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Improvement of purine and pyrimidine antimetabolite-based anticancer treatment by selective suppression of mycoplasma-encoded catabolic enzymes

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Most mycoplasmas are present as commensals, colonising the mucosa of our respiratory and gastrointestinal tract. Experimental data suggest that the long-term association of certain mycoplasma species with mammalian cells might favour host-cell transformation and malignancy. Moreover, increased mycoplasma infection has been noted in several cancers. Despite efforts to develop target-specific anticancer drugs, current cancer treatment still relies on the use of nucleobase or nucleoside-based analogues. Here, we provide experimental evidence that nucleoside-metabolising catabolic enzymes expressed by mycoplasmas substantially compromise the efficacy of nucleoside antimetabolites used in the treatment of cancer. We also suggest potential methods for improving future chemotherapy by suppressing mycoplasma-mediated catabolism of the anticancer nucleoside analogues.

What are mycoplasmas?

Mycoplasmas are the smallest (in cellular dimensions and genome size), self-replicating organisms. They are bacteria that contain a plasma membrane, but lack a rigid cell wall. Because of their limited metabolic and biosynthetic capabilities, mycoplasmas are obligate parasites, often showing host and tissue specificity. Human mycoplasmas are usually found as commensals in the mucosal surfaces of the respiratory and urogenital tracts.[1,2] Several studies have shown that mycoplasmas can be exchanged between mammalian species and can prevail in tissues different from their normal habitat. In particular, commensal mycoplasma species normally belonging to the urogenital flora, such as *Mycoplasma hominis* and *Ureaplasma urealyticum,* have been shown to spread and cause disease in the respiratory tract and joints of immunocompromised individuals.[1,3,4]

Infection by mycoplasmas is acquired through aerosol exposure and by intimate contact between mucosal surfaces, after which the mycoplasmas multiply and subsist in the host for an extended time period. In general, they remain attached to the surface of epithelial cells although some mycoplasmas—eg, *Mycoplasma penetrans*, *Mycoplasma pneumoniae* (figure 1), *Mycoplasma fermentans*, and *Mycoplasma genitalium*—can also enter and reside in non-phagocytic cells.[1] To escape the host immune response, mycoplasmas have acquired several mechanisms, including mimicry of host antigens, survival within phagocytic and non-phagocytic cells, and generation of phenotypic plasticity.[1]

Most people seem to be chronically infected with mycoplasmas without apparent clinical symptoms. When individuals become sexually active, the proportion of mycoplasma colonisation increases. In particular, about 15% of the population are infected with *M hominus* and 45–75% with *U urealyticum*. Individuals infected with mycoplasmas are often asymptomatic, but several mycoplasma species have been shown to be opportunistic pathogens.[5] Indeed, *M pneumoniae* is an important cause of upper and lower respiratory infections, *M genitalium* has been associated with pelvic inflammatory disease and urethritis, and *M fermentans* can be involved in the pathogenesis of rheumatoid arthritis.[6–8] Additionally, several mycoplasma species have been associated with HIV pathology.[9] In particular, *M fermentans* and *M penetrans* have been suggested as cofactors contributing to the development of AIDS.[10] Furthermore, a high *M genitalium* burden has been shown to be related to cervical shedding of HIV-1 DNA.[11]

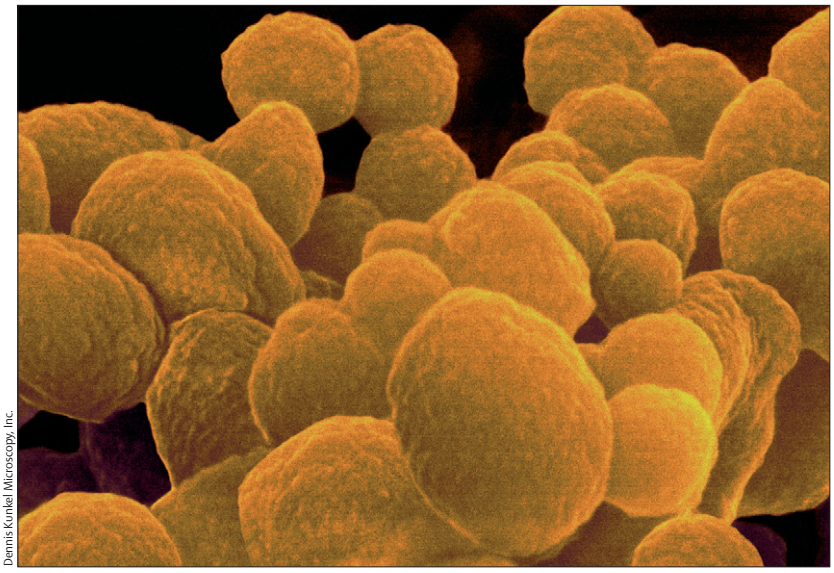

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Figure 1: Mycoplasma pneumoniae

Experimental evidence suggesting a role for mycoplasmas in oncogenesis

In view of the fact that most mycoplasmas are not pathogenic and live in close contact with mammalian cells for a large period of time, the question arises as to whether this association might affect normal host-cell behaviour. In fact, an increasing amount of data suggests the involvement of certain mycoplasma species in malignant transformation and cancer progression.

