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Review

A novel approach to develop anti-HIV drugs: adapting non-nucleoside anticancer chemotherapeutics

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Abstract

Some anticancer drugs, but not all, inhibit replication of human immunodeficiency virus (HIV) and thus, exhibit a therapeutic potential. Such drugs, unlike the traditional HIV enzyme inhibitors, could suppress HIV strains that are resistant to inhibitors of viral enzymes, decrease proviral burden in vivo, or reduce reservoirs of infection via killing infected cells. Thus, they may be an effective adjunct therapy or perhaps result in a cure. The incidence of HIV infection and AIDS mortalities continue to increase worldwide, including the United States and parts of Africa, with a parallel increase in a number of other manifestations, including AIDS defining malignancies. The basis for continual spread of HIV presumably in large part stems from the viral resistance to previously successful drugs and the lack of curative antiretroviral drugs. To reverse these trends, other approaches for AIDS therapy must be developed. One possibility is the development of potent anticancer drugs, that exhibit anti-HIV activities. At least four chemically and pharmacologically distinct classes of anticancer drugs, i.e. certain cyclin-dependent kinase inhibitors (CDKIs), topoisomerase 1 enzyme (top 1) inhibitors, non-nucleoside antimetabolites, and estrogen receptor ligands are promising candidates. These drugs, at high doses are used for cancer therapy; at lower concentrations they exhibit anti-HIV activities in cultured cells. While the antiretroviral and the anticancer activities of the cdk inhibitor flavopiridol appear to be mutually exclusive and unrelated in cells and animal model(s) of HIV disease, the top 1 inhibitor 9-nitrocamptothecin, as well as the cdk-inhibitor roscovitine inhibit replication of HIV via selective sensitization of HIV-infected cells to apoptosis. In contrast, the inhibitory effects of these compounds are different from other cancer therapeutics that, at toxic concentrations, activate HIV either in cultured cells (such as certain ingenol and butyrate derivatives) and/or in patients (such as the widely used cyclophosmamide and cisplatin). This quality may lead to the eradication of proviral reservoirs, which is not accomplished by the currently available antiretroviral drugs. In this review, relevant available clinical and in vitro data that either support or discourage using certain anticancer drugs for treatment of HIV disease, and the rationales for developing novel antiretroviral drugs that may target infected cells rather than viral proteins are discussed.

Keywords: Antiretroviral drugs; Chemotherapy; Cancer; HIV; AIDS

1. Introduction

The concept of adapting anticancer drugs for the treatment of HIV infection and acquired immunodeficiency syndrome (AIDS) can be traced to the traditional use of nucleoside, nucleotide or antimetabolite derivatives. Decades of research and clinical evidence have shown the utility of these drugs for the treatment of HIV infection and AIDS.

HIV can replicate in both dividing and non-dividing cells, and is dependent on many host proteins and nuclear enzymes that are required for viral transcription, proviral expression, and completion of its infectious life cycle. All currently available small molecule antiretroviral drugs inhibit replication of HIV via targeting viral enzymes (reverse transcriptase and protease). Even though these drugs have a profound positive effect upon morbidity and mortality due to HIV infection and improved the quality of life for AIDS patients, they (i) do not eradicate the proviral burden, (ii) do not cure the disease, (iii) entail complex therapy regimens, (iv) cause severe adverse-events in long-term therapies, (v) show evidence of poor patient compliance, and (vi) exhibit steadily reduced effectiveness because of the continued increase in the rate of drug resistance (e.g. Lange, 1998; Carosi et al., 1999; Telenti and Paolo Rizzardi, 2000; De Clercq, 2002a,b,c; Mansky, 2003; Torti et al., 2003). Further the decline in new AIDS cases and ensuing deaths that have been occurring since the dawn of clinically used anti-HIV enzyme inhibitors has plateaued. Regrettably the number of AIDS

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cases is on the rise in the United States as well as other parts of the globe (according to WHO, UNAIDS, CDC). These alarming shifts appear to be related partly to a relentless increase in the viral resistance to anti-HIV enzyme drugs and partly due to a transmission of the drug-resistant HIV. Hence, a model was recommended wherein to "delay drug treatment as long as possible," while hoping to effectively reduce toxicities associated with highly active antiretroviral therapy (HAART) and to maximize antiviral benefit (Blower et al., 2001; Velasco-Hernandez et al., 2002).

Drug resistance against HIV enzyme inhibitors generally arises as a result of drug-selected mutational changes in viral proteins. Evidence is emerging that the declining anti-HIV activities of HIV enzyme inhibitors may also be related to drug-selected changes in cellular enzymes. For example, cultured $CD4^+$ cells that express a defective thymidine kinase after prolonged treatment with AZT (Turriziani et al., 2000), and a cellular multi-drug resistance protein, such as breast gene BCRP was induced by doxorubicin (Wang et al., 2003). Ultimately, decreasing options for salvage therapy and the failure to eradicate HIV infection contribute to a current increase in the HIV epidemic (Carosi et al., 1999; Telenti and Paolo Rizzardi, 2000; CDC presses release, 2003). Moreover, a predication, based on a set of mathematical assumptions, that the rate of drug-resistant HIV transmission is a minor public health threat (Blower et al., 2001; Velasco-Hernandez et al., 2002) is optimistically phrased because: (a) the drug-resistant HIV transmission is likely to continue to increase in a non-linear fashion; (b) patients who develop drug resistance may more readily spread drug-resistant HIV than patients who have undetectable HIV; and (c) the drug resistance variants have been observed in "residual" virus pools during therapy in the absence of rebound (e.g. Martinez-Picado et al., 2000; Torti et al., 2003). Resistant strains are increasing and will increase; these, in turn will be transmitted to non-infected people (e.g. Mansky, 2003; Torti et al., 2003). The continued use of HAART as well as delaying the development of newer treatments may sustain the dynamics of drug-resistant viremia.

While both the number of licensed anti-HIV inhibitors and research on drug-based strategies to target HIV proteins have substantially increased (reviewed in De Clercq 2002a,b,c), attention should also focus on the development of drugs that effectively target host proteins that play a crucial role in HIV replication. Recently, the United States Food and Drug Administration approved Fuzeon or enfuvirtide (T-20), which prevents HIV from spreading to non-infected cells by inhibiting fusion of the viral envelope to its receptor on the target cell membrane, and is the only currently available alternative for salvage therapy of HIV disease after HIV enzyme inhibitors. However, T-20 is an invasive drug that lacks oral availability and must be injected daily. Several distinct classes of cell cycle inhibitors, such as 9-nitro-(20*S*)camptothecin (9NC), flavopiridol, and mycophenolic acid have been shown in vitro to inhibit HIV by interfering with cellular proteins regulating virus reproduction in already infected cells. These drugs, if borne useful for patients, are unlikely to generate resistant HIV strains.

The reported effects of anticancer drugs on HIV infection are surveyed in this review. Key points addressed are (a) Why do anticancer drugs inhibit human immunodeficiency virus, while most others either have no effect or activate replication of HIV? (b) Is there a hallmark or common denominator in mechanisms of actions of these compounds?, and (c) Have any of the anticancer drugs that were recently entered into clinical trials in AIDS patients with a concurrent malignancy (or with some other disease) contributed to decreasing HIV reproduction and/or improving HIV disease? To address these issues, relevant publications and recent symposia presentations about compounds that either exhibit anti-cell proliferation (based on their chemical structure/function) were analyzed for their effect on HIV replication. Furthermore, any possible anti-HIV utility of such compounds is discussed in view of concerns over their potential adverse effects that hinder further development of these drugs for HIV/AIDS indication.

Certain terminologies such as 'cell cycle inhibitor' are used hereafter to refer to compounds that can block cell division—with or without directly interfering with the proteins that regulate a cell-cycle checkpoint. Likewise, the term 'anti-proliferative' or 'anti-cell growth' compounds are used interchangeably, referring to compounds that hinder cell proliferation via, for instance, disrupting elements of mitotic spindles apparatus.

2. Survey

2.1. Cancer chemotherapies that fail to inhibit HIV replication

Most chemically and pharmacologically different class of anticancer drugs, which are used widely for treatment of various AIDS-associated cancers, at best have failed to reduce HIV viremia, and at worst have directly or indirectly activated replication of HIV in vitro and/or in clinical trials.

Several anticancer drugs exhibit contradictory antiretroviral properties. Of note, are ingenol derivatives, with diverse biological activities, such as ingenol 3,20-dibenzoate (IDB), which is an S-M phase transition blocker (Blanco-Molina et al., 2001). While ingenol-triacetate (ITA) inhibited HIV in immortalized MT-4 cells, reportedly at viral entry/adsorption level, another derivative strongly activated HIV in chronically infected cells via a mechanism independent of protein kinase C (PKC) or the transcription NFκB pathways (Fujiwara et al., 1996, 1998).

