firm dry cornified layer.	Seibel et al. (1989)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Diethylnitrosamine (DEN) and CsA							
6- to 7-wk-old F344 rats	20M	20M (basal diet alone)	CsA, purity not specified	150 ppm in diet	22, 28, or 32 wk	All rats were initiated with a single i.p. injection of DEN (250 mg/kg), followed one week later with administration of a choline-deficient diet for 7 weeks, and then followed by administration of CsA for 22, 28, or 32 additional weeks. Controls received similar treatment except they were given basal diet instead of the CsA-diet. **Liver:** When the incidences of hepatocellular carcinoma at each measured time point were combined into a cumulative incidence, this cumulative incidence was significantly greater than the incidence in controls (16/18 vs. 9/20 controls; $p < 0.05$, Fisher's exact test).	Masuhara et al. (1993)
9,10-Dimethyl-1,2-benzanthracene (DMBA) and CsA							
10-wk-old Syrian golden hamsters	effective number: 16M (LD) 15M (HD)	effective number: 15M (no treatment following DMBA application and tumor excision)	CsA, purity not specified, after DMBA cheek-pouch tumor induction	25 or 50 mg/kg/day (21 or 42 µmol/kg/day) i.v.	4 wk	All hamsters (CsA-treated and controls) received 3 applications/wk to the left cheek pouch of 0.3% (w/v) DMBA in acetone for 14 weeks. Hamsters were then held for 3 weeks without treatment. All hamsters developed well-differentiated squamous cell carcinomas in their treated cheek pouches. At the end of the 3-week period, exophytic tumors in the pouch were excised at their base. The 4-week period of CsA treatment was begun immediately after tumor excision. Hamsters were killed at the end of the 4 week CsA-treatment period. "The cheek pouches along with any regrown tumors, and the cervical lymph nodes from both sides were removed, inspected macroscopically, and then prepared for microscopic examination. The lungs, liver, heart, kidneys, adrenals, and spleen were also removed and the specimens having signs of possible metastasis were prepared for microscopic examination. Fisher's exact test was used for statistical analyses. **Cervical Lymph Nodes:** Positive (for metastasis; HD only) There was a significantly increased incidence of lymph node metastasis in HD, but not LD, hamsters (14/15 vs. 6/15 controls; $p < 0.05$). Lymph node metastasis was detected only in the ipsilateral nodes, no metastasis was detected in the contralateral nodes. Treatment with CsA had "no discernible accelerating effect on the growth of metastasized tumor cells in the lymph node."	Yamada et al. (1992)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Irradiation and CsA							
6- to 7-wk-old Swiss Webster (SW) and C57B1/6J mice	18M SW, 12M C57B1/6J (CsA alone) 39M SW, 13M C57B1/6J (350 rad γ-radiation [single whole-body] + CsA 10 days later)	18M SW, 12M C57B1/6J (basal diet alone) 26M SW, 14M C57B1/6J (radiation alone)	CsA, purity not specified, alone or after γ-irradiation	150 ppm in diet	35 wk	Mice were killed at the end of the treatment period. IARC did not specify which tissues were examined. Fisher's exact test was used to analyze tumor incidence. **Lymphatic System:** Negative (with CsA alone) Positive (for lymphoma with previous irradiation) No tumors were detected in mice that were irradiated and maintained on basal diet or in mice that were administered CsA, but were not irradiated. The incidence of lymphoid tumors was significantly increased in both strains of mice that were both irradiated and fed CsA (SW: 18/39 [$p < 0.001$]; C57B1/6J: 7/13 [$p < 0.002$]). No mention was made of tumor incidence in untreated controls.	Hattori et al. (1988; cited by IARC, 1990)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
3-Methylcholanthrene (3-MC) and CsA							
8- to 10-wk-old Sprague-Dawley rats	21M per group	*3MC and vehicle*: 21M per group	CsA, purity not specified	group A: 2.5 mg/kg (2.1 µmol/kg) s.c. daily 2 days prior to 3MC exposure and then once/wk for 2 weeks	16 days	All rats (CsA-treated and control) received a single s.c. injection of 3MC. Groups A and B and their respective controls were administered 5 mg 3MC/rat in the left flank. Group C and its respective controls were administered 1.5 mg 3MC/rat in the left flank. All rats were sacrificed at 18 weeks. It was not specified which tissues were examined.	Bussiere et al. (1991)
				group B: 5 mg/kg (4.2 µmol/kg) s.c. 2 days prior to 3MC exposure and then twice/wk for 10 wk	~ 10 wk	**All Tissues:** Negative Groups A and B did not develop tumors and the tumor incidence in group C was not significantly different from that in appropriate controls.	
				group C: 2.5 mg/kg/day (2.1 µmol/kg/day) s.c. from 5 days to 2 wk following 3MC exposure	2 wk	**Immune Function:** Immune function was assessed by measuring delayed-type hypersensitivity (DTH), natural killer cell (NK) activity, and production of interleukin 2 (IL-2), interferon (IFN), prostaglandin E_2 (PGE_2), and specific IgG antibody. "NK cytotoxicity was enhanced and antibody production was suppressed in rats treated with all doses of CsA tested. IL-2 production was elevated at the two lower doses, but antibody production, DTH reactions and synthesis of IL-2 and IFN activity seen in rats treated with the lower doses of CsA may be due to the increase in IL-2 production, while enhancement of NK activity at higher doses may be due to other mechanisms."	

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and CsA							
"young" Wistar rats	effective number: 5M (CsA alone) 9M (CsA during wk 1 + MNNG in drinking water during wk 3-28) 13M (MNNG in drinking water during wk 3-28 + CsA during wk 15) 12M (MNNG in drinking water during wk 3-28 + CsA during wk 30)	effective number: 10M (no treatment) 12M (MNNG in drinking water during wk 3-28)	CsA, purity not specified, alone or in combination with MNNG	10 mg/kg body weight/day in olive oil (8.3 µmol/kg bw/day) s.c.	1 wk	Rats were killed during week 39. None of the rats that received CsA alone and none of the controls died during the experiment. IARC did not specify which tissues were examined. **All Tissues:** Negative No tumors were detected in rats that received CsA alone or in controls. The tumor incidence in groups treated with CsA + MNNG did not differ significantly from the tumor incidence in the group treated with MNNG alone.	Johnson et al. (1984; cited by IARC, 1990)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
N-Methyl-*N*-nitrosourea (MNU) and CsA							
6- to 7-wk- old Swiss Webster mice	"groups of 28- 41M" (CsA alone) "groups of 28- 41M" (single i.p. injection of MNU [12.5 or 25 mg/kg bw] + CsA 1 wk later)	"groups of 28- 41M" (basal diet alone) "groups of 28- 41M" (MNU alone)	CsA, purity not specified, alone or after MNU	150 ppm in diet	35 wk	It was not specified when the mice were killed or which tissues, besides the thymus gland, were examined. **Lymphatic System:** Negative (with CsA alone) Positive (for thymic lymphoma with previous exposure to MNU) No thymic lymphomas were detected in mice treated with CsA alone or in untreated controls. There was a 4- to 8-fold increase in the incidence of thymic lymphoma in mice treated with MNU + CsA as compared to mice treated with either dose of MNU alone (incidences not given). IARC noted an incomplete reporting of this study.	Shinozuka et al. (1988; cited by IARC, 1990)
Sprague-Dawley rats (age not specified)	10-12M (CsA alone) 10M (single i.p. injection of 25 mg MNU/kg bw + CsA 1 wk later)	10-12M (basal diet alone) 12M (MNU alone)	CsA, purity not specified, alone or after MNU	110 ppm in diet	34 wk	Rats that were killed both during the course of the treatment period and at the end of the treatment period were necropsied. Thymus, mesenteric lymph nodes, intestinal lymphoid plaques, spleen, lungs, kidneys, and liver were examined histologically. IARC did not specify which statistical test was used to analyze tumor incidence. **Intestinal Tract:** Negative (with CsA alone) Positive (for adenocarcinoma with previous MNU-treatment) No tumors were detected in rats that received CsA alone or in untreated controls. In rats that received CsA alone, however, "atypical epithelial proliferations of the intestinal mucosa associated with hyperplasia of gut-associated lymphoid structures" were observed. There was a synergistic increase in the incidence of intestinal adenocarcinoma in rats that received MNU + CsA (6/10 vs. 1/12 MNU controls). IARC noted the small number of rats used.	Perera et al. (1986; cited by IARC, 1990)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Peripheral Blood Mononuclear Cells (PBMC) and CsA							
7- to 9-wk- old SCID mice	10 (PBMC + CsA; sex not specified)	38 (PBMC; sex not specified)	CsA, purity not specified	50 µg/g/day (42 µmol/kg/ day) i.p.	up to 36 wk	PBMC were obtained from 8 healthy human adults undergoing lymphopheresis. Mice were inoculated with 60-100 x 10^6 PBMC lymphocytes. CsA treatment of mice was begun 1 day before PBMC inoculation. Animals were necropsied when they became sick or at the end of 36 weeks. It was not specified which tissues were examined. **All Tissues:** Negative Tumor incidence in CsA-treated mice was *lower* than the incidence in controls (2/10 vs. 31/38 controls; p-value not given).	Veronese et al. (1992)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Tumor-cell Inoculation and CsA							
5- to 6-wk- old C57B1/6J mice	9M (group A) 10M (group B) 11M (group C)	11M (vehicle alone)	CsA, purity not specified	group A: 30 mg/kg bw (25 µmol/kg bw) by gavage on 7, 8, and 9th days post tumor inoculation group B: 15 mg/kg bw (12.5 µmol/kg bw) 30 min before tumor inoculation and 2 times more at 24 h intervals, by gavage group C: 30 mg/kg bw (25 µmol/kg bw) 30 min before tumor inoculation and 2 times more at 24 h intervals, by gavage	3 days	All mice were inoculated with MCA 38 colon tumor cells derived from mice bearing murine colon adenocarcinoma (mouse strain not specified). An 0.1 mL suspension of 1 x 10^5 tumor cells was injected into the portal vein of the C57B1/6J mice. Two, 3, and 4 weeks after inoculation, the abdomen of each mouse was explored for evidence of hepatic metastasis. Mice were killed 4 weeks post tumor inoculation. ANOVA was used to compare mean values between groups. A p-value of < 0.05 was considered significant. **Liver:** *Metastasis*: At 2 weeks post inoculation, the mean number of MCA 38 tumors in the liver and their mean diameter were significantly increased in group B, but not in groups A and C, as compared to controls (number: 3.3 ± 1.7 vs. 1.5 ± 0.9 in controls, $p < 0.01$; diameter: 1.5 ± 0.5 mm vs. 0.7 ± 0.5 mm in controls, $p < 0.01$). At 3 weeks post inoculation, the mean number of MCA 38 tumors in the liver and their mean diameter were significantly increased in groups B and C, but not in group A, as compared to controls (number: 4.3 ± 1.7 [group B; $p < 0.01$] and 5.5 ± 2.0 [group C; $p < 0.01$] vs. 2.8 ± 2.1 in controls; diameter: 4.8 ± 2.0 mm [group B; $p < 0.05$] and 4.4 ± 1.3 mm [group C; $p < 0.001$] vs. 2.0 ± 1.3 mm in controls). At 4 weeks post inoculation, the mean number of MCA 38 tumors in the liver and their mean diameter were significantly increased in group C, but not in groups A and B, as compared to controls (number: 19.6 ± 6.8 vs. 13.2 ± 8.3 in controls; $p < 0.01$; diameter: 13.4 ± 4.8 mm vs. 8.9 ± 4.7 mm in controls, $p < 0.05$).	Yokoyama et al. (1994)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
6-wk-old CDF1 mice	10M (LD) 6M (MD) 2M (HD)	10M (olive oil alone)	CsA, purity not specified	10, 20, or 50 mg/kg/day (8, 17, or 42 µmol/kg/day) in olive oil s.c., beginning 1 day prior to colon-26 tumor inoculation	22 days	Mice underwent laparotomy by an upper median incision. the duodenal loop was exposed, and a 0.5-mL aliquot of colon-26 tumor cells (5×10^3 cells) (from a mouse colon adenocarcinoma cell line) was inoculated into the portal vein through the superior mesenteric vein. Student's *t*-test was used to compare the number of metastases in CsA-treated and control mice. **Liver:** Positive (for increase in the number of hepatic "metastases" [authors' terminology]) All mice (CsA-treated and control) developed hepatic metastases after inoculation with colon-26 tumor cells. The mean number of hepatic metastases was significantly increased in all groups of CsA-treated mice (80.8 ± 54.2 in LD, 114.3 ± 40.4 in MD, and 169.5 ± 2.5 in HD mice vs. 4.3 ± 4.4 in controls [$p < 0.01$]). **Peritoneal Cavity:** Positive (for peritoneal metastasis) Peritoneal metastases were detected in 4/10 LD, 5/6 MD, and 2/2 HD mice (vs. none in controls; p-value not given). The number of peritoneal metastases/mouse was not specified. **Lungs:** Pulmonary metastases were detected in 1/6 MD and 2/2 HD mice (vs. none in controls; p-value not given). The number of pulmonary metastases/mouse was not specified.	Suzaki et al. (1995)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Urethan and CsA							
6- to 7-wk- old Swiss Webster mice	15M (CsA alone) 13M (single i.p. injection of 1 g urethan/kg bw + CsA 1 wk later)	14M (basal diet alone) 13M (urethan alone)	CsA, purity not specified, alone or after urethan	150 ppm in diet	22 wk	Mice were killed at the end of the treatment period. IARC did not specify which tissues were examined, besides lungs, and which statistical test was used to analyze tumor incidence. **Lungs:** Negative There was no significant difference between the number of adenomas detected in the group that received urethan + CsA and in the group that received urethane alone. Incidences were not given). No comparison was made between mice that received CsA alone and untreated controls (incidences not given). IARC noted the small number of animals used and the short duration of the study.	Shinozuka et al. (1988; cited by IARC, 1990)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Multiple Treatments							
6-wk-old CDF1 mice	6M (CsA alone) 6M (5-fluorouracil [15 mg/kg/day, every other day for 22 days] + CsA)	6M (saline alone) 6M (5-fluorouracil alone [15 mg/kg/day, every other day for 22 days])	CsA, purity not specified, alone or after 5-fluorouracil	10 mg/kg/day (8 µmol/kg/day) in olive oil s.c., beginning 1 day prior to colon-26 tumor inoculation	22 days	Mice underwent laparotomy by an upper median incision, the duodenal loop was exposed, and a 0.5-mL aliquot of colon-26 tumor cells (5 x 10^3 cells) (from a mouse colon adenocarcinoma cell line) was inoculated into the portal vein through the superior mesenteric vein. Student's *t*-test was used to compare the number of metastases in cyclosporin-treated and control mice. **Liver:** Positive (for increase in the number of hepatic "metastases" [authors' terminology]) The mean number of hepatic metastatic colonies in 5-fluorouracil-treated mice was significantly lower than the number in controls (5.3 ± 4.4 vs. 23.8 ± 10.2 in controls; $p < 0.01$). The mean number of hepatic metastatic colonies in mice treated with CsA alone (140.5 ± 50.7) was significantly increased as compared to controls. It was not clear, however, whether the comparison was made with saline controls (23.8 ± 10.2 hepatic metastatic colonies) or olive oil controls (4.3 ± 4.4 hepatic metastatic colonies). No p-value was given. The mean number of hepatic metastatic colonies in mice treated with CsA and 5-fluorouracil was not significantly different from the number in mice treated with CsA alone. The incidence of hepatic metastasis was not specified.	Suzaki et al. (1995)

