Nucleoside analogues and nucleobases in cancer treatment

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Cytotoxic nucleoside analogues and nucleobases were among the first chemotherapeutic agents to be introduced for the medical treatment of cancer. This family of compounds has grown to include a variety of purine and pyrimidine nucleoside derivatives with activity in both solid tumours and malignant disorders of the blood. These agents behave as antimetabolites, compete with physiological nucleosides, and interact with a large number of intracellular targets to induce cytotoxicity. Progress has recently been made in the identification and characterisation of nucleoside transporters and the enzymes of nucleoside metabolism. In addition, there is now greater understanding of the molecular mechanisms of anticancer nucleoside activity, which provides opportunities for potentiating their antitumour effects. Strategies to optimise intracellular analogue accumulation and to enhance cancer-cell selectivity are proving beneficial in clinical trials.

Nucleoside analogues and nucleobases are a pharmacologically diverse family, which includes cytotoxic compounds, antiviral agents, and immunosuppressive molecules. The anticancer nucleosides include several analogues of physiological pyrimidine and purine nucleosides and nucleobases. The two primary purine analogues are cladribine and fludarabine. These drugs have mostly been used in the treatment of low-grade malignant disorders of the blood. Among the currently available pyrimidine analogues, cytarabine is extensively used in the treatment of acute leukaemia; gemcitabine has activity in various solid tumours and some hematological malignant diseases; and the fluoropyrimidines fluorouracil and capecitabine have shown activity in colorectal and breast cancers.

The growing importance of nucleoside analogues as cytotoxic agents has stemmed both from the development of newer compounds with broad applicability to common cancers and from an understanding of their mechanisms of action, enabling pharmacological intervention to potentiate the antitumour effects of these compounds. In this paper we review nucleoside analogues and nucleobases commonly used in the clinic, newly described compounds, and measures to improve the therapeutic indices of these drugs.

Mechanisms of action of nucleoside analogues and drug metabolism

Cytotoxic nucleoside analogues are antimetabolites that interfere with the synthesis of nucleic acids. These agents can
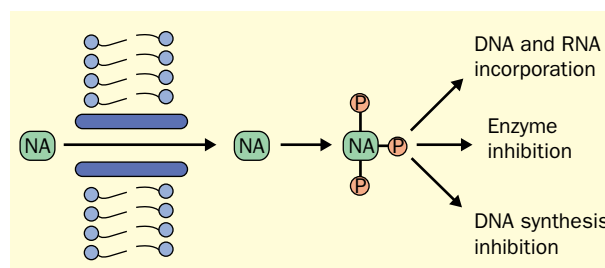

Figure 1. Common characteristics in metabolism and drug–target interactions of nucleoside analogues. Most of these agents are hydrophilic molecules and therefore require specialised transporter proteins to enter cells. Once inside, they are activated by intracellular metabolic steps to triphosphate derivatives. Active derivatives of nucleoside analogues can exert cytotoxic activity by being incorporated into and altering the DNA and RNA macromolecules or by interfering with various enzymes involved in synthesis of nucleic acids, such as DNA polymerases and ribonucleotide reductase. These actions result in inhibition of DNA synthesis and apoptotic cell death.

exert cytotoxic activity by being incorporated into and altering the DNA and RNA macromolecules themselves, by interfering with various enzymes involved in synthesis of nucleic acids, or by modifying the metabolism of physiological nucleosides (figure 1).

The nucleoside analogues share common characteristics including transport mediated by membrane transporters, activation by intracellular metabolic steps that retain the nucleotide residues in the cell, and the formation of the active phosphate derivatives.[1] Nucleoside analogues are generally hydrophilic molecules, and require specialised nucleoside transporter proteins to enter the cell. There is emerging evidence that the abundance and tissue distribution of nucleoside transport proteins contributes to cellular specificity and sensitivity to nucleoside analogues.[2] However, each of these compounds also has unique drug–target interactions that help explain their differences in activity in various diseases. For example, the cytotoxic effects of the purine analogues fludarabine and cladribine on non-dividing cells may be explained by interaction with

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targets involving DNA repair rather than replication and direct or indirect effects on mitochondria.

Purine nucleobases and purine nucleoside analogues

Thiopurines

Mercaptopurine and thioguanine are analogues of hypoxanthine and guanine, respectively, although they are more appropriately designated as nucleobases. In order to be active biologically, these molecules must be phosphorylated by the salvage enzyme hypoxanthine-guanine phosphorybosil-transferase to form thioinosine monophosphate and thioguanosine monophosphate. These compounds are subsequently converted to triphosphates, which can be incorporated into nucleic acids.[3,4] Mercaptopurine and thioguanine are principally catabolised to thiouric acid and uric acid by the enzyme xanthine oxidase.

