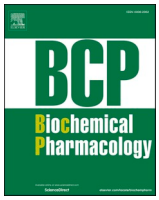
Biochemical Pharmacology 215 (2023) 115741



Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm



Review



Nucleoside-based anticancer drugs: Mechanism of action and drug resistance

Lenka Hruba [a], Viswanath Das [a], Marian Hajduch [a,b], Petr Dzubak [a,b,*]

[a] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Olomouc, Czech Republic
[b] Laboratory of Experimental Medicine, University Hospital, Olomouc 779 00, Czech Republic

ARTICLE INFO

Keywords:
Drug resistance
Cancer
Nucleosides
ABC transporters
SLC transporters
Cancer therapy
Metabolism

ABSTRACT

Nucleoside-based drugs, recognized as purine or pyrimidine analogs, have been potent therapeutic agents since their introduction in 1950, deployed widely in the treatment of diverse diseases such as cancers, myelodysplastic syndromes, multiple sclerosis, and viral infections. These antimetabolites establish complex interactions with cellular molecular constituents, primarily via activation of phosphorylation cascades leading to consequential interactions with nucleic acids. However, the therapeutic efficacy of these agents is frequently compromised by the development of drug resistance, a continually emerging challenge in their clinical application. This comprehensive review explores the mechanisms of resistance to nucleoside-based drugs, encompassing a wide spectrum of phenomena from alterations in membrane transporters and activating kinases to changes in drug elimination strategies and DNA damage repair mechanisms. The critical analysis in this review underlines complex interactions of drug and cell and also guides towards novel therapeutic strategies to counteract resistance. The development of targeted therapies, novel nucleoside analogs, and synergistic drug combinations are promising approaches to restore tumor sensitivity and improve patient outcomes.

1. Introduction

Cancer is a complex disease that affects millions of people around the world. Despite the availability of numerous treatments, curing and eradicating cancer proves challenging, largely due to the development of resistance by cancer cells to treatments. Mechanisms of chemoresistance (MOC) include several cellular alterations that significantly enhance cell survival. The first mechanism represents reduced drug uptake, particularly in the case of nucleoside-based drugs, facilitated by Concentrative Nucleoside Transporters (*CNTs/SLC28s*) and/ or Equilibrative Nucleoside Transporters (*ENTs/SLC29s*). MOC-1b refers to an increased drug efflux, which can be often mediated by MDR (multidrug resistance) proteins such as P-glycoprotein (Pgp), Multidrug Resistance-related Proteins (MRPs) or Breast Cancer Resistance Protein (BCRP), MOC-2 is characterized by a decrease in the concentration of active metabolites due to the reduction of pro-drug activation or higher active metabolite elimination. Other mechanisms include cellular changes in drug targets (MOC-3), enhanced DNA-repair mechanisms (MOC-4), decreased activity of pro-apoptotic factors (MOC-5a), and/ or increase of anti-apoptotic factors (MOC-5b), alterations in the tumor cells microenvironment (MOC-6), and activation of epithelial-mesenchymal transition (EMT) (MOC-7) [1,2]. Historically, nucleosides, compounds comprising a ribose or 2-deoxyribose sugar ring and purine (adenine and guanine) or pyrimidine (cytosine, thymine, and uracil) bases, were among the first widely used anticancer and antiviral therapeutics [3,4]. As anticancer drugs, nucleosides belong to a class of antimetabolites, a category that encompasses purine or pyrimidine analogs (nucleosides), folic acid and amino acid analogs, and ribonucleotide reductase inhibitors.[5] In 1948, the first antimetabolite, aminopterin (4-aminopteroyl-glutamic acid), was used to treat acute leukemia in children, where it was shown to induce temporary remission.[6] Nucleosides compete with physiological nucleosides and interact with a plethora of intracellular targets involved in nucleoside metabolism and signaling. The nucleoside 6-mercaptopurine (6-MP), which was synthesized in 1950 for the treatment of acute leukemia, was one of the first nucleosides to be used clinically. A few years later, structurally similar 6-thioguanine (6-TG) was synthesized, which was gradually followed by other compounds such as gemcitabine.[7,8] While the first nucleosides targeted hematological malignancies, later-developed compounds like 5-fluorouracil and gemcitabine demonstrated high efficacy in solid tumors (Fig. 1) [9].

Currently, the primary obstacles in chemotherapy include tumor cell

* Corresponding author.
E-mail address: petr.dzubak@upol.cz (P. Dzubak).

https://doi.org/10.1016/j.bcp.2023.115741
Received 8 June 2023; Received in revised form 6 August 2023; Accepted 8 August 2023
Available online 9 August 2023
0006-2952/© 2023 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

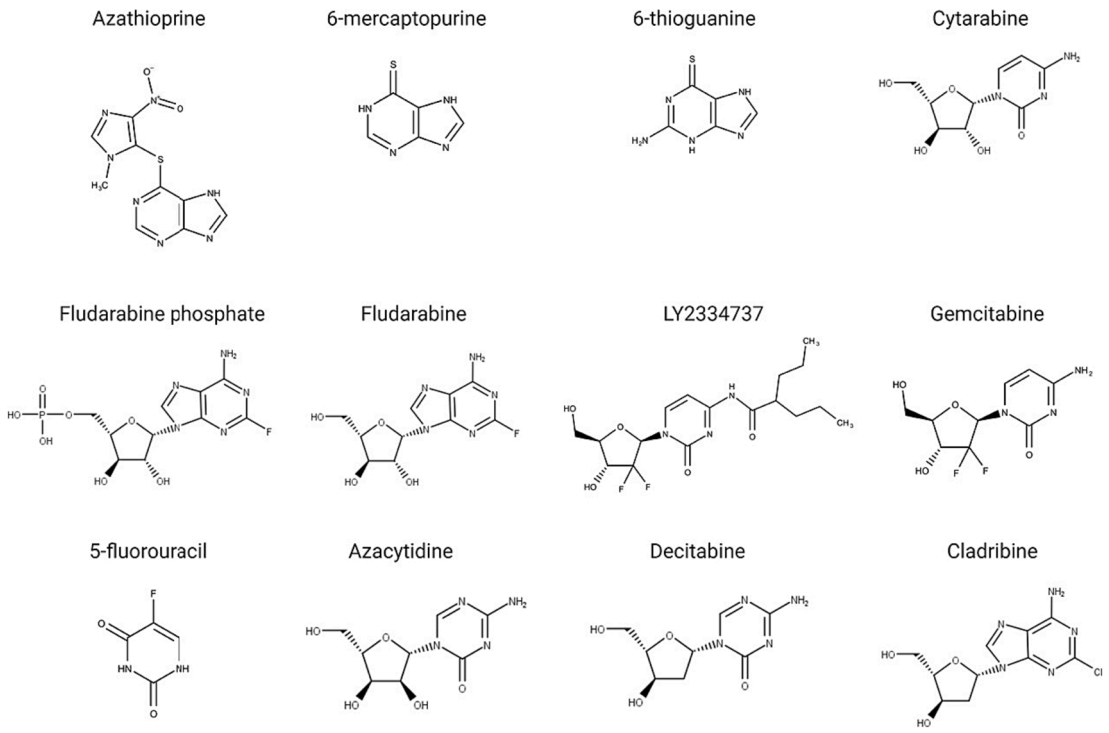


Fig. 1. Chemical structures of the most commonly used nucleoside-based anticancer drugs.

selectivity, systemic side effects, and the development of multidrug resistance due to recurrent treatment cycles, which reduce overall treatment efficacy.[10] This review summarizes the mechanisms underlying resistance of the most prevalently used nucleoside-based drugs, with a focus on drug transporters and clinically relevant resistance mechanisms, and potential strategies to overcome them.

1.1. 6-Mercaptopurine (6-MP)

6-mercaptopurine is a purine analog widely used for the treatment of acute leukemia. Its immunosuppressive properties also make it a suitable treatment option to treat autoimmune diseases and inflammatory conditions such as rheumatoid arthritis, Crohn's disease, and ulcerative colitis.[11] In clinics, 6-MP is typically administered as a pro-drug

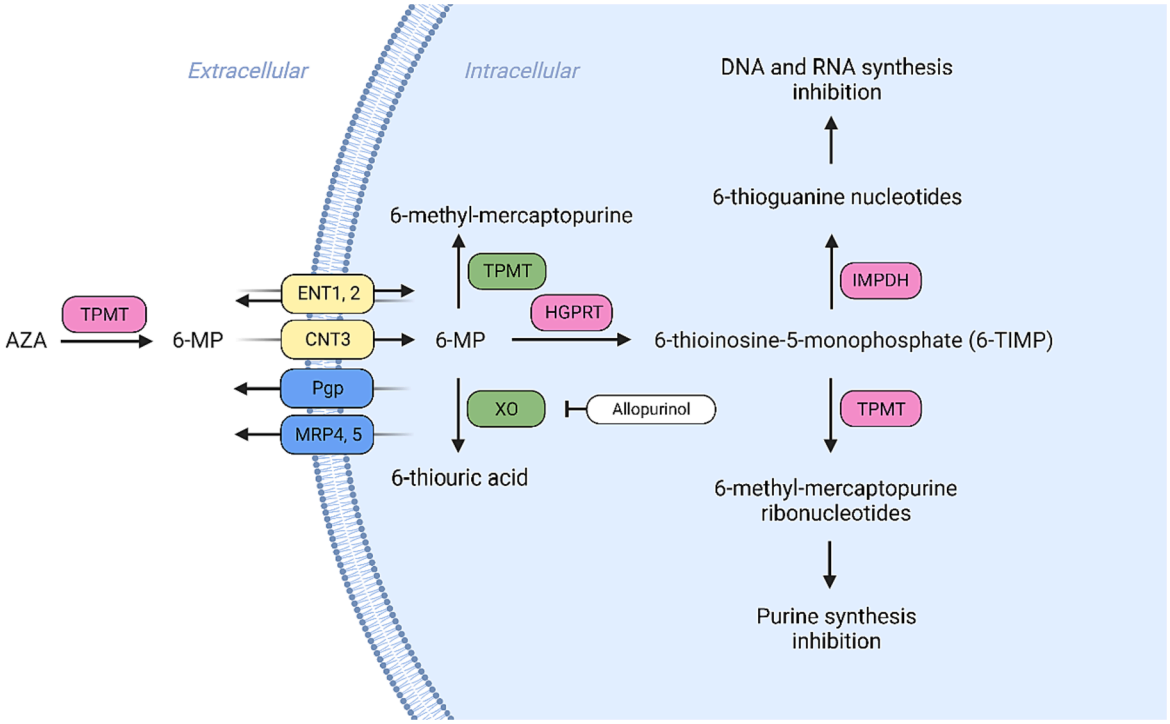


Fig. 2. Transport and metabolism of 6-mercaptopurine (6-MP). AZA: azathioprine, TPMT: Thiopurine S-methyl transferase, XO: Xanthine oxidase, HGPRT: Hypoxanthine-guanine phosphoribosyltransferase, IMPDH: Inosine-5′-monophosphate dehydrogenase.

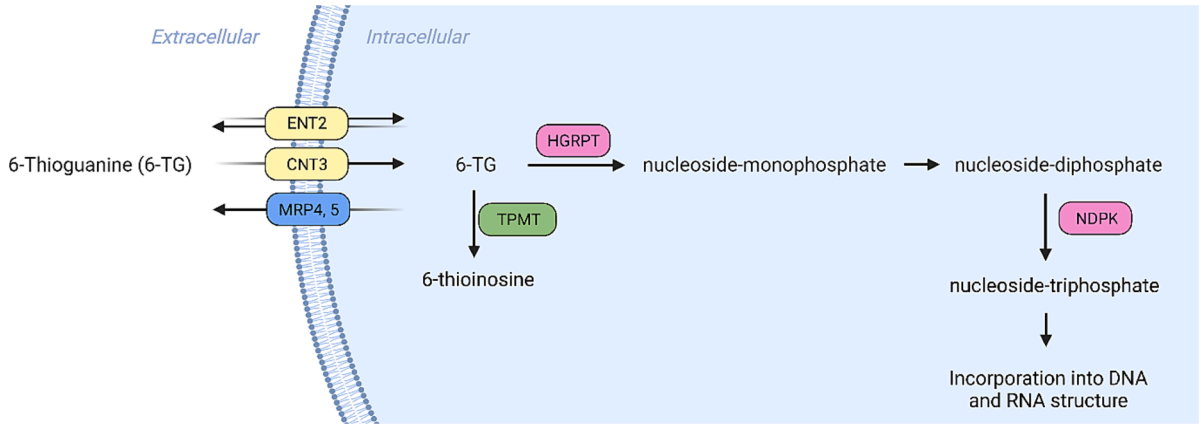


Fig. 3. Membrane transport and metabolism of 6-thioguanine (6-TG). HGRPT: Hypoxanthine-guanine phosphoribosyltransferase, TPMT: Thiopurine S-methyl transferase, NDPK: Nucleoside diphosphate kinase.

(azathioprine, AZA, Fig. 1), and both compounds are relatively slow acting, limiting their usage in monotherapy.[12] For pediatric patients with acute leukemia, 6-MP is recommended to be combined with methotrexate, resulting in a higher remission rate.[13] Recent research indicates that the addition of a low dose of 6-thioguanine (6-TG) to a 6-MP/methotrexate regimen improves disease-free survival in pediatric and adult patients newly diagnosed with acute lymphoblastic leukemia.[14] Furthermore, this combination therapy can be complemented by prednisone, which induces remission in patients with steroid-dependent inflammatory bowel disease (IBD) and Crohn's disease.[15].

The oral absorption of 6-MP is variable and incomplete, with approximately 50% of the dose entering the systemic circulation and a half-life ranging from 60 to 120 min.[16] In the case of intravenous administration, plasma half-disappearance varies from 21 min in pediatric patients to 47 min in adults. The primary catabolic pathways of 6-MP are methylation and oxidation. Other drugs, such as allopurinol, can inhibit the oxidative degradation of 6-MP by inhibition of xanthine oxidase (XO), which mediates 6-MP oxidation. However, this inhibition leads to an accumulation of 6-MP, resulting in increased toxicity without enhancing therapeutic effects.[17] Despite this, the combined therapy of allopurinol (or alternative XO inhibitor infliximab) and a low dose of thiopurines (AZA and 6-MP) has been suggested as a potential immunosuppressive strategy for treating adult patients with inflammatory bowel disease.[18] The combination of AZA/6-MP and allopurinol was initially considered controversial due to its side effects. Nonetheless, recent studies show that monitoring thiopurine metabolite levels can help adjust the dosage and reduce the adverse effects of the treatment.[19,20].

