

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}

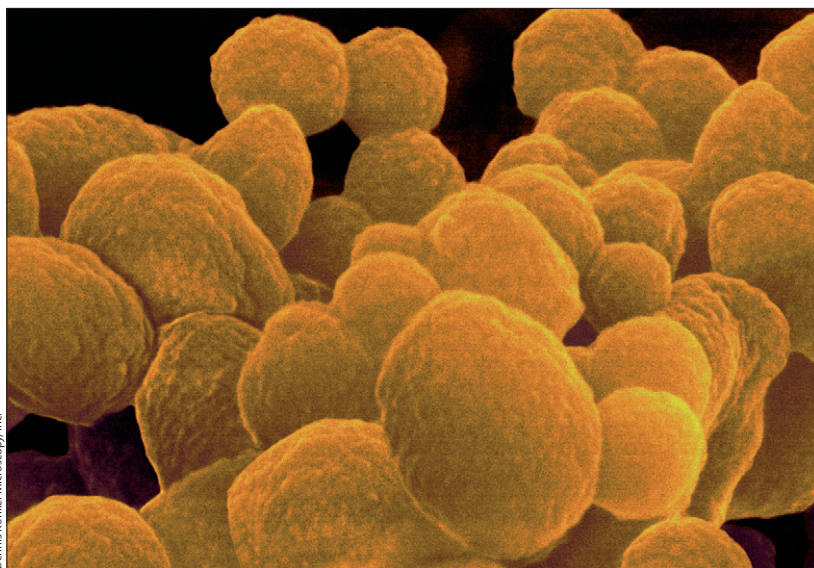
Infection by mycoplasmas is acquired through aerosol exposure and by intimate contact between mucosal

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

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.



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Figure 1: *Mycoplasma pneumoniae*