Likewise, some 'old drugs' such as sodium-*n*-butyrate, which can cause marked cell cycle arrest and apoptosis in various permanent and cultured tumorigenic cells, strongly activated replication of HIV in latent model cells (Sadaie and Hager, 1994). This compound or its derivatives, referred to as histone deacetylases inhibitors (HDACIs), or other

families of the HDACIs, such as hyroxamic acids (e.g. trichostatin) and epoxyketone-containing cyclic tetrapeptides (e.g. trapoxin) (Kouraklis and Theocharis, 2002) for reasons of their observed HIV stimulatory effects (Van Lint et al., 1996), expectedly may never be used in AIDS patients with or without malignancies. Several classes of powerful anticancer drugs have recently been used in HIV-infected patients primarily to treat an overlapping disease or an AIDS-associated cancer. None of these classes of drugs lowered viremia and some actually increased viral load. The results of these studies are described below.

2.1.1. Adriamycin (doxorubicin), bleomycin, cisplatin, cyclophosphamide, etoposide, paclitaxel, vincristine

For HIV-infected patients with newly diagnosed Hodgkin's disease, the results of standard ABVD chemotherapy (doxorubicin, bleomycin, vinblastine, dacarbazine) without any antiretroviral therapy, indicated a complete remission of their malignances in almost one-half of 21 patients while the other half had experienced life-threatening neutropenia, altogether with a median survival rate of 1.5 years. None of the patients in this study had any significant improvement in HIV progression (Levine et al., 2000). Although, the intent-to-treat in this type of clinical trial was not to evaluate anti-HIV activities of anticancer drugs, absolute lack of anti-HIV activities of these drugs conflicts with in vitro findings. Earlier laboratory studies indicated that non-toxic concentrations of adriamycin (doxorubicin), inhibited replication of HIV (by more than 90%) in cultured blood macrophages by inhibiting viral DNA synthesis. However, a similar observation did not in infected lymphocytes even at toxic concentrations (Bergamini et al., 1992; Perno et al., 1992). Likewise, the cytostatic and nucleic acid binding drug bleomycin was recently reported both to inhibit replication of HIV-1 in cultured peripheral blood lymphocytes (PBLs), possibly through a direct DNA and RNA attack, and to exhibit synergy with zidovudine, indinavir or ritonavir without an apparent increase in their toxicity (Georgiou et al., 2001; van Asbeck et al., 2001). Interestingly, bleomycin also inhibited DNA viruses, HSV-2 and vaccinia, but not coxsackievirus B5 RNA virus, indicating broad and differential antiviral activities (Norskov-Lauritsen and Ebbesen, 1993).

Could a lack of anti-HIV (as well as desirable antitumor) activities associated with a cellular drug resistance caused by prior antiretroviral therapies? This appears to be the case in cultured lymphocytic cells resistant to therapeutic doses of nucleoside inhibitor AZT (H9rAZT250) which were found to be cross-resistant to several different anticancer agents, including cisplatin, vincristine and adriamycin (doxorubicin) (Cinatl et al., 1998). Appropriate pharmacokinetic and drug interaction studies would be essential to elucidate the lack of anti-HIV activities of drugs such as adriamycin and belomycin.

Several contradictory studies have been reported with regard to the effect of HAART plus standard lymphoma regimens on the incidence or survival of patients with both HIV infection and non-Hodgkin's lymphoma (HIV-NHL) (reviewed in Wiernik, 2001). Potent regimens have usually been investigated in patients with relatively high CD4 counts (>100/μl). For patients with HIV-NHL, the clinical outcome was somewhat more promising than standard lymphoma regimens when a therapy comprised of several different compounds, either mCHOP and full-dose CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or EPOCH (etoposide, prednisone, vincristine [Oncovin] cyclophosphamide, doxorubicin HCl), plus HAART (stavudine, lamivudine, indinavir) (Levine et al., 2001). The efficacy of concomitant CHOP-HAART chemotherapy was curtailed by development of severe autonomic neurotoxicity (Vaccher et al., 2001). In contrast, selected HIV-NHL patients tolerated high dose myeloablative CBV (cyclophosphamide, carmustine, and etoposide)-HAART chemotherapy (Molina et al., 2000). "Strategies to manage such complex multiple-disease cases have often incorporated the assumption that prospects for long-term survival are poor and that intensive therapy cannot be tolerated and so is not justified" (Little et al., 2001). HIV-positive lymphoma is usually diagnosed in patients with markedly decreased CD4 cell count (Levine et al., 2001), which could in part explain why the use of HAART with standard regimens accompanied poor prognosis and lack of significant decline in viremia. These treatments failed to clear HIV, but were accompanied by a transient decline of viremia, i.e. from a median 29,000–500 copies/ml (in the AIDS Malignancy Consortium study involving 65 patients, Ratner et al., 2001). Because these patients were contemporaneously treated with HAART, no effect (positive or negative) can directly or indirectly be attributed to the anticancer therapy. Despite a favorable response rate for standard lymphoma regimens, the prognosis of patients with HIV-NHL is poor. Approximately 50% of these patients die from progressive lymphoma (Ratner et al., 2001; Spina et al., 2002; Wiernik, 2001). None of the 40 patients treated with an infusional CDE regimen [cyclophosphamide, doxorubicin, and etoposide (a topoisomerase II inhibitor)] showed any significant reduction in HIV viremia (Spina et al., 2002).

A combination of cisplatin and cyclophosphamide, used for the first time for treatment of an HIV-1-infected patient with an advanced epithelial ovarian cancer, had no significant effect on HIV-1 p24 antigen nor CD4 cells (Fishman et al., 1995). In contrast, in another anecdotal study cyclophosphamide exhibited a stimulatory effect on HIV replication, increasing circulating HIV in a patient with concomitant HIV infection and autoimmune disease (HIV + SLE) (Alonso and Lozada, 2000). In this case, after a brief therapy with cyclophosphamide, the patient's HIV RNA level was increased from undetectable level to 135,720 copies/ml, which was reversed upon cessation of the cyclophosphamide therapy. The increase in HIV replication was attributed to the immunosuppressive effect of this drug (Alonso and Lozada, 2000). In a further limited

study using combination chemotherapy with cisplatin in six patients with testicular tumors and HIV infection, two patients exhibited no changes in HIV symptoms, while three patients suffered from progressive HIV disease after therapy (Hentrich et al., 1996).

These in vivo results may or may not be related to the ability of cisplatin to activate HIV gene expression in vitro. Cisplatin treatment of HeLa cells stably transfected with an HIV-long terminal reporter construct was shown to activate the HIV promoter while decreasing cell viability (e.g. Woloschak et al., 1995; Panozzo et al., 1996). Moreover, salicylic acid was able to repress this activation (Woloschak et al., 1995). Furthermore, a paclitaxel-cisplatin regimen administered successfully to an HIV-positive patient who had developed ovarian cancer did not affect HIV viremia or cause any progression to AIDS (Knox et al., 2000). It is not quite clear whether or not the patient was on any other medication during the therapy. There is no evidence suggesting that the microtubules-targeting agents such as taxol, paclitaxel, vincristine, and nocodazole, which disturb the dynamic balance between polymerization and depolymerization of microtubules, inhibit HIV. Taxol, or its generic equivalent paclitaxel, actually activated HIV-1 promoter in cells containing LTR-reporter gene (Sweet and Hume, 1995; Mullins et al., 2001). Nocodazole, which arrests cells in G_0/M or mitosis, failed to inhibit HIV transcripts in vitro (e.g. Kootstra et al., 2000; Wang et al., 2001).

2.1.2. Methotrexate (MTX)

The folic acid analog MTX brings about secondary decreases in pyrimidine (dTTP) pools by inhibiting dihydrofolate reductase and decreasing tetrahydrofolate cofactors, exhibits a broad cytostatic effect by arresting cells in the G_1, G_2 and S phases, and is also cytotoxic; inducing apoptosis in both primary and permanent lymphoid cells. The latter is dependent upon the state of cell activation (dividing versus quiescence), drug concentration, and/or cell type (Bokkerink et al., 1988; Gorczyca et al., 1992; de Lathouder et al., 2002; Quemeneur et al., 2003). Such broad pharmacological effects are known generally for many other anticancer drugs surveyed in this review.