Azathioprine, the pro-drug of 6-MP,[12] is extracellularly metabolized to 6-MP by thiopurine S-methyl transferase (TPMT), and then transported into cells by nucleoside transporters.[21] Intracellulary is 6-MP converted to 6-methylmercaptopurine (6-MMP) by TPMT, and subsequently to 6-thiouric acid by xanthine oxidase, or alternatively, to 6-thioinosine 5-monophosphate (6-TIMP) via hypoxanthine–guanine phosphoribosyltransferase (HGPRT). 6-TIMP undergoes two potential metabolic pathways: 1) it can be converted into 6-methylmercaptopurine ribonucleotides (6-MMPR), which inhibits purine synthesis; or 2) it can be metabolized by inosine-5′-monophosphate dehydrogenase (IMPDH) into 6-thioguanine nucleotides, which inhibit DNA and RNA synthesis (Fig. 2) [22].

Resistance to 6-MP can develop through various mechanisms, including alterations in the expression of membrane transporters. Notably, 6-MP acts as a substrate for efflux pumps such as MDR1/P-glycoprotein (Pgp/*ABCB1*) and multidrug resistance-related proteins 4 and 5 (MRP4/*ABCC4*; MRP5/*ABCC5*). Overexpression of these efflux pumps can reduce 6-MP activity and lead to the development of a resistant phenotype.[23–26] Typically, a decrease in intracellular 6-MP concentration is associated with alterations in the expression of influx/uptake transporters, such as equilibrative nucleoside transporters (ENTs/*SLC29s*) and concentrative nucleoside transporters (CNTs/*SLC28s*). Approximately half of 6-MP transport is carried out via a sodium-dependent mechanism.[27] Moreover, 6-MP has been identified as a substrate for ENT1/*SLC29A1*, ENT2/*SLC29A2*, and CNT3/*SLC28A3*.[27] Historically, sensitivity to 6-MP has often been associated with a decrease or loss in the activity of enzymes such as inosinic or guanylic acid pyrophosphorylase. These enzymes facilitate the formation of mercaptopurine ribonucleotides in human cancer cells.[28].

An activating mutation in *NT5C2* (cytosolic 5́-nucleotidase II) has been recently discovered in approximately 20% of patients diagnosed with acute lymphoblastic leukemia (ALL). NT5C2, as a nucleotidase, dephosphorylates and inactivates HGPRT, effectively inhibiting conversion of 6-MP into its active form and reducing its therapeutic effectiveness.[29] Consequently, leukemia cells with *NT5C2* mutations develop resistance to 6-MP. Overcoming this resistance can be achieved either through direct targeting of NT5C2 or by the inhibition of compensatory pathways, which are notably active in cells with NT5C2 mutations. One such pathway involves the enhanced purine biosynthesis due to the excess export of purine nucleosides in cells with NT5C2 mutations. This leads to the activation of allosteric feedback loops, which in turn increases the sensitivity of these cells to the inhibition of IMPDH, a crucial enzyme in purine biosynthesis. By inhibiting IMPDH its possible to partially compensate for the purine nucleotide depletion caused by the NT5C2 mutation, thereby reducing the the resistance of these cells to 6-MP.This approach offers a promising alternative strategy for the treatment of ALL in patients with NT5C2 mutations [30].

Several studies have also identified a relationship between the inhibition of the mTOR pathway, particularly mTOR complex 1, and resistance to 6-MP in cases of B-cell acute lymphoblastic leukemia (B-ALL).[31] Another mechanism contributing to resistance involves the preferential metabolism of AZA into 6-MMP, particularly in patients with inflammatory bowel disease who exhibit a lower response to 6-MP. This effect appears to be dose-dependent; an increase in 6-MP results in a significant elevation in 6-MMP levels and minor changes in 6-thioguanine nucleotide concentrations [32].

Recent research has explored the genes *TPMT*, *NUDT15*, *ITPA,* and *APEX1* as potential predictive markers of 6-MP treatment efficacy in pediatric patients diagnosed with ALL. However, these markers have yet to find application in clinical settings.[33,34] Notably, variants of the *NUD15* have been associated with the onset of thiopurine-induced leukopenia in Asian patients suffering from Crohn's disease. In particular, the homozygous T/T variant has been linked to the development of early leukopenia in nearly all observed cases. Consequently, the administration of 6-MP and 6-TG treatments is strongly discouraged in these cases [35,36].

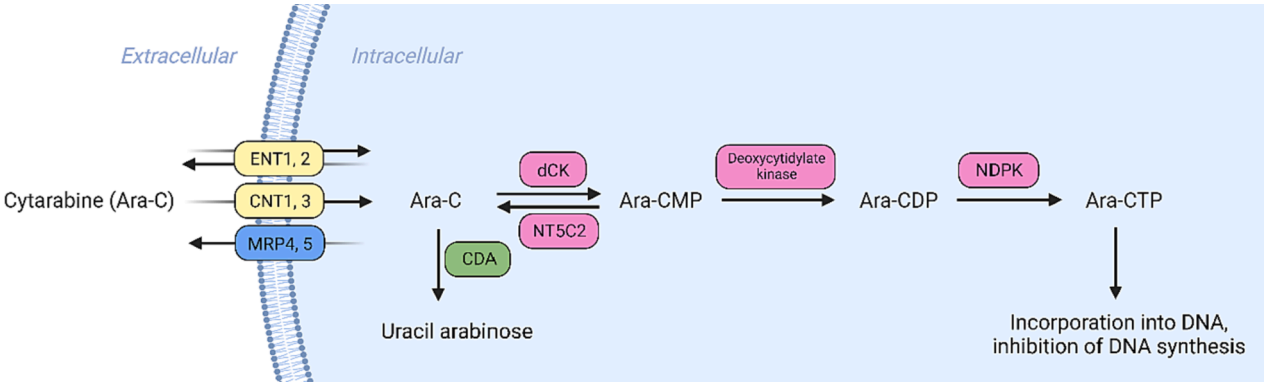


Fig. 4. Membrane transport and metabolism of cytarabine (Ara-C). Ara-CMP: Cytarabine-monophosphate, Ara-CDP: Cytarabine-diphosphate, Ara-CTP: Cytarabine-triphosphate, CDA: Cytidine deaminase, dCK: Deoxycytidine kinase, NDPK: Nucleoside diphosphate kinase.

1.2. 6-Thioguanine (6-TG)

6-Thioguanine, a metabolite structurally akin to 6-MP, can often induce cross-resistance due to its similarities. Despite this resemblance, both 6-TG and 6-MP have distinct metabolic pathways and modes of action. In instances where patients with inflammatory bowel disease (IBD) experience adverse effects from 6-MP therapy, 6-TG is often used as an alternative therapeutic agent.[37] In haemato-oncology, 6-TG, when combined with cytarabine, serves to induce remission in patients suffering from acute and chronic myeloid leukemia (ALL, CML). Preclinical studies also suggest its efficacy against prostate and pancreatic cancers.[38–41] Recent research has explored the potential of a 6-TG and disulfiram/Cu combination in inhibiting the cellular proliferation of triple-negative breast cancer. This combination seemingly disrupts DNA damage checkpoints and enhances DNA damage. [42] Furthermore, this drug combination appears to synergistically inhibit tumor-associated deubiquitinases, ubiquitin-specific protease 2 and 21 (USP2 and USP21). The activity of these enzymes is related to breast and prostate cancers, as well as hepatocellular carcinomas, making them potential targets in cancer therapy [43].

Once orally administered, 6-TG is converted into nucleoside-monophosphate by hypoxanthine–guanine phosphoribosyltransferase (HGRPT) and then phosphorylated into di- (TGDP) and tri-phosphate (TGTP) forms by nucleoside diphosphate kinase (NDPK).[44,45] Subsequently, TGTP is incorporated into the nucleic acid structure, inhibiting DNA and RNA synthesis, and leading to cellular death (Fig. 3).[46] The absorption of 6-TG by patients varies, with an average bioavailability of approximately 30% and a median half-life of 90 min. Unlike 6-MP, 6-TG metabolism does not rely on xanthine oxidase (XO), implying that a combination therapy with XO inhibitors, such as allopurinol, does not impact the activity or toxicity of the drug. 6-TG is metabolized into its inactive form, 6-thioinosine, via TPMT.[47] Pancreatic ductal adenocarcinoma cells (PDACs) exhibit low levels of TPMT, which makes 6-TG an ideal candidate for the treatment of this cancer type. The reduced inactivation via TPMT increases the efficacy of 6-TG therapy in such cases [41].

Resistance to 6-TG is often correlated with mutations in *HGPRT*, as shown in mismatch repair-deficient cell lines. These cell lines exhibit greater resistance compared to those with a fully functional DNA-repair system.[48] Mutations and inactivation of *HGPRT* impede the metabolism of 6-TG into its active form. Interestingly, such mutations have been detected in circulating human lymphocytes, with the frequency of mutated cells increasing with patient age, indicating a potential relationship between aging and mutagenesis.[49] *In vitro* experiments with melanoma cells have demonstrated that 6-TG resistant cells express approximately three times more methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme. Administration of MGMT inhibitors has been shown to enhance the sensitivity of resistant cell lines to 6-TG, suggesting a correlation between MGMT activity and resistance to 6-TG.[50] Resistance to 6-TG can also be linked to variations in the expression levels of membrane transporters. For instance, leukemia cells (MOLT-4) displaying reduced levels of ENT2 and CNT3, transporters critical to drug influx, demonstrate resistance to 6-TG.[51] It is important to note that 6-TG is also a substrate of efflux pumps, such as MRP4 and 5.[52] The relationship between MRP4 levels and 6-TG sensitivity has been observed in Japanese patients with IBD.[53] Since 6-TG is not a substrate of P-glycoprotein (Pgp), it has the potential to be used in tumors with elevated Pgp levels, such as breast and ovarian cancers with BRCA1 mutations. This could potentially overcome resistance to Poly (ADP-ribose) polymerase (PARP) inhibitors [54].

1.3. Cytarabine (Ara-C, cytosine arabinose)

Cytarabine, a cytosine analog, is a cornerstone in the treatment of ALL, Acute Non-Lymphocytic Leukemia (ANLL), the blast phase of CML, and Acute Myeloid Leukemia (AML).[55–57] The Food and Drug Administration (FDA) gave its approval to cytarabine for cancer treatment in 1969, and it has been administered in combination with other anticancer drugs to augment its efficacy since the 1990s. For example, in AML patients over 60 years, cytarabine is often used in combination with clofarabine.[58] An estimated 20–35% of AML patients harbor a mutation in the *DNMT3A* gene (DNA methyltransferase 3A), predisposing them to drug resistance (mainly to anthracyclines), a higher incidence of minimal residual disease and an increased risk of clinical relapse, particularly in an advanced-aged cohort. Yet, those carrying the DNMT3A R882 mutation exhibit heightened sensitivity to the replication stress induced by cytarabine. For patients with a wild-type variant of the *DNMT3A* gene, a combination of cytarabine and PARP inhibitors can enhance treatment efficacy [59].

Furthermore, cytarabine can be co-administered with decitabine, a combination that has shown improved prognosis in elderly patients with high-risk AML.[60] Younger and adult AML patients have also demonstrated favorable responses to the combined regimen of cytarabine, sorafenib, and idarubicine.[61,62]Recently, a combination of venetoclax with low-dose cytarabine has emerged as an effective treatment for adult AML patients who cannot undergo standard intensive chemotherapy (IC). This combined therapy can induce complete remission in 60–80 % of patients aged 16–60 years [63].

While the standard dose of cytarabine is 100–200 mg/m^2 body surface area, a higher dose (3 g/m^2 body surface area) with an elimination half-life of 3–4 h has demonstrated superior efficacy in AML treatment, although the underlying mechanism responsible for this difference remains elusive.[64,65] This high-dose cytarabine therapy is also administered to children and adolescents with ALL, ANLL, CML, and Burkitt's lymphoma. Despite serious side effects, including bone marrow suppression, nausea, vomiting, fever, and diarrhea, the increased

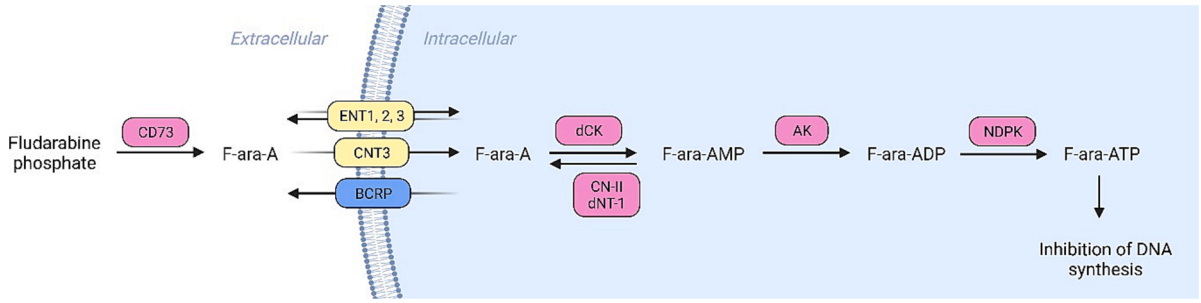


Fig. 5. Membrane transport and metabolism of fludarabine (F-ara-A). F-ara-A: Fludarabine, F-ara-AMP: Fludarabine monophosphate, F-ara-ADP: Fludarabine diphosphate, F-ara-ATP: Fludarabine triphosphate, CD73: Ecto-5́-nucleotidase, dCK: Deoxycytidine kinase, CN-II: 5́nucleotidase, dNT-1: Deoxynucleotidase-1, AK: Adenylate kinase, NDPK: Nucleoside diphosphate kinase.

cytarabine dose has proven effective in inducing remission in patients with lymphoma resistant to the standard dose [66].

Oral administration of cytarabine is less effective due to its high first-pass metabolism, with less than 20% of the drug being absorbed from the gastrointestinal tract (GIT). The primary metabolism of cytarabine into its inactive form, uracil arabinose (Ara-U), is facilitated by cytidine deaminase (CDA). Overexpression of CDA has been implicated in the development of cytarabine resistance, and it is also recognized as a valuable predictive marker of toxicity in cytarabine treatment.[67,68] Notably, lower CDA activity has been observed in adult patients suffering from severe or lethal toxicity [67].

Cytarabine is typically administered intravenously, intrathecally, or subcutaneously due to its limited absorption through the GIT. Primarily transported into cells via the ENT1 transporter, cytarabine also serves as a substrate for ENT2 CNT1 and CNT3.[69] The efflux of cytarabine is facilitated by MRP4 and 5, and the overexpression of these proteins can contribute to the development of cytarabine resistance [70,71].

Following its transport into cells, mainly via the ENT1 transporter [72], cytarabine is phosphorylated into its active form, cytarabine triphosphate (Ara-CTP). This conversion is facilitated by deoxycytidine kinase (dCK), deoxycytidylate kinase, and nucleoside diphosphate kinase (NDPK) (Fig. 4). The efficacy of cytarabine is directly related to the expression of these proteins.[64] Once activated, Ara-CTP incorporates into the nascent DNA, leading to the induction of single-strand breaks, replication fork stalling, and eventually, cell death [73].