Table 6-3. Mammalian Carcinogenicity of Cyclosporin A in Combination with Other Treatments (Continued)

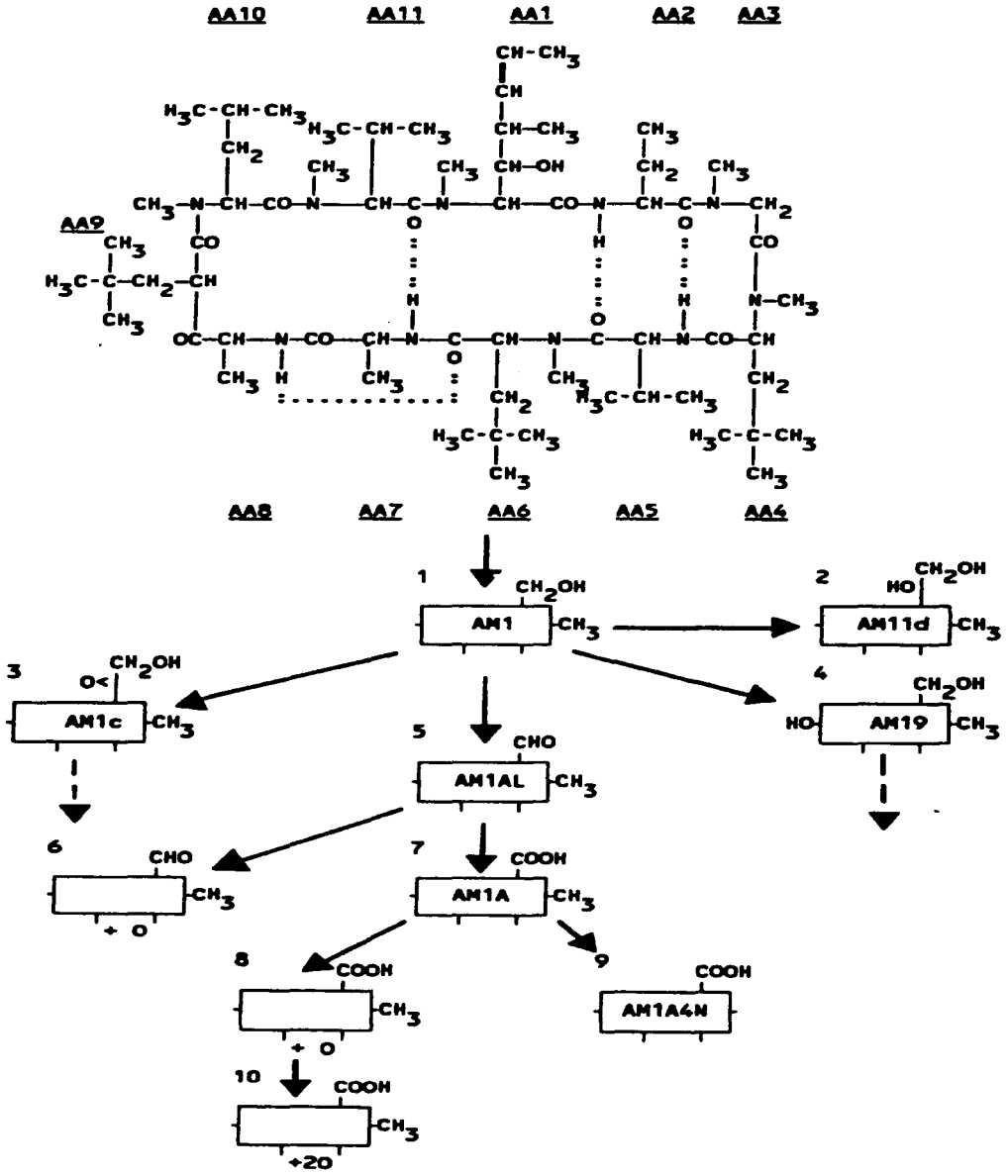
Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
macaques (age and sex not specified)	1) 16 (CsA alone) 2) 9 (concurrent CsA + azathioprine [2 mg/kg bw]) 3) 6 (rabbit antithymocyte globulin [10 mg/kg/day] on post-op days 0-7 + CsA) 4) 13 (concurrent CsA + antithymocyte globulin + azathioprine + methylpredni-solone [dose not given])	5) 11 (total lymphoid radiation alone [100 rads/day for 6–18 days]) 6) 10 (azathioprine + methylpredni-solone) 7) 23 (azathioprine + methylpredni-solone + antithymocyte globulin 8) 9 (azathioprine + antithymocyte globulin + total lymphoid radiation)	CsA, purity not specified, alone or in combination with other immuno-suppressants (see No./Sex column)	25 mg/kg bw/day (21 µmol/kg bw/day) during days 1-14; 17 mg/kg bw (14 µmol/kg bw) every day or every other day from day 15 continuously CsA was injected intra-muscularly, in myglyol (an oil base)	not specified	Prior to the treatment period, all macaques received cardiac or heart-lung allografts. Those surviving the first two post-operative weeks were included in the study. IARC did not specify which tissues were examined. **Lymphatic System:** Positive (for B-cell lymphoma when data from groups 1-5 were pooled) B-cell lymphomas were detected in 2/16 group-1, 4/9 group-2, 1/6 group-3, 2/13 group-4, and 3/11 group-5 macaques. The combined incidence of B-cell lymphoma in groups 1-5 was significantly increased (12/55 vs. none in groups 6, 7, or 8; $p < 0.001$, Fisher's exact test). Significance for individual groups was not specified. Viral particles were detected within the endoplasmic reticulum of plasmacytoid cells in 6/8 tumors from macaques treated with CsA alone or in combination with other immunosuppressive agents. The authors noted that "the incidence of spontaneous hematopoietic neoplasms in nonhuman primates is generally considered to be low, although outbreaks of lymphomas have been reported among macaques."	Bieber et al. (1982; cited by IARC, 1990)

Abbreviations: bw = body weight; LD = low dose; MD = mid dose; HD = high dose; s.c. = subcutaneously; i.p. = intraperitoneally; i.v. = intravenously

Table 6.4 Cell Proliferation Induced by Cyclosporin A in Combination with Other Treatments

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Tumor-cell Inoculation and CsA							
5- to 6-wk-old C57B1/6J mouse	9M (group A) 10M (group B) 11M (group C)	11M (vehicle alone)	CsA, purity not specified	group A: 30 mg/kg bw (25 µmol/kg bw) on 7, 8, and 9th post tumor inoculation days, by gavage group B: 15 mg/kg bw (12.5 µmol/kg bw) 30 min before tumor inoculation and 2 times more at 24-h intervals, by gavage group C: 30 mg/kg bw (25 µmol/kg bw) 30 min before tumor inoculation and 2 times more at 24-h intervals, by gavage	3 days	All mice were inoculated with colon 38 tumor cells (MCA 38) derived from mice (strain not specified) bearing murine colon adenocarcinoma. A 0.1 mL suspension of 1 x 10^5 tumor cells was injected into the portal vein of the C57B1/6J mice. In some mice, bromodeoxyuridine (BrdUrd) was injected intraperitoneally on the 7th post tumor inoculation day. Mice were then killed and their livers were removed. ANOVA was used for statistical analysis. **Liver:** Positive (for proliferative activity in groups B and C, as indicated by labeling index) The hepatic labeling index (BrdUrd-positive cell ratio) was significantly increased at 2 weeks post inoculation in groups B and C, but not in group A, as compared to controls (28.6 ± 8.2% [group B; $p < 0.01$] and 30.1 ± 12.3 % [group C; $p < 0.05$] vs. 10.0 ± 6.1% in controls). About 30% of tumor cells in groups B and C were BrdUrd-positive cells (indicating DNA-synthesizing cell), whereas < 15% of tumor cells in group A and controls were positive.	Yokoyama et al. (1994)
Topical Steroids and CsA							
55-yr-old human	1F	0	CsA, purity not specified	*wk 1–10*: 2.5 mg/kg (2.1 µmol/kg) every 12 h, 3 times/wk *wk 11–12*: 7 mg/kg (5.8 µmol/kg) every 12 h, 3 times/wk	12 wk	The patient had a 15 year history of psoriasis. She had previously been treated with topical steroids, but had never been treated with tars, psoralens, UV light, or systemic medications. **Skin:** At week 12, 14 verrucous papules were detected both within and next to psoriatic plaques as well as on uninvolved skin on extremities and trunk. Histologic examination revealed hyperkeratosis, mild hypergranulosis, and acanthosis, consistent with benign keratoses. Koilocytosis was absent, and pilar structures were not involved. Human papillomavirus DNA was not detected. The authors concluded that "CsA appears to have contributed to the induction of these benign keratoses in our patient, but the mechanism is unknown."	Ross et al. (1992)