The cytotoxicity of thiopurines is thought to depend mainly on the incorporation of their phosphorylated derivates into DNA, which interfers with the function of DNA polymerases, ligases, and endonucleases. Moreover, thiopurines may also cause toxic effects by inhibiting other enzymes such as 5-phosphoribosyl-l-pyrophosphate amidotransferase, IMP dehydrogenase, or ribonucleotide reductase which are all involved in de novo purine synthesis enzymes. In addition, the mismatch repair pathway may play a part in thiopurine-mediated cytotoxicity via the recognition of misincorporated thioguanines. These agents are sometimes referred to as "self-limiting" drugs because their biochemical effects can antagonise one another. For example, incorporation of the drug into DNA can be decreased when total DNA synthesis is inhibited by purine starvation.[5]

The thiopurines play an important role in the management of acute leukaemias. In childhood acute lymphoblastic leukaemia, they are used as part of standard consolidation and maintenance schedules. Daily maintenance doses of mercaptopurine are about 75 mg/m²,[6] compared with 50 mg for thioguanine.[7] In acute myeloid leukaemia, thioguanine is used as part of different schemes for remission induction therapy and in the post-remission phase,[8] particularly as part of the DAT (daunorubicin, cytarabine, and thioguanine) regimen at a dose of

Figure 2. Herpes zoster infection in a patient with chronic lymphocytic leukaemia after fludarabine treatment.

100 mg/m² on days 1–7. Bone-marrow depression is the most common toxic effect of both drugs. Allopurinol is sometimes added to mercaptopurine to prevent the hyperuricemia and uricosuria that follow death of leukaemic cells.

Deoxyadenosine derivatives

Two deoxyadenosine derivatives are currently used alone or in combination for the treatment of specific malignant disorders of the blood—fludarabine for refractory chronic lymphocytic leukaemia and cladribine for hairy-cell leukaemias (table 1).[9,10] These drugs share activity against other indolent lymphoid malignant disorders including low-grade non-Hodgkin lymphomas, Waldeström's macroglobulinaemia, and cutaneous T-cell lymphomas, but lack activity against multiple myeloma and most solid tumours.

Fludarabine, unlike cladribine, is administered as the soluble 5'-monophosphate form (fludarabine monophosphate) and dephosphorylated by serum phosphatases and the membrane-bound 5'-nucleotidase, CD73, before transport into the cell. Both nucleoside drugs are rapidly taken up by the target cells via nucleoside-membrane transporters and activated to their triphosphate forms. The initial step in this activation process is catalysed by deoxycytidine kinase[11] although mitochondrial deoxyguanosine kinase has also been identified as a cladribine phosphorylating enzyme.[12] Conversely, cytosolic 5'-nucleotidases dephosphorylate the monophosphate forms of fludarabine and cladribine. Once fludarabine or cladribine has been incorporated into DNA, chain elongation mediated by DNA polymerases is terminated,[13] inducing apoptosis in those cells in the S phase of the cell cycle. Both compounds also indirectly impair DNA replication by inhibiting ribonucleotide reductase,[14] consequently reducing the pool of deoxynucleotide triphosphates (dNTPs) required for DNA synthesis, and enhancing their own cytotoxicity by self-potentiation.[15]

Fludarabine and cladribine are also cytotoxic to resting cells. The most likely explanation for cytotoxicity to non-dividing cells involves inhibition of cellular DNA repair. Incorporation of the active triphosphate metabolites into DNA by the repair machinery leads to the progressive accumulation of DNA single-strand breaks eventually responsible for apoptosis by both p53-dependent and p53-independent pathways.[16] Another consequence of fludarabine and cladribine cell treatment is the direct activation of the caspase 9/caspase 3 death pathway by interaction of their active triphosphate metabolites with the pro-apoptotic factor Apaf1.[17]

Fludarabine and cladribine also alter gene transcription resulting in depletion of proteins required for cell survival.[18] In the case of fludarabine, incorporation of its monophosphate form into RNA results in premature termination of the RNA transcript, impairing its function as a template for protein synthesis.[19] Conversely, fludarabine triphosphate inhibits RNA synthesis by suppressing the activity of RNA polymerase II.[19] When cladribine metabolites, on the other hand, are present in one or both
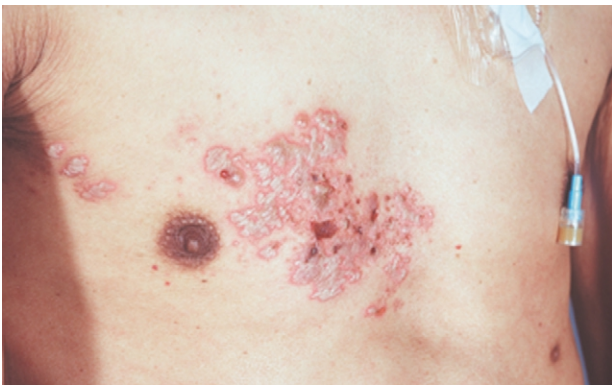
Table 1. Nucleoside analogues and their uses, doses, and adverse effects