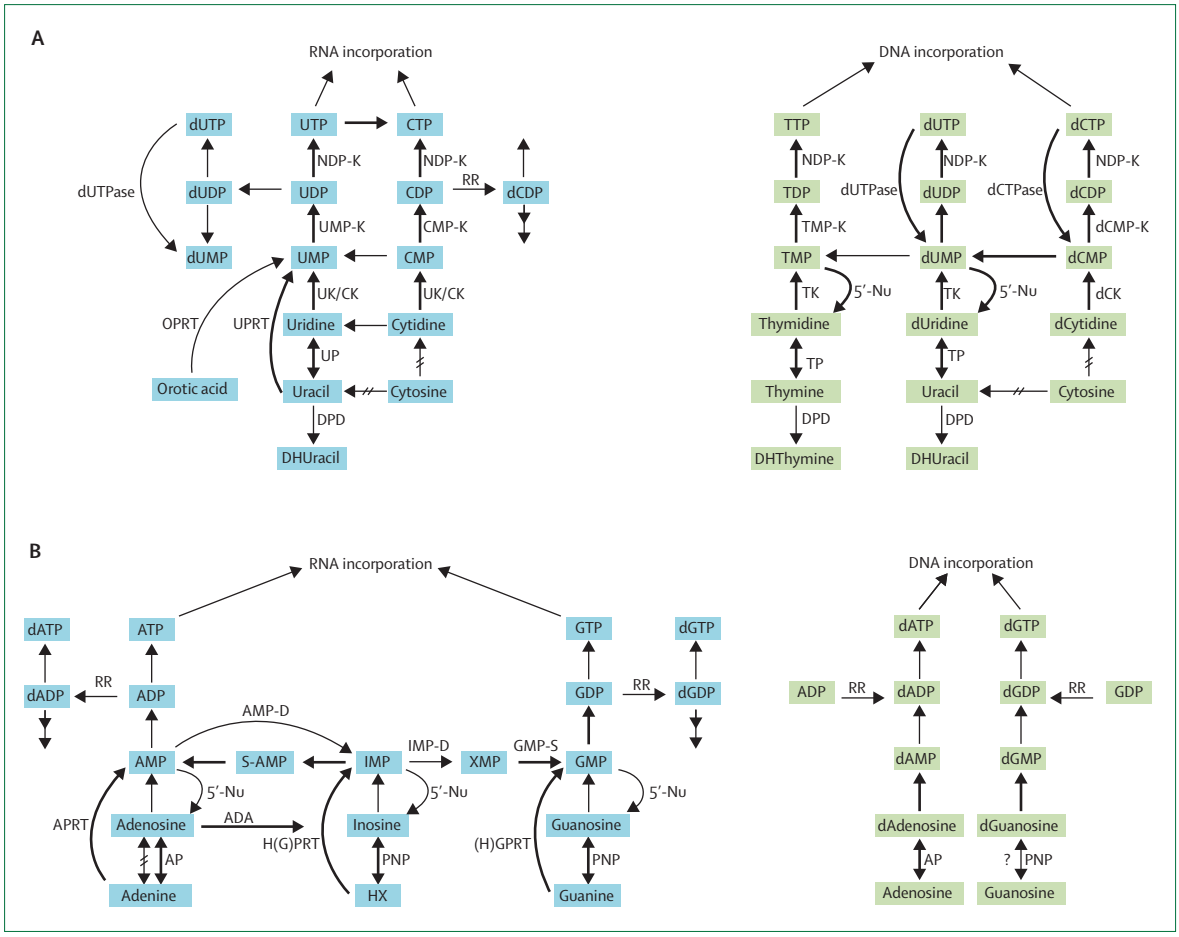

Figure 2: **Enzymatic conversions in pyrimidine (A) and purine (B) ribonucleoside and 2′-deoxyribonucleoside metabolism**
Bold arrows represent enzyme activities noted in mycoplasmas; crossed arrows represent enzyme activity not present in mammalian cells. dUTPase=2′-deoxyuridine triphosphatase. dUTP=2′-deoxyuridine triphosphate. dUDP=2′-deoxyuridine diphosphate. dUMP=2′-deoxyuridine monophosphate. OPRT=orotate phosphoribosyl transferase. UTP=uridine triphosphate. NDP-K=nucleoside diphosphate kinase. UDP=uridine diphosphate. UMP-K=uridine monophosphate kinase. UMP=uridine monophosphate. UK=uridine kinase. CK=cytidine kinase. UPRT=uracil phosphoribosyl transferase. UP=uridine phosphorylase. DPD=dihydropyrimidine dehydrogenase. DHUracil=dihydrouracil. CTP=cytidine triphosphate. CDP=cytidine diphosphate. CMP-K=cytidine monophosphate kinase. CMP=cytidine monophosphate. RR=ribonucleotide reductase. dCDP=2′-deoxycytidine diphosphate. TTP=thymidine triphosphate. TDP=thymidine diphosphate. TMP-K=thymidine monophosphate kinase. TMP=thymidine monophosphate. 5′-Nu=5′-nucleotidase. TK=thymidine kinase. TP=thymidine phosphorylase. DHThymine=dihydrothymine. dCTP=2′-deoxycytidine triphosphate. dCTPase=2′-deoxycytidine triphosphatase. dCMP=2′-deoxycytidine monophosphate. dCMP-K=2′-deoxycytidine monophosphate kinase. dCK=2′-deoxycytidine kinase. dATP=2′-deoxyadenosine triphosphate. dADP=2′-deoxyadenosine diphosphate. ATP=adenosine triphosphate. ADP=adenosine diphosphate. AMP=adenosine monophosphate. APRT=adenine phosphoribosyl transferase. AP=adenosine phosphorylase. AMP-D=adenosine monophosphate deaminase. S-AMP=succinyl-adenosine monophosphate. ADA=adenosine deaminase. HGPRT=hypoxanthine-guanine phosphoribosyl transferase. IMP=inosine monophosphate. PNP=purine nucleoside phosphorylase. HX=hypoxanthine. IMP-D=inosine monophosphate dehydrogenase. XMP=xanthine monophosphate. GMP-S=guanosine monophosphate synthetase. GTP=guanosine triphosphate. GDP=guanosine diphosphate. GMP=guanosine monophosphate. dGTP=2′-deoxyguanosine triphosphate. dGDP=2′-deoxyguanosine diphosphate. dAMP=2′-deoxyadenosine monophosphate. dGMP=2′-deoxyguanosine monophosphate.

In 1988, Dudler and colleagues[12] isolated and cloned a 37 kDa protein (P37) that was shown to be present on the surface of highly invasive FS9 mouse sarcoma cells. The invasive behaviour could be transferred to other cell lines by exposure to the culture supernatant of FS9 cells, and was shown to be caused by *Mycoplasma hyorhinis* infection.[12,13] More recent studies have shown that P37 of *M hyorhinis* binds to the surface of human prostate and melanoma-cell lines, stimulating their invasion through matrigel.[14] The P37 membrane protein can promote additional malignant changes in mammalian cells, such as inhibition of senescence, enhanced clonogenicity in soft agar, nuclear enlargement, inhibition of cell migration, and adhesion.[15,16] Stimulation of tumour progression and metastasis by P37 can involve activation of matrix metalloproteinase-2 and the subsequent activation of the epidermal growth factor receptor.[17]

Other mycoplasma species, in addition to *M hyorhinis*, can potentiate cancer aggressiveness. A metastasis-promoting protein homologous to P37 has been identified in *Mycoplasma arginini*.[18] *M fermentans* and *M penetrans* have been shown to induce chromosomal instability and malignant transformation of mouse embryonic cells.[19] Irreversible cell transformation required persistent

