5-Fluorouracil and capecitabine therapies for the treatment of colorectal cancer (Review)

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Received September 29, 2022; Accepted July 17, 2023

DOI: 10.3892/or.2023.8612

Abstract. Although 5-fluorouracil (5-FU)-based chemotherapy is the major treatment for colorectal cancer, it has disadvantages such as systemic toxicity, lack of effectiveness and selectivity, and development of resistance. Capecitabine, a prodrug form of 5-FU, was designed to overcome these drawbacks, to fulfill the need for more convenient therapy, and to improve safety, tolerability and intratumor drug concentration levels through a tumor-specific conversion to the active 5-FU drug. The purpose of the present review is to provide a comprehensive comparison between 5-FU therapy and capecitabine. In the current review, anticancer drug classification was discussed and the development of capecitabine from the original fluorinated analogue (5-FU) to overcome its drawbacks was explained. Specifically, 5-FU is compared with capecitabine therapy regarding various properties, including drug metabolism, cellular mechanism, effect on the apoptosis pathway and cell cycle phases, safety and tolerability. Moreover, three metabolizing enzymes required for the activation of capecitabine to 5-FU were discussed. Capecitabine, as monotherapy or in combination with other chemotherapies, exhibited improved drug efficacy and survival. However, the changes that mediate the chemoresistance of capecitabine treatment were classified as intracellular, extracellular or cell surface factors, or cell-phenotype state. Future studies should examine the efficacy of capecitabine combined with novel and safe drugs other than chemotherapeutic agents that play a role in the inhibition of tumor initiation, progression and metastasis.

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Key words: chemotherapeutic drugs, 5-fluorouracil, capecitabine, carboxylesterase, cytidine deaminase, thymidine phosphorylase, chemoresistance

Contents

1. Introduction

Cancer remains one of the major public health issues worldwide and the second leading cause of mortality worldwide (1). Numerous standard strategies for cancer treatment have been used to overcome this health issue, including chemotherapy, surgery and radiotherapy (2). Colorectal cancer (CRC) is a solid tumor that generates a considerable health and economic burden, as it ranks second in terms of mortality among other types of cancer (3). Among all cancers in United States in 2020, CRC is the most frequently diagnosed cancer accounting for 9% of all cases in men and 8% for those in women whereas, 9% of cancer deaths account for CRC in both sexes separately (1,3). This type of tumor begins in the colorectal tissues due to the accumulations of genetic or/and epigenetic modifications. Furthermore, tumorgenicity of CRC may develop through three defined molecular pathways: Chromosomal instability, microsatellite instability (MSI), and cytosine preceding guanine island methylator phenotype pathways (4). Several risk factors are associated with the development of CRC including genetic factors, lifestyle factors, sex, age and diet. Genetic factors mainly arise from the presence of hereditary CRC syndromes including Lynch syndrome and familial adenomatous polyposis. Mutations of oncogenes and tumor suppressor genes could also increase the risk of CRC (4,5). Moreover, red meat consumption, diet high in fat, low physical activity, obesity and cigarette smoking are examples of diet and lifestyle factors that have been linked with increase of CRC risk (3,4). Over the past few decades, a notable improvement in the treatment of CRC, whether in surgery, radiotherapy or systemic chemotherapy, has been achieved (5). With progress in antineoplastic agent identification and development over the past several decades, >100 antineoplastic agents have been identified and approved by the USA Food

and Drug Administration (FDA) (6-8). 5-Fluorouracil (5-FU) is a member of the antimetabolite group administered intravenously. As an analogue of pyrimidine, it is incorporated into DNA and RNA, thus blocking its synthesis. Capecitabine, a prodrug of 5-FU, is administered orally and exhibits the same cellular mechanism as 5-FU (5,6). Initially, capecitabine requires three-step enzymatic methods to transform it to the antimetabolite 5-FU. The variation in the presence of these metabolic enzymes between tumor and normal tissues gives capecitabine a theoretical advantage over 5-FU in terms of efficacy (5). The objective of the current review is to provide a comprehensive overview of 5-FU and capecitabine therapies regarding their physical and chemical properties, metabolism, cellular mechanism, safety and tolerability. In addition, the effects of 5-FU and capecitabine treatments on the apoptotic mechanism and cell cycle phases of certain CRC cell lines are discussed. Furthermore, the three metabolizing enzymes required for the conversion of capecitabine to 5-FU are explained, and the concept of a chemotherapeutic approach combined with capecitabine treatment is also presented.