Drug	Main uses	Doses	Main adverse effects
Purine analogues			
Fludarabine	Chronic lymphocytic leukaemia	25 mg/m^2 daily intravenously over 30 min for 5 days; repeat every 28 days	Myelosuppression, opportunistic infections, neurotoxicity
Cladribine	Hairy-cell leukaemia; non-Hodgkin lymphoma	4 mg/m^2 daily by continuous intravenous infusion for 5 consecutive days	Myelosuppression, rash, septicaemia, fever
Pyrimidine analogues			
Cytarabine	Acute myelogenous and lymphoblastic leukaemias	Conventional dose—100–200 mg/m^2 intravenously on days 1 to 7 High-dose—3 g/m^2 intravenously over 1–3 h every 12 h for 12 doses	Conventional dose—myelosuppression, vomiting, stomatitis High dose—neurotoxicity, pericarditis
Gemcitabine	Pancreatic, lung, breast, and bladder cancers	1 g/m^2 intravenously over 30 min once a week for 3 consecutive weeks every 4 weeks	Mild myelosuppression, nausea and vomiting, and skin rashes
Fluoropyrimidines			
Fluorouracil	Gastrointestinal, pancreatic, head and neck, renal, skin, prostate, and breast cancers	Mayo regimen—450–600 mg/m^2 intravenous bolus on days 1–5 every 4 weeks Roswell Park regimen—450–600 mg/m^2 intravenous bolus weekly Infusion—200–400 mg/m^2 daily continuously	Bolus—myelosuppression, stomatitis, nausea and vomiting, diarrhoea, angor pectoris Infusion—hand foot syndrome
Capecitabine	Relapsed breast and colorectal cancers	2·5 g/m^2 daily by mouth; 2 weeks on drug, 1 week of rest	Hand-foot syndrome, diarrhoea, nausea and vomiting

DNA strands, the yield of full-length transcripts is reduced impairing its function as a template for protein synthesis.[20] Cladribine has also been shown to cause direct alterations in mitochondrial function that may trigger apoptosis. It disrupts the integrity of mitochondria leading to the release of the pro-apoptotic mitochondrial protein cytochrome C, thereby initiating the caspase proteolytic cascade.[17] Cladribine also interferes either directly or indirectly with mitochondrial transcription that will eventually reduce the amounts of mitochondrial proteins that are necessary for electron transport and oxidative phosphorylation.[21]

The toxicity profiles of both drugs are similar and include moderate myelosuppression and profound and prolonged immunosuppression. One result of this immunosuppression is an increase in opportunistic infections (figure 2) and, potentially, increased risk of secondary cancers. Severe neurotoxicity occurs at higher doses.

In 1983 Cohen and colleagues[22] reported that the nucleobase arabinosyl guanine was resistant to cleavage by purine nucleoside phosphorylase and was toxic to T-lymphocytes. The development of this drug was limited by its insolubility in water. The prodrug nelarabine is ten times more water soluble than arabinosyl guanine and is rapidly converted to the active substance by plasma adenosine deaminase. In phase I clinical testing, nelarabine has shown particular promise in the therapy of T-cell malignant disorders.[23] Of particular interest, neurotoxicity is the dose limiting side-effect, with little clinical myelosuppression.[24] This feature raises the possibility of successful combination therapy with other active agents, including other haematologically active nucleoside analogues such as fludarabine. Although there have been no formal studies to analyse mechanisms of resistance to arabinosyl guanine, accumulation of arabinosyl GTP in leukaemic blasts has been associated with cytotoxic activity against malignant cells.[24] This suggests that the early steps of uptake and metabolism of arabinosyl guanine may be major determinants of cellular drug sensitivity.

Pyrimidine nucleoside analogues and nucleobases
Deoxycytidine derivatives
Cytarabine is a deoxycytidine analogue commonly used in the treatment of haematological malignant diseases, but without activity in solid tumours. This drug is one of the most active single agents in the treatment of acute myeloid leukaemia (table 1). Conventional doses of 100–200 mg/m^2 administered intravenously each day on days 1–7 lead to complete remission in about 30% of cases; when cytarabine is administered in combination with an anthracycline, the complete remission rate can be as high as 65–75% in previously untreated patients and 30–50% in patients with relapsed acute myeloid leukaemia. Similar complete remission rates have been achieved with the use of high-dose cytarabine (2–3 g/m^2 infused intravenously over 2–3 h and repeated every 12 h for as many as 12 doses).

Figure 3. Oral mucositis in a patient with acute myeloid leukaemia treated with cytarabine.

Intracellular penetration of cytarabine depends on the plasma concentration. With regimens that include conventional doses of cytarabine (plasma concentrations of 0.5–1 μM) the expression of the human equilibrative nucleoside-transport-facilitating protein 1 (hENT1) is the rate-limiting factor in cytarabine uptake. Clinical outcome is poor in patients who have myeloblasts with low expression of this transporter. Wiley and colleagues observed that low transport rates were associated with a poor clinical response to cytarabine therapy[25] but Gati and co-workers found a link between the expression of hENT1 transporters and the in vitro cytarabine sensitivity of blasts from patients with acute leukemia.[26,27] At plasma concentrations such as those reached with high-dose cytarabine treatment (>10 μmol/L) simple diffusion rates exceed those of pump-mediated transport.[28] Once inside the cell, the rate-limiting step in intracellular anabolism is conversion to arabinosyl CMP by deoxyxytidine kinase.[29] Cytarabine is broken down to the non-toxic metabolite uracil arabinoside by cytidine deaminase and arabinosyl CMP can be dephosphorylated by cytoplasmic 5'-nucleotidases.[30] Cytarabine cytotoxicity is caused by direct inhibition of DNA polymerases and incorporation of arabinosyl CTP into DNA, which leads to chain termination and DNA synthesis arrest. A low degree of incorporation of arabinosyl CTP into the DNA of blast cells in vitro is predictive of an adverse outcome in patients with AML who receive cytarabine-based therapy.[31]