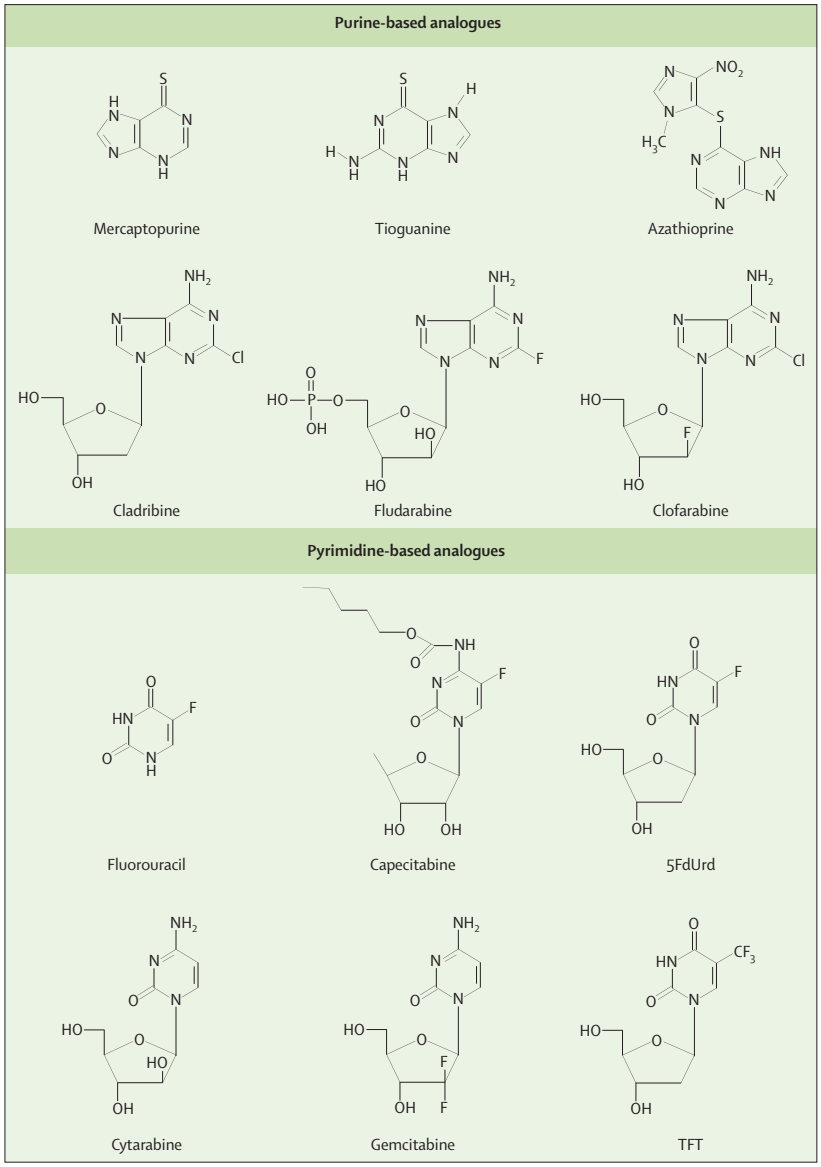

Figure 3: **Structural formulae of purine and pyrimidine nucleobase and nucleoside-based anticancer drugs**
5FdUrd=5-fluorodeoxyuridine. TFT=5-trifluorothymidine.

long-term (months) mycoplasma infection and proceeded through different stages with gradually increasing malignant properties. Additionally, several species of human mycoplasmas, including *M fermentans*, *M penetrans*, *M genitalium*, *Mycoplasma orale*, and *M pneumoniae* were shown to inhibit apoptosis and induce continued growth of interleukin-3-dependent 32D murine myeloid cells that were deprived of interleukin-3.[20] Finally, *M fermentans* was shown to inhibit tumour necrosis factor-α (TNF-α)-induced apoptosis in the human myelomonocytic U937 cell line and to promote immortalisation of peripheral-blood mononuclear cells and Epstein–Barr virus-positive B-lymphocytes.[21,22]

Additionally, the immunomodulatory properties of mycoplasmas can contribute to malignancy. Mycoplasmas have been shown to activate monocytes and macrophages to induce the secretion of various proinflammatory cytokines, chemokines, nitric oxide, and prostaglandins.[23]

Mycoplasma infections in human cancer

During the 1960s, several mycoplasma species, including *M fermentans* and *M orale*, were isolated from human leukaemic bone marrow and blood.[6,24] Although some mycoplasma isolates were able to induce leukaemia in mice, a possible link between mycoplasma infection and cancer could not be confirmed by serological and cell-culture methods.[6,25] However, more recent ecological analyses using disease surveillance data for England and Wales support a role for *M pneumoniae* in the subsequent development of childhood acute lymphoblastic leukaemia.[26] Additionally, *M penetrans* has been suggested to be a cofactor in the development of HIV-associated Kaposi's sarcoma,[27] and the presence of *M fermentans* and *M penetrans* has been noted in classic Kaposi's sarcoma.[28] Immunohistochemistry of human tissue samples with an antibody against the P37 protein of *M hyorhinis* showed that mycoplasma infection was significantly higher in gastric carcinoma and colon cancer compared with other gastric diseases.[29] Sasaki and co-workers[30] reported the detection of mycoplasma infection in 48% of DNA samples from patients with gastric cancer by PCR amplification using mycoplasma-specific primers. More recently, high mycoplasma infection in gastric cancer tissue has been confirmed and correlated to tumorigenesis.[17] Chan and colleagues[31] noted a high prevalence (59·3%) of mycoplasma DNA in malignant ovarian cancer.[31] Additionally, in oesophageal, lung, renal, and breast cancer, and in gliomas, a high infection rate of mycoplasma could be detected, suggesting an association between mycoplasma infection and certain carcinomas.[29,32]

Mycoplasmas harbour various nucleoside and nucleotide-catabolising enzymes

Mycoplasmas are unable to synthesise purine and pyrimidine bases de novo.[33] Therefore, salvage of existing nucleobases and nucleosides is essential for their homoeostasis and survival. The general metabolic pathways for purine and pyrimidine nucleosides are shown in figure 2. Mycoplasmas have been shown to contain several enzymes associated with the salvage of pyrimidine nucleosides.[34] In addition to various anabolic pyrimidine nucleo(s)-(t)ide-metabolising enzymes, *Mycoplasma mycoides* possess the catabolic 2′-deoxycytidine triphosphatase (dCTPase), 2′-deoxyuridine triphosphatase (dUTPase), 2′-deoxycytidine monophosphate (dCMP), deaminase, thymidine phosphorylase, uridine phosphorylase, and 2′-deoxyuridylate (dUMP) and thymidylate phosphatase enzymes.[34,35] In *Mycoplasma mobile*, a new nucleotide triphosphatase was recently discovered, which recognised several nucleoside triphosphates.[36]

In addition to anabolic purine nucleo(s)(t)ide-metabolising enzymes,[33,37] several catabolic enzyme activities associated with the salvage of purine nucleosides have been identified in mycoplasmas. An enzyme, that has never been detected in mammalian cells, but which seems to be commonly present in a wide variety of mycoplasmas, is adenosine phosphorylase, which catalyses the reversible conversion of adenosine (or deoxyadenosine) to adenine and (2′-deoxy)-ribose-1-phosphate.[38,39] This enzyme activity was not noted in *M pneumoniae* and *Mycoplasma lipophilium* (the latter mycoplasmas have the ability to convert adenosine into inosine by deamination). *M orale* infections of murine L1210 and P388 leukaemia cells were shown to change intracellular deoxyadenosine metabolism.[40] Ecto 5′-nucleotidase activity was found in about 50% of *M fermentans* strains, efficiently recognising adenosine monophosphate (AMP) as a substrate.[41] *M fermentans* was also noted to increase the activity of human ecto 5′-nucleotidase activity in B cells and human monocytic leukaemia (THP-1) cells, and even in Daudi and Raji Burkitt's lymphoma cells that are normally 5′-nucleotidase-negative.[8] A selective down-regulation of (anabolic) pyrimidine salvage pathways in tumour cells latently infected with mycoplasmas has also been reported.[42] Thus, mycoplasmas not only express their own catabolising enzymes, but might also be able to upregulate nucleoside catabolism or downregulate nucleoside anabolism in infected mammalian cells.