Resistance to cytarabine can primarily emerge due to: a) rapid elimination by CDA, b) restricted intracellular transport associated with reduced ENT1 expression or c) limited activation due to inhibition or reduced expression of activating kinases, notably dCK.[39] Levin et al. (2019) documented the loss of dCK function in cytarabine-resistant acute myeloblastic cells, which exhibited hypersensitivity to a combination of hydroxyurea (HU) and azidothymidine (AZT), an approach that could potentially overcome cytarabine resistance.[74] Recent *in vitro* studies have highlighted the role of CD157 (ADP-ribosyl cyclase 2) in cytarabine sensitivity in AML. CD157 induces the expression of anti-apoptotic proteins, particularly Mcl-1, while its inhibition has been shown to enhance the efficacy of cytarabine treatment in CD157-high AML cells [75].

Cytarabine resistance is also correlated with the expression of cytosolic 5́-nucleotidase II (NT5C2), which inactivates cytarabine by dephosphorylating cytarabine monophosphate into cytarabine.[76] Furthermore, SAMHD1 (SAM and HD domain-containing protein 1), a deoxynucleotide triphosphate triphosphorylase, has been identified as a predictive marker of cytarabine treatment efficacy. SAMHD1 hydrolyzes Ara-CTP, leading to a significant reduction in cytarabine activity.[77] Inhibitors of SAMHD1, such as hydroxyurea, can suppress Ara-CTP hydrolysis and enhance the efficacy of cytarabine [78,79].

An analysis of AML primary cells resistant to cytarabine treatment has revealed a link between high mitochondrial oxidation based on Bcl2 and cytarabine resistance. Consequently, these cells exhibited high sensitivity to the combined therapy of venetoclax and cytarabine. However, adaptive resistance can occur due to changes in oxidative phosphorylation, electron transport chain complex and/or the p53 pathway. Using electron transport chain inhibitors, mitochondrial ClpP protease agonists, or pyruvate dehydrogenase inhibitors could potentially offer ways to overcome this adaptive cytarabine/venetoclax resistance [80].

Recent findings suggest a role for glucose transporters (GLUTs) in determining the sensitivity of AML cells to cytarabine. Specifically, heightened glycolysis appears to diminish the sensitivity of these cells to chemotherapy in AML. Consequently, the inhibiting of glucose uptake may enhance the cells' sensitivity to cytarabine treatment [81].

1.4. *Fludarabine (F-ara-A)*

Fludarabine is a purine analog primarily employed in the treatment of lymphoproliferative malignancies, notably chronic lymphocytic leukemia (CLL). It has also proven to be useful in managing AML.[82,83] Fludarabine can be used alone or in combination with other anticancer drugs, including cytarabine, ibrutinib, bortezomib, cyclophosphamide, rituximab, clofarabine, and idarubicin.[84–86] Recent research has revealed fludarabine's potential as an inhibitor of DNA-dependent RNA polymerase in the treating of monkeypox virus [87].

Fludarabine is typically administered as the 5-O-phosphorylated prodrug, fludarabine phosphate, and it is converted into the active form, F-ara-A, by the ectoenzyme CD73 (ecto-5́-nucleotidase, S-NT). The drug has an elimination half-life of approximately 20 h.[88] The drug enters cells mainly via ENT1, ENT2, ENT3 and CNT3, after which it is phosphorylated into mono-, di-, and active triphosphate forms F-ara-AMP, F-ara-DTP, and F-ara-ATP by deoxycytidine kinase (dCK), adenylate kinase (AK), and nucleoside diphosphate kinase (NDPK), respectively (Fig. 5).[88,89] For patients with CML, fludarabine is often used in tandem with cytarabine, as fludarabine elevates the intracellular concentration of cytarabine triphosphate and augments its activity [90].

In adults, CML is frequently associated with the overexpression of MDR proteins, such as Pgp, breast cancer resistance protein (BCRP/*ABCG2*), multidrug resistance-related proteins (MRPs), and lung resistance-related protein (LRP). Higher expression of these proteins is viewed as a negative prognostic marker. Fludarabine, which is not typically associated with MDR, has shown resistance in cases with heightened expression of Pgp, MRP1, and LRP.[91] As fludarabine is a substrate for BCRP, its treatment efficacy decreases in patients with BCRP-positive CML, which also heightens the risk of relapse [92].

Fludarabine resistance primarily presents in adult patients with CLL. A study of peripheral blood mononuclear cells (PBMCs) from fludarabine-sensitive and resistant CLL patients revealed a significant increase in the expression of Pgp, glucosylceramide synthase (GSC), and CD34, a marker of leukemic stem cells. Higher GSC expression leads to the accumulation of glucosylceramide, which fosters cell proliferation, and survival, inhibits apoptosis, and stimulates resistance development.

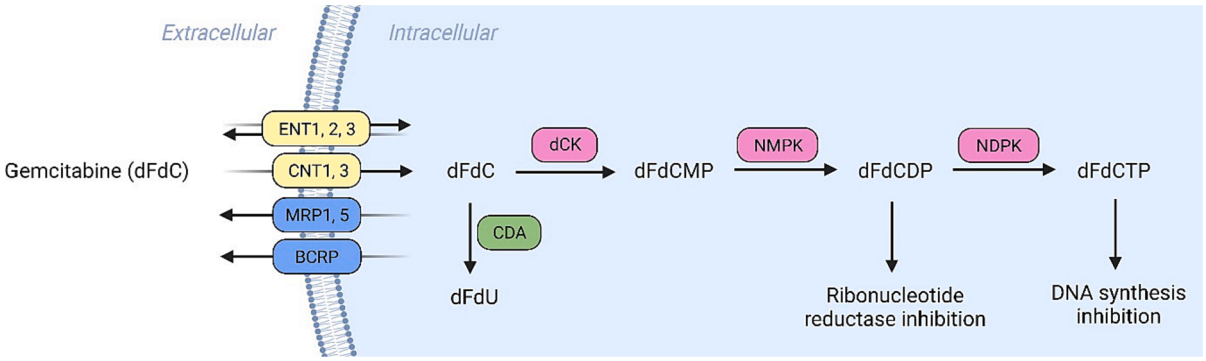


Fig. 6. Membrane transport and metabolism of gemcitabine (dFdC). dCK: Deoxycytidine kinase, CDA: Cytidine deaminase, dFdU: 2́,2́- difluoro-2́-deoxyuridine, dFdCMP: Gemcitabine-monophosphate, dFdCDP: Gemcitabine-diphosphate, dFdCTP: Gemcitabine-triphosphate, NMPK: Nucleotide monophosphate kinase, NDPK: Nucleotide diphosphate kinase.

This fludarabine resistance can be counteracted by inhibiting GSC with 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) [93].

In CLL, mitochondrial metabolism plays a key role in fludarabine sensitivity. Beyond the effect of enhanced oxidative phosphorylation and reactive oxygen species (ROS) production, recent studies have drawn a link between mitochondrial abnormalities (such as mtDNA mutations, aberrant mitochondrial biogenesis, respiration, and increased superoxide production) and a negative impact on apoptosis, which results in the development of fludarabine resistance. Moreover, malignancies showing reduced sensitivity to fludarabine seem to rely heavily on ER-Golgi protein transport. Therefore, targeting this process could present a promising therapeutic strategy that may also influence fludarabine efficacy [94–96].

CLL cell lines exhibit a more robust endoplasmic reticulum network compared to normal B-lymphocytes. Brefeldin A, an anticancer compound targeting the ER, has demonstrated its ability to induce apoptosis in fludarabine-resistant CLL cells.[94] Additionally, fludarabine resistance in CLL cell lines is linked with increased phosphorylation of ribosomal protein S6 kinase (phospho-p70S6K), activated by the mTORC1 complex implicated in translation. This mTORC1 activation is relatively prevalent across a wide range of cancers. However, mTORC1 inhibitors, including rapamycin and its analogs (rapalogs), often fail to produce the anticipated clinical effect as they only inhibit phosphatidylinositol-3-kinase (PI3K) and don't affect Akt. A compensatory surge in Akt activity has been observed, leading to a prosurvival effect. Nevertheless, in fludarabine-resistant and primary CLL cells, rapalogs succeeded in triggering cell death and inhibiting oxidative phosphorylation and glycolysis, both of which are elevated in these fludarabine-resistant cells. Thus, the inhibition of mitochondrial respiration and down-regulation of mTORC1 may prove selectively cytotoxic to fludarabine-resistant CLL cells, presenting a potential therapeutic target to counteract drug resistance [95].

CLL cells are also found to have higher levels of ROS compared to normal lymphocytes. Trachootham et al. (2008) explored a strategy to eliminate fludarabine-resistant cells using ROS and discovered that fludarabine-sensitive/resistant CLL cell lines were five times more sensitive to the anticancer and chemopreventive agent β-phenethyl isothiocyanate (PEITC) than normal lymphocytes. The exposure of fludarabine-resistant CLL cells to PEITC resulted in ROS accumulation, glutathione depletion, and the oxidation of mitochondrial cardiolipin, causing cellular death [96].

1.5. Gemcitabine (GEM, dFdC)

Gemcitabine, a deoxycytidine nucleoside analog, is deployed in the treatment of solid tumors, including breast, non-small cell lung, pancreatic, bladder, and ovarian cancers.[97–99] Its more efficient phosphorylation and activation, along with slower elimination compared to other nucleoside drugs, make gemcitabine one of the most effective nucleoside-based anticancer drugs. The half-life of gemcitabine after a brief infusion ranges from 32 to 94 min and is extended to 4 to 10 h with a longer infusion. Innovative strategies are being explored, utilizing gemcitabine in the form of a pro-drug, LY2334737, as its valproic ester [100,101].

Gemcitabine is actively transported into cells, primarily via ENT1 (SLC29A1) and CNT3 (SLC28A3). Once inside the cell, gemcitabine is phosphorylated into gemcitabine-monophosphate (dFdCMP) by deoxycytidine kinase (dCK), gemcitabine-diphosphate (dFdCDP) by nucleotide monophosphate kinase (NMPK), and gemcitabine-triphosphate (dFdCTP), the active metabolite accountable for the cytotoxic effect of gemcitabine, by nucleotide diphosphate kinase (NDPK).[102] Gemcitabine-diphosphate inhibits ribonucleotide reductase (RR), which is responsible for deoxyribonucleotide synthesis. In contrast, gemcitabine-triphosphate disrupts DNA synthesis (Fig. 6).[103,104] An portion of the incorporated triphosphate nucleosides is eliminated by the 3́-5́exonuclease. As detailed by Yang et al. (2020), gemcitabine mono/di/triphosphates can inhibit the 3́-5́exonuclease and increase the incorporation of gemcitabine-triphosphates [105].

The conversion of gemcitabine into 2́,2́-difluoro-2́-deoxyuridine (dFdU) is mediated by cytidine deaminase, which also plays a crucial role in the clearance of gemcitabine [106].

Though gemcitabine often prompts an excellent initial response, drug resistance tends to develop over time. This resistance is primarily associated with factors such as increased efflux/reduced influx (primarily via ENT1), alterations in drug metabolism, inhibition of apoptosis, changes in micro-RNA levels, and the presence of cancer stem cells (CSCs) with heightened expression of efflux pumps.[107] CDA is a protein involved in the metabolic deactivation of cytidine analogs, encompassing not only gemcitabine but also cytarabine, azacytidine, and decitabine. The expression level of CDA could serve as a reliable marker for predicting the efficacy of these analog treatments, alongside the expression level of ENT1, the primary transporter for gemcitabine influx and dCK, which mediates gemcitabine activation [108,109].

Various transporters, predominantly ENTs that function as both influx and efflux pumps, as well as ABC transporters, especially MRP5, are involved in gemcitabine efflux. The latter is associated with multidrug resistance.[110] Although the relationship between Pgp expression and gemcitabine resistance remains unclear, over-expression of Pgp seems to heighten the cells' sensitivity to gemcitabine.[111].

Recent studies have suggested an association between alterations in the Erk/Akt/Stat3, Hedgehog (Hh), Wnt and Notch pathways and the development of gemcitabine resistance.[112] Specifically, the Erk/Akt/Stat3 pathway, activated by cyclin L1 (CCNL1), promotes cell survival, thereby facilitating the development of gemcitabine resistance [113].

Gemcitabine resistance has also been linked to the Hedgehog pathway (Hh), particularly in patients with urothelial carcinoma. In

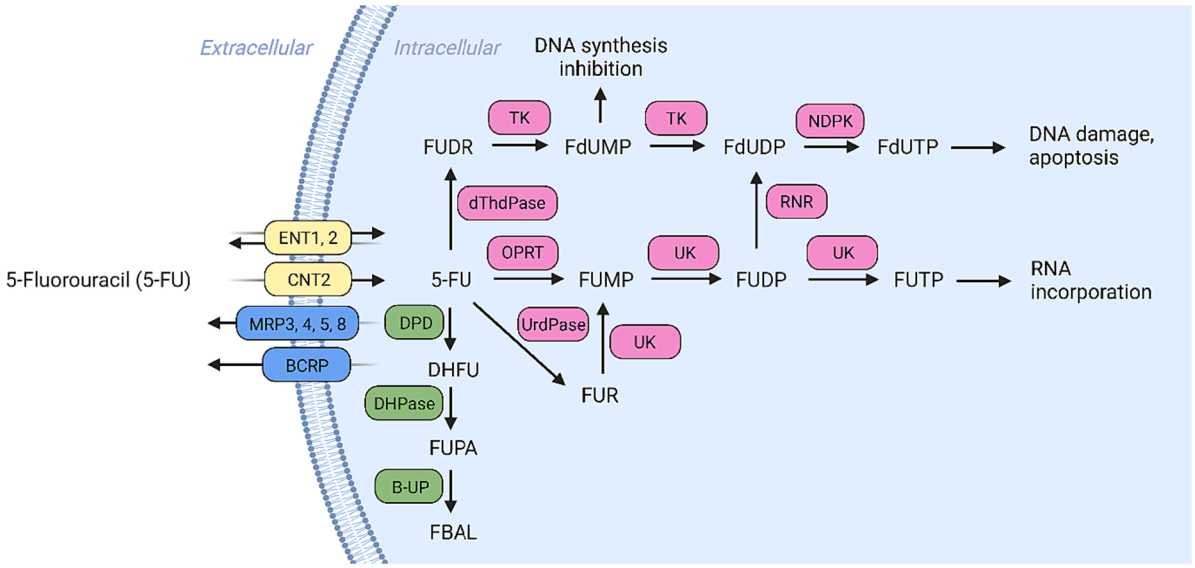


Fig. 7. Membrane transport and metabolism of 5-fluorouracil (5-FU). FBAL: α-fluoro-β-alanine, β-UP: β-ureidopropionase, FUPA: α-fluoro-β-ureido propionic acid, DHPase: Dihydropyrimidinase, DHFU: 5,6-dihydro-5-fluorouracil, DPD: Dihydropyrimidine dehydrogenase, UrdPase: Uridine phosphorylase, FUR: Fluorouridine, UK: Uridine kinase, OPRT: Orotate phosphoribosyltransferase, FUMP: Fluorouridine monophosphate, FUDP: Fluorouridine diphosphate, FUTP: Fluorouridine triphosphate, dThdPase: Thymidine phosphorylase, FUDR: Fluorodeoxyuridine, TK: Thymidine kinase, FdUMP: Fluorodeoxyuridine monophosphate, FdUDP: Fluorodeoxyuridine diphosphate, NDPK: Nucleoside diphosphate kinase, FdUTP: Fluorouridine triphosphate.

these cases, gemcitabine induces a Gli2-dependent activation of the Hh pathway, which in turn fosters cell migration and invasion, enhancing the resistant phenotype. Consequently, a potential strategy to reduce gemcitabine resistance in these patients could involve targeting the Gli2 pathway.[114] The Hh pathway also plays a significant role in the tumorigenesis of pancreatic adenocarcinoma (PC). However, clinical trials involving patients with PC treated with vismodegib, an Hh pathway inhibitor, alongside gemcitabine, have not demonstrated any significant differences in treatment efficacy when compared to a control group.[115].