2. Chemotherapeutic drugs

Depending on their mechanisms of action, chemotherapeutic drugs can be broadly categorized into two basic categories: Cytotoxic drugs and targeted drugs (8,9). Cytotoxic drugs are defined as agents that can eliminate rapidly dividing cancer cells by targeting components of the mitotic and/or DNA replication pathways. Conversely, targeted drugs are agents that can block the proliferation and spread of cancer cells by interacting with molecular targets involved in pathways linked to tumor growth, progression and metastasis (8,10). Anticancer agents are classified as chemotherapy, immunotherapy or hormonal therapy. Chemotherapy, as presented in Table I, involves a number of families defined by their chemical structure and mechanism of action, and categorized into the following eight groups: i) Alkylating agents, such as nitrogen mustards and nitrosoureas; ii) antimetabolites, including methotrexate, 5-FU, 6-mercaptopurine and hydroxyurea; iii) antibiotics, such as doxorubicin and mitomycin C; iv) mitotic inhibitors, including vincristine and vinblastine; v) topoisomerase I inhibitors, such as irinotecan; vi) topoisomerase II inhibitors, including etoposide; vii) platinum compounds, such as cisplatin; and viii) others (transcription factor inhibitors) including ecteinascidin (11,12).

Espinosa *et al* (12) proposed a method of anticancer drug classification based on targets that could be located at the DNA, RNA or protein levels. Drugs may be directed at different levels: Tumor cells or other elements included in carcinogenesis, such as the endothelium and extracellular matrix of the immune system or host cells.

The present review focused on antimetabolite agents. As aforementioned, the majority of anticancer drugs exert their cytotoxicity either by inhibiting DNA synthesis or damaging the DNA template (11). Antimetabolites, a class of chemotherapeutic agents, are the oldest designed anticancer drugs that target DNA and RNA molecules, and can be considered the first generation of targeted drugs (13). Antimetabolites are defined as substances that are structurally analogous to natural metabolites and play a role in cellular metabolism, in which they compete with or replace them, thus preventing or reducing their normal cellular utilization (13,14). Antimetabolites are used to target 'key' enzymes in the *de novo* biosynthesis pathways of purine (dATP and dGTP) and pyrimidine (UTP, dTTP and dCTP) nucleotides (11).

Antimetabolite classes include folate analogs (including aminopterin and methotrexate), pyrimidine analogs [such as 5-fluoropyrimidines (5-FU, ftorafur and capecitabine), gemcitabine and cytarabine] and purine analogs (including thiopurines, 6-mercaptopurine, pentostatin and cladribine) (11-13). According to the drug classification of Espinosa *et al* (12), antimetabolites can be grouped into drugs directed against tumor DNA (sub-class: DNA-related proteins) and drugs directed against tumor RNA. Antimetabolites can also be grouped into drugs directed at protein-DNA complexes because they do not bind directly to DNA molecules. Instead, they act by interfering with enzymes shared during DNA synthesis. This group includes antifolates, 5-fluoropyrimidines (5-FU, ftorafur and capecitabine), raltitrexed, cytarabine, gemcitabine and adenosine analogs (fludarabine, pentostatin and cladribine). Conversely, antimetabolites classified as drugs directed against tumor RNA include 5-fluoropyrimidines (5-FU, ftorafur and capecitabine) and platinum compounds (12). Antimetabolite agents are widely used to treat viral infections and multiple types of cancer. As cancer cells have abnormal proliferation compared with normal cells, blocking DNA and RNA synthesis is an efficient anticancer treatment strategy (13). Antimetabolite agents were first demonstrated to have clinical anticancer activity ~50 years ago (15). They are currently used in chemotherapy combinations for the treatment of different leukemias and solid tumors worldwide (16).