The main side-effects of conventional doses of cytarabine are leukopenia (primarily granulocytopenia), thrombocytopenia, nausea and vomiting, diarrhoea, mucositis, and hair loss (figure 3). High-dose cytarabine has commonly been associated with these side-effects and with neurotoxicity and pericarditis.[32] Adverse effects of high-dose cytarabine on the central nervous system can be separated into cerebral signs (headaches, somnolence, lethargy, concentration abnormalities, and seizures) and cerebellar signs (ataxia, dysarthria, dysdiadochokinesia, and nystagmus).[33]

The cytotoxic activity of cytarabine is limited by characteristics such as metabolic deamination, low affinity for deoxycitidine kinase, and rapid elimination of the triphosphate derivative. To address these problems, further deoxycytidine analogues were designed. Unlike cytarabine, gemcitabine has activity in solid tumours (table 1). As a single agent, gemcitabine has shown consistent acitvity in previously treated patients with responses in cancers of the pancreas, breast, lung, and ovaries. Gemcitabine is more lipophilic than cytarabine and is a better substrate for nucleoside membrane transporters; of the known substrates for deoxycytine kinase, it has the greatest affinity. These characteristics result in more efficient accumulation and longer retention of gemcitabine triphosphate in tumour cells and enhance its cytotoxicy.[34] After incorporation of gemcitabine into nucleic acids, an additional natural nucleotide is added, preventing DNA repair by base-pair excision.[35] Gemcitabine also inhibits DNA synthesis indirectly through inhibition of ribonucleotide reductase,[35] thereby blocking the de novo DNA synthesis pathway. Gemcitabine activity is self potentiating as intracellular

concentrations of normal deoxynucleotide triphosphates (particularly dCTP) decrease. Reduction in cellular dCTP results in increased incorporation of gemcitabine nucleotides into DNA and increased formation of active gemcitabine diphosphates and triphosphates, since deoxycytine kinase activity is down-regulated by high cellular dCTP concentrations. Low cellular concentrations of dCTP also decrease the metabolic clearance of gemcitabine nucleotides by deoxycytidine monophosphate deaminase (dCMP deaminase).[36,37] At high cellular concentrations, gemcitabine triphosphate directly inhibits dCMP deaminase and CTP synthetase.[38,39] Finally, gemcitabine is incorporated not only into DNA, but also into RNA.[40]

Gemcitabine is administered intravenously as a weekly 30-minute infusion for 3 weeks at doses of 0.8–1.25 g/m[2,] followed by a 1-week rest. The main toxic effects are mild myelosuppresion, mild nausea and vomiting, influenza-like syndrome, and rash. More recently, pharmacological studies have shown that administration of gemcitabine (1–1.5 g/m^{2}) in a 150-minute intravenous infusion (rate of 6–10 mg/m^{2}/min), instead of the 30-minute infusion (rate of 33 mg/m^{2}/min), maximises the rate of triphosphate format-ion.[41] Clinically, the longer infusion resulted in more object-ive responses and longer median survival in patients with advanced pancreatic cancer.[41]

Troxacitabine is a novel deoxycytidine analogue that has antitumour activity in preclinical models both against leukaemic and epithelial malignant disorders. Unlike other

Figure 4. Palmar-plantar erythrodysaesthesia, characterised by an erythematous desquamation of the palms and soles, is one of the common toxic effects of fluoropyrimidine treatment.

nucleoside analogues, troxacitabine is a poor substrate for human nucleoside transporters, and enters cells mainly by diffusion.[42] Troxacitabine is resistant to deamination by cytidine deaminase, and intracellular drug is converted to cytotoxic triphosphate derivatives by deoxycitidine kinase. Troxacitabine triphosphate can be incorporated into cellular DNA[43] and cause chain termination. Phase II testing of troxacitabine in several diseases is now underway[44] and promising activity has been seen in patients with acute myeloid leukaemia, myelodysplastic syndromes, and chronic myelogenous leukemia. At the highest tolerated doses of 8 mg/m^{2}, administered intravenously every day for 5 days, adverse effects include mucositis, rash, and painful hand-foot syndrome.[44]

Similarly to the other compounds described above, tezacitabine is phosphorylated to its active metabolites by deoxycitidine kinase and is deaminated by cytidine deaminase. After incorporation into DNA, the triphosphate of tezacitabine inhibits the subsequent addition of an additional deoxynucleotide by DNA polymerases. Tezacitabine diphosphate also inhibits ribonucleotide reductase, which, as in the case of gemcitabine, may facilitate self-potentiation of cytotoxic activity. Additionally, tezacitabine has antiangiogenic effects in mice with human tumour xenografts.[45] In a phase I study, neutropenia was the dose-limiting side-effect; influenza-like symptoms and fever were the most common non-haematological effects.[46] The dose recommended for use in phase II studies is 4 mg/m^{2} daily administered intravenously for 5 days.