The Wnt pathway, featuring β-catenin as a crucial protein in its signaling cascade, also contributes to gemcitabine resistance. Studies focused on the correlation between β-catenin expression and the efficacy of gemcitabine treatment in pancreatic carcinoma have shown that patients with lower β-catenin expression demonstrated enhanced sensitivity to gemcitabine and significantly longer disease-free and overall survival compared to those presenting high β-catenin expression [116].

Gemcitabine resistance has also been associated with dysregulation of the Notch pathway, frequently upregulated in pancreatic and ovarian cancer.[117,118] An increased expression of Notch3 is associated with reduced sensitivity to gemcitabine, which is mediated by the activation of the PI3K/Akt pathway. Consequently, a decrease in Notch3 expression reduces PI3K/Akt activity, thereby inducing apoptosis in resistant cells.[119].

Research has indicated that pancreatic cancer is not only associated with alterations in the Hh, Wnt, and Notch pathways but that gemcitabine-resistant cells also demonstrate an increase in tumor stemness.[107] While the exact mechanism remains somewhat unclear, the induction of pancreatic cell stemness seems to result from the activation of the AKT/Notch1 pathway. Hypoxia further augments the activation of this pathway, thereby promoting the development of a resistant phenotype [120].

1.6. 5-Fluorouracil (5-FU)

5-fluorouracil ranks among the earliest nucleoside-based anticancer drugs, together with 6-MP and 6-TG. First synthesized in 1957, it has since been employed in the treatment of patients with breast, colon, or head and neck cancers [121] 5-FU, an analog of uracil, is used both as a monotherapy and in combination with other anticancer drugs, such as methotrexate, leucovorin, baicalin, and oxaliplatin in order to enhance treatment efficacy [122–124] The combined use of 5-FU and curcumin has also demonstrated promising results in sensitizing 5-FU-resistant tumor cells.[125] Both *in vitro* and *in vivo* studies have provided evidence that tazemetostad (an inhibitor of the histone methyltransferase EZH2) can increase sensitivity to 5-FU treatment in cases of colorectal cancer [126].

5-FU is administered intravenously, and approximately 80%–85% of the given dosage is rapidly catabolized and deactivated (with an elimination time between 8 and 20 min) by dihydropyrimidine dehydrogenase (DPD) into 5,6-dihydro-5-fluorouracil (DHFU). Subsequently, it is processed by dihydropyrimidinase (DHPase) into α-fluoro-β-ureido propionic acid (FUPA), and then by β-ureidopropionase (β-UP) into α-fluoro-β-alanine (FBAL). The remaining molecules are mainly metabolized into fluorouridine monophosphate (FUMP) by orotate phosphoribosyltransferase (OPRT), with phosphoribosyl pyrophosphate acting as a cofactor. FUMP can also be indirectly synthesized from fluorouridine (FUR) by uridine kinase (UK).[127].

FUMP is phosphorylated by UK into fluorouridine diphosphate (FUDP), which can then be phosphorylated by UK into fluorouridine triphosphate (FUTP) and subsequently incorporated into the RNA structure. Alternatively, FUDP can be converted by ribonucleotide reductase (RNR) into fluorodeoxyuridine diphosphate (FdUDP) [128,129].

Additionally, 5-FU can be activated by thymidine phosphorylase (dThdPase) into fluorodeoxyuridine (FUDR).[127] FUDR is phosphorylated by thymidine kinase (TK) into fluorodeoxyuridine monophosphate (FdUMP), which inhibits thymidylate synthase (TS), a key enzyme in the synthesis of 2́-deoxythymidine-5́-monophosphate (dTMP). Enhancing or prolonging TS inhibition could potentially improve 5-FU treatment efficacy.[129–131] FdUMP also contributes to 5-FU myelotoxicity and cytotoxicity in the GIT.[127] FdUMP can be further phosphorylated by TK into FdUDP, which is then phosphorylated by nucleoside diphosphate kinase (NDPK) into FdUTP, another active metabolite of 5-FU that can cause DNA damage and apoptosis (Fig. 7). [132].

The limitations of 5-FU therapy include dose-dependent toxicity and the development of multidrug resistance. Several mechanisms have been

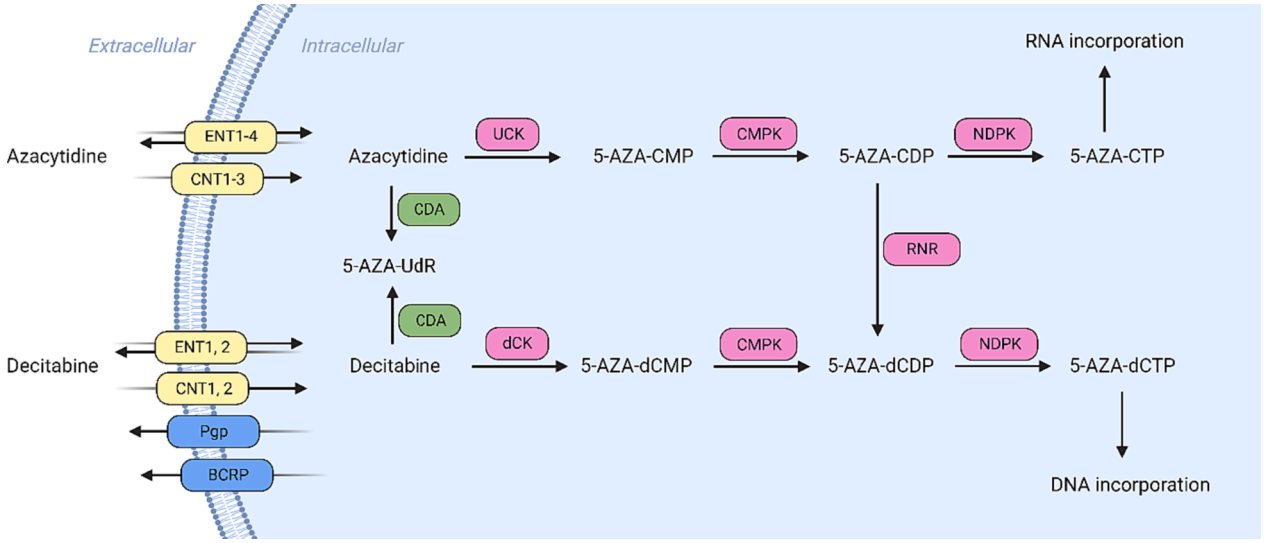


Fig. 8. Membrane transport and metabolism of azacytidine and decitabine. 5-AZA-UdR: 5-AZA-2́-deoxyuridine, CDA: Cytidine deaminase, UCK: Uridine-cytidine kinase, dCK: Deoxycytidine kinase, 5-AZA-CMP: 5-azacytidine-monophosphate, CMPK: Cytosine monophosphate kinase, 5-AZA-CDP: 5-azacytidine-diphosphate, NDPK: Nucleoside diphosphate kinase, 5-AZA-CTP: 5-azacytidine-triphosphate, 5-AZA-dCMP: 5-AZA-2́-deoxycytidine-monophosphate, 5-AZA-dCDP: 5-AZA-2́-deoxycytidine-diphosphate, 5-AZA-dCTP: 5-AZA-2́-deoxycytidine-triphosphate.

identified that contribute to altered sensitivity to 5-FU treatment. These include modifications of drug influx/efflux pumps, accelerated degradation or limited activation, and a deficiency in dihydropyrimidine dehydrogenase which can cause fatal 5-FU toxicity and alterations in various genes and molecules. These include the insulin-like growth factor-1 receptor, epidermal growth factor receptor, nuclear factor kappa B, cyclooxygenase-2, activator of transcription 3, the anti-apoptotic Bcl-2, and pro-apoptotic Bax proteins.[123,125,133] The expression of thymidylate synthase (TS) has also been recognized as one of the most important predictive markers of 5-FU sensitivity in cancer treatment. Higher TS expression is known to decrease 5-FU sensitivity. [131,134].

The overexpression of Nrf3 (nuclear factor erythroid 2-like 3) and Bcl-2 has been observed in 5-FU-resistant tissues, with Nrf3 found to promote Bcl-2 expression.[135] Another factor contributing to 5-FU resistance is the hypermethylation of the *MLH1* gene promoter. This hypermethylation inhibits MLH1 expression, which is involved in DNA mismatch repair (MMR). Cells with hypermethylated *MLH1* resist various anticancer drugs, including 5-FU. *In vitro* experiments have shown that this resistance can be overcome by the re-expression of MLH1 via the demethylation of the promoter region by decitabine. [136].

FOXM1 is another transcription factor involved in 5-FU resistance. FOXM1 is essential for DNA repair, angiogenesis, and metastasis, and the knockout of FOXM1 results in increased sensitivity to 5-FU treatment.[137,138] These studies have also highlighted an association between cancer stem cells (CSCs) and alterations in anticancer drug sensitivity. CSCs, being quiescent and poorly differentiated populations, may evade 5-FU treatment targeting cell proliferation.[137].

The disruption of the p53 pathway is a predictive marker of the efficacy of combination therapy with 5-FU and oxaliplatin in patients with gastric cancer. Defects in this pathway are associated with a poorer response to treatment.[139].

The transport of 5-FU across the cell membrane is mediated by various transporters, including ABC transporters and nucleoside transporters. A study investigating 5-FU-resistant pancreatic carcinoma cells revealed a significant increase in the expression of MRP3, 4, and 5. Furthermore, the silencing of MRP5 was found to increase sensitivity to 5-FU.[140] An association between MRP5 overexpression and 5-FU resistance has been reported in both colon and breast cancers. [141,142] An analysis of small-cell lung cancer cells resistant to 5-FU demonstrated an approximately 25-fold increase in MRP8 expression. Interestingly, the alteration in MRP8 expression was induced by 5-FU treatment. The expression level of MRP8 is directly correlated with active 5-FU efflux and resistance development.[143] Overexpression of both MRP5 and MRP8 has been observed in colorectal cancer patients. [142].

While Pgp does not transport 5-FU, *in vitro* studies using 5-FU-resistant gastric cancer cell lines have shown that increased Pgp expression can lead to cross-resistance to taxanes such as paclitaxel and docetaxel. [144].

5-FU is a substrate of the breast cancer resistance protein (BCRP), which is primarily associated with drug resistance in breast cancer. Therefore, BCRP expression could potentially serve as another

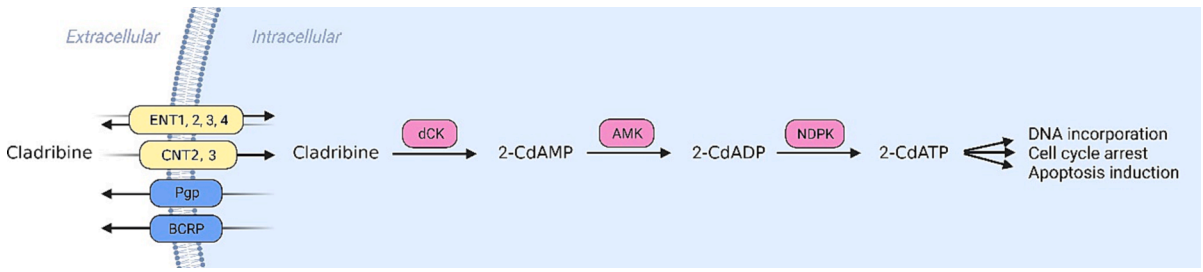


Fig. 9. Membrane transport and metabolism of cladribine. 2-CdAMP: Cladribine-monophosphate, dCK: Deoxycytidine kinase, 2-CdADP: Cladribine-diphosphate, AMK: Adenosine-monophosphate kinase, 2-CdATP: Cladribine-triphosphate, NDPK: Nucleoside diphosphate kinase.

Table 1
Nucleoside-based drugs and their predictive markers.

Drug	Disease	Predictive markers	Clinical relevance	References
5-fluorouracil	Colon cancer	ERCC1, PARP-1, AQP1	ERCC1 positivity, high expressions of PARP-1, and negative expression of AQP1 are associated with tumor progression	[203]
	Gastric cancers	p53	A defective p53 signaling pathway is associated with a worse response to combined 5-FU and oxaliplatin treatment	[139]
	Solid tumors	DPD	A DPD deficiency can cause fatal 5-FU toxicity	[133]
		TS	Lower expression/activity of TS increases 5-FU treatment efficacy	[131]
6-mercaptopurine	ALL	TPMT, NUD15, ITPA, and APEX1	Currently studied candidates for predictive markers of 6-MP therapy	[33]
	IBD	NUD15	T/T homozygous variant of *NUDT15* is strongly associated with thiopurine-induced leukopenia in Asian patients	[35,36]
6-thioguanine	IBD	HGPRT	A mutation or inactivation of *HGPRT* blocks the activating metabolism of 6-TG	[49]
		MRP4	Overexpression of the MRP4 efflux pump is associated with decreased 6-TG efficacy	[53]
		NUD15	T/T homozygous variant of *NUDT15* is strongly associated with thiopurine-induced leukopenia in Asian patients	[35,36]
	Melanoma	MGMT	DNA repair enzyme, which is overexpressed in 6-TG resistant cells	[50]
Azacytidine	Hematologic malignancies	CDA	Lower expression is associated with higher treatment efficacy	[157]
		UCK	Lower expression leads to lower azacytidine activation	[157]
Cladribine	Hematologic malignancies, MS	dCK	Cladribine activating kinase; lower expression decreases treatment efficacy	[176]
Cytarabine	AML	CDA	Overexpression causes cytarabine resistance, and lower activity is associated with severe/lethal toxicity	[67]
		dCK	Ara-C activating kinase; loss of dCK function leads to Ara-C resistance	[50]
		ENT1	Lower expression decreases the concentration of intracellular cytarabine	[72]
		NT5C2	It inactivates Ara-C by dephosphorylating Ara-C monophosphate to Ara-C and decreases cytarabine treatment efficacy	[76]
		SAMHD1	Deoxynucleotide triphosphate triphosphorylase, which hydrolases Ara-CTP and decreases Ara-C treatment efficacy	[77]
Decitabine	Hematologic malignancies	CDA	Higher expression leads to faster elimination of decitabine	[157]
		dCK	dCK phosphorylates decitabine into its active form; lower expression decreases decitabine treatment efficacy	[157]
Fludarabine	CLL	GSC	GSC overexpression leads to the accumulation of glucosylceramide, promoting proliferation, cell survival, apoptosis inhibition, and development of fludarabine resistance	[93]
	CML	BCRP	There is a decrease in fludarabine treatment efficacy in patients with BCRP-positive CML, and higher relapse risk	[92]
Gemcitabine	Solid tumors	CDA	This protein is involved in gemcitabine inactivation; a lower expression can lead to toxicity	[109]
		dCK	The gemcitabine activating kinase, the inhibition/lower expression of which decreases treatment efficacy	[109]
		ENT1	The primary gemcitabine influx transporter; a higher expression is associated with increased sensitivity to gemcitabine	[109]

Table 2
Nucleoside-based drugs as binding partners of MDR proteins and substrates for transporters: P-glycoprotein (Pgp), multidrug resistance-associated proteins (MRPs), lung resistance-related protein (LRP), breast cancer resistance protein (BCRP), and nucleoside transporters: equilibrative nucleoside transporters (ENTs) and concentrative nucleoside transporters (CNTs).