MTX has been used for treatment of many AIDS patients with various lymphomas but the virological responses in such patients are unclear, in part because most therapies were either accompanied with antiretroviral regimens or with intensive combined chemotherapies and majority of patients were pretreated with antiretroviral drugs. In one report, when MTX was offered with increasing doses of AZT to 26 AIDS patients with lymphoma (HIV-NHL), it exhibited a little decrease in CD4 cell counts (mean CD4, 132.7/μl versus 98.4/μl; $P = 0.04$) and a modest (non-significant) decrease in plasma HIV-1 p24 antigen (59.07 pg/ml versus 44.70 pg/ml), despite encouraging anticancer responses with mild adverse effects (Tosi et al., 1997). “Three patients who were p24-negative before the therapy became subsequently positive, whereas 2 positive patients had their p24 levels decrease to undetectable levels after AZT + MTX treatment (Tosi et al., 1997).” Unlike mycophenolic acid, however, MTX cannot prevent T cell activation (de Lathouder et al., 2002). Moreover, in contrast to its antiviral effect against DNA viruses such as human cytomegalovirus (Shanley and Debs, 1989), MTX activated HIV production in latent model cells (O’Brien et al., 1995). Because of its ability to inhibit dTTP pools, the authors of an in vitro study proposed that MTX could be used as an adjunct drug to increase antiretroviral activities of some NRTIs, such as stavudine (D4T, 2′,3′-didehydro-3′-deoxythymidine), to inhibit replication of both mutant and wild type HIV isolates (Ahluwalia et al., 1996). Taken together, it is still unclear weather MTX can be useful anti-HIV drug.

2.1.3. Retinoids

The retinoid metabolites 9-*cis*-retinoic acid (9cRA, alitretinoin; Ligand Pharmaceuticals, Inc.), 13-*cis*-retinoic acid (13cRA) and all-*trans*-retinoic acid (ATRA) are structurally related to retinol (Vitamin A) and are a borderline class of cytostatic compounds that appear to be less potent than gold standard anticancer drugs. Retinoids modulate many cellular factors, including inhibition of ornithine decarboxylase, and exhibit antitumor activities (reviewed in Camacho, 2003; Verma, 2003). Retinoids can induce growth arrest of cultured leukemic cells at G_0/G_1, inhibit cdks (such as cdk2, -4, -6), and induce apoptosis in a cell type and concentration-dependent manner (e.g. Cariati et al., 2000). HIV-1 replication was inhibited in cultured primary macrophages ($EC_{50} = 0.1$–1 μg/ml) when cells were treated before infection with physiological concentrations of either retinol (Vitamin A) or retinoic acid, but not after infection that in contrast stimulated HIV production (Maciaszek et al., 1998). This observation suggests that retinoids could either slow HIV entry to susceptible cells (and thus exert a prophylactic effect) or fail as anti-HIV drug in which case it could even exasperate HIV disease in already infected people). Numerous independent prospective case studies suggested that a moderate dietary intake of Vitamin A improve CD4 levels and reduce HIV disease progression, but the evidence-based randomized trials, including an intake of high dose Vitamin A, indicated the opposite that retinoids have no significant effect on viral load, CD4 cell counts or HIV disease progression (reviewed in Dreyfuss and Fawzi, 2002). Interestingly, oral intake of ATRA in six AIDS patients was associated with a considerable decreases in spontaneous apoptosis of PBLs in ex vivo (Yang et al., 1995). In an open label dose escalation study, available data from a cohort of 40 patients with Kaposi’s sarcoma (KS) indicated that oral intake of 9cRA as single anticancer agent indicated insignificant changes in levels of plasma HIV RNA or CD4 cells within 5 months of treatment despite 37% positive KS pathogenic responses, three patients exhibited >1 log 10 decreases in plasma HIV (two of these had undetectable levels of HIV RNA) (Miles et al., 2002). However, it is ambiguous weather or not

Table 1
Effect of well-known cell growth inhibitors on HIV-infection/AIDS

Inhibitors of HIV replication	Non-inhibitors	Activators
9-Nitro-(20*S*)camptothecin	Adriamycin	Cisplatin
Betulinic acid	Bleomycin	Cyclophosphomide
Flavopiridol	Carboplatin	Ingenoids
Hydroxyurea	Dacarbazine	Methotrexate
Ingenol-triacetate	Etoposide	Nucleoside analogs (5-AZC, 5-FU)
Leflunomide	Methotrexate	Sodium-*n*-butyrate
Mycophenolate	Paclitaxel	Taxol or paclitaxel
Nucleoside analogs (5-AZC, etc.)	Retinoids	Trapoxin
Roscovitine	Vincristine	Trichostatin
Tamoxifen and it analog PBPE	Vinblastine	Vinblastine
Topotecan		

Drugs are listed according to their direct (or indirect) main effect reported in the literature toward HIV in cultured cells and/or in vivo. All non-inhibitors represent results of studies with AIDS cancer patients. Adriamycin and beleomycin are inhibitors of HIV infection in vitro, but not in patients, although the related clinical trials were designed to evaluate anticancer properties of these drugs, and not their antiviral effects. Activators represent primarily in vitro results, except limited clinical studies on cisplatin and clophosphomide. See text for detail.

retinoids could increase the survival rate of the infected and/or non-infected lymphocytes in patients with AIDS to the extent that could delay time to AIDS progression (TAP) and/or increase quality of life.

Exceptions to the drugs surveyed in Section 2.1 are compounds that exhibit different biological activities or modulate other cellular targets. The most prominent examples are hydroxyurea mycophenolic acid, tamoxifen and topotecan that are in various stages of clinical trials. The rest of the compounds with dual anticancer and anti-HIV activities are at various stages of in vitro and/or animal model studies. These are included in Tables 1 and 2, and are described below.

2.2. Anticancer drugs that also inhibit HIV replication

In contrast to the many anticancer drugs or DNA damaging agents, such as sodium-butyrate, paclitaxel and platinum-based chemotherapies that either stimulate HIV gene expression or lack anti-HIV activity, several classes of drugs have been reported to exhibit positive anti-HIV activities, yet differ from each other in terms of chemical structure–activity, i.e. biochemical target, pharmacokinetics and pharmacodynamics, and potency in terms of their effective dose (EC_{50}) for inhibiting HIV in cultured cells (Table 2), even though some cause overlapping or different adverse effects in vivo (Table 3).

2.2.1. Nucleoside analogs

Purine/pyrimidine derivatives have shown both antiviral and anticancer activities (reviewed in Gumina et al., 2001). Available anti-HIV nucleoside reverse transcriptase inhibitors (NRTIs) belong to the class of cytotoxic nucleoside inhibitors. For instance, zidovudine (3′-azidothymidine, AZT), has also been used to inhibit cell growth in patients with colorectal cancer or various malignancies (Posner et al., 1992; Falcone et al., 1997; Galmarini et al., 2002). A high plasma concentration of AZT, in combination with

Table 2
Apoptosis-inducing non-nucleoside anticancer drugs: effects on cell cycle check points, HIV replication, and clinical status

Compound	Cell cycle check point[a]	Cellular target	Anti-HIV activity	EC_{50} (nM)	Clinical study	
					Cancer	HIV/AIDS
9-Nitrocamptotehcin	G_2, S blocker	Top 1	Inhibitor	3–4	In trial	N/T
Topotecan	G_2, S	Top 1	Inhibitor	2–5	Available	In trial
Hydroxyurea	G_1/S	RNR, dNTP	Inhibitor	≥100000	In trial	In trial
Flavopiridol	G_1/S, G_2/M	P-TEFb	Inhibitor	8–15	In trial	N/T
Roscovitine	G_1/S, G_2/M	cdk1, -2, -7	Inhibitor	3000–5000	In trial	N/T
Mycophenolate	G_0/G_1, S	IMPDH, dGTP	Inhibitor	≥100	In trial	In trial
Leflunomide	G_0/G_1	DHODH	Inhibitor	20000–25000	In trial	N/T
Ingenol-triacetate	S/M?	Non-PKC, NFκB	Inhibitor	≥50	N/T	N/T
Tamoxifen	G_0/G_1	PKC, ER	Inhibitor	≥10000	In trial	In trial
Ingenol analogs	S/M	PKC, NFκB	Activator	N/A	N/T	N/A
Na-butyrate	G_1/G_2, G_2/M	HDAC	Activator	N/A	In trial	N/A
Trichostatin	G_0/G_1, G_2/M	HDAC, cdk2	Activator	N/A	N/T	N/A
Trapoxin	G_0/G_1, G_2/M	HDAC	Activator	N/A	N/T	N/A
Cisplatin	M	Tubulin	Activator	N/A	Available	N/A
Nocodazole	G_0/M	MAPK	None	N/A	N/A	N/A

[a] Cell cycle arrests can be transient (cytostatic and reversible) or permanent (stable and apoptotic), depending upon drug concentration, frequency of treatment and cell type. The abbreviations used are: Top 1: the cellular enzyme topoisomerase type one; RNR: ribonucleotide reductase (primary target); dNTP: deoxyribonucleoside triphosphates (secondary decreases); P-TEFb: positive transcription elongation factor b, an enzyme complex comprised of cyclin T1 and cdk9; cdk: cyclin-dependent kinase; IMPDH: inosine 5′-monophosphate-dehydrogenase; dGTP: deoxyribonucleoside guanosine triphosphate or guanosine nucleotides (secondary decreases); DHOD: dihydro-orotate dehydrogenase; NFκB: nuclear factor kappa B; HDAC: histone deacetylase; MAPK: mitogen-activated protein kinase; PKC: protein kinase C; ER: estrogen receptor; EC_{50}: 50% effective inhibitory concentration in the absence of apparent toxicity (mostly in primary lymphoid cells in culture); N/A: may not be applicable; N/T: test not performed. See text for detail.