Figure 6-1. Metabolic Pathways of CsA via AM1 as Elucidated by Human Microsomal Metabolism



A – Compound (Cyclosporin A)
M – Metabolite
A and M are followed by a sequence of Arabic numbers and letters, indicating position and type of metabolic changes:

x – Hydroxylation of the indicate amino acid
xA1 – Oxidation of the indicated amino acid to an aldehyde
xA - Oxidation of the indicated amino acid to carboxylic acid
xN – *N*-Demethylation of the indicated amino acid
XOX – Formation of an epoxide at the indicated amino acid

Source: Christians and Sewing (1993)

Figure 6-2a. Metabolic Pathways via AM9

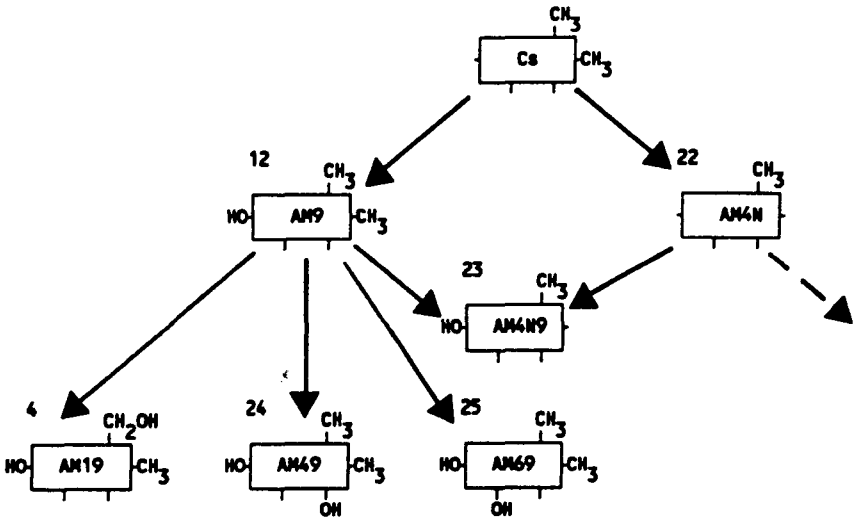
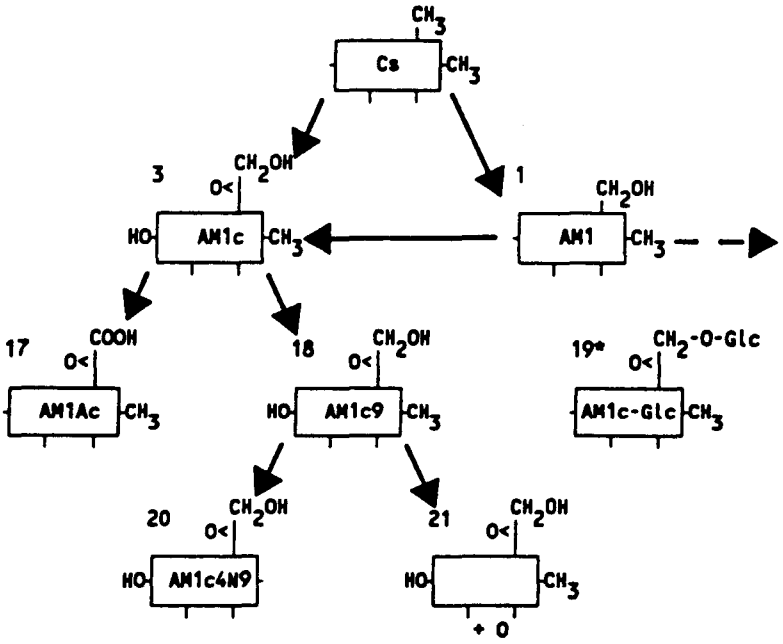


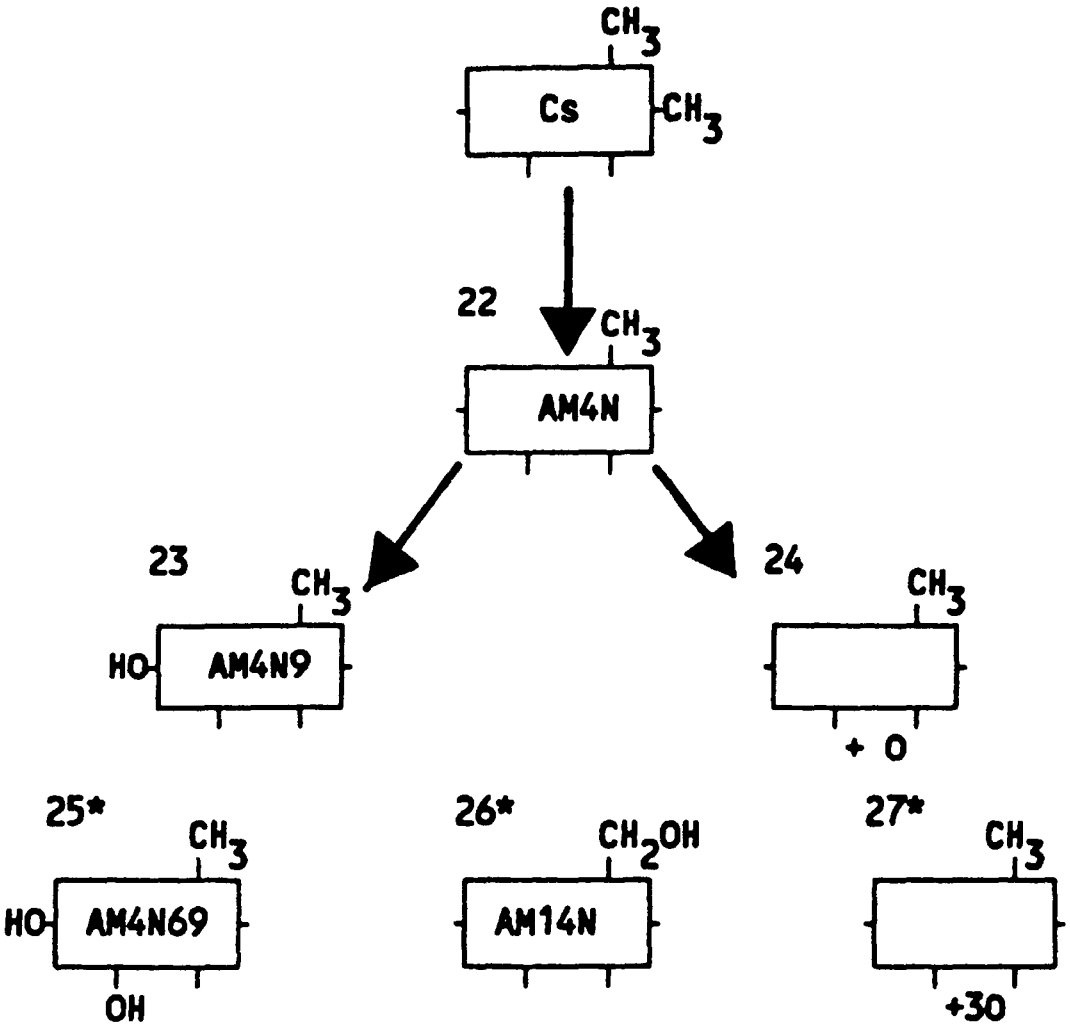
Figure 6-2b. Metabolic Pathways via AM1c



* unidentified position in the metabolic pathway

Source for both figures: Christians and Sewing (1993)

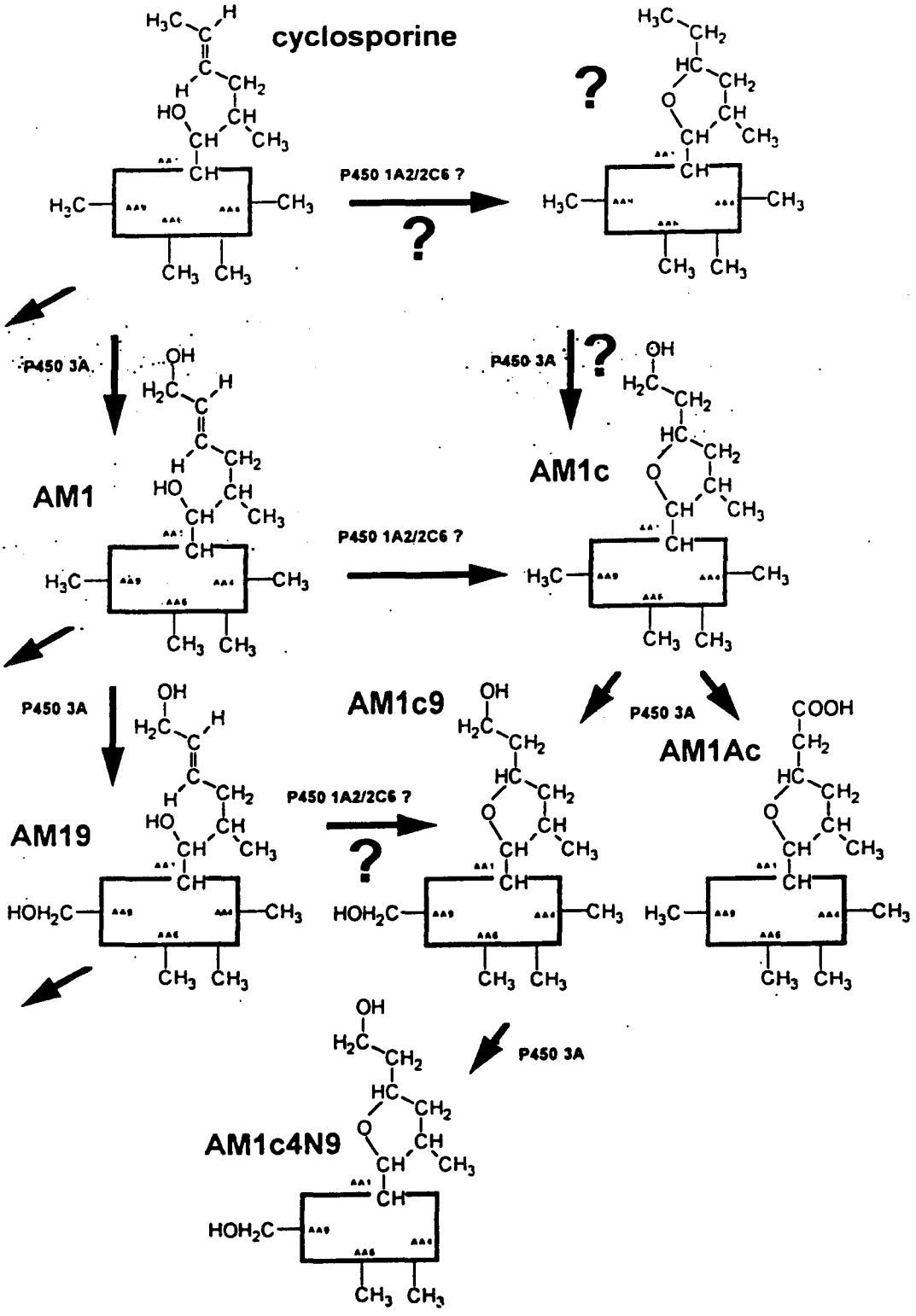
Figure 6-2c. Metabolic Pathways via AM4N



* unidentified position in the metabolic pathway

Source: Christians and Sewing (1993)

Figure 6-2d. Proposed Metabolic Pathways of Cyclized Cyclosporin Metabolites



Source: Christians and Sewing (1995)

7.0 REFERENCES

Akagi, H., A. Reynolds, and M. Hjelm. 1991. Cyclosporin A and its Metabolites: Distribution in Blood and Tissues. J. Int. Med. Res. 19(1):1-18.