Fluoropyrimidine nucleobases and nucleosides

The nucleobase fluorouracil and the nucleoside floxuridine have activity in patients with colorectal, pancreatic, breast, and head and neck cancers (table 1). Fluorouracil is activated by conversion to floxuridine monophosphate, fluorouridine triphosphate, or floxuridine triphosphate.

Direct inhibition of thymidylate synthase by floxuridine monophosphate is the best characterised biochemical effect of fluorouracil biochemical effects. Thymidylate synthase catalyses the reductive methylation of deoxyuridine monophosphate into deoxythymidine monophosphate in the presence of the folate cofactor 5,10 methylene-tetrahydrofolate. Deoxythymidine monophosphate is further metabolised to deoxythymidine triphosphate for DNA synthesis. This process is the sole de novo source of deoxythymidine monophosphate in the cell. After binding of floxuridine monophosphate, thymidylate synthase is blocked in a covalent ternary complex (thymidylate synthase, floxuridine monophosphate, and folate). Since this complex is stable and the steady state pools of thymidine nucleotides are small, DNA synthesis is inhibited until new enzyme can be synthesised.

Fluorouracil can also be incorporated into nucleic acids causing other effects; it is incorporated into RNA much more readily than DNA and affects several processes including transcription, intracellular distribution, and translation.[47] Thymidine synthase inhibition also leads to a rapid depletion in the deoxythymidine triphosphate pool and an expansion in the dUMP pool that is in turn
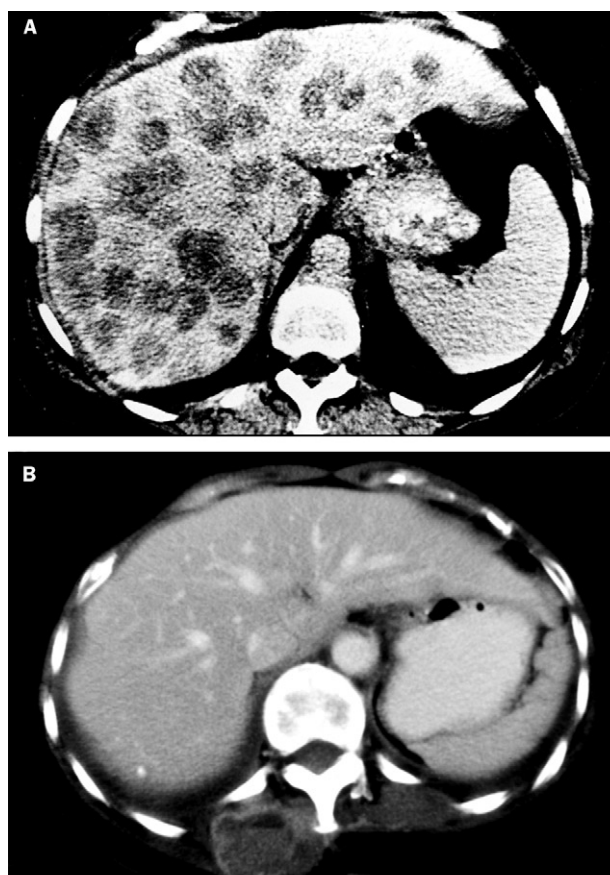

Figure 5. Complete response in a patient with breast cancer and liver metastases after three cycles of capecitabine.

phosphorylated to dUTP. In the absence of dTTP, uracil can also be incorporated into DNA and excised by uracil-DNA glycosylase leading to single or double DNA strand breaks.[48] This imbalance in dTTP/dUTP concentrations and subsequent DNA damage results in the induction of apoptosis.

Fluorouracil is the mainstay of treatment for many common malignant diseases. In colorectal cancer, fluorouracil is generally administered intravenously as a 450–600 mg/m^{2} bolus injection either daily for 5 days every 4–5 weeks (Mayo schedule) or weekly (Roswell Park schedule). It is quite well tolerated at standard bolus doses; side-effects typically involve the gastrointestinal mucosa and bone marrow. Fluorouracil has several other effects that are less well understood, including those on the nervous system (cerebellar ataxia and somnolence), cardiovascular system, and skin; some of these may be due to fluorouracil catabolites.

Fluorouracil given by long-duration infusion has greater antitumour activity than bolus fluorouracil and induces less myelosuppression. Continuous infusion regimens commonly include 750–1000 mg/m^{2} fluorouracil for 5 days. However, some patients develop a painful rash on the palms and soles called palmar-plantar erythrodysesthesia (figure 4). A meta-analysis of six randomised trials in advanced colorectal cancer confirmed that fluorouracil administered by long-

duration infusion results in better responses and less severe side-effects than bolus administration; however, there was no overall survival benefit.[49] Since patients receiving infusions need central venous catheters, benefits may be outweighed by factors such as longer stays in hospital and the added risks of catheter-related complications.

