GENERAL REMARKS ON THE SUBSTANCES CONSIDERED

This seventy-sixth volume of *IARC Monographs* comprises evaluations of some pharmaceutical agents, including some antiviral agents (aciclovir, zidovudine, zalcitabine and didanosine), some DNA topoisomerase II inhibitors (teniposide, etoposide, mitoxantrone and amsacrine), and others (hydroxyurea, phenolphthalein and vitamin K substances). These agents have not been evaluated by previous IARC working groups.

For several compounds evaluated in this volume, no data were available on carcinogenicity in experimental animals, and this limited the possibility of a comprehensive evaluation of their carcinogenic risks to humans. Although they may not have been tested for carcinogenicity, the Working Group suspected that studies might have been conducted by pharmaceutical companies but never published. The Working Group encourages pharmaceutical companies and cognizant governmental agencies to make available all studies of carcinogenesis that have been, or will be, carried out on pharmaceutical agents in the form of publications or technical reports.

Two chemicals were tested for carcinogenicity in genetically engineered mice which are particularly susceptible to induction of tumours at certain sites through specific mechanisms. The use of such animals for evaluation of the carcinogenicity of chemicals has been reviewed (McGregor *et al.*, 1999). Some, but not all, of these transgenic ('knockout') models can be considered the laboratory counterparts of certain rare human genetic syndromes, and the models may be particularly useful for testing drugs to be administered to individuals with such syndromes. Also, genetically engineered mice that lack one copy of an essential tumour suppressor gene, such as *p53*, model the situation in which a functioning copy of the suppressor gene has been lost in the somatic cells of a normal individual through a stochastic process. The transgenic models may therefore also be useful for studying the mechanism or mode of action of chemicals and, in particular, to test genetic targets of carcinogenicity. Because of the limited database on the responses of particular genetically engineered mice to chemical carcinogens, however, the results of bioassays with these animals must be interpreted with caution.

1. Antiretroviral agents

Although studies on three antiretroviral agents (zidovudine, zalcitabine and didanosine) and on one agent used in adjunctive therapy (hydroxyurea) are reviewed in this volume, numerous drugs are approved for the treatment of human immunodeficiency

virus (HIV) infections. Furthermore, the anti-herpesvirus drug, aciclovir, is often given to patients with HIV infections. The drugs evaluated in this volume are usually combined with other nucleoside reverse transcriptase inhibitors, protease inhibitors and/or non-nucleoside reverse transcriptase inhibitors in order to constitute a regimen likely to suppress HIV replication fully. Combination regimens have been proven repeatedly to be superior to therapy with single drugs for effective and durable treatment of HIV infection (Graham *et al.*, 1996; Hammer, 1996; Englund *et al.*, 1997; Hammer *et al.*, 1997; Phillips *et al.*, 1998). In two large, well-conducted trials, initial therapy with multiple drugs followed by simplification of the regimen in a maintenance phase has been shown to be less effective than continued multi-agent therapy (Havlir *et al*., 1998; Pialoux *et al*., 1998).

The field of HIV therapy continues to evolve rapidly, and guidelines are available (e.g. Centers for Disease Control and Prevention, 1998; Gazzard & Moyle, 1998) on the goals of combination therapy, monitoring and recommended combinations. Recent revisions of treatment and prevention guidelines are available on-line from the HIV AIDS treatment information service (http://www.hivatis.org/atisinfo.html).

Data on the possible carcinogenic effects of antiretroviral agents in humans have been obtained predominantly in studies in developed countries, where HIV-1 is the main agent in HIV infection. In this volume, therefore, the term HIV should be assumed to refer to HIV-1.

The incidence of non-Hodgkin lymphoma is greatly increased in persons with HIV infection (IARC, 1996), and in AIDS patients the rate may be increased at least 100-fold. The vast majority of AIDS-related non-Hodgkin lymphomas are B-cell neoplasms. No substantial variation in risk by mode of transmission has been observed. The association appears to be mediated by HIV-related immune dysregulation and to involve Epstein-Barr virus (human herpesvirus 4) infection, which is widely prevalent (IARC, 1997). The risk for Kaposi sarcoma of persons infected with HIV is much higher than that of uninfected persons (IARC, 1996), and, in developed countries, homosexual and bisexual men have a 5-10-fold greater risk for Kaposi sarcoma than other groups infected with HIV. There is now evidence that this neoplasm results from co-infection with another herpesvirus, human herpesvirus 8 (IARC, 1997). Effective combination therapy in HIV-infected patients can result in improved immunocompetence, which has led to reduced viraemia and decreased incidences of opportunistic malignancies, especially Kaposi sarcoma and to a lesser extent B-cell lymphomas (Rabkin *et al.*, 1999). These beneficial results may, however, mask potential carcinogenic effects of the antiviral agents.