Purine and pyrimidine nucleoside and nucleobase analogues with anticancer activity

Several purine-based and pyrimidine-based drugs have been shown to exert anticancer activity against various solid tumours, leukaemias, and lymphomas (figure 3). Among the pyrimidine-based drugs are the deoxycytidine analogues cytarabine, gemcitabine, troxacitabine, and sapacitabine, and the uracil-based fluorouracil and its nucleoside analogue-based prodrug capecitabine. Two additional 5-substituted uracil-based nucleoside analogues (5-fluoro-dUrd [5FdUrd] and 5-trifluorothymidine [TFT]) have not yet been approved for clinical use (figure 3). Among the purine-based analogues are thioguanine, mercaptopurine and its prodrug azathioprine; and the deoxyadenosine analogues fludarabine, cladribine, and clofarabine. The deoxyguanosine derivatives nelarabine (which is a water-soluble prodrug of β-D-arabinofuranosyl-guanine), and 2′,2′-difluoroguanosine have not yet been formally approved for clinical use. The different cancers that are targeted by the clinically approved drugs are shown in the table.

	Targeted cancer
Purine analogues	
Mercaptopurine	Acute lymphoblastic leukaemia
Thioguanine	Acute lymphoblastic and myeloid leukaemia
Azathioprine	Acute lymphoblastic leukaemia
Fludarabine	Chronic lymphocytic leukaemia
Cladribine	Hairy-cell leukaemia, non-Hodgkin lymphoma
Clofarabine	Relapsed or refractory acute lymphoblastic leukaemia
Pyrimidine analogues	
Cytarabine	Acute lymphoblastic and myeloid leukaemia
Gemcitabine	Pancreatic, lung, breast, and bladder cancers
Fluorouracil	Gastrointestinal, pancreatic, head and neck, renal, skin, and breast cancers
Capecitabine	Breast and colorectal cancers

Table: **Nucleobase and nucleoside analogues used in cancer chemotherapy**

Metabolic properties and mechanisms of action of anticancer drugs

The nucleobase fluorouracil is converted to 5-fluorouridine monophosphate by orotate phosphoribosyl transferase.[43] 5-fluorouridine monophosphate can then generate 5-fluorouridine diphosphate, and 5-fluorouridine triphosphate, and also 5-fluoro-2′-deoxyuridine-5′-monophosphate (5FdUMP) by ribonucleotide reductase and cellular kinases. The anticancer activity of fluorouracil is predominantly due to thymidylate synthase inhibition (by 5FdUMP) resulting in the inhibition of de-novo synthesis of dTMP, thymidine diphosphate (TDP), and thymidine triphosphate (TTP), and eventual inhibition of DNA synthesis.[44] However, fluorouracil can also inhibit RNA synthesis (by 5-fluorouridine triphosphate).[43,45] Capecitabine, a nucleoside analogue prodrug of the nucleobase fluorouracil, needs thymidine phosphorylase activity to eventually release fluorouracil that can then be anabolised to its active metabolite as described above.[46–48] It is generally believed that bolus fluorouracil and leucovorin treatment acts by inhibition of RNA synthesis whereas prolonged infusions of fluorouracil or capecitabine act via inhibition of thymidylate synthase and thus DNA synthesis. The nucleoside analogues 5FdUrd and TFT are activated (phosphorylated) by thymidine kinase to their 5′-monophosphate derivatives, which strongly inhibit thymidylate synthase.[49] However, TFT monophosphate is efficiently further converted to its 5′-triphosphate and TFT triphosphate incorporation into DNA is the main mechanism of antitumour action of TFT rather than thymidylate synthase inhibition. Thymidine phosphorylase acts as a catabolising enzyme for 5FdUrd and TFT. Thus, thymidine phosphorylase activity is obligatory for capecitabine activity, but deleterious for the cytotoxic activity of 5FdUrd and TFT.

Cytarabine is phosphorylated by 2′-deoxycytidine kinase to its 5′-monophosphate form.[50] After subsequent phosphorylation by monophosphate and diphosphate kinases, cytarabine triphosphate inhibits DNA polymerase α and gets incorporated into the elongating DNA strands, causing DNA chain termination.[51] For gemcitabine, 2′-deoxycytidine kinase is also the

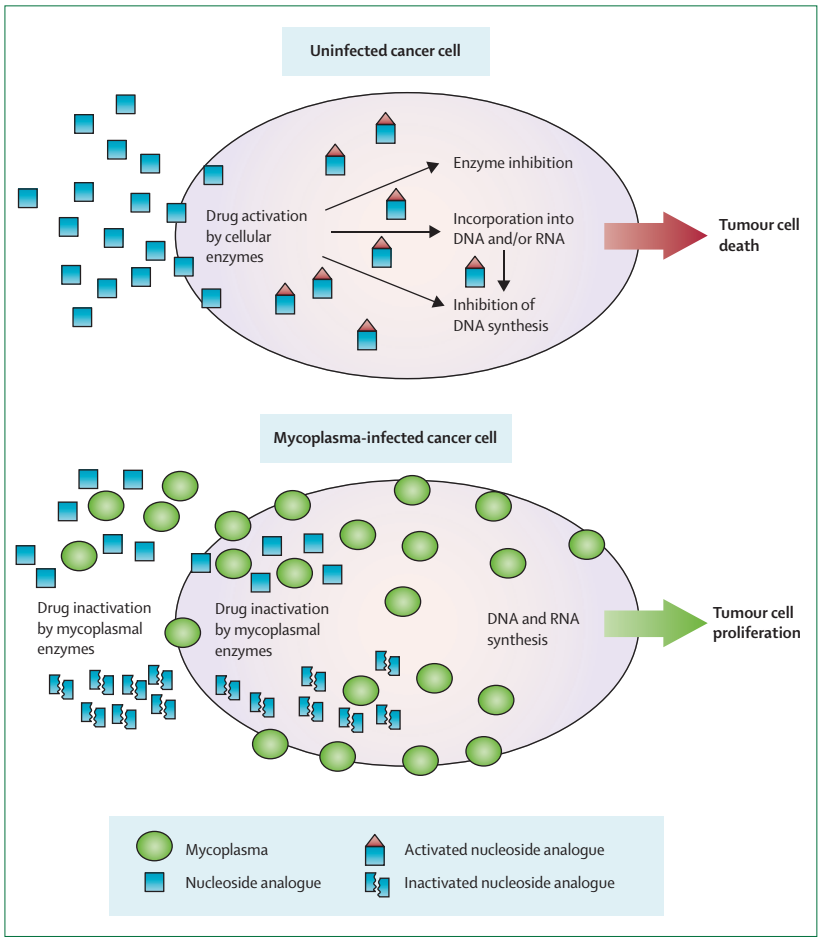

Figure 4: **Schematic representation of the potential effect of mycoplasma infection on anticancer drug therapy**

rate-limiting enzyme for active metabolite formation. The triphosphate of gemcitabine is incorporated into DNA (resulting in a so-called masked DNA chain termination), which is considered the most important mechanism of antitumour action.[52] However, inhibition of ribonucleotide reductase by its diphosphate metabolite, cytidine triphosphate synthetase by its triphosphate, and thymidylate synthase by its deaminated monophosphate metabolite, can also contribute to the eventual antiproliferative activity of gemcitabine.[52]