Table 3
Overlapping toxicities[a] of non-nucleoside anticancer drugs (with anti-HIV activities) vs. licensed anti-HIV drugs

Drug	Serious adverse events	Other side effects
NRTIs		
Zidovidine (AZT)	Anemia, myelosuppression, neutropenia, mitochondrial DNA depletion	Nausea
NNRTIs		
Nevirapine, activity delavirdine, efavirnez	Immunological (allergic, rash)	Elevated liver activity
Protease inhibitors		
Saquinavir, ritonavir, indinavir	Immunological (allergic reactions)	Gastrointestinal: nausea, vomiting and/or diarrhea
Cell cycle inhibitors		
Hydroxyurea	Myelosuppression	Gastrointestinal
Flavopiridol	Neutropenia	Gastrointestinal
Roscovitine	Anemia, skin reactions	Gastrointestinal
9NC or topotecan	Anemia, myelosuppression, neutropenia	Gastrointestinal
Mycophenolate	Myelosuppression	Gastrointestinal
Leflunomide	Myelosuppression	Gastrointestinal

[a] These drugs are generally well tolerated within the potential therapeutic ranges. The most common and maximum dose-limiting toxicities (dlts) summarized above are for comparison purpose, and could vary among patients depending upon duration, frequency of treatments, etc. For cell cycle inhibitors, dlts have primarily been observed in non-AIDS cancer patients. The reader is referred to the original articles for details.

fluorouracil or methotrexate, produced desirable anticancer effect; was confirmed in further clinical studies (Danesi et al., 1998).

For many reasons, most notably differences in the mechanism of action, certain nucleoside derivates as well as certain non-nucleoside anticancer drugs cause conflicting pharmacological effects on HIV replication (Table 1). For instance, the cytosine nucleoside analogs 5-azacytidine and 5-aza-2′-deoxycytidine (5AZA), in stark contrast to their anti-HIV activities in vitro (e.g. Bouchard et al., 1990), and 5-flurouracil (5-FU) activated HIV replication in chronically infected cultured cells (Laurence et al., 1993; O'Brien et al., 1995). 5AZA also activated latent HIV in cultured primary lymphocytes of transgenic mice; that was reportedly mediated via demethylation of CpG motifs (Tanaka et al., 2003). 5AZA arrested lymphoid cells in G_0/G_1 phases, which was correlated with reactivation of tumor suppressor genes via CpG demethylations (e.g. Yang et al., 2002).

NRTIs block the formation of a 3′,5′-phosphodiester linkage in DNA synthesis, a key point in rendering this class of drugs effective anti-HIV inhibitors. Long-term low dose maintenance therapies are generally required to control HIV viremia and AIDS. However, the pharmacological and general mutagenic properties of NRTIs, alone or in nucleoside-containing HAART, can contribute to serious adverse effects such as inhibition of mitochondrial DNA polymerase (Johnson et al., 2001), mitochondrial DNA depletion or dysfunction leading to cardiotoxicity, especially with AZT and protease inhibitors (reviewed in Barbaro, 2002; Barbaro and Klatt, 2003), to neurotoxicity (polyneuropathy) (Peltier and Russell, 2002), to toxicity to bone marrow progenitor cells or myleosuppresion (e.g. Dornsife et al., 1991; Middleton and Lau, 1992), and to induction of secondary neoplasia (e.g. Middleton and Lau, 1992).

Furthermore, differential anti-HIV activities of NRTIs (such as AZT, ddA, ddC, ddI) in resting PBLs and macrophages (Richman et al., 1987; Watson and Wilburn, 1992) as well as insufficient bioavailability (or limited tissue distribution) of these drugs in vivo, i.e. to macrophages of deep tissues and central nervous system, in which HIV often takes refuge (Aquaro et al., 1998) have diminished the long-term effectiveness of the licensed NRTIs.

One way to avoid these drawbacks, a combination therapy with other available drugs, i.e. non-nucleoside reverse transcriptase inhibitors (NNRTIs) containing HAART (in place of NRTI-containing HAART) has been proposed, albeit used less commonly, because of: (i) cross-resistance among NNRTIs; (ii) lack of efficacy to HIV-1 group O and HIV-2; (iii) adverse pharmacokinetic reactions; (iv) not to mention drug selected HIV RT mutants (reviewed in Joly et al., 2002). Another obvious way has been the relentless research toward developing novel derivatives of NRTIs (reviewed in Gumina et al., 2001; De Clercq, 2002a,b,c).

2.2.1.1. NRTIs, 5-fluorouracil, 5-fluorocytosine, CPE-C, D-d4FC, etc. The most recent derivatives of NRTIs are mostly at various stages of non-clinical and preclinical research. 5-Fluorouracil analogs such as beta-L-d4FU showed both anti-HIV-1 and antitumoral activities (Shi et al., 1999), while N4-nicotinoyl, *o*-nitrobenzoyl and *n*-butyryl substitutions showed reduced mitochondrial toxicity compared to the parent compound (Shi et al., 2003).

Dideoxy-2′-fluoro-4′-thionucleosides (cytosine 17, 5-fluorocytosine 18, and adenine 27), showed potent anti-HIV activities (EC_{50} = 0.12, 0.15, and 1.74 μM, respectively) without considerable cytotoxicity up to 100 μM to cultured lymphoid cells (Choo et al., 2003). Likewise, a number of cytidine analogs [beta-D-2′,3′-dideoxy (D2) and 2′,3′-didehydro-2′,3′-dideoxy (D4) 5-fluorocytosine (D-d2FC and D-d4FC)], containing either bulky benzoyl or various alkyl substitutions, were synthesized and tested for anti-HIV activities in cultured MT2 lymphoid cells.

Cyclopentenyl nucleoside analogs [adenine (neplanocin A), cytosine (CPE-C), and 5-fluorocytosine] showed modest to potent anti-HIV activities (EC_{50} = 0.1, 0.06, and 5.34 μM, respectively), and were cytotoxic to both primary and permanent lymphoid cells in culture (Song et al., 2001).

Several of these analogs showed higher antiviral activities, especially N4-*p*-iodobenzoyl-D2FC (~46-fold), without increasing cytotoxicity (Shi et al., 2003). D-d4FC

(DPC817, Reverset; DuPont Pharmaceuticals) in combination with other nucleosides or protease inhibitors showed additive synergistic antiviral effect on infected cells (Erickson-Viitanen et al., 2003). Moreover, it inhibited 3TC- and AZT-resistant HIV strains as well as a laboratory strain of HIV-1 in culture, but not multi-NRTI-resistant HIV, when tested against a panel of 50 viruses carrying RT mutations (Geleziunas et al., 2003).

Nevertheless, ability of HIV to readily acquire resistance to the novel NRTI analogs, such D-d4FC (upon selection in cultured cells) (Geleziunas et al., 2003) conjures up yet another uncanny trait of HIV, which would be hard to overcome by all these new derivatives that target HIV enzyme(s). While complementary clinical trials should be useful in evaluating their anti-HIV potency, the classes of cytostatic drugs other than the derivatives of the already approved NRTIs must be studied as alternative ways.

2.2.2. Non-nucleoside antimetabolites

2.2.2.1. Hydroxyurea or hydroxycarbamide. For HIV therapy with hydroxyurea, the strategy used was to treat with drug levels sufficient to inhibit cellular dNTP synthesis, which blocks cells in G_1 and S phases. Hydroxyurea decreased nearly 90% of HIV release and viral DNA synthesis in cultured cells by inhibiting ribonucleotide reductase, a key cellular enzyme essential for DNA synthesis and repair, and synergized with nucleoside inhibitors (ddI, AZT, ddC) (Lori et al., 1994; Gao et al., 1994). Hydroxyurea is by far the most studied non-nucleoside anticancer drug toward HIV infection (reviewed in Ravot et al., 1999), yet its clinical use is currently limited, and not recommended by international guidelines. In HIV-infected children, hydroxyurea + ddI and/or d4T, was reportedly safe with evidence of antiviral activity in some patients (Kline et al., 2000). Sanne et al. (2001) asserted that the combination of hydroxyurea and didanosine (HU + ddI) is an affordable alternative prescription for HIV-infected persons in economically underdeveloped countries. However, switching from HAART (IDV + ZDV + d4T) to HAART plus HU, did not reportedly exhibit any additive patient benefit; consequently the therapy was discontinued because of increased toxicity (e.g. Havlir et al., 2001; Zala et al., 2002).