Nucleoside-based drug	Pgp	MRPs	LRP	BCRP	ENTs	CNTs	Ref.
5-fluorouracil	No	MRP3, 4, 5, 8	Yes	Yes	ENT1, 2	CNT2	[140,141,143–148]
6-mercaptopurine	Yes	MRP4, 5	Nd	Yes	ENT1, 2	CNT3	[23–27,198]
6-thioguanine	No	MRP4, 5	Nd	Nd	ENT2	CNT3	[51,52]
Azacytidine	Nd	Nd	Nd	Nd	ENT 1, 2, 3, 4	CNT1, 2, 3	[158]
Cladribine	Yes	No	Nd	Yes	ENT1, 2, 3, 4	CNT3	[64,173,177]
Cytarabine	No	MRP4, 5	Nd	No	ENT1, 2	CNT1, 3	[69–72,147,199]
Decitabine	Yes	Nd	Nd	Yes	ENT1, 2	CNT1, 2	[158,167,168]
Fludarabine	No	Nd	Nd	Yes	ENT1, 2, 3	CNT3	[198,200]
Gemcitabine	No	MRP1, 5	Nd	Yes	ENT1, 2, 3	CNT1, 3	[102,110,202,202]

Nd: Not described.

prognostic marker of 5-FU treatment efficacy.[145] Research has shown that the lung resistance protein (LRP), also known as the human major vault protein (MVP), is involved in the development of resistance to 5-FU.[146] 5-FU uptake is mediated mainly via nucleoside transporters, specifically ENT1, ENT2, and CNT2. The expression levels of these transporters could potentially help predict the efficacy of 5-FU treatment. However, resistance to 5-FU is typically associated with low expression or inhibition of these transporters.[147–149].

1.7. 5-Azacytidine

5-Azacytidine, a pyrimidine analog of cytidine, was firstly synthesized in 1963. Since then, azacytidine has primarily been used in the treatment of AML, chronic myelomonocytic leukemia, and myelodysplastic syndrome (MDS), which transforms into leukemia in approximately 30% of patients.[150,151].

Azacytidine can be administered both as a monotherapy and in combination therapy. In adult patients, azacytidine, when combined with venetoclax, has shown promising results for the treatment of AML.[152] This therapeutic combination also appears to be effective in managing high-risk MDS or chronic myelomonocytic leukemia.[153] The bioactivation process of azacytidine begins with its phosphorylation by the uridine-cytidine kinase (UCK) into 5-azacytidine-monophosphate (5-AZA-CMP). This compound is then phosphorylated by the cytosine nucleoside monophosphate kinase (CMPK) into 5-azacytidine-diphosphate (5-AZA-CDP). Subsequently, the nucleoside diphosphate kinase (NDPK) phosphorylates this diphosphate into 5-azacytidine-triphosphate (5-AZA-CTP), which is incorporated into RNA.[154] Azacytidine exhibits a relatively short elimination half-life, approximately 41 min, and its metabolism is similar to that of decitabine.[151] 5-AZA-CDP can be further metabolized by the ribonucleotide reductase (RNR) into 5-AZA-dCDP, which represents a phosphorylated form of decitabine (Fig. 8).[155] The inactivation of azacytidine can occur either by spontaneous hydrolysis or by the action of the cytidine deaminase (CDA), which metabolizes azacytidine into 5-AZA-2́-deoxyuridine (5-AZA-UdR).[154].

Resistance to azacytidine can arise from various factors, such as the decreased activation of kinases (particularly uridine-cytidine kinase 2 [UCK2]), diminished expression levels of CDA, alterations in the pyrimidine metabolism pathway, or the expression of specific membrane transporters.[156,157] Although azacytidine is transported by ENT1-4 and CNT1-3, CNT3 seems to play a pivotal role.[158] Consequently, a reduction in expression or inhibition of these transporters may diminish the efficacy of azacytidine. While no direct correlation has been reported between azacytidine resistance and Pgp over-expression, cell-based experiments have revealed an enhancement in azacytidine efficacy following the inhibition of ABC transporters (Pgp and MRPs) by erlotinib.[159,160].

Next-generation sequencing of patients with AML and MDS treated with azacytidine has uncovered numerous genetic mutations. However, none of these were significantly associated with treatment response. The only clinical marker linked with shorter survival was a platelet count of less than 50,000/μl.[161] The expression of UCK, which phosphorylates azacytidine into its active form, and CDA, which is responsible for the deactivation and elimination of azacytidine, could potentially serve as predictive markers for azacytidine treatment efficacy.[157].

1.8. Decitabine (5-AZA-2́-deoxycytidine, 5-AZA-CdR)

Decitabine is a compound structurally similar to azacytidine, synthesized a year later in 1964. Like azacytidine, decitabine is indicated for the treatment of adult patients diagnosed with AML and MDS.[151,162] Decitabine is used as a monotherapy or in combination with talacotuzumab for AML patients, and with cedazuridine for patients suffering from MDS or CMML.[163].

Decitabine is administered intravenously and transported into cells primarily via ENT1 and CNT1, and to a lesser extent, via ENT2 and CNT2.[158] Within the cell, decitabine is phosphorylated into 5-AZA-2́-deoxycytidine-monophosphate (5-AZA-dCMP) by deoxycytidine kinase (dCK). Following this, 5-AZA-dCMP undergoes phosphorylation by cytosine monophosphate kinase (CMPK) to form diphosphate (5-AZA-dCDP) and subsequently into triphosphate (5-AZA-dCTP) by nucleoside diphosphate kinase (Fig. 8).[155] The 5-AZA-2́-deoxycytidine triphosphate (5-AZA-dCTP) is incorporated into DNA, thereby inhibiting DNA methylation.[164] The half-life of decitabine ranges between 37 and 47 min.[151].

Our understanding of the mechanism behind decitabine resistance remains incomplete. However, in cases of MDS, non-responders have been observed to exhibit higher expression of CDA compared to responders, leading to increased elimination of decitabine.[165] Decitabine treatment efficacy is also related to the expression of dCK, which plays a role in its activating phosphorylation. Lower expression levels of this kinase may contribute to reduced efficacy of decitabine treatment.[166] Both decitabine and azacytidine resistance have been linked with alterations in the pyrimidine metabolism pathway.[157] Although decitabine is a substrate for BCRP protein, over-expression of this protein has not been reported in decitabine-resistant cell lines.[159,167] Decitabine is a poor Pgp substrate, and *in vitro* studies show, that decitabine treatment decreases Pgp expression leading to increasing sensitivity to drugs, which are Pgp substrates.[168].

1.9. Cladribine (2-CdA)

Cladribine, an analog of deoxyadenosine, is used to treat multiple sclerosis (MS) and hairy cell leukemia.[169,170] Cladribine is a prodrug and is metabolized into its active form, cladribine-triphosphate. The oral bioavailability of cladribine varies from 37% to 51%, but reaches 100% when administered subcutaneously.[171] The drug has an elimination half-life of approximately 10 h.[172] Cladribine enters cells via ENT1, 2, 3, 4, CNT2, and 3,[173] and is subsequently

phosphorylated by deoxycytidine kinase (dCK) into cladribine-monophosphate (2-CdAMP). Following this, 2-CdAMP is phosphorylated by adenosine-monophosphate kinase (AMK) into cladribine-diphosphate (2-CdADP), and then by nucleoside diphosphate kinase (NDPK) into cladribine-triphosphate (2-CdATP) (Fig. 9).[174] Upon integration into DNA, 2-CdATP inhibits ribonuclease reductase and arrests the cell cycle. Cladribine also induces apoptosis in non-dividing cells.[169].

The resistance to cladribine is multifaceted, attributed to factors such as increased activity of 5́-nucleotidase, which dephosphorylates cladribine-monophosphate into its inactive form, decreased activity of the activating dCK, or alterations in the apoptotic pathway.[175,176] Cladribine is a substrate of Pgp and BCRP but not MRPs, and elevated expression of these efflux pumps may adversely impact cladribine's efficacy.[172,173,177].

2. Current strategies to overcome nucleoside-based drug resistance

The development of drug resistance is a multifactorial process, including cellular changes improving cancer cell viability and proliferation.[10] Drug resistance can be connected with the altered expression of enzymes involved in the metabolic pathways, which activate or eliminate drugs. This is called cross-resistance, which arise among drugs with similar structure or mechanism of action. This kind of resistance is typical, for example, between 6-mercaptopurine and 6-thioguanine, [178] or among cytarabine, gemcitabine, and fludarabine, which are intracellularly activated by the same kinase (dCK).[179] Therapeutic strategy how to over-come this resistance can be choosing a drug with a different structure, and/ or mechanism of action or to employ combinatory therapy, which can enhance treatment efficacy.

Multidrug resistance (MDR) rising between drugs with completely different chemical structures and mechanisms of action is typically associated with altered expression of ABC-transporters (Pgp, MRPs, BCRP).[180,181] Since these proteins were identified as risk factors for MDR development, they became potential therapeutic targets. Until now, four generations of Pgp inhibitors were synthetized, but clinical trials did not demonstrate significantly improved therapeutic effects. [182] Reason for this failure can be the fact that ABC-transporters are very often co-expressed, and their substrate specificity is overlapping, so inhibition of one of them can be compensated by the others. Recently dual Pgp/BCRP inhibitors, such as flavonoids, their precursors and derivatives, curcumin, chalcone, and quinazoline derivatives etc., have been investigated with promising results.[183–186] In contrast, some MRP inhibitors show clinical relevance by themselves. For example, Ibrutinib, an MRP1 inhibitor, is improving the cytotoxicity of drugs that are substrates of this transporter.[187].

Other cellular mechanisms of chemoresistance are not connected only with the expression of membrane transporters or with the activity of metabolic enzymes. These therapeutic targets include changes at the genomic or proteomic level, which can be heterogenous among patients. Identifying biomarkers is important in finding new therapeutic approaches based on synthesizing small drug molecules, antibiotics, and monoclonal antibodies, which are the main focus of current research. [188] please in this position insert (te'In addition to direct binding to a cellular target and eliciting an immune response, monoclonal antibodies can also be applied as conjugates with anticancer drugs leading to the accumulation of drugs in the tumor tissue or with radionuclides enabling focus cytotoxic effect of radiation only at a specific location.[189,190] Specific binding and reducing the side effect caused by the off-target interactions is the biggest advantage of using monoclonal antibodies in cancer treatment.

The first antibody-drug conjugate (ADC), vindesine-anti-CEA, was successfully prepared in 1983, and many others followed.[191] However, finding the optimal combination of drug and antibody is challenging, and not all ADCs have successfully passed the clinical trial. Currently, more than 11 FDA-approved ADCs are utilized in clinical settings, for example, gemtuzumab ozogamicin, which is used in the treatment of acute myeloid leukemia, brentuximab vedotin used in the treatment of Hodgkin lymphoma or ado-trastuzumab emtansine for breast cancer treatment.[192–193].

Nucleoside-based drugs are also being studied in their conjugated form. In this case, aptamer-drug conjugates (ApDCs) are being developed, which utilize nuclei acids instead of monoclonal antibodies.[195] Aptamers have been constructed for many nucleoside drugs. For example, the RNA aptamer (P19) was developed for gemcitabine and 5-fluorouracil, and these ApCDs significantly inhibited the proliferantion of gemcitabine-resistant pancreatic cells.[196] In 2022, a new class of drug-constituted DNA-like oligomers, known as "drugtamers", were developed for clofarabine, gemcitabine, floxuridine and ara-guanosine. Compared to ApDCs drugtamers are expected to have a higher loading ratio, active targeting capability, and enzymatically controlled release of active compounds. This represents a promising advancement in the field of drug conjugates.[197].

The ultimate goal of current research is to advance personalized medicine, which can identify the optimal treatment for each individual patient. The identification of predictive markers is a useful tool, enabling the prediction of treatment efficacy and the implementation of the most effective cancer therapy.

3. Conclusion

Nucleosides, as delineated in Table 1, represent a versatile group of antimetabolites that have been successfully applied in the treatment of a broad spectrum of conditions, including diverse forms of cancer, myelodysplastic syndromes (MDSs), multiple sclerosis, and viral infections. The mechanism of action of this class of drugs typically involves the activation of a phosphorylation cascade, culminating in the formation of triphosphate forms that interact with nucleic acids. However, as these drugs take effect, cancer cells can evolve strategies to resist their impacts. While the pathways leading to resistance are diverse and multifactorial, several common pathways emerge, including a) alterations in membrane transporters, which can be characterized by reduced expression or activity of ENTs, CNTs, and/or overexpression of efflux pumps such as Pgp, MRPs, and BCRP (refer to Table 2); b) reduced activity of activating kinases, which may result in decreased phosphorylation of the drug and its capacity to interact with nucleic acids; and c) accelerated drug elimination through the activation of metabolic processes or increased renal clearance (see Table 1). Alongside these mechanisms, cancer cells may also develop resistance to nucleoside drugs by activating DNA repair mechanisms. This approach enables cancer cells to repair the damage caused by nucleoside drugs, thereby reducing their effectiveness.