Fludarabine, cladribine, and clofarabine are predominantly activated (phosphorylated) by 2′-deoxycytidine kinase.[53–55] Although the 5′-triphosphate of fludarabine mainly inhibits DNA synthesis after incorporation into DNA, it also inhibits ribonucleotide reductase. By contrast, the 5′-triphosphate of cladribine is especially inhibitory to ribonucleotide reductase. Clofarabine triphosphate has the mechanistically favourable properties of both agents.[53,56–59]

Anabolic–catabolic balance of anticancer drugs

The eventual antiproliferative activity of the antimetabolite cancer drugs greatly depends on the balance between activating and inactivating enzymes present in the plasma and tumour cells. Indeed, catabolic enzymes, such as 5′-nucleotidases, pyrimidine and purine nucleoside phosphorylases, pyrimidine and purine nucleoside and nucleotide deaminases, and nucleotide triphosphatases, can prevent efficient conversion of the nucleoside drugs into their active metabolite(s) and, thus, can hamper their eventual cytotoxic or anticancer activity. Several studies have clearly shown decreased efficacy of cladribine, fludarabine, cytarabine, and gemcitabine in patients with cancer due to increased 5′-nucleotidase activity, eventually resulting in lower 5′-triphosphate metabolite levels available for subsequent incorporation of the drug into DNA (or RNA).[60–62] Patients with acute myelocytic leukaemia (AML), whose blasts express high levels of 5′-nucleotidase, have been shown to have a worse prognosis than patients with normal 5′-nucleotidase levels.[63] Inactivation of 5FdUrd and TFT is mainly modulated by thymidine phosphorylase followed by dihydropyrimidine dehydrogenase, which further catabolises fluorouracil and trifluorothymine.[64,65] Increased dihydropyrimidine dehydrogenase expression in patients has been shown to be related to resistance to fluorouracil and fluoropyrimidine nucleosides.[64] Cytarabine and gemcitabine are broken down into the non-toxic uracil derivatives by cytidine deaminase and dCMP-deaminase, and cytarabine monophosphate and gemcitabine monophosphate can be dephosphorylated by cytoplasmic 5′-nucleotidases.[63,66] 2′-Deoxycytidine kinase deficiency has also been reported as a contributing factor to cytarabine resistance.[67,68] Such tumour cells show cross-resistance to other deoxycytidine kinase-dependent drugs, such as gemcitabine, cladribine, clofarabine, and fludarabine.[60,69–71] Clofarabine is much less susceptible to purine nucleoside phosphorylase inactivation than cladribine and the nucleoside of fludarabine. Each of the pyrimidine or purine-based drugs shows unique characteristics with regard to its susceptibility to the catabolic versus anabolic enzymes and their molecular mechanisms of drug resistance. Such individual drug properties make them selectively effective against certain types of tumours and ineffective, or poorly cytotoxic, to other types of tumours and untransformed cells.

Efforts have been devoted to the development of prodrugs of antitumour agents to optimise their pharmacological profile and anticancer activity by: circumventing their degradation by catabolic enzymes; rendering them more tumour selective; or lowering their toxic side-effects. For example, capecitabine is a nucleoside analogue prodrug of the nucleobase fluorouracil that can be absorbed through the gastrointestinal tract, after which it is metabolised by a cascade of enzymes to fluorouracil.[46] Conversion of 5′-deoxy-5-fluorouridine to fluorouracil by thymidine phosphorylase is the last and necessary step in the activation of capecitabine. Therefore, it is not surprising

that thymidine phosphorylase expression has been associated with an antitumour response to capecitabine in breast cancer and to capecitabine plus irinotecan in patients with metastatic colorectal cancer.[47,48]

A new concept

As outlined in previous sections, a wide variety of purine-based and pyrimidine-based nucleobase and nucleoside analogues are currently available in the clinic for anticancer treatment and several promising nucleoside analogues are in preclinical development. The efficacy of such anticancer drugs predominantly depends on the balance between their anabolism (activation) and catabolism (inactivation) in tumour cells. Additionally, tumours can become resistant to chemotherapeutic drugs by down-regulating the activating enzymes (ie, nucleoside kinases), or by upregulating the target enzymes (ie, thymidylate synthase) or catabolic enzymes (ie, cytidine deaminase). Catabolic enzymes are present in all mammalian cells including tumour cells, but such enzymes are also, often abundantly, present in mycoplasmas. Most people are infected with such (usually non-pathogenic) mycoplasmas, which contain a depot form of nucleo(s)-(t)ide-inactivating enzymes. Moreover, mycoplasmas have been associated with different kinds of cancers and have been shown to be preferentially present in various cancer tissues.[29–31] Therefore, we believe that mycoplasmas might exert a continuous latent threat that compromises the efficacy of purine-based and pyrimidine-based anticancer chemotherapy. Thus, mycoplasma infections might have an underestimated role in therapy failure and might contribute to individual differences and variability in the efficacy of anticancer drugs (figure 4).

Our experimental data show that the cytostatic activity of thymidine derivatives, such as 5FdUrd and TFT, is dramatically decreased (10-times to 150-times) in mycoplasma-infected MCF-7 breast cancer cell cultures.[72] This decrease in cytostatic activity could be fully restored by coadministration of a thymidine phosphorylase-specific inhibitor (ie, thymidine phosphorylase inhibitor [TPI]).[72,73] In mycoplasma-infected tumour-cell cultures, the intracellular levels of the active metabolites of the above-mentioned thymidine antimetabolites were much lower than in non-infected tumour-cell cultures, which is in full agreement with the decreased cytostatic activity of the anticancer drugs in the mycoplasma-infected cells.[72] However, it is of particular importance to notice that short exposure of the mycoplasma-infected cell cultures (ie, 1–3 days) to an antibiotic that targets mycoplasmas (ie, plasmocin) proved sufficient to restore the cytostatic activity of the above-mentioned anticancer drugs in the mycoplasma-infected MCF-7 cell cultures.[72]