5-Fluoropyrimidine compounds, a class of antimetabolite agents, are the result of a fluorine substitution at a specific site of the pyrimidine ring; specifically, replacement of a hydrogen atom at position 5 of the pyrimidine ring by a similarly sized fluorine (F) atom (17). Naturally, to convert uracil (U) nucleotides to thymine (T) nucleotides, the insertion of the methyl group ($-CH_3$) should take place at the 5-position of the U ring instead of the hydrogen atom. During the development of 5-fluoropyrimidine compounds, the 5-position of the U ring, which occupies the hydrogen atom, was selected as the specific site of substitution with the F atom because it was considered to be more efficient in inhibiting the subsequent conversion of the U nucleotide to the T nucleotide, thus preventing DNA synthesis and cellular proliferation (17). 5-Fluoropyrimidines are a class of anticancer drugs, specifically antimetabolites (18). Clinically, they are still used as a standard treatment regimen for solid tumors, including breast cancer, CRC and cancers of the aerodigestive tract (18,19). Examples of 5-fluoropyrimidine drugs include 5-FU, ftorafur and capecitabine (12).

3. 5-FU

5-FU was specifically created to resemble pyrimidine bases (U and T) (11). It is a fluorinated analog of the nitrogenous base U, and it was originally synthesized in 1957 (20) and sold under the brand name Adrucil® (21). It was the first pyrimidine analog registered for cancer treatment (16). Approved by the USA FDA in 1962, 5-FU is an antimetabolite commonly used as a chemotherapeutic agent, with activity against several types

Table I. Classical classification of anticancer drugs.

Anticancer drug	Classification
Chemotherapy	Alkylators
	Antibiotics
	Antimetabolites
	Topoisomerase inhibitors
	Mitosis inhibitors
	Others (inhibitors of transcription factors)
Hormonal therapy	Steroids
	Anti-estrogens
	Anti-androgens
	LH-RH analogues
	Anti-aromatase agents
Immunotherapy	Interferon
	Interleukin 2
	Vaccines

Data adapted from reference 12. LH-RH, luteinizing hormone-releasing hormone.

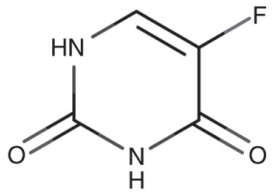

Figure 1. Chemical structure of 5-fluorouracil (26).

of solid tumors, including head and neck, breast, prostate, pancreas, liver, genitourinary, gastrointestinal tract and ovary cancer, and CRC (20,22). It has been the main chemotherapeutic agent for the treatment of CRC for ~50 years (23,24), and has been used as a single agent and in combination with other agents (24). In a 5-FU molecule, the hydrogen atom at position 5 of the U base is replaced with a similarly sized F atom (25-27), as shown in Fig. 1. This molecule was designed to occupy the active sites of target enzymes, thereby blocking metabolism in tumor cells (25). The International Union of Pure and Applied Chemistry (IUPAC) name for 5-FU is 5-fluoropyrimidine-2,4(1*H*,3*H*)-dione (27). Various physical and chemical properties of the 5-FU compound are listed in Table II.

Metabolism of 5-FU. 5-FU can enter efficiently a cell using a facilitated transmembrane carrier system known as a nucleoside transporter system, specifically the human equilibrative nucleoside transporter (36), where it is then transformed into several metabolites. This drug requires enzymatic conversion into a nucleotide through ribosylation and phosphorylation reactions to perform its cytostatic activity. Moreover, conversion to nucleotides enhances intracellular retention and further metabolism (37). The level of 5-FU available for transformation into an active nucleotide is based on the extent to which it is catabolized (22). After penetration into a cell, 5-FU is metabolized via two routes; either an anabolic or a catabolic route in competition with each other (25). The anabolic route (activation route) occurs through thymidine phosphorylase (TP), which forms the active metabolites of 5-FU that are responsible for cytotoxic activity. The catabolic route (inactivation route) occurs through dihydropyrimidine dehydrogenase (DPD), which is responsible for the inactivation, degradation, detoxification and subsequent elimination of 5-FU from an organism (20,25).