Much of the evidence on the possible carcinogenic effects of antiretroviral agents in humans is derived from trials designed to evaluate the efficacy of these agents in the treatment of patients with immunosuppression of varying severity. Many of the studies are based on data from a time when these drugs were used singly for treatment of HIV infection. In consequence, the survival of infected patients was relatively poor and the opportunity for long-term follow-up to assess cancer risk was limited.

Difficulties were encountered in seeking to evaluate cancer risk from most of these studies, which was not their primary objective, because the length of follow-up was too short, the numbers of participants were too small, and there was insufficient information about the severity of immunodeficiency. In addition, the occurrence of cancer may have been underascertained, and in many of the studies, no formal, appropriate analyses of cancer rates were presented.

Transplacental exposure to single or combinations of antiretroviral drugs, given during pregnancy to prevent maternal transmission of HIV, may constitute a carcinogenic risk for the children. This inference is based on the transplacental carcinogenicity of zidovudine (AZT), the most widely used such agent, in mice. Approximately 7000 HIV-positive women in the USA become pregnant yearly (Mofenson, 1998), and most receive antiretroviral therapy during pregnancy. While the situation is similar in other developed countries, most developing countries are currently unable to offer these therapies because of their cost.

The number and complexity of antiretroviral drug regimens given to HIV-positive pregnant women is increasing, and the potential carcinogenic risk to children exposed *in utero* may become apparent only many years after birth. The remarkable success of the antiretroviral treatments in saving the lives of thousands of children born annually to HIV-infected women supports continuing use of these drugs during pregnancy, but data from experimental studies suggest that long-term surveillance of these children for cancer risk would be appropriate.

2. DNA topoisomerase II inhibitors

DNA topoisomerase II is a nuclear enzyme that transiently cleaves and re-ligates double-stranded DNA, thereby changing its topology. The structure and biological functions of DNA topoisomerase II have been reviewed (Baguley & Ferguson, 1998; Berger, 1998; Burden & Osheroff, 1998; Isaacs *et al*., 1998; Nitiss, 1998). Type II DNA topoisomerases are located at the base of chromosomal scaffolds during mitosis, but can also be detected free in the cell throughout the cell cycle. The topological changes mediated by these enzymes are important for chromosomal replication and condensation, and disruption of their function may prevent accurate chromosomal segregation and increase recombination. DNA topoisomerase II-catalysed cleavage is necessary to separate the multiply intertwined daughter DNA strands after DNA replication and before or during the progressive chromatin condensation that precedes mitosis.

The DNA topoisomerase II inhibitors that are evaluated in this volume of the *Monographs* trap the DNA topoisomerase II reaction intermediate at the point where the enzyme has formed a double-strand break in DNA and is covalently bound to the 5′ ends of the broken strands (Woynarowski *et al*., 1994). This decreases the rate of re-ligation catalysed by the enzyme and has the overall effect of enhancing DNA strand cleavage. Especially during replication, enhanced DNA strand cleavage leads to accumulation of DNA–protein cross-links and double-stranded DNA breaks

(Pommier *et al*., 1985), which may be relevant to translocations. It is important to note that the drugs that target DNA topoisomerase II which are evaluated in this volume are not classical inhibitors in the enzymological sense, inasmuch as they do not inhibit the activity of the free enzyme but rather decrease the rate of re-ligation. Although widely referred to as DNA topoisomerase II inhibitors they are more strictly DNA topoisomerase II poisons (Corbett & Osheroff, 1993).

Antineoplastic treatment of patients with regimens that include DNA topoisomerase II inhibitors has been associated with the occurrence of acute myeloid leukaemia (AML) and other forms of leukaemia. The subtypes of AML are classified according to the French–American–British (FAB) system (Bennett *et al*., 1985; Cheson *et al*., 1990), which is summarized in Table 1. The types associated with exposure to DNA topoisomerase II inhibitors are typically acute myelomonocytic (FAB subtype M4) and monoblastic (FAB subtype M5a) leukaemias, but other AML subtypes, myelodysplastic syndrome, acute lymphoblastic leukaemia and chronic myeloid leukaemia have also been described.