Given these complex resistance mechanisms, several strategies for countermeasures have been proposed. These include targeted therapies, the development of innovative nucleoside drugs, and the formulation of unique drug combinations yielding synergistic effects. Such strategies hold the potential to prevent and overcome the resistant phenotype, thereby restoring tumor sensitivity and ultimately enhancing patient survival rates. The development of new strategies to combat resistance to nucleoside drugs continues to be a vibrant area of research, promising further breakthroughs in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported in parts from infrastructural projects (CZ-OPENSCREEN – LM2023052; EATRIS-CZ – LM2023053) and the project National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102), and by project nr. LX22NPO5107 (MEYS): Financed by EU – Next Generation EU, and by the IGA_LF_2023_025.

References

[1] J.J.G. Marin, et al., Molecular bases of drug resistance in hepatocellular carcinoma, Cancers (Basel) 12 (2020) 1–26.
[2] H.C. Zheng, The molecular mechanisms of chemoresistance in cancers, Oncotarget 8 (2017) 59950.
[3] K.L. Seley-Radtke, J.E. Thames, C.D. Waters, Broad spectrum antiviral nucleosides—Our best hope for the future, Annu. Rep. Med. Chem. 57 (2021) 109–132.
[4] M. Guinan, C. Benckendorff, M. Smith, G.J. Miller, Recent advances in the chemical synthesis and evaluation of anticancer nucleoside analogues, Molecules 25 (2020).
[5] S.J. Clarke, A.L. Jackman, K.R. Harrap, Antimetabolites in cancer chemotherapy, Adv. Exp. Med. Biol. 309 (1991) 7–13.
[6] S. Farber, L.K. Diamond, R.D. Mercer, R.F. Sylvester, J.A. Wolff, Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid, N. Engl. J. Med. 238 (1948) 787–793.
[7] M. Pannico, P. Musto, SERS spectroscopy for the therapeutic drug monitoring of the anticancer drug 6-Mercaptopurine: Molecular and kinetic studies, Appl. Surf. Sci. 539 (2021), 148225.
[8] M. Tu, A. Zhang, L. Hu, F. Wang, A retrospective cohort study of the efficacy, safety, and clinical value of 6-TG versus 6-MP maintenance therapy in children with acute lymphoblastic leukemia, Biomed Res. Int. 2022 (2022).
[9] C.M. Galmarini, J.R. Mackey, C. Dumontet, Nucleoside analogues and nucleobases in cancer treatment, Lancet Oncol. 3 (2002) 415–424.
[10] A.F. Read, T. Day, S. Huijben, The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy, Proc. Natl. Acad. Sci. 108 (2011) 10871–10877.
[11] Mercaptopurine. *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury* (2017).
[12] N.K. Yeo, W.J. Park, D.W. Eom, M.Y. Oh, J.H. Lee, Effects of azathioprine and its metabolites on inflammatory cytokines in human nasal polyp organ cultures, Int Forum Allergy Rhinol 9 (2019) 648–655.
[13] E. Frei, et al., Studies of sequential and combination antimetabolite therapy in acute leukemia: 6-mercaptopurine and methotrexate, Blood 18 (1961) 431–454.
[14] L.N. Toksvang, et al., Thiopurine Enhanced ALL Maintenance (TEAM): study protocol for a randomized study to evaluate the improvement in disease-free survival by adding very low dose 6-thioguanine to 6-mercaptopurine/methotrexate-based maintenance therapy in pediatric and adult patients (0–45 years) with newly diagnosed B-cell precursor or T-cell acute lymphoblastic leukemia treated according to the intermediate risk-high group of the ALLTogether1 protocol, BMC Cancer 22 (2022).
[15] J. Maté-Jiménez, C. Hermida, 6-mercaptopurine or methotrexate added to prednisone induces and maintains remission in steroid-dependent inflammatory bowel disease, Eur. J. Gastroenterol. Hepatol. 12 (2000) 1227–1233.
[16] van Laar, J. M. Immunosuppressive Drugs. *Kelley and Firestein's Textbook of Rheumatology* 983-998.e4 (2017) doi:10.1016/B978-0-323-31696-5.00062-0.
[17] S.E. Conneely, S.L. Cooper, R.E. Rau, Use of allopurinol to mitigate 6-mercaptopurine associated gastrointestinal toxicity in acute lymphoblastic leukemia, Front. Oncol. 10 (2020) 1129.
[18] M.L. Seinen, K.h. De Boer, A.A. Van Bodegraven, S.B. Hanauer, F. Hoentjen, Allopurinol-thiopurine combination therapy in inflammatory bowel disease, Clin. Investig. (2014) 873–879, https://doi.org/10.4155/CLI.14.81.
[19] J. Amin, B. Huang, J. Yoon, D.Q. Shih, Update 2014: advances to optimize 6-mercaptopurine and azathioprine to reduce toxicity and improve efficacy in the management of IBD, Inflamm. Bowel Dis. 21 (2015) 445–452.
[20] E.L.S.A. van Liere, et al., Azathioprine with allopurinol is a promising first-line therapy for inflammatory bowel diseases, Dig. Dis. Sci. 67 (2022) 4008–4019.
[21] C. Cuffari, A Physician's guide to azathioprine metabolite testing, Gastroenterol. Hepatol. (N Y) 2 (2006) 58.
[22] G.L.C. Chan, G.R. Erdmann, S.A. Gruber, A.J. Matas, D.M. Canafax, Azathioprine metabolism: pharmacokinetics of 6-mercaptopurine, 6-thiouric acid and 6-thioguanine nucleotides in renal transplant patients, J. Clin. Pharmacol. 30 (1990) 358–363.
[23] X.X. Peng, et al., Up-regulation of P-glycoprotein confers acquired resistance to 6-mercaptopurine in human chronic myeloid leukemia cells, Oncol. Lett. 2 (2011) 549.
[24] Y. Chen, et al., Discovery of novel multidrug resistance protein 4 (MRP4) inhibitors as active agents reducing resistance to anticancer drug 6-Mercaptopurine (6-MP) by structure and ligand-based virtual screening, PLoS One 13 (2018) e0205175.
[25] J. Wijnholds, et al., Multidrug-resistance protein 5 is a multispecific organic anion transporter able to transport nucleotide analogs, Proc. Natl. Acad. Sci. 97 (2000) 7476–7481.
[26] H. Zeng, Z.P. Lin, A.C. Sartorelli, Resistance to purine and pyrimidine nucleoside and nucleobase analogs by the human MDR1 transfected murine leukemia cell line L1210/VMDRC.06, Biochem. Pharmacol. 68 (2004) 911–921.
[27] L.S. Conklin, et al., 6-mercaptopurine transport in human lymphocytes: Correlation with drug-induced cytotoxicity, J. Dig. Dis. 13 (2012) 82.
[28] R.W. Brockman, Biochemical aspects of mercaptopurine inhbition and resistance - PubMed, Cancer Res. 23 (1963) 1191–1201.
[29] G. Tzoneva, et al., Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL, Nat. Med. 19 (2013) 368–371.
[30] C.L. Dieck, A. Ferrando, Genetics and mechanisms of NT5C2-driven chemotherapy resistance in relapsed ALL, Blood 133 (2019) 2263–2268.
[31] T.T.T. Vo, et al., mTORC1 inhibition induces resistance to methotrexate and 6-mercaptopurine in Ph + and Ph-like B-ALL, Mol. Cancer Ther. 16 (2017) 1942–1953.
[32] M.C. Dubinsky, et al., 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease, Gastroenterology 122 (2002) 904–915.
[33] Lee, J. M., Shim, Y. J., Kim, D. H., Jung, N. & Ha, J. S. The Effect of NUDT15, TPMT, APEX1, and ITPA genetic variations on mercaptopurine treatment of pediatric acute lymphoblastic leukemia. *Children 2021, Vol. 8, Page 224* 8, 224 (2021).
[34] T.W. Kouwenberg, et al., Dosage of 6-mercaptopurine in relation to genetic TPMT and ITPA variants: toward individualized pediatric acute lymphoblastic leukemia maintenance treatment, J. Pediatr. Hematol. Oncol. 42 (2020) e94–e97.
[35] J.H. Lee, et al., Measurements of 6-thioguanine nucleotide levels with TPMT and NUDT15 genotyping in patients with Crohn's disease, PLoS One 12 (2017) e0188925.
[36] K. Matsuoka, NUDT15 gene variants and thiopurine-induced leukopenia in patients with inflammatory bowel disease, Intest Res 18 (2020) 275.
[37] B. Jharap, et al., Biotransformation of 6-thioguanine in inflammatory bowel disease patients: a comparison of oral and intravenous administration of 6-thioguanine, Br. J. Pharmacol. 163 (2011) 722.
[38] L. Chen, H.X. Yan, X.W. Liu, W.X. Chen, Clinical efficacy and safety of 6-thioguanine in the treatment of childhood acute lymphoblastic leukemia: A protocol for systematic review and meta-analysis, Medicine 99 (2020) e20082.
[39] C. Arthur, et al., Prolonged administration of low-dose cytarabine and thioguanine in elderly patients with acute myeloid leukaemia (AML) achieves high complete remission rates and prolonged survival, Leuk. Lymphoma 61 (2020) 831–839.
[40] Laera, L., Guaragnella, N., Giannattasio, S. & Moro, L. 6-Thioguanine and its analogs promote apoptosis of castration-resistant prostate cancer cells in a BRCA2-dependent manner. *Cancers 2019, Vol. 11, Page 945* 11, 945 (2019).
[41] I. Kim, et al., A drug-repositioning screen for primary pancreatic ductal adenocarcinoma cells identifies 6-thioguanine as an effective therapeutic agent for TPMT-low cancer cells, Mol. Oncol. 12 (2018) 1526–1539.
[42] M. Chu, et al., Combination of the 6-thioguanine and disulfiram/Cu synergistically inhibits proliferation of triple-negative breast cancer cells by enhancing DNA damage and disrupting DNA damage checkpoint. Biochimica et Biophysica Acta (BBA) - Molecular, Cell Res. 1869 (2022), 119169.
[43] H.C. Lin, et al., Disulfiram and 6-Thioguanine synergistically inhibit the enzymatic activities of USP2 and USP21, Int. J. Biol. Macromol. 176 (2021) 490–497.
[44] S. Karner, et al., Determination of 6-thioguanosine diphosphate and triphosphate and nucleoside diphosphate kinase activity in erythrocytes: novel targets for thiopurine therapy? Ther. Drug Monit. 32 (2010) 119–128.
[45] L. Zhang, D.J. Hinz, G.S.M. Kiruba, X. Ding, J.K. Lee, Gas-phase experimental and computational studies of human hypoxanthine-guanine phosphoribosyltransferase substrates: Intrinsic properties and biological implications, J. Phys. Org. Chem. 35 (2022) e4343.
[46] L. Ding, et al., Hypoxanthine guanine phosphoribosyltransferase activity is related to 6-thioguanine nucleotide concentrations and thiopurine-induced leukopenia in the treatment of inflammatory bowel disease, Inflamm. Bowel Dis. 18 (2012) 63–73.
[47] L. Dean, Thioguanine therapy and TPMT and NUDT15 genotype, Medical Genetics Summaries (2020).
[48] W.E. Glaab, et al., Resistance to 6-thioguanine in mismatch repair-deficient human cancer cell lines correlates with an increase in induced mutations at the HPRT locus, Carcinogenesis 19 (1998) 1931–1937.
[49] A.A. Morley, S. Cox, R. Holluday, Human lymphocytes resistant to 6-thioguanine increase with age, Mech. Ageing Dev. 19 (1982) 21–26.
[50] N. Gefen, et al., Acquired resistance to 6-thioguanine in melanoma cells involves the repair enzyme O 6-methylguanine-DNA methyltransferase (MGMT), Cancer Biol. Ther. 49 (2010) 49–55.
[51] A.K. Fotoohi, M. Lindqvist, C. Peterson, F. Albertioni, Involvement of the concentrative nucleoside transporter 3 and equilibrative nucleoside transporter 2 in the resistance of T-lymphoblastic cell lines to thiopurines, Biochem. Biophys. Res. Commun. 343 (2006) 208–215.
[52] P.R. Wielinga, et al., Thiopurine metabolism and identification of the thiopurine metabolites transported by MRP4 and MRP5 overexpressed in human embryonic kidney cells, Mol. Pharmacol. 62 (2002) 1321–1331.
[53] H. Ban, et al., The multidrug-resistance protein 4 polymorphism is a new factor accounting for thiopurine sensitivity in Japanese patients with inflammatory bowel disease, J. Gastroenterol. 45 (2010) 1014–1021.
[54] N. Issaeva, et al., 6-thioguanine selectively kills BRCA2-defective tumors and overcomes PARP inhibitor resistance, Cancer Res. 70 (2010) 6268–6276.