The anabolic route, which can be complex, demonstrates the mechanism of 5-FU cytotoxicity (20). Both cancerous and non-cancerous cells metabolize 5-FU drugs intracellularly via three routes into three different active metabolites that cause cell injury: 5-Fluoro-2'-deoxyuridine-5'-monophosphate (5-FdUMP); 5-fluoro-2'-deoxyuridine-5'-triphosphate (5-FdUTP) and 5-fluorouridine-5'-triphosphate (5-FUTP) (18,19,38), as presented in Fig. 2.

The first route, which is the conversion of 5-FU into 5-fluoro-2'-deoxyuridine (5-FdUrd), is performed through the TP enzyme, followed by the subsequent phosphorylation of 5-FdUrd through the thymidine kinase enzyme, which results in the formation of the cytotoxic nucleotide 5-FdUMP (18,22). In the presence of a cofactor, reduced folate 5-FdUMP, which is a powerful inhibitor of the thymidylate synthase (TS) enzyme, binds to TS to form a covalent complex. This binding blocks the production of thymidylate, which is a necessary precursor of deoxy thymidine triphosphate (dTTP), which is essential for DNA synthesis (19,22,38), thus inhibiting cell division (19). In addition, the clinical response to 5-FU treatment correlates with the level of TS inhibition of 5-FdUMP in patient tumors (38).

The second route, which is the formation of 5-fluorouridine-5'-monophosphate (5-FUMP) from 5-FU, is carried out either through direct conversion by orotate phosphoribosyl transferase in the presence of 5'-phosphoribosyl-1-pyrophosphate as a co-substrate or through intermediate conversion into a ribonucleoside 5-fluorouridine by uridine and subsequent conversion into 5-FUMP by uridine kinase. The formed 5-FUMP can undergo two steps of phosphorylation to form 5-fluorouridine-5-diphosphate (5-FUDP) and 5-FUTP through pyrimidine monophosphate kinase and pyrimidine diphosphate kinase (PDPK), respectively (25,39). Nuclear transcriptional enzymes can mistakenly incorporate 5-FUTP instead of uridine triphosphate during the synthesis of RNA molecules (19,25). The previous mistake can overlap with RNA processing (post-transcriptional process) and protein synthesis (translation) (19). Moreover, 5-FUTP can be conjugated to sugars through UDP glucose phosphorylase, producing 5-FU-nucleotide sugars (5-FUDP-sugars) that are incorporated into the cell membrane, thereby altering the membrane function (25).

In the third route, both 5-FUDP and 5-FdUMP can be converted into 5-fluoro-2'-deoxyuridine-5'-diphosphate, which is phosphorylated by PDPK to 5-FdUTP. 5-FdUTP acts as a substrate for DNA polymerases, which are enzymes responsible for DNA replication, and can therefore be incorporated into a DNA molecule, leading to the production of single-strand breaks and DNA fragmentation (which inhibits DNA replication) (11,25,40,41).

Table II. Physical and chemical properties of 5-flourouracil.

Property name	Description	(Refs.)
Form (Appearance)	Crystalline powder	(28)
Color	White to nearly white	(28)
Solubility	Less than 1 mg/ml (at 66˚F=18.89˚C)	(29)
Molecular weight	130.08 g/mol	(30)
Molecular formula	$C_4H_3FN_2O_2$	(26)
Formal charge	0	(30)
Hydrogen bond donor count	2	(30)
Hydrogen bond acceptor count	3	(30)
Rotatable bond count	0	(30)
Number of rings	1	(31,32)
Acid/Base property	A weakly acidic compound	(33)
pK_a	7.93 or 8.05	(34,35)