There has been progress towards improving the therapeutic index of fluorouracil through biochemical modulation. One strategy has been the enhancement of fluorouracil activity against colorectal tumours by combination with folinic acid, which is not cytotoxic in itself, but enhances the cytotoxic effects of fluorouracil by promoting formation of the ternary complex containing thymidylate synthase. A meta-analysis of nine randomised clinical trials in patients with advanced colorectal cancers[50] showed that therapy with fluorouracil plus folinic acid yielded better response rates than single-agent fluorouracil (23% vs 11%) but did not improve survival. However, the combination of fluorouracil and folinic acid is associated with an increase in severe gastrointestinal toxic effects. As the fluorouracil and folinic acid regimen was being developed, there was a debate about the optimum folinic acid dose; most physicians now accept that high-dose folinic acid (200 mg/m^2 daily) should be used in the weekly schedule whereas low-dose folinic acid (20 mg/m^2 daily) is sufficient in the daily-times-five schedule. Both regimens are comparable in terms of response and survival; however, more patients experience leucopaenia and stomatitis with

the daily-times-five schedule and more severe diarrhoea is observed with the weekly schedule.

New oral fluoropyrimidine nucleosides achieve higher concentrations of fluorouracil in tumours than in normal tissues, so are more effective and less toxic. The most active agent in this class is capecitabine (table 1). After oral administration, capecitabine passes intact through the intestinal mucosa and is rapidly and extensively metabolised into 5'-deoxy-5-fluorocytidine and 5'-deoxy-5-fluorouridine. Fluorouracil is generated secondarily by thymidine phosphorylase; this enzyme is more highly expressed in tumour cells than in normal cells.[51]

The recommended dose of capecitabine is 2·5 g/m^2 divided into two doses and given daily by mouth for 2 weeks, every 3 weeks, on an intermittent dosing schedule. Mild to moderate diarrhoea and palmar-plantar erythrodysesthesia are the main toxic effects, and myelosuppression is rare. Phase II/III trials have established the efficacy and tolerability of this regimen in patients with metastatic colorectal and breast cancers (figure 5).[52,53] In these trials, capecitabine resulted in response rates of 20–25%. In metastatic colorectal cancer, capecitabine is as effective as and less toxic than bolus fluorouracil plus folinic acid. Trials are underway to investigate capecitabine as a single-agent, or in combination with other cytotoxic agents, in a range of tumours, including those of the pancreas, gastrointestinal tract, ovaries, cervix, and head and neck. The preclinical synergy of capecitabine and taxane combinations seems to be due to paclitaxel and docetaxel-induced upregulation of intratumoural thymidine phosphorylase.[54] Phase I studies showed that intermittent or continuous infusions of capecitabine plus intravenous paclitaxel (175 mg/m^2) or docetaxel (100 mg/m^2) are well tolerated for the treatment of patients with metastatic breast cancer.[55]

Fluorouracil is primarily catabolised by dihydropyrimidine dehydrogenase to the inactive metabolite 5-fluorodihydrouracil. Inhibition of dihydropyrimidine dehydrogenase can affect both toxicity to normal tissue and the antitumour efficacy of fluorouracil. Several studies have shown that dihydropyrimidine dehydrogenase expression in cell lines and human tumour xenografts is related to resistance to fluorouracil and fluoropyrimidine nucleosides.[56] In patients who achieve complete responses to fluorouracil therapy, the activity of dihydropyrimidine dehydrogenase in the tumour tends to be lower than in patients who don't respond or those who have only a partial response.[57] The recent development of several inhibitors of dihydropyrimidine dehydrogenase provides the possibility of increasing the cytotoxic activity of fluoropyrimidines in tumour cells that contain high concentrations of this enzyme, while improving the pharmacokinetics of fluorouracil-based therapies. Preclinical and clinical studies with some of these compounds have shown that inhibiting pyrimidine catabolism in normal and neoplastic tissues enables the dose of fluorouracil to be lowered by about 300 times, while maintaining clinically efficacy.[58]

Eniluracil is an irreversible inactivator of dihydropyrimidine dehydrogenase. This agent significantly changed the disposition of fluorouracil resulting in a mean
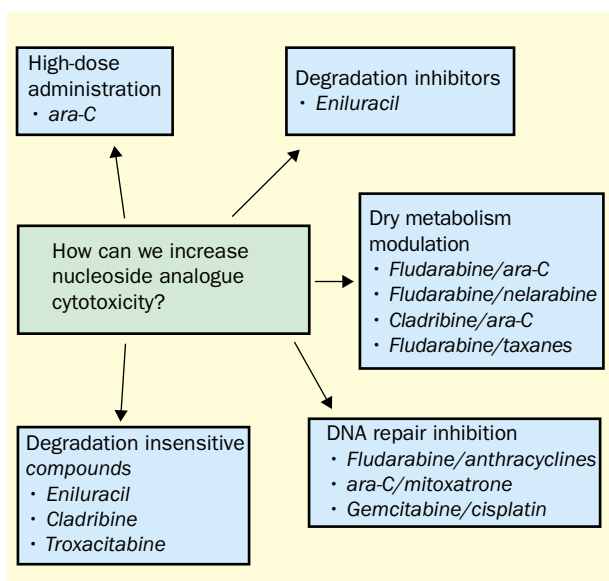