[55] L. Möllgård, et al., High single dose of mitoxantrone and cytarabine in acute non-lymphocytic leukemia: A pharmacokinetic and clinical study, Ther. Drug Monit. 20 (1998) 640–645.
[56] J.E. Cortes, et al., Glasdegib in combination with cytarabine and daunorubicin in patients with AML or high-risk MDS: Phase 2 study results, Am. J. Hematol. 93 (2018) 1301–1310.
[57] R. Hehlmann, *et al.* Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. *Leukemia 2017 31:11* 31, 2398–2406 (2017).
[58] S. Faderl, et al., Clofarabine and cytarabine combination as induction therapy for acute myeloid leukemia (AML) in patients 50 years of age or older, Blood 108 (2006) 45–51.
[59] K. Venugopal, et al., DNMT3A Harboring Leukemia-Associated Mutations Directs Sensitivity to DNA Damage at Replication Forks, Clin. Cancer Res. 28 (2022) 756–769.
[60] M. Hong, et al., Decitabine in combination with low-dose cytarabine, aclarubicin and G-CSF tends to improve prognosis in elderly patients with high-risk AML, Aging (Albany NY) 12 (2020) 5792.
[61] A.B. Halpern, et al., Addition of sorafenib to cladribine, high-dose cytarabine, G-CSF, and mitoxantrone (CLAG-M) in adults with newly-diagnosed acute myeloid leukemia (AML) and high-grade myeloid neoplasms independent of FLT3-mutation status: final results of a phase 1/2 study, Blood 140 (2022) 8999–9001.
[62] F. Ravandi, et al., Final report of phase II study of sorafenib, cytarabine, and idarubicin for initial therapy in younger patients with acute myeloid leukemia, Leukemia 28 (2014) 1543.
[63] R. Dillon, et al., Venetoclax combined with low dose cytarabine compared to standard of care intensive chemotherapy for the treatment of favourable risk adult acute myeloid leukaemia (VICTOR): Study protocol for an international, open-label, multicentre, molecularly-guided randomised, phase II trial, BMC Cancer 22 (2022) 1–13.
[64] Z. Li, et al., Exploring the antitumor mechanism of high-dose cytarabine through the metabolic perturbations of ribonucleotide and deoxyribonucleotide in human promyelocytic Leukemia HL-60 Cells, Molecules 22 (2017).
[65] J.F. Zeidner, et al., Final clinical results of a phase II study of high dose cytarabine followed by pembrolizumab in relapsed/refractory AML, Blood 134 (2019) 831.
[66] N.J. Barrios, C.K. Tebbi, A.I. Freeman, M.L. Brecher, Toxicity of high dose Ara-C in children and adolescents, Cancer 60 (1987) 165–169.
[67] R. Fanciullino, et al., CDA as a predictive marker for life-threatening toxicities in patients with AML treated with cytarabine, Blood Adv. 2 (2018) 462–469.
[68] D. Malani, *et al.* Enhanced sensitivity to glucocorticoids in cytarabine-resistant AML. *Leukemia 2017 31:5* 31, 1187–1195 (2016).
[69] J.H. Gray, R.P. Owen, K.M. Giacomini, The concentrative nucleoside transporter family, SLC28, Pflugers Arch. 447 (2004) 728–734.
[70] A.D. Adema, et al., Overexpression of MRP4 (ABCC4) and MRP5 (ABCC5) confer resistance to the nucleoside analogs cytarabine and troxacitabine, but not gemcitabine, Springerplus 3 (2014) 1–11.
[71] A.C. Jaramillo, et al., Ex vivo resistance in childhood acute lymphoblastic leukemia: Correlations between BCRP, MRP1, MRP4 and MRP5 ABC transporter expression and intracellular methotrexate polyglutamate accumulation, Leuk. Res. 79 (2019) 45–51.
[72] P. Macanas-Pirard, et al., Resistance of leukemia cells to cytarabine chemotherapy is mediated by bone marrow stroma, involves cell-surface equilibrative nucleoside transporter-1 removal and correlates with patient outcome, Oncotarget 8 (2017) 23073.
[73] W. Qi, et al., The effects of cytarabine combined with ginsenoside compound K synergistically induce DNA damage in acute myeloid leukemia cells, Biomed. Pharmacother. 132 (2020), 110812.
[74] M. Levin, M. Stark, B. Berman, Y.G. Assaraf, Surmounting Cytarabine-resistance in acute myeloblastic leukemia cells and specimens with a synergistic combination of hydroxyurea and azidothymidine. *Cell Death & Disease 2019 10:6* 10, 1–14 (2019).
[75] Y. Yakymiv, *et al.* CD157 signaling promotes survival of acute myeloid leukemia cells and modulates sensitivity to cytarabine through regulation of anti-apoptotic Mcl-1. *Sci. Rep. 2021 11:1* 11, 1–16 (2021).
[76] A.K. Mitra, et al., Genetic variants in cytosolic 5'-nucleotidase II are associated with its expression and cytarabine sensitivity in HapMap cell lines and in patients with acute myeloid leukemia, J. Pharmacol. Exp. Ther. 339 (2011) 9–23.
[77] C. Schneider *et al.* SAMHD1 is a biomarker for cytarabine response and a therapeutic target in acute myeloid leukemia. *Nature Medicine 2016 23:2* 23, 250–255 (2016).
[78] S.G. Rudd, et al., Ribonucleotide reductase inhibitors suppress SAMHD1 ara-CTPase activity enhancing cytarabine efficacy, EMBO Mol. Med. 12 (2020) e10419.
[79] M. Jädersten, et al., Targeting SAMHD1 with hydroxyurea in first-line cytarabine-based therapy of newly diagnosed acute myeloid leukaemia: results from the HEAT-AML trial, J. Intern. Med. 292 (2022) 925–940.
[80] C. Bosc *et al.* Mitochondrial inhibitors circumvent adaptive resistance to venetoclax and cytarabine combination therapy in acute myeloid leukemia. *Nature Cancer 2021 2:11* 2, 1204–1223 (2021).
[81] H. Åbacka, et al., Targeting GLUT1 in acute myeloid leukemia to overcome cytarabine resistance, Haematologica 106 (2021) 1163.
[82] M.J. Keating, et al., Fludarabine: A new agent with marked cytoreductive activity in untreated chronic lymphocytic leukemia, J. Clin. Oncol. 9 (1991) 44–49.
[83] G. Borthakur, et al., Retrospective comparison of survival and responses to Fludarabine, Cytarabine, GCSF (FLAG) in combination with gemtuzumab ozogamicin (GO) or Idarubicin (IDA) in patients with newly diagnosed core binding factor (CBF) acute myelogenous leukemia: MD Anderson experience in 174 patients, Am. J. Hematol. 97 (2022) 1427–1434.
[84] M.S. Davids, et al., Ibrutinib plus fludarabine, cyclophosphamide, and rituximab as initial treatment for younger patients with chronic lymphocytic leukaemia: a single-arm, multicentre, phase 2 trial, Lancet Haemato.l 6 (2019) e419–e428.
[85] L. Smolej, et al., Low-dose fludarabine and cyclophosphamide combined with rituximab in the first-line treatment of elderly/comorbid patients with chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL): long-term results of project Q-lite by the Czech CLL Study Group, Br. J. Haematol. 193 (2021) 769–778.
[86] X.X. Wang, et al., Bortezomib in combination with fludarabine plus cyclophosphamide for patients with relapsed or refractory mantle-cell lymphoma: results of the LYM-4003 study, Ann. Hematol. 100 (2021) 2961–2968.
[87] H.N. Altayb, Fludarabine, a potential DNA-dependent RNA polymerase inhibitor, as a prospective drug against monkeypox virus: a computational approach, Pharmaceuticals 15 (2022) 1129.
[88] V. Gandhi, W. Plunkett, Cellular and clinical pharmacology of fludarabine, Clin. Pharmacokinet. 41 (2002) 93–103.
[89] M. Gorzkiewicz, et al., Glycodendrimer nanocarriers for direct delivery of fludarabine triphosphate to leukemic cells: improved pharmacokinetics and pharmacodynamics of fludarabine, Biomacromolecules 19 (2018) 531–543.
[90] A. Michelutti, et al., Effect of fludarabine and arabinosylcytosine on multidrug resistant cells - PubMed, Haematologica 82 (1997) 143–147.
[91] A. Candoni, et al., Flai (fludarabine, cytarabine, idarubicin) plus low-dose Gemtuzumab Ozogamicin as induction therapy in CD33-positive AML: Final results and long term outcome of a phase II multicenter clinical trial, Am. J. Hematol. 93 (2018) 655–663.
[92] D. Damiani, et al., Fludarabine-based induction therapy does not overcome the negative effect of ABCG2 (BCRP) over-expression in adult acute myeloid leukemia patients, Leuk. Res. 34 (2010) 942–945.
[93] C. Huang, Y. Tu, C.E. Freter, Fludarabine-resistance associates with ceramide metabolism and leukemia stem cell development in chronic lymphocytic leukemia, Oncotarget 9 (2018) 33124.
[94] J.S. Carew, et al., Targeting endoplasmic reticulum protein transport: a novel strategy to kill malignant B cells and overcome fludarabine resistance in CLL, Blood 107 (2006) 222–231.
[95] A. Sharma, et al., Targeting mTORC1-mediated metabolic addiction overcomes fludarabine resistance in malignant B cells, Mol. Cancer Res. 12 (2014) 1205–1215.
[96] D. Trachootham, et al., Effective elimination of fludarabine-resistant CLL cells by PEITC through a redox-mediated mechanism, Blood 112 (2008) 1912–1922.
[97] L. Toschi, G. Finocchiaro, S. Bartolini, V. Gioia, F. Cappuzzo, Role of gemcitabine in cancer therapy, Future Oncol. 1 (2005) 7–17.
[98] T. Conroy, et al., FOLFIRINOX or Gemcitabine as Adjuvant Therapy for Pancreatic Cancer, N. Engl. J. Med. 379 (2018) 2395–2406.
[99] D.A. Yardley, et al., nab-Paclitaxel plus carboplatin or gemcitabine versus gemcitabine plus carboplatin as first-line treatment of patients with triple-negative metastatic breast cancer: results from the tnAcity trial, Ann. Oncol. 29 (2018) 1763–1770.
[100] S. Gesto, M.F.S.A. Cerqueira, N.A.P. Fernandes, J.M. Ramos, Gemcitabine: a critical nucleoside for cancer therapy, Curr. Med. Chem. 19 (2012) 1076–1087.
[101] E.J.B. Derissen, A.D.R. Huitema, H. Rosing, J.H.M. Schellens, J.H. Beijnen, Intracellular pharmacokinetics of gemcitabine, its deaminated metabolite 2′,2′-difluorodeoxyuridine and their nucleotides, Br. J. Clin. Pharmacol. 84 (2018) 1279–1289.
[102] J. Ciccolini, C. Serdjebi, G.J. Peters, E. Giovannetti, Pharmacokinetics and pharmacogenetics of Gemcitabine as a mainstay in adult and pediatric oncology: an EORTC-PAMM perspective, Cancer Chemother. Pharmacol. 78 (2016) 1.
[103] H. Xu, C. Faber, T. Uchiki, J. Racca, C. Dealwis, Structures of eukaryotic ribonucleotide reductase I define gemcitabine diphosphate binding and subunit assembly, PNAS 103 (2006) 4028–4033.
[104] W. Plunkett, P. Huang, V. Gandhi, Preclinical characteristics of gemcitabine, Anticancer Drugs 6 (Suppl 6) (1995) 7–13.
[105] S. Yang, et al., New mechanism of gemcitabine and its phosphates: DNA polymerization disruption via 3′-5′ exonuclease Inhibition, Biochemistry 59 (2020) 4344–4352.
[106] T.K. Bjånes, et al., Intracellular cytidine deaminase regulates gemcitabine metabolism in pancreatic cancer cell Lines, Drug Metab. Dispos. 48 (2020) 153–158.
[107] J.Y. Lee, S.Y. Song, J.Y. Park, Notch pathway activation is associated with pancreatic cancer treatment failure, Pancreatology 14 (2014) 48–53.
[108] G.J. Peters, E. Giovannetti, R.J. Honeywell, J. Ciccolini, Can cytidine deaminase be used as predictive biomarker for gemcitabine toxicity and response? Br. J. Clin. Pharmacol. 85 (2019) 1213.
[109] M. Yamamoto, et al., Roles for hENT1 and dCK in gemcitabine sensitivity and malignancy of meningioma, Neuro Oncol. 23 (2021) 945–954.
[110] W. Hagmann, R. Jesnowski, J.M. Löhr, Interdependence of gemcitabine treatment, transporter expression, and resistance in human pancreatic carcinoma cells, Neoplasia 12 (2010) 740.
[111] A.M. Bergman, et al., Increased sensitivity to gemcitabine of P-glycoprotein and multidrug resistance-associated protein-overexpressing human cancer cell lines, Br. J. Cancer 88 (2003) 1963.
[112] Y. Jia, J. Xie, Promising molecular mechanisms responsible for gemcitabine resistance in cancer, Genes Dis 2 (2015) 299–306.