Not all classes of HIV-infected cell reservoirs exhibit equal sensitivity to the available treatments. In cultured cells, hydroxyurea was more effective in dividing PBLs, while protease inhibitors worked better in non-dividing monocyte-derived dendritic cells in culture (Piccinini et al., 2002). Clinical studies performed around the world exhibited substantive HIV proviral DNA reduction and thus, some patient benefit. This effect was observed when either HU + ddI or a multi-drug regimen of HU + ddI + a protease inhibitor were administered (e.g. Lafeuillade et al., 2002; Youle et al., 2002). Although hydroxyurea could exert a dual therapeutic benefit (antiviral and anticancer), pushing the limits of its antiviral therapeutic index may result in an adverse pharmacokinetic interaction or an excessive impact on the cellular retention of the nucleoside inhibitors that could exacerbate dose limiting serious adverse events such as myelo-suppression or toxicity to the liver and kidneys (Lafeuillade et al., 2002; Lisziewicz et al., 2003). Dose-dependent toxicity by hydroxyurea on pancreatic cells was demonstrated in both cell culture (Foli et al., 2001) and in patients when high doses or more anti-HIV enzyme inhibitors were used (Havlir et al., 2001; Lafeuillade et al., 2002; Zala et al., 2002). While its long-term safety is unclear, recent clinical evidence suggested that the low dose hydroxyurea (600 mg daily) therapy improved patient benefit (Lisziewicz et al., 2003).

2.2.2.2. Mycophenolate. Mycophenolate mofetil or CellCept® (Roche Pharma), or its active metabolite mycophenolic acid (*N*-(trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonic acid amide) is a cytostatic, antiproliferative, and immunosuppressive drug (Mirmohammadsadegh et al., 1998; e.g. reviewed in Allison and Eugui, 2000; Moder, 2003). It is a non-competitive inhibitor of inosine monophosphate dehydrogenase, a key enzyme involved in the synthesis of purine nucleotides, especially in the conversion of inosine monophosphate to guanosine monophosphate (Sintchak and Nimmesgern, 2000; Moder, 2003). It suppresses T cell activation by arresting the cell cycle in G_0/G_1 and S phases in cultured PBLs (Heinschink et al., 2000; de Lathouder et al., 2002). This form of therapy is not fully developed to treat cancer. As an immunomodulator, mycophenolate is used for the prevention of rejection of organ (kidney, heart) transplants, treatment of arthritis, and for experimental therapy of a variety of inflammatory and autoimmune disorders (reviewed in Sintchak and Nimmesgern, 2000; Moder, 2003).

Mycophenolate exhibited anti-HIV activity in cultured cells, primarily at the viral pre-integration stage, in cells pretreated with 1–10 μM concentrations that also inhibited proliferation of activated lymphocytes. Complete suppression of HIV and proliferation occurred in purified CD4 T cells with 4 and 1.25 μM, respectively (Chapuis et al., 2000). It synergized with NRTIs, and inhibited replication of multi-drug-resistant HIV-1 (MDRV1 and ORSM34) in a dose-dependent manner at low concentrations ($EC_{50} \geq 100$ nM) with no apparent antiproliferative effect (Hossain et al., 2002). It was recently administered as an adjunct therapy to chronic AIDS patients, to reduce activated T cells that support HIV infection and deplete residual virus (Chapuis et al., 2000; Press et al., 2002). Preliminary safety studies indicated that mycophenolate, in combination with HAART (Abacavir (ddG) + amprenavir), was well tolerated in a small cohort of AIDS patients (Chapuis et al., 2000). Overall, there were no meaningful changes in the viral load measurements and $CD4^+/CD8^+$ T cell counts between the study and control subjects, presumably because both cohorts also received antiretroviral regimens. However, five of six mycophenolate-treated patients exhib-

ited almost complete inhibition (up to 98%, $P = 0.05$) in the titers of cell-associated infectious HIV in purified $CD4^+$ T cells ex vivo, and exhibited delayed decreases (approximately one-third after 24 weeks) in the numbers of proliferating ($Ki67^+CD4^+CD8^+$) T cells in vivo (Chapuis et al., 2000). In an independent safety study, mycophenolate as a salvage therapy was tolerated over 107–154 days of follow-up, with 3 of 13 AIDS patients exhibiting decreases in T cell counts, in addition to decreases in viremia (Press et al., 2002). The most obvious deficiencies in these studies are: (a) the lack of study cohorts who could receive only mycophenolate; and (b) lack of pharmacokinetic data to assess the bioavailability and turnover of mycophenolate in the presence and absence of concomitant antiretroviral regimens.

2.2.2.3. Leflunomide. Leflunomide (*N*-[4(trifluoromethyl)-phenyl]-5-methylisoxazole-4-carboxamide) primarily blocks the activity of dihydro-orotate dehydrogenase, an essential enzyme for the synthesis of pyrimidine nucleotides (reviewed in Breedveld and Dayer, 2000). It is a cytostatic compound that blocks T cell and B cell proliferation in the G_0/G_1 phases of the cell cycle (Breedveld and Dayer, 2000; Cory and Cory, 2001). The study of anti-HIV activity of leflunomide was recently begun in vitro with the hope of preventing HIV-infected lymphocytes from accumulating sufficient pyrimidines to support viral transcription or viral genomic RNA biosynthesis. Leflunomide inhibited HIV replication up to approximately 75% in cultured non-activated tonsillar lymphoid cells as well as activated PBLs. It also decreased the integration rate of HIV in PBLs, at concentrations having no apparent changes in cell metabolism, proliferation, and viability (Schlapfer et al., 2003). Its inhibitory effect was comparable to those of mycophenolate and hydroxyurea, with a slight additive effect when PBLs were concurrently treated with mycophenolate (Schlapfer et al., 2003). Interestingly, the inhibition was accompanied with a selective loss of either $CD4^+CCR5^+$ density or number of the T cells harboring these HIV receptors, but not $CD4^+CXCR4^+$ cells (Schlapfer et al., 2003). Because the primary lymphoid cells were pretreated for 2–3 days with various concentrations of leflunomide (before HIV inoculation), this could have, in reality, hindered the CCR5-tropic virus from entering cells or reduced pools of the available non-infected cells for infection, thus exerting a prophylactic effect. Discrepancies notwithstanding, because (HIV susceptible) activated T cells expand pyrimidine pools faster than purine pools (Breedveld and Dayer, 2000), leflunomide is potentially an important alternative strategy in deterring propagation of residual virus after conventional therapy; such clinical studies remain. The anticancer activity of leflunomide appears to be independent of its inhibitory effect on pyrimidine biosynthesis, because it also blocks other cellular factors, including tyrosine kinase and the transcription factor NFκB (Xu et al., 1999; Breedveld and Dayer, 2000). These changes occur at high concentrations of leflunomide, which could dismantle the platelet-derived growth factor receptor-signaling pathway and/or cell cycle progression (Xu et al., 1999). Leflunomide was recently shown to have a modest success in a phase II clinical trial in non-AIDS prostate cancer (Ko et al., 2001) and in a phase I trial of glioma patients (Vlassenko et al., 2000).

2.2.3. Estrogen receptor ligands

Tamoxifen (triphenylethylene) is a non-steroidal antitumoral compound that modulates the activities of a number of cellular proteins such as acetyl coenzyme acetyltransferases in a dose dependant manner (Lu et al., 2001), arrests permanent lymphoid cells in G_0–G_1, and blocks cell proliferation (e.g. Scambia et al., 1990). It has been used extensively for treatment of breast cancer because of its anti-estrogenic antitumor activity (e.g. reviewed in Brown, 2002; Jordan, 2003). However, its serious adverse effects, especially its contradictory gynecologic carcinogenicity and/or estrogenic activities are worrisome (Brown, 2002; Jordan, 2003). Tamoxifen, at concentrations that were $\leq$20% cytotoxic ($EC_{50} = 1$–$2\,\mu M$), inhibited activation (HIV-1 release) of latently infected monocytoid U1 cells by up to 90% in phorbol ester (PMA)-induced, but not in 5-AZC-induced cells (Laurence et al., 1990). Its anti-HIV mechanism appeared to be independent of cellular estrogen receptor (Mesange et al., 1996), but was attributed to inhibition of the steroid responsive element in HIV LTR promoter and/or cellular protein kinase C (PKC) activity (Laurence et al., 1990). In PBL cells, freshly infected with HIV in culture, tamoxifen inhibited HIV p24 production in the absence of anti-proliferative activity ($EC_{50} = 10\,\mu M$) (Mesange et al., 1996). The diphenylmethane derivative of tamoxifen, pyrolidino-benzyl-phenoxy-ethanamine (PBPE) or 1-benzyl-4-[*N*-2-pyrrolidinylethoxy]benzene was surprisingly as potent as AZT and better than tamoxifen in inhibiting HIV production in PBLs at concentrations with no adverse effect to viability or proliferation of non-infected control cells (Mesange et al., 1996). Unlike tamoxifen, PBPE fails to inhibit PKC, has no undesirable estrogenic activity and is a high affinity ligand for anti-estrogen binding site, which is a hetero-oligomeric complex involved in the biosynthesis of cholestrol (Marc Poirot, personal communication). On the other hand, like tamoxifen, PBPE in a dose dependent manner arrests tumor cells in G_0-G_1 followed by apoptosis (Marc Poirot, personal communication). Whether these compounds blocked proliferation or induced apoptosis of HIV-infected PBLs was not reported. However, tamoxifen treatment of cultured PBLs of patients with AIDS inhibited the HIV-associated spontaneous apoptosis of $CD4^+$ T cells (Pan et al., 1998). Moreover, it blocked the HIV gp120-dependent activation-induced (FAS-mediated) apoptosis of permanent $CD4^+$ T cells in culture (Micoli et al., 2000). Taken together, tamoxifen derivatives might be useful in decreasing viral load and increasing $CD4^+$ T cells in AIDS. Tamoxifen therapy in AIDS is rare. In a