Figure 6. Currently applied strategies to increase the cytotoxicity of nucleoside analogues. One approach is to use high-dose administration of nucleoside analogues. Another is the use of compounds that are insensitive to the effect of degradative enzymes or specific inhibitors of degradative enzymes. Fludarabine, cladribine, and troxacitabine are resistant to degradation by adenosine deaminase and cytidine deaminase, respectively, whereas eniluracil inhibits fluorouracil degradation by dihydropyrimidine dehydrogenase. Different combinations of nucleoside analogues or other cytotoxic drugs potentiate activity of nucleoside analogues by increasing drug metabolism. Finally, combination of nucleoside analogues with DNA-damaging agents agents inhibits DNA-repair induction.

terminal half-life value of about 4·5 h (instead of 8–20 minutes after bolus injection) and complete oral bioavailability.[58] Moreover, the principal route of fluorouracil elimination was shifted from dihydropyrimidine dehydrogenase metabolism to renal-excretion. In combination with twice-daily eniluracil (10–20 mg daily), fluorouracil can be administered orally once a day for 5 days at low doses (20–25 mg/m^{2}), or twice a day for 28 days, every 35 days, at 1 mg/m^{2}; these regimens show highly reproducible bioavailability and high antitumour activity.[58,59] In patients with metastatic colorectal cancer, adminstration of eniluracil plus fluorouracil with or without oral folinic acid seems as effective as traditional schedules of intravenous fluorouracil and folinic acid.[59] The main toxic effects of eniluracil plus fluorouracil are myelosuppression and diarrhoea. Combinations of eniluracil and fluorouracil are also effective in cancer of the breast and advanced cancers of the head and neck.

Other agents have been developed for oral use in combination with fluorouracil prodrugs and inhibitors of dihydropyrimidine dehydrogenase. UFT is a combination of ftorafur (a prodrug of fluorouracil) and uracil in a molar ratio of 4:1. Ftorafur is converted to fluorouracil by the hepatic cytochrome P450 pathway and the uracil component prevents fluorouracil degradation by competitive inhibition of dihydropyrimidine dehydrogenase. This action increases the half life of the converted fluorouracil, which leads to longer exposure, higher intracellular concentrations, and increased antitumour activity. Plasma fluorouracil concentrations obtained after oral administration of UFT are similar to those obtained with equimolar doses of fluorouracil administered as a continuous infusion.

In clinical trials, UFT has been administered alone or with folinic acid. If given alone, the most frequently used dose is 300–400 mg/m^{2} daily divided into two or three administrations, over 21–28 days, followed by a 7-day rest period. When combined with oral folinic acid the recommended schedule is UFT 300 mg/m^{2} plus 75–150 mg folinic acid daily, over 28 days, followed by a 1-week rest period.[60] The dose-limiting toxic effect of UFT is generally diarrhoea; other commonly described toxic effects include nausea and vomiting, fatigue, and stomatitis. Myelosuppression occurs infrequently. Hand-foot syndrome and neurological toxic effects are not generally observed with UFT treatment.

UFT is being extensively studied in combination with folinic acid and, to date, has shown impressive activity in colorectal, gastric, and head and neck cancers.[60] In cancer of the large bowel, oral UFT plus folinic acid resulted in objective responses in about 40% of patients.[61] When UFT was administered alone or with lower doses of folinic acid, about 25% of patients achieved a response. UFT plus folinic acid seems to have equivalent antitumor efficacy to a regimen of intravenous fluorouracil plus folinic acid, with less severe toxic effects.[62]

S-1 is a treatment that combines ftorafur with two modulators of fluorouracil: 5-chloro-2,4-dihydroxypyridine and potassium oxonate. 5-chloro-2,4-dihydroxypyridine is a potent inhibitor of dihydropyrimidine dehydrogenase, much more so than uracil, and potassium oxonate selectively inhibits fluorouracil phosphorylation in the gastrointestinal mucosa, thus reducing the severity of diarrhoea caused by ftorafur. In rat colorectal xenograft models, this combination had improved tumour-selective toxicity and reduced systemic toxic effects, compared with fluorouracil.[63] In a clinical setting, S-1 has shown activity in gastric, colon, and head and neck cancers.[64]

Oral fluorouracil prodrugs in combination with fluorouracil modulators, particularly dihydropyrimidine dehydrogenase inhibitors, offer the possibility of simplifying fluorouracil administration while maintaining or improving on the efficacy of intravenous fluorouracil plus folinic acid. Clinical trials are underway that will help define the role of each of these approaches in various clinical situations.

Strategies to improve nucleoside analogue antitumour efficacy

Greater understanding of the metabolism and mechanisms of action of nucleoside analogues has created opportunities for improving their antitumour efficacy (figure 6). Some new approaches, such as use of combination regimens, have already been proven effective in clinical settings. Furthermore, studies are underway to investigate other approaches such as those that modulate metabolic pathways or intracellular pools of nucleotides.