In light of our preliminary findings, we believe that the combination of several purine and pyrimidine nucleoside-based anticancer drugs with specific inhibitors of catabolic mycoplasma-encoded enzymes could greatly improve the outcome of anticancer drug therapy (figure 4). Furthermore, we believe that administration of specific antibiotics against mycoplasmas could reverse the decreased cytotoxic activity of several types of purine and pyrimidine (nucleoside)-based anticancer drugs, even in cases where the nature of the mycoplasmal catabolic enzyme has not been firmly identified. For example, plasmocin, a new generation of bactericidal antibiotic was strongly active in mycoplasma-infected cell cultures.[72] It contains two bactericidal components: one acts on the bacterial-protein synthesis by interfering with ribosome translation and the other interferes with the DNA replication fork. These mycoplasma-specific targets are absent in mammalian cells. Additionally, plasmocin has been shown to be active against extracellular mycoplasmas, cell-associated mycoplasmas, and intracellular forms of mycoplasmas.[74] The interesting feature of using mycoplasma-targeting antibiotics is that several drugs have already been established for the treatment of mycoplasma infections, including tetracyclines (ie, doxycycline), macrolide antibiotics (ie, erythromycin, azithromycin, and clarithromycin), and fluoroquinolines (ie, ciprofloxacin and levofloxacin). However, not all types of purine and pyrimidine analogue-based anticancer drugs would benefit from combination with inhibitors of mycoplasma-derived catabolic enzymes and antimycoplasma antibiotics. For example, mycoplasma-derived enzymes can have a beneficial effect on the cytostatic potential of fludarabine and capecitabine.[38,48]

Testing the concept

In immunodeficient mice (ie, severe combined immunodeficiency [SCID] mice), both tumour cells and mycoplasmas can be grown without being suppressed by the immune system. This small-animal model represents an ideal environment for investigating the efficacy of anticancer drugs in the presence or absence of a mycoplasmal infection. Such experiments could allow further exploration of the effect of specific inhibitors of mycoplasma-encoded catabolic enzymes, and specific mycoplasma-targeting antibiotics, on the efficacy of purine-based and pyrimidine-based anticancer chemotherapy.

Additionally, in view of the fact that clinically approved, orally available antibiotics against mycoplasma infections, such as doxycycline, erythromycin, and ciprofloxacin, are available, and that TPI, a potent inhibitor of thymidine phosphorylase, is currently the subject of phase II clinical studies, it would be feasable to set up clinical trials that combine antitumour nucleoside-based drug therapy with such antibiotics or TPI. Thus, this new therapeutic principle to improve and optimise current nucleoside-based anticancer therapy could be explored and validated in the clinic in a relatively short time period.

Search strategy and selection criteria

Information for this Personal View was obtained by searches of Medline and PubMed, by use of the following search terms (alone and in combination): "mycoplasma", "cancer", "cancer chemotherapy", "purine and pyrimidine nucleobase/nucleoside analogues", "nucleoside enzymes", "catabolic enzymes", "thymidine/uridine/adenosine phosphorylase", "nucleotidase", "cytidine/cytidylate deaminase", "mercaptopurine", "thioguanine", "azathioprine", "cladribine", "fludarabine", "clofarabine", "fluorouracil", "capecitabine", "TFT", "fluorodeoxyuridine", "cytarabine", and "gemcitabine". Information from publications, such as Deoxynucleoside Analogs in Cancer Therapy (Peters GJ, ed. Totawa, NJ: Humana Press Inc, 2006), was also used to search for peer-reviewed international journals. Only papers published in English from 1970 onwards were used.

Conclusion

We have provided experimental evidence that mycoplasma infections seriously compromise the cytostatic potential of several anticancer nucleoside analogues in cell culture. Therefore, we believe that the presence of (non)pathogenic mycoplasmas in patients with cancer can also lower the efficacy of cancer treatment due to the abundance of catabolic enzymes expressed by mycoplasmas. Administration of specific inhibitors of the mycoplasmal catabolic enzymes or antibiotics that suppress mycoplasma infections in patients with cancer could have a beneficial effect on the efficacy of anticancer therapy with pyrimidine and purine nucleoside analogues.

Contributors

SL and JB provided figures, did the literature search, and wrote the manuscript. AB provided data and helped with the literature search.

Conflicts of interest

The authors declared no conflicts of interest.