Anonymous. 1990. Consensus Document: Hawk's Cay Meeting on the Therapeutic Drug Monitoring of Cyclosporine. Transplant. Proc. 22:1357-1361.

Arico, M., M. Bosco, and A. Galeone. 1987. Manifestazioni Cutanee in Trapiantati Renali. Due Case di Sarcoma di Kaposi. [Cutaneous Manifestations in Renal Transplant Patients. Two Cases of Kaposi's Sarcoma (Ital.)]. G. Ital. Dermatol. Venerol. 122: 637-642. Cited by IARC Vol. 50, 1990.

Armitage, J.M., R.L. Kormos, R.S. Stuart, F.J. Fricker, B.P. Griffith, M. Nalesnik, R.L. Hardesty, and J.S. Dummer. 1991. Posttransplant Lymphoproliferative Disease in Thoracic Organ Patients: Ten Years of Cyclosporine-Based Immunosuppression. J. Heart Lung Transplant. 10:877-887.

Blohmé, I. and O. Larkö. 1992. No Difference in Skin Cancer Incidence With or Without Cyclosporine—A 5-Year Perspective. Transplant. Proc. 24(1):313.

Bos, J.D., and M.M. Meinardi. 1989. Two Distinct Squamous Cell Carcinomas in a Psoriasis Patient Receiving Low-dose Cyclosporine Maintenance Treatment. J. Am. Acad. Dermatol. 21(6):1305-1306.

Bourget, P., H. Fernandez, H. Bismuth, and E. Papiernik. 1990. Transplacental Passage of Cyclosporine After Liver Transplantation. Transplantation 49(3):663-664.

Bourget, P., H. Fernandez, and C. Delouis. 1991. Accumulation of Cyclosporine in the Conceptus During the First Trimester of Pregnancy after Liver Transplantation. Transplantation 51(6):1306-1307.

Bowers, L.D., D.D. Norman, X.X. Yan, D. Scheeler, and K.L. Carlson. 1990. Isolation and Structural Identification of ^{9}Hydroxy-^{9}desmethyl-cyclosporine. Clin. Chem. 36:1875-1879.

Brooks C.A, S.M. Cramer, and T.G. Rosano. 1993. Preparative Chromatographic Purification of Cyclosporine Metabolites. Clin. Chem. 39(3):457-466.

Budavari, S. (Ed.). 1996. Monograph 6682. *o*-Nitroanisole. The Merck Index, Twelfth Edition. Whitehouse Station, NJ: Merck Research Laboratories.

Burdmann, E.A., B. Young, T.F. Andoh, A. Evans, C.E. Alpers, J. Lindsley, R.J. Johnson, W. Couser, and W.M. Bennett. 1994. Mechanisms of Cyclosporine-Induced Interstitial Fibrosis. Transplant. Proc. 26(5):2588-2589.

Busnach, G., G. Civatti, B. Brando, M.L. Broggi, G. Gecchini, G. Ragazzi, A. Canino, and L. Minnetti. 1993. Viral and Neoplastic Changes of the Lower Genital Tract in Women With Renal Allografts. Transplant. Proc. 25(1):1389-1390.

Bussiere, J.L., G.G. Mather, and J.H. Exon. 1991. Effects of Cyclosporine on 3-Methylcholanthrene-Induced Carcinogenesis and Immune Responses in the Rat. Immunobiol. 182:205-215.

Chem Sources. 1996. U.S. suppliers selected from STN International online database files CSCHEM and CSCORP, which are equivalent to the printed directories CHEM SOURCES-USA and CHEM SOURCES-INTERNATIONAL. Directories Publishing Company, Inc.

Christians, U., and K.-F. Sewing. 1993. Cyclosporin Metabolism in Transplant Patients. Pharmacol. Ther. 57:291-345.

Christians, U., and K.-F. Sewing. 1995. Alternative Cyclosporine Metabolic Pathways and Toxicity. Clin. Biochem. 8(6):547-559.

Christians, U., H.J. Schlitt, J.S. Bleck, R. Kownatzki, H.M. Schiebel, G. Maurer, S. Strohmeyer, R. Schottmann, K. Wongeit, R. Pichlmayr, and K.F. Sewing. 1988. Measurement of Cyclosporine and 18 Metabolites in Blood, Bile and Urine by High-Performance Liquid Chromatography (HPLC). Transplant. Proc. 20:609-613.

Christians, U., S. Strohmeyer, R. Kownatzki, H.-M. Schiebel, J. Bleck, J. Greipel, K. Kohlhaw, R. Schottmann, and K.F. Sewing. 1991a. Investigations on the Metabolic Pathways of Cyclosporin. I. Excretion of Cyclosporin and its Metabolites in Human Bile—Isolation of 12 New Cyclosporine Metabolites. Xenobiotica 21:1199-1210.

Christians, U., S. Strohmeyer, R. Kownatzki, H.-M. Schiebel, J. Bleck, K. Kohlhaw, R. Schottmann, and K.F. Sewing. 1991b. Investigations on the Metabolic Pathways of Cyclosporin: II. Elucidation of the Metabolic Pathways *in Vitro* by Human Liver Microsomes. Xenobiotica 21:1185-1198.

Chu, S.-H., M.-K. Lai, C.-C. Huang, and C.-K. Chuang. 1992. Lymphoma in Cyclosporin-Treated Renal Transplant Recipients. Transplant. Proc. 24(4):1594-1595.

Cockburn, I. 1987. Assessment of the Risks of Malignancy and Lymphomas Developing in Patients Using Sandimmune. Transplant. Proc. 19(1):1804-1807.

Copeland K, M. Lukowski, R. Yatscoff, J. Thliveris, and N. Honcharik. 1990a. Pharmacokinetics and Biotransformation of the Cyclosporine Metabolite M-17 in the Rabbit. Drug Metab. Dispos. 18(2):226-230.

Copeland, K.R., R.W. Yatscoff, and R.M. McKenna. 1990b. Immunosuppressive Activity of Cyclosporine Metabolites Compared and Characterized by Mass Spectroscopy and Nuclear Magnetic Resonance. Clin. Chem.36(2): 225-229.

Delbello, M.W., W.H. Dick, C.B. Carter, and F.O. Butler. 1991. Polyclonal B Cell Lymphoma of Renal Transplant Ureter Induced by Cyclosporin: Case Report. J. Urol. 146:1613-1614.

Demetris, A.J., M.A. Nalesnik, H.W. Kunz, T.J. Gill, and H. Shinozuka. 1984. Sequential Analysis of the Development of Lymphoproliferative Disorders in Rats Receiving Cyclosporine. Transplantation 38:239-246.

Ellis, C.N. 1993. Cyclosporin in the Treatment of Psoriasis. In: Weinstein, G.D., and A.B. Gottleib (Eds.). Therapy of Moderate to Severe Psoriasis. Stamford, CT, Haber and Flora, Inc., pp. 106-124. Cited in Sandoz Pharmaceuticals Corporation, personal communication from M.D. Grebenau to R.W. Tennant, May 31, 1996

Fahr, A. 1993. Cyclosporin Clinical Pharmacokinetics. Clin. Pharmacokinet. 24(6):472-495.

Frei, U., U. Bode, H. Repp, R. Schindler, R, Brunkhorst, P. Vogt, J. Hauss, and R. Pichlmayr. 1993. Malignancies Under Cyclosporine After Kidney Transplantation: Analysis of a 10-Year Period. Transplant. Proc. 25(1):1394-1396.

Fry, L. 1992. Psoriasis: Immunopathology and Long-term Treatment with Cyclosporin. J. Autoimmun. 5(Suppl. A):277-283.

Giacalone, B., A. Mastroianni, C. Ferraro, L. D'Auria, A. Amantea, S. Bucher, and A. Pittarello. 1996. B Lymphoma During a Cyclosporine Treatment in an Erythrodermic Psoriatic Patient. Giornale Italiano di Dermatologia e Venereologia 131(1):39-43. Abstract from TOXLINE TOXBIB-92-398229 (Italian).

Gold, L.S., N.B. Manley, T.H. Slone, G.B. Garfinkel, B.N. Ames, L. Rohrbach, B.R. Stern, and K. Chow. 1995. Sixth Plot of the Carcinogenic Potency Database: Results of Animal Bioassays Published in the General Literature 1989 to1990 and by the National Toxicology Program 1990 to 1993. Environ. Health Perspect. 103(Suppl. 8):3-122.

Green, C., and J.L. Hawk. 1993. Cutaneous Malignancy Related to Cyclosporin A Therapy. Clin. Exp. Dermatol. 18:30-31.

Gruber, S.A., K. Gillingham, R.B. Sothern, E. Stephanian, A.J. Matas, and D.L. Dunn. 1994. *De Novo* Cancer in Cyclosporine-Treated and Non-Cyclosporine-Treated Adult Primary Renal Allograft Patients. Clin. Transplant. 8:388-395.

Guerci, A.P., P. Burtin, S. Mattei, and P. Lederlin. 1992. Lymphoproliferative Syndromes after Cardiac Transplantation and Treatment with Cyclosporin. Report of 4 Cases. Ann. Med. Interne 143(3):1559-159. Abstract from TOXLINE TOXBIB-92-398229.

Hartman, N.R., L.A. Trimble, J.C. Vederas, and I. Jardine. 1985. An Acid Metabolite of Cyclosporin. Biochem. Biophys. Res. Commun. 133:964-971.

Hashem, H., R. Venkataramanan, G.J. Burckart, L. Makowka, T.E. Starzl, E. Fu, and L.K. Wong. 1988. Identification of the Aldehydic Metabolites. Transplant. Proc. 20:176-178.

Henricsson, S. A 1990. Sulfate Conjugate of Cyclosporin. Pharmacol. Toxicol. 66(1):53-55.

Henricsson, S., A. Lindholm, and M. Aravoglou. 1990. Cyclosporin Metabolism in Human Liver Microsomes and Its Inhibition by Other Drugs. Pharmacol. Toxicol. 66(1):49-52.

Hiesse, C., J.R. Larue, F. Kriaa, P. Blanchet, J. Bellamy, G. Benoit, and B. Charpentier. 1995. Incidences and Type of Malignancies Occurring After Renal Transplantation in Conventionally and in Cyclosporine-Treated Recipients: Single-Center Analysis of a 20-year period in 1600 Patients. Transplant. Proc. 27(4):2450-2451.

Humes, H.D., and N.M. Jackson. 1988. Cyclosporine Effects on Isolated Membranes, Proximal Tubule Cells, and Interstitium of the Kidney. Transplant. Proc. 20(Suppl. 3):748-758.

IARC (International Agency for Research on Cancer). 1990. Ciclosporin. IARC Monogr. Eval. Carcinog. Risks Hum. 50(Pharmaceutical Drugs):77-114.

Jackson, N.M., C.-H. Hsu, G.E. Visscher, M.A. Venkatachalam, and H.D. Humes. 1987. Alterations in Renal Structure and Function in a Rat Model of Cyclosporine Nephrotoxicity. J. Pharmacol. Exp. Ther. 242(2):749-756.

Johansson, A., S. Henricsson, and E. Moeller. 1990. A Novel Sulfate Conjugate of Cyclosporine Occurring in High Concentrations *in Vivo*. Transplantation 49(3):619-6.

Kehinde, E.O., A. Petermann, J,D. Morgan, Z.A. Butt, P.K. Donnelly, P.S. Veitch, and P.R.F. Bell. 1994. Triple Incidence of De Novo Cancer in Renal Transplant Recipients. Brit. J. Surg. 81:985-986.

Kohler, L.D., F. Kautzky, H.J. Vogt. 1995. Multiple Cutaneous Neoplasms in Cyclosporin Therapy After Kidney Transplantation. Hautarzt 46(9):638-642. Abstract from TOXLINE TOXBIB-92-398229 (German).