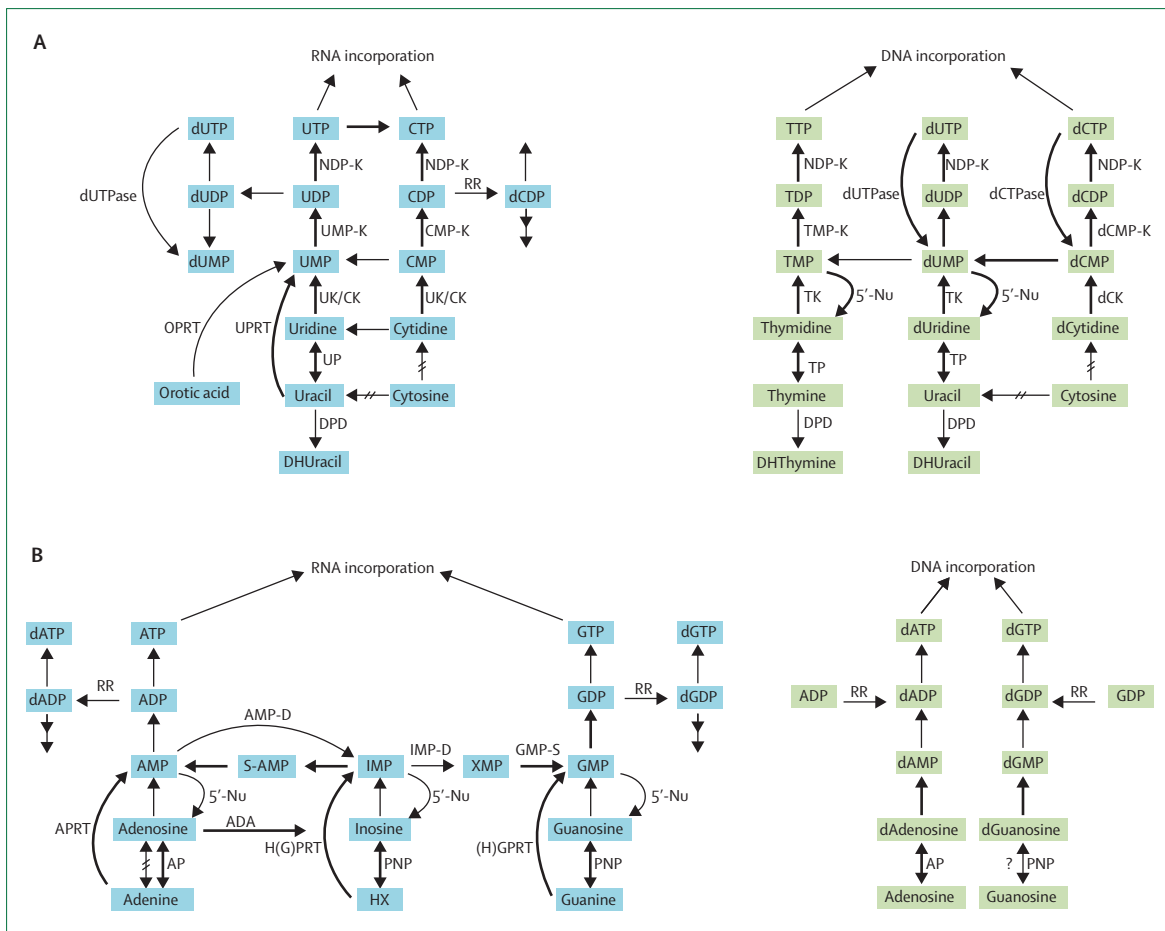


Figure 2: Enzymatic conversions in pyrimidine (A) and purine (B) ribonucleoside and 2'-deoxyribonucleoside metabolism
 Bold arrows represent enzyme activities noted in mycoplasmas; crossed arrows represent enzyme activity not present in mammalian cells. dUTPase=2'-deoxyuridine triphosphatase. dUTP=2'-deoxyuridine 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. DHTThymine=dihydrothymine. dCCTP=2'-deoxycytidine triphosphate. dCCTPase=2'-deoxycytidine triphosphatase. dCCTP=2'-deoxycytidine monophosphate. dCCTP-K=2'-deoxycytidine monophosphate kinase. dCCK=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 colleagues¹² 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