[113] H. Yang, et al., Genome-wide CRISPR screening identifies DCK and CCNL1 as genes that contribute to gemcitabine resistance in pancreatic cancer, Cancers (Basel) 14 (2022).
[114] Y.H. Chang, H.L. Tam, M.C. Lu, H.S. Huang, Gemcitabine-induced Gli-dependent activation of hedgehog pathway resists to the treatment of urothelial carcinoma cells, PLoS One 16 (2021).
[115] D.V.T. Catenacci, et al., Randomized phase Ib/II study of gemcitabine plus placebo or vismodegib, a hedgehog pathway inhibitor, in patients with metastatic pancreatic cancer, J. Clin. Oncol. 33 (2015) 4284–4292.
[116] Q. Zhang, et al., The Wnt/β-catenin signaling pathway mechanism for pancreatic cancer chemoresistance in a three-dimensional cancer microenvironment, Am. J. Transl. Res. 8 (2016) 4490.
[117] V. Kumar, et al., The role of notch, hedgehog, and wnt signaling pathways in the resistance of tumors to anticancer therapies, Front. Cell Dev. Biol. 9 (2021) 857.
[118] M.T. Rahman, et al., Notch3 overexpression as potential therapeutic target in advanced stage chemoresistant ovarian cancer, Am. J. Clin. Pathol. 138 (2012) 535–544.
[119] J. Yao, C. Qian, Inhibition of Notch3 enhances sensitivity to gemcitabine in pancreatic cancer through an inactivation of PI3K/Akt-dependent pathway, Med. Oncol. 27 (2010) 1017–1022.
[120] Z. Zhang, et al., Hypoxia potentiates gemcitabine-induced stemness in pancreatic cancer cells through AKT/Notch1 signaling, J. Exp. Clin. Cancer Res. 37 (2018) 1–13.
[121] L.M.P. Verissimo, et al., Transport properties of aqueous solutions of the oncologic drug 5-fluorouracil: A fundamental complement to therapeutics, J. Chem. Thermodyn. 161 (2021), 106533.
[122] H. Blomstrand, U. Scheibling, C. Bratthäll, H. Green, N.O. Elander, Real world evidence on gemcitabine and nab-paclitaxel combination chemotherapy in advanced pancreatic cancer, BMC Cancer 19 (2019) 1–9.
[123] N.H. Shehatta, T.M. Okda, G.A. Omran, M.M. Abd-Alhaseeb, Baicalin; a promising chemopreventive agent, enhances the antitumor effect of 5-FU against breast cancer and inhibits tumor growth and angiogenesis in Ehrlich solid tumor, Biomed. Pharmacother. 146 (2022), 112599.
[124] S. Chakrabarti, et al., Bolus 5-fluorouracil (5-FU) In Combination With Oxaliplatin Is Safe and Well Tolerated in Patients Who Experienced Coronary Vasospasm With Infusional 5-FU or Capecitabine, Clin. Colorectal Cancer 18 (2019) 52–57.
[125] S. A. Hosseini, H. Zand, M. Cheraghpour, The Influence of Curcumin on the Downregulation of MYC, Insulin and IGF-1 Receptors: A Possible Mechanism Underlying the Anti-Growth and Anti-Migration in Chemoresistant Colorectal Cancer Cells. *Medicina 2019, Vol. 55, Page 90* 55, 90 (2019).
[126] X. Tan, *et al.* Inhibition of EZH2 enhances the therapeutic effect of 5-FU via PUMA upregulation in colorectal cancer. *Cell Death & Disease 2020 11:12* 11, 1–13 (2020).
[127] K. Miura, et al., 5-FU metabolism in cancer and orally-administrable 5-FU drugs, Cancers (Basel) 2 (2010) 1717.
[128] T.J. Wigle, E. Tsvetkova, S.A. Welch, R.B. Kim, DPYD and fluorouracil-based chemotherapy: Mini review and case report, *Pharmaceutics* 11 (2019).
[129] W.W. Ma, et al., Emergency use of uridine triacetate for the prevention and treatment of life-threatening 5-fluorouracil and capecitabine toxicity, Cancer 123 (2017) 345–356.
[130] W.B. Parker, Y.C. Cheng, Metabolism and mechanism of action of 5-fluorouracil, Pharmacol. Ther. 48 (1990) 381–395.
[131] G.J. Peters, et al., Induction of thymidylate synthase as a 5-fluorouracil resistance mechanism, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1587 (2002) 194–205.
[132] S. Blondy, et al., 5-Fluorouracil resistance mechanisms in colorectal cancer: From classical pathways to promising processes, Cancer Sci. 111 (2020) 3142–3154.
[133] S.S. Fidai, A.E. Sharma, D.N. Johnson, J.P. Segal, R.R. Lastra, Dihydropyrimidine dehydrogenase deficiency as a cause of fatal 5-Fluorouracil toxicity, Autops. Case Rep. 8 (2018).
[134] S.L. Showalter, et al., Evaluating the drug-target relationship between thymidylate synthase expression and tumor response to 5-fluorouracil: Is it time to move forward? Cancer Biol. Ther. 7 (2008) 986.
[135] B.Q. Cai, W.M. Chen, J. Zhao, W. Hou, J.C. Tang, Nrf3 promotes 5-FU resistance in colorectal cancer cells via the NF- κ B/BCL-2 signaling pathway in vitro and in vivo, J. Oncol. 2021 (2021).
[136] C.N. Arnold, A. Goel, C.R. Boland, Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines, Int. J. Cancer 106 (2003) 66–73.
[137] C. Sethy, C.N. Kundu, 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: Implication of DNA repair inhibition, Biomed. Pharmacother. 137 (2021), 111285.
[138] V. Varghese, et al., FOXM1 modulates 5-FU resistance in colorectal cancer through regulating TYMS expression, Sci. Rep. 9 (2019).
[139] D. Na, et al., Predictive biomarkers for 5-fluorouracil and oxaliplatin-based chemotherapy in gastric cancers via profiling of patient-derived xenografts, Nat. Commun. 12 (2021).
[140] W. Hagmann, R. Jesnowski, R. Faissner, C. Guo, J.M. Löhr, ATP-binding cassette C transporters in human pancreatic carcinoma cell lines: upregulation in 5-fluorouracil-resistant cells, Pancreatology 9 (2009) 136–144.
[141] Y.D. Kartika, I. Astuti, W.R. Pratiwi, The expression of multidrug resistance protein 5 and thymydilate synthase on fluorouracil resistance WiDr colon cancer cell line, J. Kedokteran Kesehatan Indonesia 7 (2015) 59–64.
[142] S. Pratt, et al., The multidrug resistance protein 5 (ABCC5) confers resistance to 5-fluorouracil and transports its monophosphorylated metabolites, Mol. Cancer Ther. 4 (2005) 855–863.
[143] T. Oguri, et al., MRP8/ABCC11 directly confers resistance to 5-fluorouracil, Mol. Cancer Ther. 6 (2007) 122–127.
[144] R. Mori, *et al.* P-glycoprotein plays an important role in the cross-resistance to taxanes in 5FU-resistant gastric cancer cells. *Transl. Gastrointest. Cancer* 4, 12330–12130 (2015).
[145] J. Yuan, et al., Role of BCRP as a biomarker for predicting resistance to 5-fluorouracil in breast cancer, Cancer Chemother. Pharmacol. 63 (2009) 1103–1110.
[146] U. Stein, et al., YB-1 facilitates basal and 5-fluorouracil-inducible expression of the human major vault protein (MVP) gene, Oncogene 24 (2005) 3606–3618.
[147] S.M. Naes, S. Ab-Rahim, M. Mazlan, A.A. Rahman, Equilibrative nucleoside transporter 2: properties and physiological roles, Biomed Res. Int. 2020 (2020).
[148] M. Tsujie, et al., Human equilibrative nucleoside transporter 1, as a predictor of 5-fluorouracil resistance in human pancreatic cancer, Anticancer Res 27 (2007) 2241–2249.
[149] C.E. Cass, S.A. Baldwin, J. Young, CNT2, concentrative nucleoside transporter 2. *xPharm: The Comprehensive Pharmacology Reference* 1–4 (2007) doi:10.1016/B978-008055232-3.60462-3.
[150] K. Raj, G.J. Mufti, Azacytidine (Vidaza®) in the treatment of myelodysplastic syndromes, Ther. Clin. Risk Manag. 2 (2006) 377.
[151] E.J.B. Derissen, J.H. Beijnen, J.H.M. Schellens, Concise drug review: azacitidine and decitabine, Oncologist 18 (2013) 619.
[152] C.D. DiNardo, et al., Azacitidine and venetoclax in previously untreated acute myeloid leukemia, N. Engl. J. Med. 383 (2020) 617–629.
[153] A. Bazinet, et al., Azacitidine plus venetoclax in patients with high-risk myelodysplastic syndromes or chronic myelomonocytic leukaemia: phase 1 results of a single-centre, dose-escalation, dose-expansion, phase 1–2 study, Lancet Haematol 9 (2022) e756–e765.
[154] G. Leone, F. D'Alo, G. Zardo, M. Voso, C. Nervi, Epigenetic treatment of myelodysplastic syndromes and acute myeloid leukemias, Curr. Med. Chem. 15 (2008) 1274–1287.
[155] R. L. Momparler, A Perspective on the Comparative Antileukemic Activity of 5-Aza-2′-deoxycytidine (Decitabine) and 5-Azacytidine (Vidaza). *Pharmaceuticals 2012, Vol. 5, Pages 875-881* 5, 875–881 (2012).
[156] P. Sripayap, et al., Mechanisms of resistance to azacitidine in human leukemia cell lines, Exp. Hematol. 42 (2014).
[157] X. Gu, *et al.* Decitabine- and 5-azacytidine resistance emerges from adaptive responses of the pyrimidine metabolism network. *Leukemia 2020 35:4* 35, 1023–1036 (2020).
[158] V.L. Damaraju, et al., Role of human nucleoside transporters in the uptake and cytotoxicity of azacitidine and decitabine, Nucleosides Nucleotides Nucleic Acids 31 (2012) 236–255.
[159] E.-H. Hur, et al., Establishment and characterization of hypomethylating agent-resistant cell lines, MOLM/AZA-1 and MOLM/DEC-5, Oncotarget 8 (2016) 11748–11762.
[160] E. Lainey, et al., Erlotinib increases efficacy of 5-azacitidine (AZA) by inhibiting drug efflux via ABC-transporters, Blood 116 (2010) 974.
[161] A. Kuendgen, et al., Efficacy of azacitidine is independent of molecular and clinical characteristics - an analysis of 128 patients with myelodysplastic syndromes or acute myeloid leukemia and a review of the literature, Oncotarget 9 (2018) 27882.
[162] S. Dhillon, Decitabine/Cedazuridine: First Approval. *Drugs 2020 80:13* 80, 1373–1378 (2021).
[163] P. Montesinos, *et al.* Safety and efficacy of talacotuzumab plus decitabine or decitabine alone in patients with acute myeloid leukemia not eligible for chemotherapy: results from a multicenter, randomized, phase 2/3 study. *Leukemia 2020 35:1* 35, 62–74 (2020).
[164] J. Bouchard, R.L. Momparler, Incorporation of 5-Aza-2'-deoxycytidine-5'-triphosphate into DNA. Interactions with mammalian DNA polymerase alpha and DNA methylase - PubMed, Mol. Pharmacol. 24 (1983) 109–114.
[165] T. Qin, et al., Mechanisms of resistance to decitabine in the myelodysplastic syndrome, PLoS One 6 (2011).
[166] X. Gu, et al., Decitabine- and 5-azacytidine resistance emerges from adaptive responses of the pyrimidine metabolism network, Leukemia 35 (2021) 1023.
[167] C. Säll, C. Fogt Hjorth, In vitro drug-drug interactions of decitabine and tetrahydrouridine involving drug transporters and drug metabolising enzymes, Xenobiotica 52 (2022) 1–15.
[168] T. Ando, M. Nishimura, Y. Oka, Decitabine (5-Aza-2'-deoxycytidine) decreased DNA methylation and expression of MDR-1 gene in K562/ADM cells, Leukemia 14 (2000) 1915–1920.
[169] H. M. Bryson, E.M. Sorkin Cladribine. *Drugs 1993 46:5* 46, 872–894 (2012).
[170] G. Giovannoni, et al., Safety and efficacy of cladribine tablets in patients with relapsing-remitting multiple sclerosis: Results from the randomized extension trial of the CLARITY study, Mult. Scler. 24 (2018) 1594–1604.
[171] J. Liliemark, The clinical pharmacokinetics of cladribine, Clin. Pharmacokinet. 32 (1997) 120–131.
[172] R. Hermann, et al., The clinical pharmacology of cladribine tablets for the treatment of relapsing multiple sclerosis, Clin. Pharmacokinet. 58 (2019) 283–297.
[173] R. Hermann, et al., Review of transporter substrate, inhibitor, and inducer characteristics of cladribine, Clin. Pharmacokinet. 60 (2021) 1509.
[174] K.W. Rammohan, J. Shoemaker, J. Emerging multiple sclerosis oral therapies. *undefined* 74, (2010).
[175] K. Lotfi, G. Juliusson, F. Albertioni, Pharmacological basis for cladribine resistance, Leuk. Lymphoma 44 (2003) 1705–1712.

[176] F. Aybar, et al., 2-Chlorodeoxyadenosine (Cladribine) preferentially inhibits the biological activity of microglial cells, Int. Immunopharmacol. 105 (2022), 108571.
[177] N. Chen, et al., Xenobiotica the fate of foreign compounds in biological systems In vitro drug-drug interactions of budesonide: inhibition and induction of transporters and cytochrome P450 enzymes In vitro drug-drug interactions of budesonide: inhibition and induction of transporters and cytochrome P450 enzymes, Xenobiotica 48 (2018) 637–646.
[178] A.C. Sartorelli, G.A. LePage, E.C. Moore, Metabolic effects of 6-thioguanine: I. studies on thioguanine-resistant and sensitive ehrlich ascites cells, Cancer Res. 18 (1958) 1232–1239.
[179] M. Klanova, et al., Downregulation of deoxycytidine kinase in cytarabine-resistant mantle cell lymphoma cells confers cross-resistance to nucleoside analogs gemcitabine, fludarabine and cladribine, but not to other classes of anti-lymphoma agents, Mol. Cancer 13 (2014) 1–14.
[180] S. Dei, L. Braconi, M.N. Romanelli, E. Teodori, Recent advances in the search of BCRP- and dual P-gp/BCRP-based multidrug resistance modulators, Cancer Drug Resistance 2 (2019) 710.
[181] K. Sodani, A. Patel, R.J. Kathawala, Z.S. Chen, Multidrug resistance associated proteins in multidrug resistance, Chin. J. Cancer 31 (2012) 58.
[182] J. Dong, et al., Medicinal chemistry strategies to discover P-glycoprotein inhibitors: An update, Drug Resist. Updat. 49 (2020), 100681.
[183] J. Lee, et al., Dual inhibition of P-gp and BCRP improves oral topotecan bioavailability in rodents, Pharmaceutics 13 (2021).
[184] S. Zhang, X. Yang, M.E. Morris, Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport, Mol. Pharmacol. 65 (2004) 1208–1216.
[185] A. Pick, M. Wiese, Tyrosine kinase inhibitors influence ABCG2 expression in EGFR-positive MDCK BCRP cells via the PI3K/Akt signaling pathway, ChemMedChem 7 (2012) 650–662.
[186] C.Y. Cai, et al., Biological evaluation of non-basic chalcone CYB-2 as a dual ABCG2/ABCB1 inhibitor, Biochem. Pharmacol. 175 (2020), 113848.
[187] H. Zhang, et al., In vitro, in vivo and ex vivo characterization of ibrutinib: a potent inhibitor of the efflux function of the transporter MRP1, Br. J. Pharmacol. 171 (2014) 5845–5857.
[188] K. Sechidis, et al., Distinquishing prognostic and predictive biomarkers: an information theoretic approach, Bioinformatics 34 (2018) 3365–3376.
[189] J.M. Lambert, Drug-conjugated antibodies for the treatment of cancer, Br. J. Clin. Pharmacol. 76 (2013) 248.
[190] F. Buchegger, et al., Radiolabeled fragments of monoclonal antibodies against carcinoembryonic antigen for localization of human colon carcinoma grafted into nude mice, J. Exp. Med. 158 (1983) 413–427.
[191] C.H.J. Ford, et al., Localisation and toxicity study of a vindesine-anti-CEA conjugate in patients with advanced cancer, Br. J. Cancer 47 (1983) 35–42.
[192] J. Hitzler, E. Estey, Gemtuzumab ozogamicin in acute myeloid leukemia: act 2, with perhaps more to come, Haematologica 104 (2019) 7.
[193] N.W.C.J. Van De Donk, E. Dhimolea, Brentuximab vedotin, *MAbs* 4 (2012) 458.
[194] P.F. Peddi, S.A. Hurvitz, Ado-trastuzumab emtansine (T-DM1) in human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer: latest evidence and clinical potential, Ther Adv Med Oncol 6 (2014) 202.
[195] M.R. Dunn, Jimenez, R. M., Chaput, J. C. Analysis of aptamer discovery and technology. *Nat. Rev. Chem. 2017 1:10* 1, 1–16 (2017).
[196] S. Yoon, et al., Aptamer-drug conjugates of active metabolites of nucleoside analogs and cytotoxic agents inhibit pancreatic tumor cell growth, Mol. Ther. Nucleic Acids 6 (2017) 80–88.
[197] L. Zhu, J. Yang, Y. Ma, X. Zhu, C. Zhang, Aptamers entirely built from therapeutic nucleoside analogues for targeted cancer therapy, J. Am. Chem. Soc. 144 (2022) 1493–1497.
[198] K. Natarajan, Y. Xie, M.R. Baer, D.D. Ross, Role of breast cancer resistance protein (BCRP/ABCG2) in cancer drug resistance, Biochem. Pharmacol. 83 (2012) 1084.
[199] E. Yagüe, et al., P-glycoprotein (MDR1) expression in leukemic cells is regulated at two distinct steps, mRNA stabilization and translational initiation, J. Biol. Chem. 278 (2003) 10344–10352.
[200] E.L. Woodahl, J. Wang, S. Heimfeld, A.G. Ren, J.S. McCune, Imatinib inhibition of fludarabine uptake in T-lymphocytes, Cancer Chemother. Pharmacol. 62 (2008) 735–739.
[201] M. Pastor-Anglada *et al.* Nucleoside transporters in chronic lymphocytic leukaemia. *Leukemia 2004 18:3* 18, 385–393 (2004).
[202] J. Gu, et al., Hsa-miR-3178/RhoB/PI3K/Akt, a novel signaling pathway regulates ABC transporters to reverse gemcitabine resistance in pancreatic cancer, Mol. Cancer 21 (2022).
[203] A.E. Abdelrahman, D.A. Ibrahim, A. El-Azony, A.A. Alnagar, A. Ibrahim, ERCC1, PARP-1, and AQP1 as predictive biomarkers in colon cancer patients receiving adjuvant chemotherapy, Cancer Biomark. 27 (2020) 251–264.