Kolars, J.C., P.L. Stetson, B.D. Rush, M.J. Ruwart, P. Schmiedlin-Ren, E.A. Duell, J.J. Voorhees, and P.B. Watkins. 1992. Cyclosporine Metabolism by P450IIIA in Rat Enterocytes—Another Determinant of Oral Bioavailability? Transplantation 53(3):596-602.

Koo, J.Y., J.N. Kadonaga, B.V. Wintroub, and F.I. Lozada-Nur. 1992. The Development of B-cell Lymphoma in a Patient with Psoriasis Treated with Cyclosporine. J. Am. Acad. Dermatol. 26:836-840.

Krupp, P., and C. Monka. 1990. Side-Effect Profile of Cyclosporin A in Patients Treated for Psoriasis. Brit. J. Dermatol. 122(Suppl. 36):47-56.

Lindholm, A. 1991. Therapeutic Monitoring of Cyclosporin—An Update. Eur. J. Clin. Pharmacol. 41(4):273-283.

Lucey, M.R., J.C. Kolars, R.M. Merion, D.A. Campbell, P.B. Watkins, et al. 1990. Cyclosporin Toxicity at Therapeutic Blood Levels and Cytochrome P-450 IIIA, Lancet 335:11-15.

MacDonald, J.S., G.R. Lankas, and R.E. Morrissey. 1994. Toxicokinetic and Mechanistic Considerations in the Interpretation of the Rodent Bioassay. Toxicol. Pathol. 22(2):124-140.

Mahe, B., P. Moreau, S. Le Tortorec, M. Hourmant, J.-L. Harousseau, and N. Milpied. 1994. Autologous Bone Marrow Transplantation for Cyclosporin-related Lymphoma in a Renal Transplant Patient. Bone Marrow Transplant. 14:645-646.

Marcén, R., J. Pascual, P. Serrano, L. Orofino, F.J. Burgos, J.L. Teruel, and J. Ortuño. 1992. Renal Cell Carcinoma of the Native Kidney in a Female Renal Allograft Patient without Acquired Cystic Kidney Disease. Nephron 61:238-239.

Masouyé, I., D. Salomon, and J-H. Saurat. 1993. B-Cell Lymphoma After Cyclosporine for Keratosis Lichenoides Chronica. Arch. Dermatol. 129:914-915.

Masuhara, M., H. Ogasawara, S.L. Katyal, T. Nakamura, and H. Shinozuka. 1993. Cyclosporine Stimulates Hepatocyte Proliferation and Accelerates Development of Hepatocellular Carcinomas in Rats. Carcinogenesis 14(8):1579-1584.

Maurer, G., and M. Lemaire. 1986. Biotransformation and Distribution in Blood of Cyclosporin and Its Metabolites. Transplant. Proc. 18:25-34.

Maurer, G., H.R. Loosli, E. Schreier, and B. Keller. 1984. Disposition of Cyclosporin in Several Animal Species and Man. I. Structural Elucidation of Its Metabolites. Drug Metab. Dispos. 12:120-126.

Meier, G.P., S.B. Park, G.C. Yee, D.J. Gmur, and J. Carlin. 1990. Isolation and Identification of a Novel Human Metabolite of Cyclosporin A: Dihydro-CsA M17. Drug Metab. Disp. 18:68-71.

Melosky, B., M. Karim, A. Chui, M. McBride, E.C. Cameron, C.K. Yeung, D. Landsberg, C. Shackleton, and P.A. Keown. 1992. Lymphoproliferative Disorders After Renal Transplantation in Patients Receiving Triple or Quadruple Immunosuppression. J. Am. Soc. Nephrol. 2:S290-S294.

Motagnino, G., P.L. Bencini, A. Tarantino, R. Caputo, and C. Ponticelli. 1994. Clinical Features and Course of Kaposi's Sarcoma in Kidney Transplant Patients: Report of 13 Cases. Am. J. Nephrol. 14:121-126.

Moody, A.R., S.R. Wilson, and P.D. Greig. 1992. Non-Hodgkin Lymphoma in the Porta Hepatis after Orthotopic Liver Transplantation: Sonographic Findings. Radiology 182(3):867-870.

Nakamoto, T., M. Igawa, S. Mitani, M. Ueda, A. Usui, and T. Usui. 1994. Metastatic Renal Cell Carcinoma Arising in a Native Kidney of a Renal Transplant Recipient. J. Urol. 152:943-945.

Nakamura, M., S. Imaoka, K. Miura, E. Tanaka, S. Misawa, and Y. Funae. 1994. Induction of Cytochrome P450 Isozymes in Rat Renal Microsomes by Cyclosporin A. Biochem. Pharmacol. 48(9):1743-1746.

Nandakumaran, M., and A.S. Eldeen. 1990. Transfer of Cyclosporin in the Perfused Human Placenta. Dev. Pharmacol. Ther. 15:101-115.

NIOSH (National Institute for Occupational Safety and Health). 1976. National Occupational Hazard Survey (1972-74). Cincinnati, OH: Department of Health, Education, and Welfare.

NIOSH (National Institute for Occupational Safety and Health). 1984. National Occupational Exposure Survey (1980-83). Cincinnati, OH: Department of Health and Human Services.

Penn, I. 1983. Lymphomas Complicating Organ Transplantation. Transplant. Proc. 15(4):2790-2797.

Penn, I. 1994. Posttransplant Malignancies in Pediatric Organ Transplant Recipients. Transplant. Proc. 26(5):2763-2765.

Pham-Huy, C., N. Sadeg, T. Becue, C. Martin, G. Mahuzier, J.M. Warnet, M. Hamon, and J.R. Claude. 1995. *In Vitro* Metabolism of Cyclosporin A with Rabbit Renal or Hepatic Microsomes: Analysis by HPLC-FPIA and HPLC-MS. Arch. Toxicol.69(5):346-349.

Pichard, L., I. Fabre, M. Daujat, J. Domergue, H. Joyeux, and P. Maurel. 1992. Effect of Corticosteroids on the Expression of Cytochromes P450 and on Cyclosporin A Oxidase Activity in Primary Cultures of Human Hepatocytes. Mol. Pharmacol. 41(6):1047-1055.

Rappaport, D.C., G.L. Weisbrod, and S.J. Herman. 1989. Cyclosporine-Induced Lymphoma Following a Unilateral Lung Transplant- The Toronto Transplant Group. Can. Assoc. Radiol. J. 40(2):110-111. Abstract from TOXLINE TOXBIB-92-398229.

Reddi, A.S., G.N. Jyothirmayi, B. DeAngelis, V. Kabaria, O. Frank, and H. Baker. 1991. Vitamin and Micronutrient Concentrations in Cyclosporine-Induced Renal Tumor from Diabetic Rats. Life Sci. 49:1531-1537.

Reents, S. (Ed.). 1996. Cyclosporin monograph. Clinical Pharmacology. Version 1.8, 9th quarterly ed. Gainesville, FL: Gold Standard Multimedia Inc. Available at URL http://www.gsm.com/resources/cponline.

Regev, E., R. Zeltser, and J. Lustmann. 1992. Lip Carcinoma in Renal Allograft Recipient with Long-term Immunosuppressive Therapy. Oral Surg. Oral Med. Oral Pathol. 73:412-414.
Ritters, B., B. Grabensee, and P. Heering. 1994. Malignancy Under Immunosuppressive Therapy Including Cyclosporine. Transplant. Proc. 26(5):2656-2657.

Rosenkranz, H.S., and G.O. Klopman. 1992. A Structural Analysis of the Genotoxic and Carcinogenic Potentials of Cyclosporin A. Mutagenesis 7(2):115-118.

Ross, M., M.M. Goodman, R.J. Barr, and S.Y. Liao. 1992. Multiple Eruptive Benign Keratoses Associated with Cyclosporine Therapy for Psoriasis. J. Am. Acad. Dermatol. 29(1):128-129.

Ryffel, B., M.J. Mihatsch, and G.L. Fisher. 1992. Immunosuppression and Cancer: The Ciclosporin Case. Drug Chem. Toxicol. 15(2):95-115.

Ryffel, B. 1992. The Carcinogenicity of Ciclosporin. Toxicology 73:1-22.

Sandoz Pharmaceuticals Corporation. 1996. Personal Communication Between Raymond Tennant and Mark D. Greenbau.

Schwinghammer, T.L., D. Przepiorka, R. Venkataramanan, C.P. Wang, G.J. Burckart, C.S. Rosenfeld, and R.K. Shadduck. 1991. The Kinetics of Cyclosporine and its Metabolites in Bone Marrow Transplant Patients. Br. J. Clin. Pharmacokin. 32:323-328.

Seibel, W., J.P. Sundberg, L.J. Lesko, J.J. Sauk, L.B. McCleary, and T.M. Hassell. 1989. Cutaneous Papillomatous Hyperplasia in Cyclosporine A Treated Beagles. J. Invest. Dermatol. 93:224-230.

Serino, F., J. Grevel, K.L. Napoli, B.D. Kahan, and H.W. Strobel. 1993. Generation of Oxygen Radicals During the Metabolism of Cyclosporine A: A Cause-Effect Relationship with Metabolism Inhibition. Mol. Cell. Biochem. 122:101-112.

Serino, F., J. Grevel, K.L. Napoli, B.D. Kahan, and H.W. Strobel. 1994. Oxygen Radical Formation by the Cytochrome P450 System as a Cellular Mechanism for Cyclosporine Toxicity. Transplant. Proc. 26(5):2916-2917.

Sigal, N.H., C.S. Lin, and J.J. Siekierka. 1991. Inhibition of Human T-Cell Activation by FK 506, Rapamycin, and Cyclosporine A. Transplant. Proc. 23(2, Suppl. 2):1-5.

Suzaki, N., S. Fuchimoto, H. Iwagaki, and K. Orita. 1995. Effects of Cyclosporine A on Experimental Hepatic Metastases of Mouse Colon-26 Tumour. J. Int. Med. Res. 23:112-118.

Swoboda, A., and V. Fabrizii. 1993. Tonsillar Carcinoma in a Renal Graft Recipient Treated with Cyclosporine A. Clin. Nephrol. 39(5):272-274.

Thomas, D.W., S.V. Seddon, and J.P. Shepherd. 1993. Systemic Immunosuppression and Oral Malignancy: A Report of a Case and Review of the Literature. Br. J. Oral Maxillofac. Surg. 31:391-393.

Tomson, C.R., S. Graham, R.M. Hutchinson, A.J. Flower, M.E. Edmunds, and J. Feehally. 1991. Sezary Cell Lymphoma Following Cyclosporin Immunosuppression for Renal Transplantation. Nephrol. Dial. Transplant. 6:896-898.

Uhing, M. R., A.S. Goldman, and M.P. Goto. 1993. Cyclosporin A-Induced Embryopathy in Embryo Culture is Mediated Through Inhibition of the Arachidonic Acid Pathway. Proc. Soc. Exp. Biol. Med. 202(3):307-314.

Valicenti, J.M., A. Pakula, W.A. Caro, and F.D. Malkinson. 1993. Papilloma Development in Cyclosporine-Treated Patients. Arch. Dermatol. 129:794-795.

Veronese, M.L., A. Veronesi, E. D'Andrea, A. Del Mistro, S. Indraccolo, M.R. Mazza, M. Mion, R. Zamarchi, C. Menin, M. Panozzo, A. Amdori, and L. Chieci-Bianchi. 1992. Lymphoproliferative Disease in Human Peripheral Blood Mononuclear Cell-Injected SCID Mice. I. T Lymphocyte Requirement for B Cell Tumor Generation. J. Exp. Med. 176:1763-1767.

Vickers, A.E.M. 1994. Use of Human Organ Slices to Evaluate the Biotransformation and Drug-Induced Side-Effects of Pharmaceuticals. Cell Biol. Toxicol. 10(5-6)407-414.