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References

1 Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. *Microbiol Mol Biol Rev* 1998; **62:** 1094–156.
2 Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. *Clin Microbiol Rev* 2004; **17:** 697–28.
3 Mian AN, Farney AC, Mendley SR. Mycoplasma hominis septic arthritis in a pediatric renal transplant recipient: case report and review of the literature. *Am J Transplant* 2005; **5:** 183–88.
4 Pinna GS, Skevaki CL, Kafetzis DA.The significance of *Ureaplasma urealyticum* as a pathogenic agent in the paediatric population. *Curr Opin Infect Dis* 2006; **19:** 283–89.
5 Murray PR, Pfaller MA, Kobayashi GS. Medical microbiology, 4th edn. Chapter 42. St. Louis, MO: Mosby CV, 2002.
6 Cimolai N. Do mycoplasmas cause human cancer? *Can J Microbiol* 2001; **47:** 691–97.
7 Haggerty CL. Evidence for a role of *Mycoplasma genitalium* in pelvic inflammatory disease. *Curr Opin Infect Dis* 2008; **21:** 65–69.
8 Johnson SM. The importance of B-cells and ecto-5\nucleotidase in *Mycoplasma fermentans* infection and the relevance to rheumatoid arthritis. *Immunology* 2008; **123:** 187–96.
9 Lo SC, Tsai S, Benish JR, Shih JW, Wear DJ, Wong DM. Enhancement of HIV-1 cytocidal effects in CD4+ lymphocytes by the AIDS-associated mycoplasma. *Science* 1991; **251:** 1074–76.
10 Montagnier L, Blanchard A. Mycoplasmas as cofactors in infection due to the human immunodeficiency virus. *Clin Infect Dis* 1993; **17:** S309–15.
11 Manhart LE, Mostad SB, Baeten JM, Astete SG, Mandaliya K, Totten PA. High Mycoplasma genitalium organism burden is associated with shedding of HIV-1 DNA from the cervix. *J Infect Dis* 2008; **197:** 733–36.
12 Dudler R, Schmidhauser C, Parish RW, Wettenhall RE, Schmidt T. A mycoplasma high-affinity transport system and the in vitro invasiveness of mouse sarcoma cells. *EMBO J* 1988; **7:** 3963–70.
13 Schmidhauser C, Dudler R, Schmidt T, Parish RW. A mycoplasmal protein influences tumour cell invasiveness and contact inhibition in vitro. *J Cell Sci* 1990; **95:** 499–506.
14 Ketcham CM, Anai S, Reutzel R, et al. p37 induces tumor invasiveness. *Mol Cancer Ther* 2005; **4:** 1031–38.
15 Liu W, Ren T, Jiang B, Gong M, Shou C. Mycoplasmal membrane protein p37 promotes malignant changes in mammalian cells. *Can J Microbiol* 2007; **53:** 270–76.
16. Goodison S, Nakamura K, Iczkowski KA, Anai S, Boehlein SK, Rosser CJ. Exogenous mycoplasmal p37 protein alters gene expression, growth and morphology of prostate cancer cells. *Cytogenet Genome Res* 2007; **118:** 204–13.
17 Gong M, Meng L, Jiang B, et al. p37 from *Mycoplasma hyorhinis* promotes cancer cell invasiveness and metastasis through activation of MMP-2 and followed by phosphorylation of EGFR. *Mol Cancer Ther* 2008; **7:** 530–37.
18 Ushio S, Iwaki K, Taniai M, et al. Metastasis-promoting activity of a novel molecule, Ag 243-5, derived from mycoplasma, and the complete nucleotide sequence. *Microbiol Immunol* 1995; **39:** 393–400.
19 Tsai S, Wear DJ, Shih JW, Lo SC. Mycoplasmas and oncogenesis: persistent infection and multistage malignant transformation. *Proc Natl Acad Sci USA* 1995; **92:** 10197–201.
20 Feng SH, Tsai S, Rodriguez J, Lo SC. Mycoplasmal infections prevent apoptosis and induce malignant transformation of interleukin-3-dependent 32D hematopoietic cells. *Mol Cell Biol* 1999; **19:** 7995–8002.
21 Gerlic M, Horowitz J, Horowitz S. *Mycoplasma fermentans* inhibits tumor necrosis factor alpha-induced apoptosis in the human myelomonocytic U937 cell line. *Cell Death Differ* 2004; **11:** 1204–12.
22 Zhang S, Tsai S, Wu TT, Li B, Shih JW, Lo SC. *Mycoplasma fermentans* infection promotes immortalization of human peripheral blood mononuclear cells in culture. *Blood* 2004; **104:** 4252–59.
23 Yang J, Hooper WC, Phillips DJ, Talkington DF. Cytokines in *Mycoplasma pneumoniae* infections. *Cytokine Growth Factor Rev* 2004; **15:** 157–68.
24 Girardi AJ, Hayflick L, Lewis AM, Somerson NL. Recovery of mycoplasmas in the study of human leukaemia and other malignancies. *Nature* 1965; **205:** 188–89.
25 Murphy WH, Bullis C, Dabich L, Heyn R, Zarafonetis CJ. Isolation of mycoplasma from leukemic and nonleukemic patients. *J Natl Cancer Inst* 1970; **45:** 243–51.
26 Alexander FE. Is *Mycoplasma pneumonia* associated with childhood acute lymphoblastic leukemia? *Cancer Causes Control* 1997; **8:** 803–11.
27 Wang RY, Shih JW, Weiss SH, et al. *Mycoplasma penetrans* infection in male homosexuals with AIDS: high seroprevalence and association with Kaposi's sarcoma. *Clin Infect Dis* 1993; **17:** 724–29.
28 Cuccuru MA, Cottoni F, Fiori PL, Masala MV, Cerimele D. PCR analysis of *Mycoplasma fermentans* and *M penetrans* in classic Kaposi's sarcoma. *Acta Derm Venereol* 2005; **85:** 459–60.
29 Huang S, Li JY, Wu J, Meng L, Shou CC. Mycoplasma infections and different human carcinomas. *World J Gastroenterol* 2001; **7:** 266–69.
30 Sasaki H, Igaki H, Ishizuka T, Kogoma Y, Sugimura T, Terada M. Presence of Streptococcus DNA sequence in surgical specimens of gastric cancer. *Jpn J Cancer Res* 1995; **86:** 791–94.
31 Chan PJ, Seraj IM, Kalugdan TH, King A. Prevalence of mycoplasma conserved DNA in malignant ovarian cancer detected using sensitive PCR-ELISA. *Gynecol Oncol* 1996; **63:** 258–60.

32 Pehlivan M, Pehlivan S, Onay H, Koyuncuoglu M, Kirkali Z. Can mycoplasma-mediated oncogenesis be responsible for formation of conventional renal cell carcinoma? *Urology* 2005; **65:** 411–14.

33 Wang L, Westberg J, Bölske G, Eriksson S. Novel deoxynucleoside-phosphorylating enzymes in mycoplasmas: evidence for efficient utilization of deoxynucleosides. *Mol Microbiol* 2001; **42:** 1065–73.

34 Neale GA, Mitchell A, Finch LR. Enzymes of pyrimidine deoxyribonucleotide metabolism in *Mycoplasma mycoides* subsp *mycoides*. *J Bacteriol* 1983; **156:** 1001–05.

35 Mitchell A, Finch LR. Enzymes of pyrimidine metabolism in *Mycoplasma mycoides*. *J Bacteriol* 1979; **137:** 1073–80.

36 Ohtani N, Miyata M. Identification of a novel nucleoside triphosphatase from Mycoplasma mobile: a prime candidate motor for gliding motility. *Biochem J* 2007; **403:** 71–77.

37 Mitchell A, Sin IL, Finch LR. Enzymes of purine metabolism in *Mycoplasma mycoides* subsp *mycoides*. *J Bacteriol* 1978; **134:** 706–12.

38 Hatanaka M, Del Giudice R, Long C. Adenine formation from adenosine by mycoplasmas: adenosine phosphorylase activity. *Proc Natl Acad Sci USA* 1975; **72:** 1401–05.

39 Bonissol C, Stoiljkovic B. AdoP assay detection of mycoplasmal contamination in biological media. *Res Virol* 1989; **140:** 241–51.

40 Plagemann PG, Woffendin C. Mycoplasma contamination alters 2\'-deoxyadenosine metabolism in deoxycoformycin-treated mouse leukemia cells. *J Cell Biochem* 1990; **43:** 161–72.

41 Johnson S, Pitcher D. Distribution of ecto 5\'-nucleotidase on mycoplasma species associated with arthritis. *FEMS Microbiol Lett* 2000; **192:** 59–65.

42 Merkenschlager M, Kardamakis D, Rawle FC, Spurr N, Beverley PC. Rate of incorporation of radiolabelled nucleosides does not necessarily reflect the metabolic state of cells in culture: effects of latent mycoplasma contamination. *Immunology* 1988; **63:** 125–31.

43 Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003; **3:** 330–38.

44 Danenberg PV. Thymidylate synthetase—target enzyme in cancer chemotherapy. *Biochim Biophys Acta* 1977; **473:** 73–92.

45 Ingraham HA, Tseng BY, Goulian M. Nucleotide levels and incorporation of 5-fluorouracil and uracil into DNA of cells treated with 5-fluorodeoxyuridine. *Mol Pharmacol* 1982; **21:** 211–16.

46 Villalona-Calero MA, Weiss GR, Burris HA, et al. Phase I and pharmacokinetic study of the oral fluoropyrimidine capecitabine in combination with paclitaxel in patients with advanced solid malignancies. *J Clin Oncol* 1999; **17:** 1915–25.