The degradation of 5-FU (the catabolic route) is mediated by the DPD enzyme, resulting in the reduction of 5-FU to the markedly less toxic 5-fluoro-5,6-dihydrouracil compound (20,25,41), which then undergoes two cleavage reactions. In the first reaction, the dihydropyrimidinase enzyme cleaves the pyrimidine to produce α-fluoro-β-ureidopropionic acid (FUPA) (19,41). In the second step, the β-ureido-propionase enzyme cleaves FUPA to produce α-fluoro-β-alanine, which is the major catabolite of 5-FU and is cleared by urinary elimination (19,42). Neither of these metabolites has antiproliferative effects (20,25), as shown in Fig. 3. DPD is the initial rate-limiting enzyme of 5-FU catabolism (i.e., the first enzyme in the catabolic cycle of 5-FU), and it is abundant in the liver, intestinal mucosa, pancreas, lungs, kidneys and peripheral blood (22,23). ~90% of 5-FU drugs are catabolized by DPD, while only 10% are excreted without a change in urine (22). Due to the role of DPD in the detoxification of 5-FU, deficiency of this enzyme in patients can result in severe and even lethal 5-FU toxicity (18,20).

5-FU cytotoxicity is caused by interference with RNA and DNA synthesis (22,25). Specifically, it interferes with RNA synthesis through the inclusion of a drug metabolite (5-FUTP) in replicating RNA molecules, thus impeding RNA processing and subsequent protein synthesis (inhibition of RNA synthesis and function) (19,22,40). It also interferes with DNA synthesis by inhibiting the TS enzyme, resulting in the depletion of the thymidine nitrogenous base, which is necessary for DNA synthesis (19,20,43). Moreover, the cellular damage resulting from 5-FU induces three modes of cell proliferation modulation: i) Accumulation or loss of S-phase cells; ii) blocking of the G_2/M phase; iii) and arrest of the G_1/S phase (22). The cause of the accumulation or loss of S-phase cells is the blocking of the *de novo* synthesis of T nucleotides, which leads to a lack of T in tumor cells, resulting in the inability of cells to create DNA molecules, which accumulate at the beginning of the S-phase (44). Another mechanism that could be partly responsible for the cytotoxic effect of 5-FU was reported, which involves the alteration of membrane function after the treatment of cells with 5-FU. This alteration is associated with the formation of 5-FUDP-sugars after 5-FUTP conjugation to sugars and their incorporation into the cell membrane (25). The extent to which any of these mechanisms predominate in human cancer is unknown, and varies depending on the cancer type, route of administration and dose of drug. It has been suggested that longer exposure to low doses of 5-FU [as intravenous (IV) treatment] results in a TS-inhibited mechanism (DNA damage), which mediates the process of cell death, whereas the bolus route of 5-FU results in an RNA-mediated process of cell death (RNA damage) (18,25).

The present review used DrugBank 5.1.8 (https://www.drugbank.com) to identify the direct cellular targets (referred as DTs) of 5-FU. DrugBank is a comprehensive online database that provides specific drug data and information on drug actions and targets. 5-FU was output as DB00544 in DrugBank 5.1.8, and various DTs of 5-FU that involved DNA, RNA, TS, TP, DPD, and multidrug resistance-associated proteins 4 and 5 were selected (32), as demonstrated in Table III.

The SwissADME (http://www.swissadme.ch/) and DrugBank 5.1.8 databases were used to represent certain pharmacokinetic properties. 5-FU was found to be readily (highly) absorbed from the human intestine and did not cross the blood-brain barrier (BBB) (32,45). Therefore, it could not cause toxicity to the central nervous system (CNS).

Effect of 5-FU treatment on the apoptotic pathway and cell cycle phases of CRC cell lines. Apoptosis is the mechanism of programmed cell death that normally occurs during development and aging. It is a homeostatic process that maintains cell populations in tissues and a defense mechanism when cells are damaged. There are two main apoptotic pathways, namely the extrinsic and intrinsic pathways, in addition to a third new pathway, which is the perforin/granzyme pathway. The extrinsic pathway is called the death receptor pathway, and is triggered by the binding of a death ligand to death receptors, such as Fas and tumor necrosis factor receptors, which are expressed on the cell surface. The intrinsic pathway is called the mitochondrial-dependent pathway, and is initiated by mitochondria. The perforin/granzyme pathway involves perforin/granzyme-dependent cell killing and T-cell-mediated cytotoxicity. Each pathway has a cascade of molecular events