Vickers, A.E.M., V. Fischer, S. Connors, R.L. Fisher, J.P. Baldeck, G. Maurer, and K. Brendel. 1992. Cyclosporin A Metabolism in Human Liver, Kidney, and Intestine Slices. Comparison to Rat and Dog Slices and Human Cell Lines. Drug Metab. Dispos. 20(6):802-809.

Vickers, A.E.M., R.L. Fisher, K. Brendel, J. Guertler, R. Dannecker, B. Keller, and V. Fischer. 1995. Sites of Biotransformation for the Cyclosporin Derivative SDZ IMM 125 Using Human Liver and Kidney Slices and Intestine: Comparison with Rat Liver Slices and Cyclosporin A Metabolism. Drug Metab. Dispos. 23(3):327-333.

Vilardell, J., F. Oppenheimer, A.M. Castelao, J.M. Puig, M.C. Cantarell, R. Solá, and R. Lauzurica. 1992. Cancer after Transplantation in Catalonia. Transplant. Proc. 24(1):124.

Walker, P.M.B. 1995. Fick's Law of Diffusion. In: Larousse Dictionary of Science and Technology. Walker, P.M.B. (Ed.). Larousse Kingfisher Chambers, Inc., NY, pp.415-416.

Walker, R.J., J.S. Horvath, D.J. Tiller, and G.G. Duggin. 1989. Malignant Lymphoma in a Renal Transplant Patient on Cyclosporin A Therapy, Aust. N.Z. J. Med. 19:154-155. Cited by IARC Vol. 50, 1990.

Webber, I.R., W.H.M. Peters, and D.J. Back. 1992. Cyclosporin Metabolism by Human Gastrointestinal Mucosal Microsomes. Br. J. Clin. Pharmacol. 33(6):661-664.

Wenger, R.M. 1990. Structures of Cyclosporine and its Metabolites. Transplant. Proc. 22(3):1104-1108.

Wu, C.Y., L.Z. Benet, M.F. Hebert, S.K. Gupta, M. Rowland, D.Y. Gomez, and V.J. Wacher. 1995. Differentiation of Absorption and First-Pass Gut and Hepatic Metabolism in Humans: Studies with Cyclosporine. Clin. Pharmacol. Ther. 58(5):492-497.

Yamada, T., M. Mogi, T. Kage, A. Ueda, J. Nakajima, and T. Chino. 1992. Enhancement by Cyclosporin A of Metastasis from Hamster Cheek Pouch Carcinoma. Arch. Oral Biol. 37(7):593-596.

Yokoyama, I., S. Hayashi, E. Sato, T. Kobayashi, M. Negita, K. Uchida, and H. Takagi. 1994. Enhancement of Tumor Proliferation by Cyclosporine A in Early Phase of Experimental Hepatic Metastasis. Jpn. J. Cancer Res. 85:704-709.

Zanke, B.W., D.N. Rush, J.R. Jeffery, and L.G. Israels. 1989. HTLV-1 T Cell Lymphoma in a Cyclosporine-treated Renal Transplant Patient. Transplantation 48(4):695-697. Abstract from TOXLINE TOXBIB-92-398229.

Zijlmans, J.M., A.W. Van Rijthoven, P.M. Kluin, N.M. Jiwa, B.A. Dijkmans, and J.C. Kluin-Nelemans. 1992. Epstein-Barr Virus-associated Lymphoma in a Patient with Rheumatoid Arthritis Treated with Cyclosporin. New Engl. J. Med. 326(20):1363.

Zwanenburg, T.S., and A. Cordier. 1994. No Cyclosporin-Induced Chromosomal Aberrations in Human Peripheral Blood Lymphocytes *in Vitro*. Mutat. Res. 320(3):217-221.

APPENDIX A

DESCRIPTION OF ONLINE SEARCHES FOR CYCLOSPORIN A

DESCRIPTION OF ONLINE SEARCHES FOR CYCLOSPORIN A (IARC Monograph in Vol. 50, 1990)

The searches described below were conducted between March and October 1996. An exhaustive search of all pertinent databases was not attempted, but the ones chosen were expected to provide citations for most of the relevant recently published literature. No attempt was made in the search strategy to find toxicity information for metabolites and other structural analogs.

Generally, if an IARC monograph or another authoritative review had been published, literature searches were generally restricted from the year before publication to the current year.

Older literature that needed to be examined was identified from the reviews and original articles as they were acquired. Current awareness was maintained by conducting weekly searches of Current Contents on Diskette® Life Sciences 1200 [journals] edition.

In the searches, two variant spellings for the name were used besides cyclosporin—cyclosporine and ciclosporin. In the March 1996 TOXLINE records, the ratio for the occurrence of the names cyclosporine, cyclosporin (the spelling in *The Merck Index*, 11th ed.), and ciclosporin was 30:17:1. In CANCERLIT, the ratio was 68:49:1. When indexing for a particular database was available by Chemical Abstracts Service Registry Number (CASRN), use of the CASRN was preferred.

TOXLINE (on STN International): A total of 7481 records comprised 3131 indexed by the CASRN 59865-13-3 and records containing all variant spellings of the name. A total of 4233 records were for publications after 1989. Of these, 965 records were indexed by the MESH (Medical Subject Heading) term metabolism+all (92 terms); 411 records, by the MESH term neoplasms+all (654 terms); and 930 records included the free text terms (question mark indicates truncation so that all forms of the word are included) carcinog? or mechanis? or pharmacokinetic? or toxicokinetic? or metab?. When the latter three sets were combined, an answer set of 1710 records resulted. Titles were printed for 995 of these records that were indexed by the CASRN. About 260 citations were selected and printed. From the 260 citations, about 190 publications were tentatively selected for acquisition. Later reconsideration of the value of examining the numerous pharmacokinetics studies in patients reduced this number considerably. TOXLINE had another 55 records of interest, which were noted later when comparing the 995 titles with the titles of the selected CANCERLIT records. Dr. John Bucher examined the results of the TOXLINE searches and selected another 24 records for retrieval.

CANCERLIT: Use of the CASRN alone found 1938 records in the database. Combining the CASRN with the MESH term neoplasms+all restricted the retrievals (624) to publications from 1991 and after although the database coverage extends back to 1963. Because CANCERLIT (NCI produced) and TOXLINE are NLM databases in which substantial overlap might be expected, a close comparison was made of the titles of records from both. Only about 200 records were common to these two databases. Of the 93 CANCERLIT records of interest,

however, 55 were in TOXLINE, but most of the 55 records had not been selected in the initial examination of the TOXLINE titles. Some of the other CANCERLIT titles had been selected from other databases. About two-thirds of the 76 records whose citations were printed pertained to the incidence of cancer in groups or individual patients treated with the drug. Case reports as well as cohort studies were cited in the 1990 IARC monograph (about 20 publications). About half of the 76 publications were selected for acquisition.

EMIC/EMICBACK: Nineteen records were indexed by the CASRN.

IRIS: No profile was found in this EPA risk assessment database.

NTIS: Of the 49 records retrieved by use of the term "Cyclosporin?", 3 of interest were selected.

EMBASE: The CASRN was used to index 13,560 records, and 1113 of these records included the terms represented by "mechanis?". The term "carcinogen?" added 281 more records. Of the 1394 records, 1152 represented publications after 1989. This large set was pared to 233 records as follows. The searcher (the technical support contractor P.I.) chose from among the 1152 records those for which mechanis? was in the controlled vocabulary for indexing or appeared in titles and those which included "carcinogen?" (48). The searcher compared the titles of 233 records to the selected TOXLINE records and selected 24 EMBASE records to print the full citations. Of these, 2 records were recognized as having been already acquired. The searcher also looked at another set of titles from 185 records that included the term "review?", printed citations of 6 of the 185 records, and decided 5 were of interest.

TOXLIT: Of the 5135 records indexed by the CASRN, 2147 were published after 1990. The searcher printed the titles of 362 records for studies published since 1994, selected 18 unique records, printed their full citations, and chose 17 for library acquisition. In another approach to limit the retrievals, records were eliminated that included the terms treat? or therap? or preparation? or drug delivery. The result was a set of 2686 records. Requiring that the free text terms used in the TOXLINE search be present in the records reduced the set to 843. Of these, 432 records have been published since 1989. From the 432 titles, the searcher selected 80 and printed full citations. After identification of duplicates and elimination of foreign-language reviews, 64 were selected for library retrieval (20 on metabolism, 15 on pharmacokinetics, 4 on structure-activity relationships, 19 on other mechanistic considerations, and 7 on topics possibly related to carcinogenesis mechanisms).

Occupational Safety and Health (NIOSHTIC): Among 14 records retrieved by use of only the name, 3 new reviews were identified.

In September 1996, the contractor performed searches for updating sections 1 and 2, which had been last updated in 1994 with regulatory information from print sources and REGMAT (May 1993 version). REGMAT had broad coverage of EPA regulations, but it is no longer available. Databases searched in 1996 included CSCHEM and CSCORP for U.S. suppliers (databases produced by Chem Sources); HSDB; the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System) and ISHOW (for physical-

chemical properties); Chemical Abstracts Service's (CAS) File CHEMLIST for TSCA and SARA updates in 1996; and CAS's CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information.

In further attempts to identify pertinent FDA regulations and the current usage status (approved or investigational), another series of searches in September 1996 was performed in pharmaceuticals and other regulatory databases. The databases included the following:

- 21 CFR (via Internet access)
- Clinical Pharmacology (drug monographs available on the Internet from Gold Standard Media Inc.)
- Derwent Drug File (DIALOG File 376 for nonsubscribers) (covers 1964-1982)
- Diogenes (DIALOG File 158) (covers 1976-1996; file includes FDA regulatory information from news stories and unpublished documents, including listings of approved products, documentation of approval process for specific products, recall, and regulatory action documentation)
- Drug Information Fulltext (DIALOG File 229) (current, updated quarterly; includes information on at least 1000 commercially available drugs and 57 investigational injectable drugs)
- Federal Register (DIALOG File 669) (covers 1988-1996) (full text)
- Federal Register Abstracts (DIALOG File 136) (covers 1977-1993)
- International Pharmaceutical Abstracts (DIALOG File 74) (covers 1970-1996, all phases of drug development including laws and state regulations)
- NCI/PDQ. National Cancer Institute's menu-driven online database available from the National Library of Medicine and via the Internet. File contains state-of-the-art cancer treatment protocols and clinical trials. 1996

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Cnromosomal aberrations, human lymphocytes in vivo

Test Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test Code	Definition
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test Code	Definition
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange

APPENDIX C

CASE REPORTS ANNOTATED BIBLIOGRAPHY

CASE REPORTS ANNOTATED BIBLIOGRAPHY

Arico, M., M. Bosco, and A. Galeone. Manifestazioni Cutanee in Trapiantati Renali. Due Case di Sarcoma di Kaposi. [Cutaneous Manifestations in Renal Transplant Patients. Two Cases of Kaposi's Sarcoma (Ital.)]. G. Ital. Dermatol. Venerol. 122: 637-642, 1987; cited in IARC (1990), vol. 50

Two cases of Kaposi's sarcoma were reported in two Italian kidney transplant recipients.

Bencini, P.L., L. Marchesi, T. Cainelli, and C. Crosti. Kaposi's Sarcoma in Kidney Transplant Recipients Treated with Cyclosporin. Br. J. Dermatol. 118:709-714, 1988; cited in IARC (1990), vol. 50

This study followed the clinical course and histological features of Kaposi's sarcoma in 3 renal transplant recipients treated with cyclosporin in combination with other immunosuppressive agents. Lesions completely healed in all 3 patients within 4 months after discontinuation of the immunosuppressive therapy.