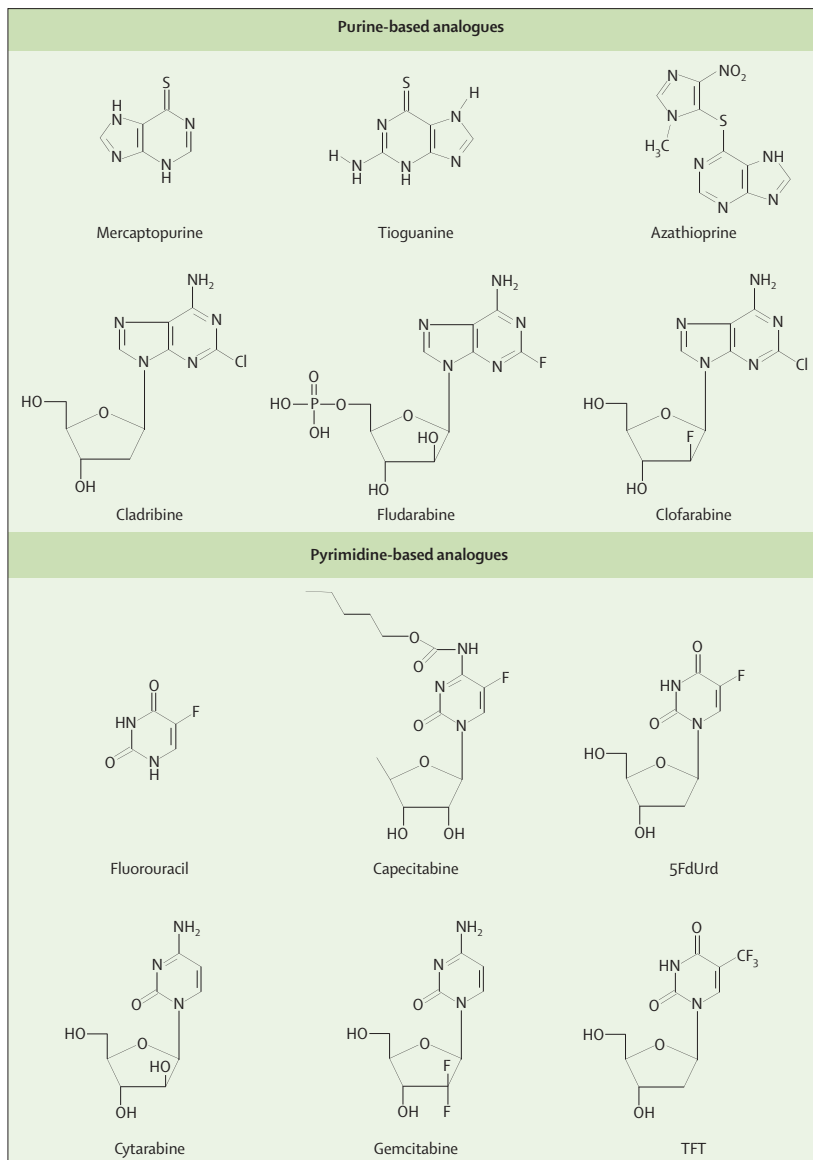


Figure 3: Structural formulae of purine and pyrimidine nucleobase and nucleoside-based anticancer drugs. 5FdUrd=5-fluorodeoxyuridine. TFT=5-trifluorothymidine.

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-workers³⁰ 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 colleagues³¹ 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 homeostasis 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 nucleoside(s)-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'-deoxyuridylylate (dUMP) and thymidylylate 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)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 lipophilum* (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.

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

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

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

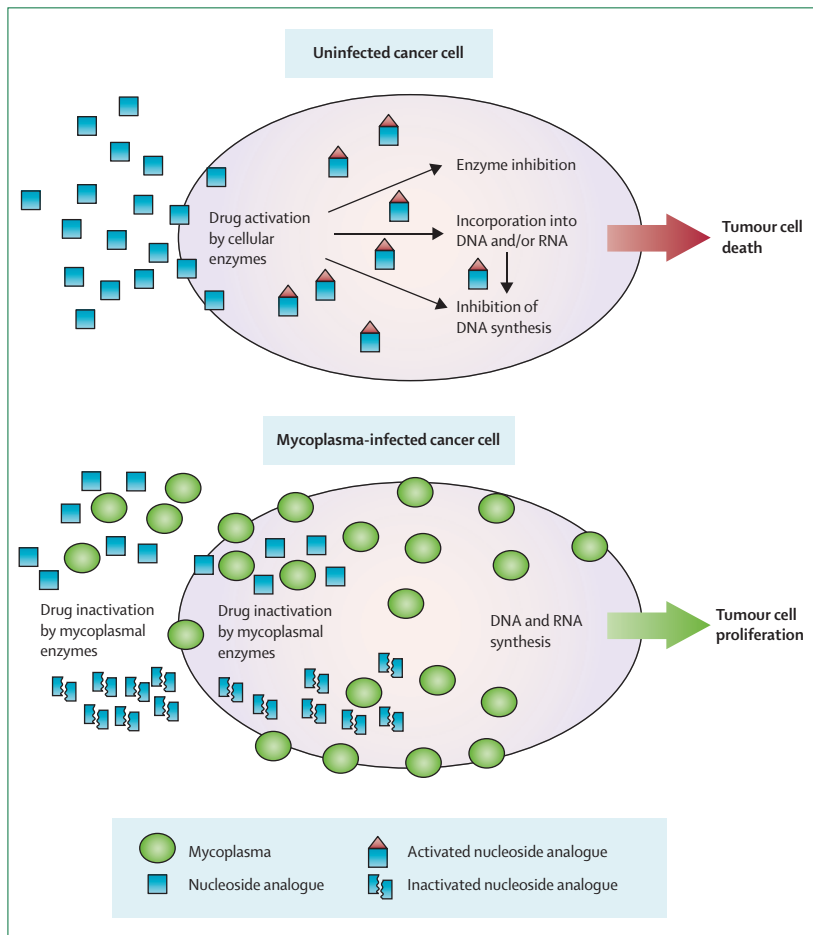


Figure 4: Schematic representation of the potential effect of mycoplasma infection on anticancer drug therapy

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 nucleoside-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 mycoplasma 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 fluoroquinolones (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 mycoplasma 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 feasible 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", "mercaptapurine", "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. Totowa, 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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