retrospective study, it was offered as anticancer adjuvant to 10 AIDS patients with ER^+ breast tumors, with previous or concurrent HIV infection (Hurley et al., 2001). The data on CD4 cell counts, viral loads, and AIDS progression during tamoxifen therapy and any follow-ups (after cessation of the tamoxifen therapy) are unavailable. Much debated undesirable adverse effects of tamoxifen might deter further studies on its potential anti-HIV utility. However, research in this area is also needed to fully elucidate anti-HIV activities of its derivatives such as PBPE, raloxifene, toremifene and idoxifene that are predicted to have lower undesirable toxicities (e.g. Brown, 2002).

2.2.4. Cyclin-dependent kinase inhibitors (CDKIs)

CDKIs generally exhibit broad-spectrum antiviral activity, and can inhibit replication of HIV-1, as well as HSV-1 in infected cultured cells, that are resistant to conventional antiretroviral drugs, by targeting cellular rather than viral proteins (Chao et al., 2000, 2003; Schang, 2002; Schang et al., 2002). They have a relative specificity for viral strains that depend on cellular cdks to replicate such as HIV-1, HSV-1, and HSV-2, human cytomegalovirus, varicella-zoster virus, but not vaccinia virus or lymphocytic choriomeningitis virus 2 (Schang, 2002; Schang et al., 2002).

2.2.4.1. Flavopiridol. Flavopiridol is a small semi-synthetic plant alkaloid derived from rohitokine. It inhibits all CDKs [cdk1, -2, -4, and P-TEFb (cyclin T1 and cdk9)] in a dose-dependent manner, causes cell cycle arrest in G_1/S and G_2/M, and binds to duplex DNA via possibly intercalation (reviewed in Senderowicz and Sausville, 2000; Sausville, 2002; Fischer and Gianella-Borradori, 2003). Flavopiridol inhibited replication of HIV (10–20-fold) in nanomolar concentrations without any apparent toxicity on the growth of freshly infected lymphoid cells in culture (Chao et al., 2000, 2003; Schang, 2002; Schang et al., 2002). In a HIV-induced transgenic mouse model, it suppressed HIV-1 transcripts in the kidneys during 20 days of treatment (0.742 ± 0.078 versus. 0.149 ± 0148, $P < 0.01$), and reduced HIV-1-induced nephropathy. Changes in overall gene expression pattern of the kidney cells were also observed by sensitive microarray analysis (Nelson et al., 2003). The anti-HIV effect of flavopiridol correlated with its tight binding to the cell cycle enzyme complex P-TEFb and inhibition of its kinase activity (Chao et al., 2000, 2003). P-TEFb is a component of the cellular transcription factors required for phosphorylating C-terminal domain of RNA polymerase Pol II and subsequent elongation of cellular and viral transcripts (Chao et al., 2000, 2003; Fischer and Gianella-Borradori, 2003).

Interestingly, flavopiridol is also capable of blocking Bcl-2 phosphorylation induced by microtubule-disrupting drugs (paclitaxel, vincristine, nocodazole) (Pathan et al., 2001). This type of effect might actually bring about a favorable outcome by reducing toxicity of one chemotherapy while sustaining the antiviral activity of another.

2.2.4.2. Roscovitine. Roscovitine is a purine derivative, and inhibitor of cdk7 and cdk9, as well as cyclin A- and E-associated histone H1 kinases (Wang et al., 2001). It blocked DNA synthesis in non-dividing rat brain cortex cells by a mechanism independent of CDK inhibition (Yakisich et al., 1998). The purified enantiomeric R-roscovitine (ROS, CYC202; Cyclacel, Ltd.) inhibits all cdks, but it is less potent than flavopiridol (reviewed in Fischer and Gianella-Borradori, 2003). ROS is more specific against cdk1 and cdk2/cyclin E kinase activities (McClue et al., 2002). Preliminary safety studies suggested that ROS is well tolerated up to 2000 mg per day; at 800 mg twice daily, it reached plasma C_{max} of 2630 ng/ml (Laurence et al., 2002). It is currently in phase-II clinical trials for treating breast cancer and lung carcinomas (Fischer and Gianella-Borradori, 2003).

Roscovitine inhibits replication of HIV-1 in cultured lymphoid cells (Wang et al., 2001; Schang et al., 2002; Chao et al., 2003). Consistent with previous studies with the cellular topoisomerase 1 (top 1) enzyme inhibitor 9NC (Moulton et al., 1998), roscovitine exhibited a differential anti-HIV and antiproliferative activities. It inhibited reactivation of HIV-1 in latently infected model cell lines, such as lymphocytic ACH-2 cells and induced apoptosis to a considerable extent (30%), but to a lesser extent (5%) in non-infected parental CEM cells (Wang et al., 2001). Its anti-HIV activity was also evident in cultured PBLs infected with either subtype A or E primary HIV-1 isolates, these strains were inhibited approximately 85 and 95%, respectively, after a one time treatment of the cells with 10 μM roscovitine (Wang et al., 2001), the concentration that is quite close to the non-toxic plasma C_{max} of ROS in cancer patients (Laurence et al., 2002).

Unlike flavopiridol, which appears to interact tenaciously with ATP-binding site in a non-reversible manner (ATP antagonist) (Chao et al., 2000), roscovitine appears to bind reversibly to the ATP site utilized by cdks (Wang et al., 2001). Such differences might, in part, account for the much higher doses of roscovitine required to reach to the same level of inhibition of flavopiridol. Despite downstream biochemical similarities, whether such differences would affect the effective dose and/or therapeutic index of these compounds in clinical settings remain to be determined.

2.2.5. Topoisomerase 1 enzyme inhibitors

2.2.5.1. Betulinic acid. In an effort to separate the HIV inhibition from a possible harm to non-infected cells, non-camptothecin topoisomerase 1 enzyme inhibitors have recently been investigated. One such example is betulinic acid, a potent pentacyclic plant-derived compound. It inhibited both HIV replication and cell cycle (Chowdhury et al., 2002). Betulinic acid inhibited top 1 interaction with DNA via a mechanism other than formation of 'ternary cleavable complex' (DNA–enzyme–drug) (Chowdhury et al., 2002). Betulinic derivatives, such as IC9564, also inhibited HIV-1

in cultured cells at early and late stages of the viral replicative cycle, i.e. cell entry (fusion) level, virion assembly and/or budding of virions (Sun et al., 2002; reviewed in De Clercq, 2002a).

2.2.5.2. Camptothecin (CPT) derivatives. The hallmark of the plant pentacyclic alkaloid camptothecin (CPT) and its derivatives is their ability to inhibit the function of cellular top 1 enzyme (reviewed in O'Leary and Muggia, 1998; Denis and Rowinsky, 1999; Lerchen, 1999; Kellner et al., 2000). The need to improve the pharmacodynamics of these drugs for AIDS treatment, by altering the chemical structure of the active drug, its formulation, or delivery system may be necessary to make them broadly suitable for AIDS patients.

Camptothecin derivatives differ from each other in terms of their pharmacodynamics, pharmacokinetics, strength of binding to topoisomerase cleavage complex, DNA cleavage characteristics, stability, metabolism (rate of clearance and conversion of lactone to active inactive metabolites), and dependence upon multi-drug resistance (reviewed in O'Leary and Muggia, 1998; Denis and Rowinsky, 1999; Lerchen, 1999; Garcia-Carbonero and Supko, 2002). The actual mechanisms of HIV inhibition by CPT derivatives, although attributed to their possible interaction with top 1 enzyme (Priel et al., 1991; Sadaie et al., 1999), may differ depending upon the chemical structure of drug as well as its possible interaction with cellular (Zhang et al., 1997a; Sadaie et al., 1999) and viral proteins. Known examples of the latter are the association of top 1 enzyme with HIV-1 gag nucleocapsid protein (p15) (Takahashi et al., 1995) and/or viral particles (Jardine et al., 1993).