Bencini, P.L., G. Montagnino, C. Crosti, and F. Sula. Squamous-Cell Epithelioma and Cyclosporine Treatment. Br. J. Dermatol. 113:373-374, 1985; cited in IARC (1990), vol. 50

The authors question the role of cyclosporin in the development of squamous-cell carcinoma in 1/67 kidney transplant recipients treated with the drug from 1 to 17 months at 15 mg/kg/day (route not specified) for the first 2 weeks, and tapered to 7 mg/kg/day 97 days post-transplantation. The 62-year-old patient had a history of psoriasis and had undergone multiple phototherapy sessions over a 12-year period. The authors suggested that squamous-cell carcinoma developed as a result of pre-existing factors (old age and prolonged UV exposure). See also Bencini et al. (1986).

Bencini, P.L., G. Montagnino, A. DeVecchi, C. Crosti, and A. Tarantino. Cutaneous Lesions in 67 Cyclosporin-Treated Renal Transplant Recipients. Dermatologica 172:24-30, 1986; cited in IARC (1990), vol. 50

This study followed 67 renal transplant patients treated with cyclosporin and methylprednisolone over a period of 1-17 months (mean = 3.2 months) with a maximum follow-up period of 1.5 years. A squamous epithelioma was reported in one patient and skin nodules thought to be a lymphoma were reported for another. See also Bencini et al. (1985).

Beveridge, T., P. Krupp, and C. McKibbin. Lymphomas and Lymphoproliferative Lesions Developing under Cyclosporin Therapy. Lancet I:788, 1984; cited in IARC (1990), vol. 50

A case report of an organ transplant recipient receiving only cyclosporin A, without azathioprine or cytotoxic agents, and developing lymphomas in the gastrointestinal tract.

Bloom, R.E., J.K. Brennan, J.L. Sullivan, R.S.K. Chiganti, R. Dinsmore, and R. O'Reilly. Lymphoma of Host Origin in a Marrow Transplant Recipient in Remission of Acute Myeloid Leukemia and Receiving Cyclosporin A. Am. J. Hematol. 18:73-83, 1985; cited in IARC (1990), vol. 50

A 21-year old woman developed a poorly differentiated lymphocytic lymphoma 6 months after full body radiation, bone marrow transplantation for acute myeloid leukemia in remission, and treatment with cyclosporin A. Post-marrow transplant lymphomas are rare, generally of donor cell origin, and usually contain Epstein-Barr Viral (EBV) DNA. The reported lymphoma was of host origin, yet did not contain EBV DNA. Cyclosporin was discontinued approximately 132 days post-transplantation after the patient developed a nonproductive cough, pruritic skin rash, and became afebrile. The patient, placed on antibiotics and chemotherapy, died of paradoxical cerebral emboli 4 days after initiation of chemotherapy. The autopsy revealed little evidence of tumor.

Bos, J.D., and M.M. Meinardi. Two Distinct Squamous Cell Carcinomas in a Psoriasis Patient Receiving Low-dose Cyclosporine Maintenance Treatment. J. Am. Acad. Dermatol. 21(6):1305-1306, 1989

Squamous cell carcinoma was detected in a 48-year-old woman treated with cyclosporin for psoriasis. The patient was initially treated for 4 months with 5 mg/kg/day as part of a double-blind, placebo-controlled study. Maintenance treatment (4.65 mg/kg/day) was continued for an additional 6 months before the detection of a well-differentiated squamous cell carcinoma of the skin which was completely excised. Cyclosporin treatment was continued for another 18 months. One year following the detection of the first squamous cell carcinoma, another squamous cell carcinoma of the skin, minimally infiltrating, was detected. The authors noted that the patient's skin had been previously exposed to UVB, PUVA, and perhaps arsenic compounds.

Castro, C.J., P. Klimo, and A. Worth. Unifocal Aggressive Lymphoma in the Gastrointestinal Tract in a Renal Transplant Patient Treated with Cyclosporin A and Prednisone. Cancer 55:1665-1667, 1985; cited in IARC (1990), vol. 50

This case report described a renal transplant recipient maintained on cyclosporin A (20 mg/kg/day) and prednisone (0.4 mg/kg/day). Four months later, laparotomy revealed multiple lesions present in the gastrointestinal tract diagnosed as an aggressive and extensive form of lymphoma. The authors pointed out the short latency between the initiation of cyclosporin therapy and the clinical manifestation of the lymphoma.

Chu, S.-H., M.-K. Lai, C.-C. Huang, and C.-K. Chuang. Lymphoma in Cyclosporin-Treated Renal Transplant Recipients. Transplant. Proc. 24(4):1594-1595, 1992

In 205 cases of renal transplant recipients (154 received cyclosporin, 51 received azathioprine), 3 cyclosporin-treated patients (1.9%) developed lymphoma, whereas none of the azathioprine-

treated patients did. B-cell monoclonal malignant lymphoma was detected in the stomach of a 28-year-old man who had received a kidney transplant 2 years before and was treated with cyclosporin and prednisolone. Epstein-Barr virus-deoxyribonucleic acid was detected in the tumor tissue. A monoclonal malignant lymphoma was detected in the paranasal sinus of a woman who had received a kidney transplant 3 years before and was treated with cyclosporin and prednisolone. Immunoblastic malignant lymphoma was detected in the gums of a man who had received a kidney transplant 2 years before and was treated with cyclosporin and prednisolone.

Civati, G., G. Busnach, B. Brando, M.L. Broggi, C. Brunati, G.P. Casadei, and L. Minetti. Occurrence of Kaposi's Sarcoma in Renal Transplant Recipients with Low Doses of Cyclosporine. Transplant. Proc. 20:924-928, 1988; cited in IARC (1990), vol. 50

Three percent of all *de novo* neoplasms noted in solid organ transplant recipients are Kaposi's sarcoma (KS). This figure represents a 50-fold increase in the general population incidence (0.06%). The authors noted a decreased latency (5-10 months) for the appearance of KS and an increased incidence (2.6%) in 4/150 cyclosporin-treated kidney graft recipients as compared to 0/100 patients treated with azathioprine, steroids, and antilymphocyte globulin. Patients received cyclosporin A and 6-mercaptopurine. KS stabilized, or almost healed in 3/4 patients after cyclosporin was reduced or removed from their therapy. The fourth patient died of acute respiratory failure within 3 weeks of KS diagnosis, although no toxic side effects were noted. The authors were of the opinion that such malignancies were a complication of immunosuppression *per se,* rather than specific to cyclosporin treatment, and suggested that this drug should be contraindicated in KS or patients so disposed.

Cockburn, I.T.R., and P. Krupp. The Risk of Neoplasms in Patients Treated with Cyclosporine A. J. Autoimmunol. 2:723-731, 1989; cited in IARC, vol. 50

The occurrence of 186 neoplasms, primarily lymphomas of the gastrointestinal tract and leukemia (55 cases), and Kaposi's sarcoma (26 cases) in organ transplant recipients on cyclosporin monotherapy were described and reported to the drug manufacturer. The mean latency from time of treatment to neoplasm diagnosis was 10 months.

Delbello, M.W., W.H. Dick, C.B. Carter, and F.O. Butler. Polyclonal B Cell Lymphoma of Renal Transplant Ureter Induced by Cyclosporin: Case Report. J. Urol. 146:1613-1614, 1991

Polyclonal B cell lymphoma was detected in a 51-year-old man who had received a kidney transplant 3 months before and was treated with prednisone and cyclosporin. After transplantation, prednisone was started at 2 mg/kg and tapered to 1 mg/kg. Cyclosporin was initially administered intravenously (dose not specified) and then changed to 14 mg/kg (12 μmol/kg) once per day orally. Upon discharge from the hospital, the dose of cyclosporin had been decreased to 7.5 mg/kg (6 μmol/kg) and this dose was continued until the lymphoma was detected.

Ellis, C.N. Cyclosporin in the Treatment of Psoriasis. In: Weinstein, G.D., and A.B. Gottleib (Eds.). Therapy of Moderate to Severe Psoriasis. Stamford, CT, Haber and Flora, Inc.:106-124, 1993; cited in Sandoz Pharmaceuticals Corporation, personal communication from M.D. Grebenau to R.W. Tennant, May 31, 1996

Original article could not be located. "The risk of developing lymphoma or other cancers as a direct result of treating psoriasis with cyclosporin is negligible, and may be related to the degree of immunosuppression induced by the treatment regimen."

Fry, L. Psoriasis: Immunopathology and Long-term Treatment with Cyclosporin. J. Autoimmun. 5 (Suppl. A):277-283, 1992

A report on 58 patients treated for psoriasis with cyclosporin. Thirty patients received short-term treatment (up to 12 weeks) and 28 patients received long-term treatment. Of those that received long-term treatment, 18 were treated for more than 1, but less than 3 years and 10 were treated for 4.0 to 5.5 years. In all patients, the dose ranged from 1 to 6 mg/kg/day. Three patients developed malignancies (1 had basal and squamous cell cancers of the skin, 1 had squamous cell carcinoma of the skin, and 1 had intraepithelial neoplasia of the cervix; 1 treatment group not specified).

Giacalone, B., A. Mastroianni, C. Ferraro, L. D'Auria, A. Amantea, S. Bucher, and A. Pittarello. B Lymphoma During a Cyclosporine Treatment in an Erythrodermic Psoriatic Patient. G. Ital. Dermatol. Venereol. 131(1):39-43, 1996; from TOXLINE abstract TOXBIB-92-398229 (Italian)

B-cell lymphoma was detected in a psoriasis patient treated for 16 months with CsA.

Gorg, K., C. Gorg, K. Havemann, and H. Lange. Hodgkinsche Erkrankung nach Niertransplantation unter Cyclosporin A [Hodgkin's Disease after Kidney Transplantation and Cyclosporin A (Ger.)]. Klin. Wochensr. 64:663-665, 1986; cited in IARC (1990), vol. 50

A renal transplant recipient treated with cyclosporin A in combination with prednisolone developed Hodgkin's disease of the mediastum 9 months after immunosuppressive therapy. (Details in Dutch/German)

Green, C., and J.L. Hawk. Cutaneous Malignancy Related to Cyclosporin A Therapy. Clin. Exp. Dermatol. 18:30-31, 1993

Five basal cell carcinomas on the face and a squamous cell carcinoma on the neck were detected in a 61-year-old man treated for psoriasis for 6 months with cyclosporin. Previous therapy had included PUVA for ~ 15 years and methotrexate for ~ 5 years.

Guerci, A.P., P. Burtin, S. Mattei, and P. Lederlin. Lymphoproliferative Syndromes after Cardiac Transplantation and Treatment with Cyclosporin. Report of 4 Cases. Ann. Med. Interne 143(3):1559-159, 1992; from TOXLINE abstract TOXBIB-92-398229

Four case reports of cancer in heart transplant recipients treated with triple-drug immunosuppression (CsA, azathioprine, prednisolone) (3 lymphomas, 1 multiple myeloma).

Kohler, L.D., F. Kautzky, H.J. Vogt. Multiple Cutaneous Neoplasms in Cyclosporin Therapy After Kidney Transplant. Hautarzt 46(9):638-642, 1995; from TOXLINE abstract TOXBIB-92-398229 (German)

Cutaneous neoplasms (squamous cell carcinoma, basal cell carcinoma, Bowen's disease, actinic keratosis, sebaceous hyperplasia, a dysplastic naevus, and finally, a nodular malignant melanoma) were detected in a kidney transplant recipient receiving CsA.

Koo, J.Y., J.N. Kadonaga, B.V. Wintroub, and F.I. Lozada-Nur. The Development of B-cell Lymphoma in a Patient with Psoriasis Treated with Cyclosporine. J. Am. Acad. Dermatol. 26:836-840, 1992

B-cell lymphoma was detected in the left maxillary sinus, extending to the orbit, the palate, and the infratemporal fossa, in a 67-year-old man treated for psoriasis with cyclosporin. Cyclosporin was administered at doses of 5 mg/kg/day or less for 8 months. It was discontinued because of progressive nephrotoxicity. Lymphoma was detected 7 months after the treatment was stopped.