47 Honda J, Sasa M, Moriya T, et al. Thymidine phosphorylase and dihydropyrimidine dehydrogenase are predictive factors of therapeutic efficacy of capecitabine monotherapy for breast cancer-preliminary results. *J Med Invest* 2008; **55:** 54–60.

48 Meropol NJ, Gold PJ, Diasio RB, et al. Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. *J Clin Oncol* 2006; **24:** 4069–77.

49 Myers CE. The pharmacology of the fluoropyrimidines. *Pharmacol Rev* 1981; **33:** 1–15.

50 Liliemark JO, Plunkett W. Regulation of 1-beta-D-arabinofuranosylcytosine 5\'-triphosphate accumulation in human leukemia cells by deoxycytidine 5\'-triphosphate. *Cancer Res* 1986; **46:** 1079–83.

51 Kufe DW, Spriggs DR. Biochemical and cellular pharmacology of cytosine arabinoside. *Semin Oncol* 1985; **12** (2 suppl 3)**:** 34–48.

52 Bergman AM, Peters GJ. Gemcitabine. Mechanism of action and resistance. In: Peters GJ, ed. Cancer drug discovery and development: deoxynucleoside analogs in cancer therapy. Totawa, NJ: Humana Press Inc, 2006: 225–51.

53 Parker WB, Shaddix SC, Chang CH, et al. Effects of 2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5\'-triphosphate. *Cancer Res* 1991; **51:** 2386–94.

54 Bonate PL, Arthaud L, Cantrell WR Jr, Stephenson K, Secrist JA 3rd, Weitman S. Discovery and development of clofarabine: a nucleoside analogue for treating cancer. *Nat Rev Drug Discov* 2006; **5:** 855–63.

55 Kantarjian HM, Jeha S, Gandhi V, Wess M, Faderl S. Clofarabine: past, present, and future. *Leuk Lymphoma* 2007; **48:** 1922–30.

56 Parker WB, Bapat AR, Shen JX, Townsend AJ, Cheng YC. Interaction of 2-halogenated dATP analogs (F, Cl, and Br) with human DNA polymerases, DNA primase, and ribonucleotide reductase. *Mol Pharmacol* 1988; **34:** 485–91.

57 Parker WB, Shaddix SC, Rose LM, et al. Comparison of the mechanism of cytotoxicity of 2-chloro-9-(2-deoxy-2- fluoro-beta-D-arabinofuranosyl)adenine, 2-chloro-9-(2-deoxy-2-fluoro-beta-D-ribofuranosyl)adenine, and 2-chloro-9-(2-deoxy 2,2-difluoro-beta-D-ribofuranosyl)adenine in CEM cells. *Mol Pharmacol* 1999; **55:** 515–20.

58 Xie C. Metabolism and actions of 2-chloro-9-(2-deoxy-2-fluoro-beta-D- arabinofuranosyl)-adenine in human lymphoblastoid cells. *Cancer Res* 1995; **55:** 2847–52.

59 Xie KC, Plunkett W. Deoxynucleotide pool depletion and sustained inhibition of ribonucleotide reductase and DNA synthesis after treatment of human lymphoblastoid cells with 2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl) adenine. *Cancer Res* 1996; **56:** 3030–37.

60 Dumontet C, Fabianowska-Majewska K, Mantincic D, et al. Common resistance mechanisms to deoxynucleoside analogues in variants of the human erythroleukaemic line K562. *Br J Haematol* 1999; **106:** 78–85.

61 Schirmer M, Stegmann AP, Geisen F, Konwalinska G. Lack of cross-resistance with gemcitabine and cytarabine in cladribine-resistant HL60 cells with elevated 5\'-nucleotidase activity. *Exp Hematol* 1998; **26:** 1223–28.

62 Carson DA, Carrera CJ, Wasson DB, Iizasa T. Deoxyadenosine-resistant human T-lymphoblasts with elevated 5\'nucleotidase activity. *Biochim Biophys Acta* 1991; **1091:** 22–28.

63 Galmarini CM, Mackey JR, Dumontet C. Nucleoside analogues and nucleobases in cancer treatment. *Lancet Oncol* 2002; **3:** 415–24.

64 Harris BE, Song R, Soong SJ, Diasio RB. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. *Cancer Res* 1990; **50:** 197–201.

65 Spector T, Cao S, Rustum YM, Harrington JA, Porter DJ. Attenuation of the antitumor activity of 5-fluorouracil by (R)-5-fluoro-5,6-dihydrouracil. *Cancer Res* 1995; **55:** 1239–41.

66 Bergman AM, Giaccone G, Van Moorsel CJ, et al. Cross-resistance in the 2\',2\'-difluorodeoxycytidine (gemcitabine)-resistant human ovarian cancer cell line AG6000 to standard and investigational drugs. *Eur J Cancer* 2000; **36:** 1974–83.

67 Bergman AM, Pinedo HM, Jongsma AP, et al. Decreased resistance to gemcitabine (2\',2\'-difluorodeoxycytidine) of cytosine arabinoside-resistant myeloblastic murine and rat leukemia cell lines: role of altered activity and substrate specificity of deoxycytidine kinase. *Biochem Pharmacol* 1999; **57:** 397–406.

68 Bhalla K, Nayak R, Grant S. Isolation and characterization of a deoxycytidine kinase-deficient human promyelocytic leukemic cell line highly resistant to 1-beta-D- arabinofuranosylcytosine. *Cancer Res* 1984; **44:** 5029–37.

69 Ruiz van Haperen VWT, Veerman G, Eriksson S, et al. Development and molecular characterization of a 2\',2\'-difluorodeoxycytidine-resistant variant of the human ovarian carcinoma cell line A2780. *Cancer Res* 1994; **54:** 4138–43.

70 Montgomery JA, Shortnacy-Fowler AT, Clayton SD, Riordan JM, Secrist JA III. Synthesis and biologic activity of 2\'-fluoro-2-halo derivatives of 9-β-D-arabinofuranosyladenine. *J Med Chem* 1992; **35:** 397–401.

71 Löfgren C, Albertioni F, Paul C. High activity and incomplete cross resistance of nucleoside analogues cladribine and fludarabine versus Ara-C on leukemic cells from patients with AML. *Ther Drug Monit* 2005; **27:** 641–46.

72 Bronckaers A, Balzarini J, Liekens S. The cytostatic activity of fluoropyrimidine nucleosides is strongly modulated by *Mycoplasma hyorhinis* infection: implications for cancer therapy. *Biochem Pharmacol* 2008; **76:** 188–97.

73 Fukushima M, Suzuki N, Emura T, et al. Structure and activity of specific inhibitors of thymidine phosphorylase to potentiate the function of antitumor 2'-deoxyribonucleosides. *Biochem Pharmacol* 2000; **59:** 1227–36.

74 InVivoGen product information for Plasmocin. Mechanism of action, properties. http://www.plasmosin.com/mecanism.htm (accessed Feb 19, 2009).