Table 1. French–American–British (FAB) classification of acute myeloid leukaemia subtypes

Subtype	Morphology
M0	Acute undifferentiated leukaemia
M1	Acute undifferentiated myeloid leukaemia
M2	Acute differentiated myeloid leukaemia
M3	Acute promyelocytic leukaemia
M4	Acute myelomonocytic leukaemia
M5a	Acute monoblastic leukaemia
M5b	Acute differentiated monocytic leukaemia
M6	Acute erythroleukaemia
M7	Acute megakaryoblastic leukaemia

The term ‘secondary leukaemia’ indicates that the disease did not develop spontaneously or *de novo* (Larson *et al*., 1996). Leukaemia preceded by myelodysplastic syndrome was recognized as a risk associated with anti-cancer chemotherapy with alkylating agents long before the association of DNA topoisomerase II inhibitors with leukaemias was described. The leukaemias seen after alkylating agent therapy are characterized by antecedent myelodysplastic syndrome, a mean latency of 5–7 years and deletion of chromosomes 5 or 7, while those associated with administration of DNA topoisomerase II inhibitors have a mean latency of 2 years and certain balanced chromosomal translocations, usually but not always involving the *MLL* gene at chromosome 11q23 (Felix *et al.*, 1998). The particular mechanisms of DNA damage by these different agents may underlie differences in the chromosomal aberrations with

which they are associated. Leukaemias associated with translocations involving *MLL* have a propensity to express both myeloid- and lymphoid-associated phenotypic cell-surface markers. The translocation process seems central to leukaemogenesis, and translocations involving *MLL* are associated with progression to leukaemia over a short interval.

The *MLL* gene undergoes rearrangement by fusion with various partner genes in the course of specific chromosomal rearrangements which, with few exceptions, are involved in both therapy-related and *de novo* cases. Although hyperleukocytosis and extramedullary disease occur less often in treatment-related cases, the clinical features, FAB types and immunophenotypes are otherwise similar to those of *de novo* cases. These similarities between *de novo* and treatment-related leukaemias may sometimes make it difficult to ascribe the leukaemia to the treatment; however, most *de novo* leukaemias associated with *MLL* gene translocations occur in infants and young children (reviewed by Felix & Lange, 1999), in whom the translocations have been shown to occur *in utero* (Ford *et al.*, 1993; Gill-Super *et al*., 1994; Gale *et al.*, 1997; Megonigal *et al.*, 1998).

DNA topoisomerase II inhibitors are usually used in combination chemotherapy regimens that may include alkylating agents or other DNA topoisomerase II inhibitors, which themselves may be leukaemogenic. Such combinations may confound analysis of the association of specific agents with leukaemia. Furthermore, in some studies in which patients were treated with etoposide and/or teniposide, the authors used various empirical conversion factors to derive an 'equivalent dose' of etoposide from that of teniposide. The conversions were based, however, on the therapeutic effects rather than on metabolic considerations or on possible leukaemogenic potency at a given dose. Such studies do not allow evaluation of the carcinogenicity of either compound as a single agent.

Additional chromosomal and genetic changes may occur in secondary leukaemias (Corral *et al*., 1996). Certain individuals may be at higher risk for secondary leukaemia because they have a DNA repair deficiency or another heritable predisposition such as polymorphisms in the gene encoding cytochrome P450 3A4, an important drug-metabolizing enzyme (Felix *et al*., 1998). Some individuals with the form of leukaemia associated with anti-tumour treatment with alkylating agents have germ-line mutations in the *TP53* cell cycle checkpoint gene as a predisposing factor (Felix *et al.*, 1996).

There are close structural homologies between the DNA topoisomerase II enzymes in higher eukaryotes, but bacterial gyrase and topoisomerase IV play the relevant role in bacteria (Levine *et al*., 1998). Inhibitors of mammalian DNA topoisomerase II are not necessarily effective inhibitors of these bacterial enzymes and, as a result, may not be detected in microbial assays for mutagenic and clastogenic effects.

A number of drugs target DNA topoisomerase I, a different enzyme, which introduces transient single-stranded nicks into DNA. These drugs are not considered in this volume.

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