The exact mechanism of action of the inhibition of HIV replication by camptothecin derivatives remains an enigma. For instance, 9NC concentrations, which were both toxic to HIV-infected cells and highly inhibitory to HIV replication, had little effect on non-infected PBLs (Hung et al., 2001). Further investigations are needed to determine whether the anti-HIV activities of the various CPT derivatives are all related specifically to the drug interaction with top 1 enzyme–DNA cleavage site. For a further review of the possible antiviral activities of camptothecins see Pantazis et al. (1999).

Camptothecin (CPT). In early studies, CPT inhibited HIV-1 replication in infected cultured cell lines (Priel et al., 1991), but failed to inhibit at non-toxic concentrations (Jardine et al., 1993). Therefore, the anti-HIV research on the prototype CPT did not continue. Most subsequent viral studies were limited to using CPT as a research tool and/or chemical precursor to make improved derivatives. On the other hand, several cell cycle (G_2-arrest) dependent changes in the cellular gene expression blue print were observed in CPT-treated cancer cells. These effects correlated with the extent of the drug-induced toxicity, analyzed by a microarray (Zhou et al., 2002).

Topotecan. Topotecan (Hycamtin^{TM}, SmithKline Beecham), a water-soluble derivative of CPT, was among the most extensively studied camptothecin derivates in the anticancer arena. The US-FDA approved it in 1996 for the treatment of refractory ovarian cancer. When used in antiviral studies, topotecan inhibited the replication of HIV-1 in cultured lymphoid cell lines at non-toxic concentrations (Li et al., 1993) through a mechanism independent of its interaction with top 1 enzyme (Zhang et al., 1997b). Treatment in AIDS was recently initiated on 12 highly selected patients suffering from the progressive multifocal leukoencephalopathy (PML), the brain disorder caused by the JC polyomavirus (Royal et al., 2003). Available results from 11 patients indicated three positive responders to PML. Regrettably seven patients died within 1 month of therapy and the study was closed because of the hard to control adverse effects of the infusional topotecan such as bone marrow suppression (Royal et al., 2003). All participants had low CD4 cell counts at the baseline, with either low or high viremia, and majority showed either sustained or marginal decreases in HIV RNA levels, and CD4 counts, possibly because of topotecan's adverse effects. The responders had sustained viral loads (one undetectable) from the baseline at the start of treatment to marginally decreased CD4 levels at the end of study after up to nine courses of continuous infusional treatment (each course comprised of 3 weeks on, 1 week off, at a dose of 0.3–0.6 mg/m^2 per day) (Royal et al., 2003). However, it is impossible to extract any significance from the data because the subject numbers of responders are too small and the viral load measurements are too variable between the responders and non-responders for every category. Also, a big deficiency in this study was the lack of a control group and lack of pharmacokinetic data. More subjects are needed, preferably at early stages of the disease, to determine any effect of topotecan on HIV disease. No further antiretroviral studies have been reported and no reports of its 'off label' use for AIDS patients with or without cancers have surfaced.

9-Nitro-(20S)camptothecin (9NC). 9NC is a semi-synthetic, lipophilic, cytostatic and cytotoxic congener of CPT. It was made bioavailable orally, and was well tolerated in phase-I/II clinical trials in non-AIDS cancer patients (e.g. Verschraegen et al., 1998; Stehlin et al., 1999; Patel et al., 2003). It has shown moderate efficacy for pancreatic cancer (Verschraegen et al., 1998; Stehlin et al., 1999), and less for various soft tissue sarcomas (Patel et al., 2003).

In freshly infected cultured PBLs, dose dependent anti-HIV effect of 9NC occurred with low concentrations (EC_{50} = 3 nM). At 5 nM, 9NC inhibited approximately 80% of HIV, and accelerated the cell death (27%) in infected PBLs, but not in non-infected controls (Hung et al., 2001). At higher concentrations (up to 30 nM), HIV level dropped further to <5% while HIV-induced apoptosis increased further to ≥75%. The same doses induced ≤10% apoptosis in non-infected controls (as observed in mito-

chondrial and flow cytometric toxicity assays) (Hung et al., 2001). Furthermore, 9NC blocked lymphocytic cell lines in the G_2 phase of cell cycle (Moulton et al., 1998), but demonstrated no similar effect in PBLs (Hung et al., 2001). Therefore, in addition to its potential antitumor effect, it was proposed that 9NC could have the potential to eliminate reservoirs of infected cells (Hung et al., 2001). The differential anti-HIV and anti-proliferative activities of 9NC in PBLs are consistent with those in latently HIV-1-infected permanent lymphoid cells ACH-2, and with its cytostatic effect to parental non-infected cells (Moulton et al., 1998).

The inhibition of HIV by 9NC via a possible interaction with cellular top 1 enzyme was suggested in studies using cell lines with experimentally mutated top 1 genes (Sadaie et al., 1999). In these cells, the resistance to 9NC correlated with a similar resistance to HIV inhibition. On the other hand, the failure of 9NC to inhibit HIV-1 replication in the resistant cells could also be related to multi-drug resistance genes or perturbations in cellular efflux, because these cells had acquired spontaneous resistance to other different alkaloids such as tubulin binding vincristine (Pantazis et al., 1995) and cisplatin (Saleem et al., 1997). Moreover, it is unknown whether other potential molecular targets of camptothecin for instance mitochondria top 1 (top 1mt) (Zhang et al., 2001) were also affected.

The contribution of other HIV-affected cellular targets should also be considered. For instance, 9NC-treated cells exhibited changes in various cellular proteins, such as a transcription-independent activation of NFκB (Sadaie et al., 1999), up-regulation of cyclin B1 and cdc2 (Chatterjee et al., 2000a) or down-regulation of telomerase activity, c-Myc and Bcl-2 (Chatterjee et al., 2000b). Whether such perturbations could be linked to 9NC's cytostatic effect rather than to its anti-HIV activity is unknown. Another possibility is that 9NC might directly wreak havoc on both HIV transcription and overall cellular transcriptional machinery of the infected cells. It is possible that HIV transcription might be relatively more sensitive to 9NC than the normal cell transcription machinery. Consistent with this scenario, nuclear top 1 enzyme, an ATP-independent DNA single-strand endonuclease and ligase, is known to release the torsional stress of supercoiled DNA during the replication, recombination, transcription, repair of DNA, and chromatin remodeling (reviewed in Kellner et al., 2000; Champoux, 2001; Garcia-Carbonero and Supko, 2002). The possibility remains that the stable binding of 9NC to the topoisomerase-DNA cleavage site could have placed the top 1 enzyme on a default collision course with RNA polymerase and/or DNA polymerase. This would result in a premature or incomplete termination of viral transcription (Collins et al., 2001), and/or abortive/damaged DNA strands (O'Leary and Muggia, 1998; Denis and Rowinsky, 1999). However, it is unclear why this would preferentially occur in infected cells.

Consistent with the apparent lack of 9NC-induced cytotoxicity in (proliferating) non-infected PBLs (Hung et al., 2001), both CPT-induced apoptosis and inter-nucleosomal DNA strand-breaks were absent in G_0 (resting) PBLs (Daza et al., 2002). Moreover, CPT-induced single strand-DNA breaks were repaired in these cells a day after CPT treatment (Daza et al., 2002).

ST1481, BN-80915, and BN-80927. A number of new (fourth) generation camptothecin derivatives have been proposed for cancer indications. These appear to have superior oral bioavailability, improved lactone stability, and possibly with reduced side effects to bone marrow progenitor cells. These highly lipophilic 7-modified camptothecins, the unusually potent derivatives such as the 7-oxyimino methyl derivative of camptothecin, ST-1481 (7-[(*E*)-*tert*-butyloxyimniomethyl]-camptothecin) (e.g. Dallavalle et al., 2000, 2001; De Cesare et al., 2001; Pratesi et al., 2002), and silicon-containing camptothecin BNP1350 or 7-[(2-trimethylsilyl)ethyl]-20(*S*)-camptothecin (Van Hattum et al., 2000) need to be tested for anti-HIV activities. Likewise, the hydrophilic homocamptothecins: such as BN-80915 (difluoro-hCPT, diflomotecan) (Demarquay et al., 2001), and related analogs have recently been entered to phase-I clinical studies in various cancer patients (reviewed in Ulukan and Swaan, 2002; Bailly, 2003). However, antiretroviral activities of these interesting compounds have not yet been reported.