Figure 2. Intracellular anabolic route of 5-FU. This drug is converted via three routes into three different cytotoxic metabolites: 5-FdUmp, 5-FdUTP and 5-FUTP. Thereafter, the cytotoxic effects of 5-FU can occur through the incorporation of these cytotoxic metabolites into DNA, RNA and the cell membrane. These mechanisms inhibit DNA and RNA synthesis, and alter the cell membrane function. 5-FU, 5-fluorouracil; 5-FUrd, 5-fluorouridine; 5-FUMP, 5-fluorouridine monophosphate; 5-FUDP, 5-fluorouridine-5-diphosphate; 5-FdUDP, 5-fluoro-2'-deoxyuridine-5'-diphosphate; 5-FUTP, 5-fluorouridine-5'-triphosphate; 5-FdUTP, 5-fluoro-2'-deoxyuridine-5'-triphosphate; 5-FUDP-Sugar, 5-FU-nucleotide sugar; 5-FdUrd, 5-fluoro-2'-deoxyuridine; 5-FdUmp, 5-fluoro-2'-deoxyuridine-5'-monophosphate; dTMP, deoxythymidine monophosphate.

and activates its own initiator caspases (8-10), which in turn activates the executioner caspase 3 (46,47). The cell cycle is a complicated process involved in cell proliferation and development, regulation of DNA repair mechanisms, tissue hyperplasia, and diseases such as cancer (48). Cell cycle arrest has been considered a target for cancer therapy, in which cancer cell proliferation and metastasis can be prevented by specific cell cycle regulation (49). Cyclin-dependent kinases (CDKs) are regulatory proteins that act in specific pathways to determine whether the cell cycle may remain arrested between stages or proceed (50). The cell cycle transition is an ordered, regulated process that includes multiple checkpoints, which assess extracellular growth signals, cell size and DNA integrity (51). Cell division has three stages: i) The interphase (G_0, G_1, S and G_2 phases); ii) mitotic phase (M phase); and iii) cytokinesis. The interphase is the first and longest stage of the cell cycle, and consists of four steps: i) Gap 0 phase (G_0); ii) growth phase 1 (G_1); iii) synthesis phase (S); and iv) growth phase 2 (G_2). Overall, these steps take 12-24 h in mammalian tissues, in which the cell increases in size and produces proteins that synthesize RNA (52). The G_0 phase is the arresting time when the cell stops dividing and leaves the cycle, while the G_1 phase is when the cell increases in size and starts producing RNA and synthesizing proteins. This step is important to ensure that

Table III. Identification of certain direct cellular targets of 5-FU in humans using DrugBank 5.1.8.

Drug name	DrugBank Identity (DB-ID)	Target name	Type of target	Gene name	Uniprot ID
5-FU	DB00544	DNA	Nucleotide	-	-
		RNA	Nucleotide	-	-
		Thymidylate synthase	Protein	*TYMS*	P04818
		Thymidine phosphorylase	Protein	*TYMP*	P19971
		Dihydropyrimidine dehydrogenase [NADP (+)]	Protein	*DPYD*	Q12882
		Multidrug resistance-associated protein 4	Protein	*ABCC4*	O15439
		Multidrug resistance-associated protein 5	Protein	*ABCC5*	O15440

5-FU, 5-fluorouracil.