Mahe, B., P. Moreau, S. Le Tortorec, M. Hourmant, J.-L. Harousseau, and N. Milpied. Autologous Bone Marrow Transplantation for Cyclosporin-related Lymphoma in a Renal Transplant Patient. Bone Marrow Transplant. 14:645-646, 1994

Eighty-eight months (7.3 years) after receiving a kidney transplant, a 37-year-old woman was diagnosed with abdominal lymphoma. Immunosuppressive therapy consisted of antithymoglobulin for 14 days following transplantation, prednisolone for 28 days following transplantation, and cyclosporin (5 mg/kg/day) from the time of transplantation to the time of detection of lymphoma.

Marcén, R., J. Pascual, P. Serrano, L. Orofino, F.J. Burgos, J.L. Teruel, and J. Ortuño. Renal Cell Carcinoma of the Native Kidney in a Female Renal Allograft Patient without Acquired Cystic Kidney Disease. Nephron 61:238-239, 1992

Renal cell carcinoma was detected in a 37-year-old woman who had been treated with cyclosporin (3 mg/kg/day) and prednisone (10 mg/day) for approximately 4 years. Renal cysts were not detected via histologic examination or computerized tomography, although there was the possibility that cysts were present before the renal transplant.

Masouyé, I., D. Salomon, and J-H. Saurat. B-Cell Lymphoma After Cyclosporine for Keratosis Lichenoides Chron. Arch. Dermatol. 129:914-915, 1993

Four months following the cessation of cyclosporin treatment (5 mg/kg/day for 5 months) for severe keratosis, a 57-year-old man developed B-cell lymphoma. Complete staging revealed abdominal and thoracic lymphadenopathies and bowel infiltration. A serological test was positive for Epstein-Barr virus. Five years prior to cyclosporin treatment, the patient had received etretinate (50 mg/day) for 3 months. The authors noted that before this case, no case of keratosis lichenoides chronica had been complicated by lymphoma.

Maung, R., A. Pinto, A. Robertson, G.L.E. Stuart, J.K. Klassen, and R.B. Hons. Development of Ovarian Carcinoma in a Cyclosporin A Immunosuppressed Patient. Obstet. Gynecol. 66(Suppl.):895-925, 1985; cited in IARC (1990), vol. 50

A kidney transplant patient immunosuppressed by cyclosporin A and prednisone developed an ovarian carcinoma 13 months post-transplant that was fatal 5 weeks after diagnosis. This was the first reported ovarian carcinoma in a cyclosporin- immunosuppressed patient. The literature on malignant transformation in the immunosuppressed patient with emphasis on a gynecologic perspective was reviewed.

Moody, A.R., S.R. Wilson, and P.D. Greig. Non-Hodgkin Lymphoma in the Porta Hepatis after Orthotopic Liver Transplantation: Sonographic Findings. Radiology 182(3):867-870, 1992

Non-Hodgkin lymphoma was detected in the porta hepatis of 3/196 (1.5%) patients who had received liver transplants between January 1985 and July 1991 at Toronto General Hospital. Immunosuppressive therapy consisted of induction therapy with Minnesota antilymphoblast globulin and steroids for 7–14 days followed by cyclosporin and prednisolone (doses and durations of administration not given). The 3 patients were men who ranged in age from 38–67 years (mean, 53) at the time of transplantation. The lymphomas were detected in the 3 patients 4, 6, and 9 months after transplantation.

Nakamoto, T., M. Igawa, S. Mitani, M. Ueda, A. Usui, and T. Usui. Metastatic Renal Cell Carcinoma Arising in a Native Kidney of a Renal Transplant Recipient. J. Urol. 152:943-945, 1994

Metastatic renal cell carcinoma was detected in a 39-year-old man who had received a kidney transplant at age 32. Cyclosporin (60 mg; 50 μmol), mizoribine (150 mg), azathioprine (50 mg), and prednisolone (10 mg) were administered daily to maintain immunosuppression (duration not specified). In 1992, the patient complained of paraplegia of the leg and was subsequently found to have a bone tumor in a lumbar vertebra. Pathological examination of this tumor revealed it to be a metastatic adenocarcinoma and a mass was detected in the right native kidney. The bone and kidney tumors were removed, cyclosporin was discontinued, and interferon-α was given.

One year after surgery, the bone tumor had completely resolved. At the patient's request, the kidney was not biopsied.

Penn, I., and M.E. Brunson. Cancers after Cyclosporine Therapy. Transplant. Proc. 20(Suppl.):885-892, 1988; cited in IARC (1990), vol. 50

The most recent registry of organ transplant patients that developed tumors indicates the development of 412 tumors in cyclosporin-treated patients (total not given). The most frequently reported tumor incidence was lymphoma (29%), followed by skin cancer (22%), and Kaposi's sarcoma (11%). IARC noted that compared to the low incidence of Kaposi's sarcoma in the general population, whatever the total number of patients, the "number of cases in this registry is strikingly large."

Penn, I. Posttransplant Malignancies in Pediatric Organ Transplant Recipients. Transplant. Proc. 26(5):2763-2765, 1994

Tumor incidence was evaluated for data collected by the Cincinnati Transplant Tumor Registry from the fall of 1968 to March 1994. Cancers were detected in 7596 recipients (the total number of transplant recipients was not specified). Malignancies occurred in 7213 adults and in 383 children. Forty-eight percent of children who developed malignancies had been treated with cyclosporin. In these cyclosporin-treated children, lymphoma was the most common tumor type. It was not specified whether other immunosuppressive therapies were used in combination with cyclosporin. Cyclosporin use among adult transplant recipients was not investigated.

Rappaport, D.C., G.L. Weisbrod, S.J. Herman. Cyclosporine-Induced Lymphoma Following a Unilateral Lung Transplant- The Toronto Transplant Group. Can. Assoc. Radiol. J. 40(2):110-111, 1989; from TOXLINE abstract TOXBIB-92-398229

One lymphoma was detected in 24 patients who received single or double lung transplants. A 16-year-old man developed multiple, rapidly appearing pulmonary nodules 4 months after transplantation. No mention was made of immunosuppressive therapy for this patient.

Regev, E., R. Zeltser, and J. Lustmann. Lip Carcinoma in Renal Allograft Recipient with Long-term Immunosuppressive Therapy. Oral Surg. Oral Med. Oral Pathol. 73:412-414, 1992

Squamous cell carcinoma of the lower lip was detected in a 42-year-old woman who had received a kidney transplant 5 years earlier. From the time of transplantation, the patient had been treated daily with 200 mg cyclosporin, 50 mg azathioprine, 7.5 mg prednisone, and 50 mg atenolol.

Swoboda, A., and V. Fabrizii. Tonsillar Carcinoma in a Renal Graft Recipient Treated with Cyclosporine A. Clin. Nephrol. 39(5):272-274, 1993

A squamous cell carcinoma was detected on the right tonsil of a 33-year-old man who had received a kidney transplant and was treated with cyclosporin and prednisolone. The tumor was detected after 5 years of cyclosporin treatment. Cyclosporin doses ranged from 140 mg twice per day to 750 mg three times per day.

Thiru, S., R.Y. Calne, and J. Nagington. Lymphoma in Renal Allograft Patients Treated with Cyclosporin-A as One of the Immunosuppressive Agents. Transplant. Proc. 13:359-364, 1981; cited in IARC (1990), vol. 50

The author cites Penn's [Cincinnati] Tumor Transplant Registry with regard to increased tumor incidence among transplant recipients, 75% of which have been lymphoproliferative malignancies such as carcinomas of the lip, skin or cervix. A greater than 10-fold increase in lymphomas (33%) was noted as compared to the general population (3%-4%). Three of 57 patients treated with cyclosporin in combination with two other immunosuppressive drugs (protocol not defined) developed lymphomas within 4 to 11 months after renal transplantation. The author concluded that cyclosporin alone was unlikely to be carcinogenic, but its potent immunosuppressive action served as a cofactor in patients with viral activation and chronic allogenic stimulation.

Thomas, D.W., S.V. Seddon, and J.P. Shepherd. Systemic Immunosuppression and Oral Malignancy: A Report of a Case and Review of the Literature. Br. J. Oral Maxillofac. Surg. 31:391-393, 1993

Squamous cell carcinoma of the lower lip was detected in a 34-year-old man who had received a renal transplant 4 years before. At the time of detection, therapy included cyclosporin (200 mg/day), prednisolone (7.5 mg/day), and atenolol (dose not given). Therapy prior to tumor detection was not specified.

Thompson, J.F., R. Allen, P.J. Morris, and R. Wood. Skin Cancer in Renal Transplant Patients Treated with Cyclosporin. Lancet I:158-159, 1985; cited in IARC (1990), vol. 50

This report is particularly concerned with the development of skin cancer in 2/8 patients who were long-term survivors of renal transplants after conversion from prednisolone/azathioprine to cyclosporin plus prednisolone therapy. Squamous cell carcinomas developed in each patient within 9 months of conversion to cyclosporin, whereas patients on cyclosporin monotherapy had not developed tumors. The authors suggested a possible involvement of UV-light and human papilloma virus infection as co-factors in the risk of squamous cell carcinoma development, and suggested that cyclosporin's potent immunosuppression increases a patient's susceptibility to this viral infection.

Tomson, C.R., S. Graham, R.M. Hutchinson, A.J. Flower, M.E. Edmunds, and J. Feehally. Sezary Cell Lymphoma Following Cyclosporin Immunosuppression for Renal Transplantation. Nephrol. Dial. Transplant. 6:896-898, 1991

Two years after receiving a kidney transplant, a 59-year-old woman was diagnosed with Sezary cell lymphoma. Following transplantation, the patient was treated with cyclosporin (17 mg/kg/day, reduced to 7 mg/kg/day over 6 months) and prednisolone (60 mg/day, reduced to 25 mg over the first weeks and to 10 mg on alternate days by 6 months).

Valicenti, J.M., A. Pakula, W.A. Caro, and F.D. Malkinson. Papilloma Development in Cyclosporine-Treated Patients. Arch. Dermatol. 129:794-795, 1993

Two cases were described of patients who developed multiple papillomatous facial lesions following treatment with cyclosporin. In case 1, a 36-year-old man had received a kidney transplant 1 years before the onset of the lesions. The patient's treatment had included cyclosporin (3.6 mg/kg/day), minoxidil, propranolol, furosemide, nifedipine, and prednisone. In case 2, a 29-year-old man had received a kidney transplant. The latency time for papillomatous lesions was not given. This patient's treatment had included cyclosporin (2.6 mg/kg/day), minoxidil, captopril, furosemide, prednisone, and theophylline. The duration of exposure to cyclosporin was not specified for either case.

Walker, R.J., J.S. Horvath, D.J. Tiller, and G.G. Duggin. Malignant Lymphoma in a Renal Transplant Patient on Cyclosporin A Therapy, Aust. N.Z. J. Med. 19:154-155, 1989; cited in IARC(1990), vol. 50

A case report of an organ transplant recipient receiving only cyclosporin A, without azathioprine or cytotoxic agents, and developing lymphomas in the gastrointestinal tract.

Zanke, B.W., D.N. Rush, J.R. Jeffery, L.G. Israels. HTLV-1 T Cell Lymphoma in a Cyclosporine-treated Renal Transplant Patient. Transplantation 48(4):695-697, 1989; from TOXLINE record TOXBIB-92-398229

No abstract given.

Zijlmans, J.M., A.W. Van Rijthoven, P.M. Kluin, N.M. Jiwa, B.A. Dijkmans, and J.C. Kluin-Nelemans. Epstein-Barr Virus-associated Lymphoma in a Patient with Rheumatoid Arthritis Treated with Cyclosporin. New Engl. J. Med. 326(20):1363, 1992

A Burkitt-type lymphoma was detected in a 58-year-old man who was treated for rheumatoid arthritis with cyclosporin. Treatment with 5 mg/kg body weight was initiated in 1985. Four months later, this dose was decreased to 2.5 mg/kg body weight. In May 1990, the multiple ulcerations and tumors in the small bowel were first detected. Epstein-Barr virus-specific genomic sequences were detected the tumor cell.