3. Perspective

There are compelling reasons to seek alternative antiretroviral drugs that would be able to purge reservoirs of HIV that remain resistant to traditional antiretroviral drugs as well as to inhibit a myriad of emerging, immunologically divergent, HIV subtypes which pause more challenging issues to develop an HIV vaccine.

The feasibility of adapting new class of anticancer drugs as anti-HIV drugs, which target cellular factors rather than viral proteins, may be afforded via a careful clinical study(s) in AIDS patients with cancer. It is crucial to encourage the use of such new drugs for treatment of at least moderately-immunocompromised HIV-infected patients. In a worst-case scenario, a dose-limiting toxicity of chemotherapeutic drugs can be reversed upon cessation of the therapy. On the contrary, the patient benefit might be via effectively reducing, and potentially eliminating, reservoirs of infected cells analogous to a cancer therapy.

The ability of certain anticancer drugs to inhibit replication of HIV, while simultaneously blocking proliferation of the infected T cells at a given cell-cycle check point, may be mutually exclusive activities of the pharmacologically different anticancer drugs. The type and degree a cell-cycle perturbation(s) by a pharmacologically active cdk inhibitor, as well as a non-cdk inhibitor, may depend on the underlying biochemical defect(s) or existing cellular functions. For instance, flavopiridol and roscovitine block cells at [G_1/S],

[G_2/M] and [G_1/S], [G_2/M], respectively, depending on the cell type.

Chemo-sensitivity of HIV replication and apoptotic commitment of the infected cells following treatment with an anticancer drug(s) are mutually exclusive for certain drugs, but not all. Furthermore, anti-HIV activities of the cdk-inhibitors flavopiridol and roscovitine, and non-cdk-inhibitor 9NC are irrespective of their abilities to block cell cycle. However, while flavopiridol may be more effective in reducing replicating virus, drugs such as 9NC and roscovitine might purge reservoirs of infected cells by means of apoptosis. To these authors' knowledge, these drugs have not yet been tested to determine their dual anti-HIV and antitumor efficacy in clinical trials with AIDS patients having a newly diagnosed cancer.

In non-AIDS cancer patients who had received 2 mg/m^2 per day had tolerated 9NC up to 6 months (Stehlin et al., 1999). The effective dose 50% (ED_{50}) of 9NC in freshly HIV-infected PBLs in culture was about 3 nM (1.2 ng/ml) (Hung et al., 2001). This was 100-fold less than the mean plasma level of 120 ng/ml achieved at the maximum tolerated dose (1.5 mg/m^2 per day) of 9NC given to cancer patients for up to 18 months (Stehlin et al., 1999). However, 9NC is unlikely to be administered to the AIDS patients with poor prognosis such as the stage-III AIDS ($CD4^+$ cell count = 50–200/μl), stage-IV advanced AIDS ($CD4^+$ cell count = 0–50/μl) and heavily pretreated AIDS patients, who have a dire prognosis or poor tolerability profile to the 9NC hematological toxicities.

Many believe that anti-HIV drugs affect people differently depending on how damaged their immune systems are, and which therapy models they have already exhausted. There is no compelling reason to believe that individuals with a persistent immunosuppression or severe AIDS will tolerate 9NC better than the marketed HIV enzyme inhibitors. Similarly, there is no data yet to support the safety and potential efficacy of flavopiridol in AIDS individuals, although the concentration used to inhibit HIV replication in cultured cells was lower than doses used in cancer patients.

It is tempting to postulate that a combined regimen consisting of flavopiridol and a camptothecin derivative would display an improved therapeutic index; i.e. diminishing camptothecin-induced adverse effects, restraining overall side effects, while sustaining anti-HIV effects of both drugs. In this theme, flavopiridol, might have a dual anti-HIV and palliative functions. Recent studies in cultured neurons indicated that certain CDKIs block camptothecin-induced apoptosis via antagonizing its effect on cell cycle regulatory factors. This was observed also for the flavopirdiol rescue of the camptothecin-induced cell death, which was mediated by flavopirdiol suppression of pRb and p107 phosphorylation and loss, or neutralizing camptothecin-induced activation of pRb and p107 phosphorylation at a cdk4/6 phosphorylation site and subsequent loss of Rb and p107 (Park et al., 1997, 2000).

Notably, kinetic cell cycle analyses suggested that S phase cells in HIV-infected patients are more likely to undergo apoptosis than G_0–G_1 cells (Patki et al., 2000). A comparison of the PBLs of HIV-1-infected patients and those of healthy controls indicated that patients' $CD4^+$ cells were more frequently in S phase, while no difference was observed in $CD8^+$ T cells (Patki et al., 2000). On the other hand, analysis of PBL dynamics during HIV infection and HAART therapy revealed a significant increase in DNA synthesis and proliferation of PBLs in patients who were at the late stages of the disease (<100/μl $CD4^+$ cells), in response to opportunistic infections (Tissot et al., 1998). Therefore, moderately immunocompromised individuals as well as asymptomatic HIV-infected individuals are likely to tolerate potent anticancer drugs.

Because 9NC exerts its dose-limiting-toxicity and subsequent induction of apoptosis upon a stable interaction in the ternary complex (drug–enzyme–DNA) in proliferating cells that enter to S phase, conceivably, this class of compound can be administered safely in patients having CD4 cell counts more than 200/μl. It should be mentioned that continuous high dose or intermittent infusional drugs, such as flavopiridol, might be needed to control threshold bioavailability and achieve a better response against cancer. However, aggressive cancer dosing regimens, using drugs such as 9NC and flavopiridol, may not be necessary to lower HIV replication. A convenient daily oral administration and a modified dosing schedule might positively impact clinical outcome and adverse effects.

It is possible that HIV regulatory and/or accessory proteins, such as Tat and Nef, capable of interfering with cell cycle, or the multifunctional Vpr protein, which is involved in the induction of G_2 arrest and apoptosis (e.g. Zhu et al., 2001) augmented the camptothecin-induced apoptosis. For instance, CPT-induced apoptosis was enhanced in transfected lymphocytic cells expressing Nef (Rasola et al., 2001). Consistent with this, HTLV-I Tax protein sensitized the lymphocytic CEM cells to camptothecin-induced apoptosis (Worley and Feuer, 2003).

An additive contributory role of the apoptogenic HIV-1 accessory protein Vpr to the observed cytotoxic effects of camptothecin (CPT or 9NC) in infected cells cannot be ruled out. In primary PBL cells, however, it could involve Vpr in a G_2-arrest independent manner (Hung et al., 2001; Somasundaran et al., 2002). Of note, the mechanism of the enhanced cytotoxicity in HIV-infected and drug-treated cells might have involved shared or independent pro-apoptogenic pathways (Chatterjee et al., 2001; Muthumani et al., 2003; Roumier et al., 2002), which can be elucidated in an experimental model using Vpr-deleted viruses (in the presence of drug) and gene profiling techniques.

Immortalized cells, with chaotic neoplastic processes and various cell cycle check point defects, if used as model HIV-infected cells, may not yield much useful information to test anti-HIV activity of anticancer drugs. Such cells are likely to exhibit an undesirable therapeutic index that may

be construed as the response to a toxic drug and this may hinder continued studies. Therefore, extensive clinically relevant studies in human primary cells and appropriate animal modes may be better indicators toward the proof of concept in clinical trials.

4. Conclusion

Novel anti-HIV drug(s) are sorely needed worldwide, especially drugs that would be affordable within the developing countries, where AIDS and related disease are on the rise. Non-nucleoside anticancer compounds with dual therapeutic activities, such as topoisomerase-1 inhibitors, CDKIs, and non-CDK inhibitors, could be used as single agents, in combination with conventional antiretroviral drugs, or as salvage therapy. It is currently unclear whether or not such regimens would positively influence clinical outcomes because of the potential(s) for pharmacokinetic interactions, synergism, and/or cross-toxicity. Several such drugs exert strong anti-HIV activities regardless of their ability to slow down cell proliferation or block cell cycle at a given cycle check point. In contrast, not all antiproliferative or cell cycle arresting compounds exhibit anti-HIV activity. However, those drugs that inhibit HIV and simultaneously kill infected cells should specially be useful in decreasing infected cells and/or residual HIV in vivo. To determine therapeutic potentials of these distinct classes of compounds, an appropriate animal model of AIDS as well as clinical studies early in the HIV disease should be considered using improved analogs, modified doses, or formulation of these drugs. Moreover, the evaluation of their impacts on important determinants of viral pathogenesis, such as HIV-associated spontaneous apoptosis of primary T cells, in addition to HIV RNA and T cell counts, and other clinical end points should be performed. Such studies would provide useful information toward establishing the safety and effectiveness of these compounds for HIV therapy and bring the current therapeutic interventions a major step closer to eradicate HIV.

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