The potential of nucleoside analogues to be incorporated into nucleic acids by the DNA repair machinery makes them interesting candidates for combination with DNA-damaging agents. Once incorporated, nucleoside analogues are fairly resistant to repair excision and cause irreversible damage that is recognised by the cell. This is particularly true of gemcitabine, which causes "masked chain termination" where an additional nucleotide is incorporated into the DNA chain before replication is interrupted. Inhibition of DNA repair by nucleoside analogues may also increase accumulation of DNA lesions induced by DNA-damaging agents, and slow their removal, thereby potentiating cytotoxic effects. The effectiveness of combining gemcitabine and cisplatin has been confirmed in lung and bladder cancers. Mosconi and colleagues reported a 54% response in stage III/IV non-small-cell lung cancer.[65] In bladder cancer, gemcitabine plus cisplatin (gemcitabine 1 g/m^{2} on days 1, 8, 15, plus cisplaltin 70 mg/m^{2} every 28 days) is now recommended for treatment of advanced disease; this regimen has a better safety profile and is more tolerable than the classical M-VAC (methotrexate, vinblastine, doxorubicin, and cisplatin) combination, with similar survival.[66] Tolerability can be improved by use of carboplatin instead of cisplatin (gemcitabine 1 g/m^{2} on days 1 and 8, and carboplatin[67] on day 1, every 21 days).[68] Combinations of fludarabine and cyclophosphamide may soon become the gold standard for treatment chronic lymphocytic leukaemia.[69] Other approaches that combine agents directed against microtubules, such as the taxanes, with gemcitabine, have also shown promising results in patients with breast cancer.[70]

Metabolic modulation of the cytotoxicity of nucleoside analogues has been approached with the aim of reducing

intracellular pools of nucleotides; this strategy has been studied extensively by Plunkett, Gandhi and their colleagues.[71,72] Inhibition of ribonucleotide reductase by low doses of a nucleoside analogue has been achieved with concurrent administration of a therapeutic dose of a nucleoside analogue. Nucleoside-analogue combinations have achieved responses in patients with untreated and refractory acute leukaemias.[73,74] In the FLAG regimen (fludarabine, cytarabine, and G-CSF), the combination of fludarabine and cytarabine resulted in substantially increased incorporation of cytarabine into DNA.[75] This modulatory effect has also been shown with other nucleoside analogues such as nelarabine.[23] The combination of nelarabine (1.2 g/m^2 intravenous infusion on days 1, 3, and 5) and fludarabine (30 mg/m^2 administered 4 hours before the nelarabine infusion, on days 3 and 5) is an effective and well-tolerated regimen for fludarabine-refractory indolent diseases.[23]

Better understanding of the processes of nucleoside-analogue transport[76] may uncover more therapeutic possibilities. Pharmacological inhibitors of hENT1 and hENT2 protect bone-marrow progenitors in vitro from the cytotoxic effects of nucleoside analogues.[77] There remains, however, a considerable amount of work to do before the clinical relevance of the many nucleoside-analogue transporters is fully understood in both normal and neoplastic tissues.

Another approach consists of increasing the concentrations of active derivatives of nucleoside analogues by modulating the enzymes that facilitate their activation and inactivation. In vitro, exposure of tumour cells to etoposide resulted in an increase in deoxycytidine kinase, a key enzyme in nucleoside-analogue activation. Inhibition of overexpressed nucleotidases could also enhance the therapeutic activity of nucleoside analogues. We found that patients with AML whose blasts have high expression of cN-II 5'-nucleotidase, have a worse prognosis[30] than patients with normal cN-II 5'-nucleotidase, suggesting that inhibition of this enzyme may enhance the cytotoxic effect of cytarabine in these patients. Gosselin and colleagues adopted the strategy of developing monophosphorylated nucleotides which are "protected" by a hydrophobic group allowing them to enter cells, where they are activated by non-specific esterases in the cytoplasm.[78] This strategy bypasses the deoxycitidine-kinase-monophosphorylation step and works best if 5'-monophosphate forms are delivered to the cell.

An important aspect of the antitumour effects of nucleoside analogues relies on their activity on non-dividing tumour cells. We have disucssed some of the hypotheses that might explain the apoptosis-inducing effect of nucleoside analogues in quiescent tumour cells. This effect could be increased by combining nucleoside analogues with compounds that target mitochondria. Although mitochondria are involved in apoptosis, as induced by chemotherapeutic agents, few compounds are thought to target the mitochondria specifically.[79] However, some agents, such as arsenic trioxide, lonidamine, and diazepam, have the ability to induce apoptosis in tumour cells.[80,81] The study of

combination of such compounds with nucleoside analogues is clearly warranted.

Given the growing number of anticancer nucleoside analogues, the abundance of their molecular targets, and the multitude of drug-resistance mechanisms, nucleoside analogues are among the most complex and promising anticancer agents. During the past decade, there has been dramatic progress in the understanding of the properties of these compounds including their uptake by cells, pharmacokinetics, metabolism, interaction with cellular targets, and their apoptotic effects. This knowledge underscores the importance of a multifactorial assessment of the molecular and clinical determinants of nucleoside drug activity. Current efforts should aim to identify the key mechanisms of resistance for the range of nucleoside analogues in various diseases, so that they may be circumvented.

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