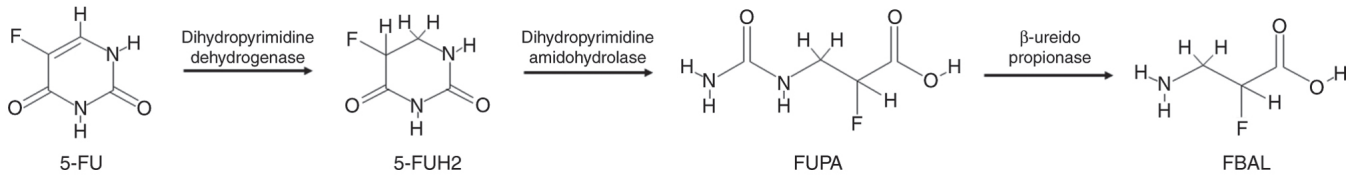

Figure 3. Catabolic route of 5-FU before urinary elimination. Initially, 5-FU is degraded by the dihydropyrimidine dehydrogenase enzyme, resulting in the reduction of 5-FU to a markedly less toxic metabolite, which then undergoes two cleavage reactions and subsequent elimination from the body through the urine. The figure was created by using Chem4Word tool (www.chem4word.co.uk) in Microsoft Word. 5-FU, 5-fluorouracil; 5-FUH_2, 5-fluoro-1,3-diazinane-2,4-dione; FUPA, 3-carbamoylamino-2-fluoropropanoic acid; FBAL, 3-amino-2-fluoropropanoic acid.

everything is prepared for DNA synthesis (S phase) (51). The S phase is where DNA replication begins, and it aims to produce new daughter cells that have two identical chromosomes. Accuracy is important in preventing any genetic abnormalities that can lead to disease or cell death (52). In the G_2 phase, the cells proliferate and produce new proteins. The checkpoint of this phase determines whether the cell is ready to enter a new stage (mitotic M phase) and divide (51). The mitosis phase, which is the second stage, is shorter than the interphase, in which the chromosomes split between the two daughter cells. In the middle of this phase there is a checkpoint similar to the G_1 and G_2 phases, which ensures the cell is ready to complete the division (52). Cytokinesis, the final stage of the cell cycle, is initiated during the late phase of mitosis, during which the two daughter cells are completely separated (51,53).

DNA-damaging chemotherapeutic agents, including antimetabolites, generally trigger cell death in tumor cells by inducing apoptosis. The extent to which cells are susceptible to apoptosis depends on the status of the genes (e.g., tumor suppressor gene p53) that regulate the critical components of the cell death mechanism (54,55). These antimetabolites, including 5-FU and the prodrug capecitabine, are considered to stimulate the intrinsic apoptotic pathway (mitochondria-dependent pathway) through caspase-8 and/or caspase-3 activation. Moreover, they cause cell cycle arrest in the S phase (56).

The following section discusses the effects of 5-FU treatment on apoptosis and the changes in the cell cycle phases of CRC cell lines reported by several studies. The human colon cancer cell line Caco-2 was treated with 5-FU (2 and 5 μM) and underwent the intrinsic apoptotic mechanism through the activation of caspase 9. This treatment significantly increased the intracellular reactive oxygen species (ROS) level, which caused oxidative stress apoptotic pathways in cancer cells (57). The percentage of the human colon cancer cell line HCT-116 in the G_1 phase of the cell cycle decreased after 5-FU treatment (10 μg/l), whereas that in the G_2/M phase increased. No significant apoptotic effect of 5-FU chemotherapy was observed in the same cancer cells (58). 5-FU treatment was added to *TP53* 5-FU-resistant cell lines (ContinB and ContinD) generated from the HCT-116 human colon cancer cell line to determine its effects on cell cycle and apoptosis. The results revealed that 5-FU treatment caused S phase arrest, accumulation in the G_2/M phase, upregulation of cell cycle regulation (*CDKN1A*) and apoptosis induction in both parental and resistant cell lines with variable levels of response (high or low). Additionally, it resulted in the upregulation of *p53*-target genes in the DNA damage response and apoptosis-regulatory pathways (Fas) in the same cells (59). Analysis of the SW-620 colorectal adenocarcinoma cell line treated with the half-maximal inhibitory concentration of 5-FU indicated that 5-FU therapy resulted in apoptosis and increased percentage of cells in the S phase of the cell cycle (60). The cell cycle alterations in human colon cancer cell lines (HCT-116 and metastatic LoVo) treated with 5-FU were evaluated as follows: In HCT-116 cells, 5-FU induced significant arrest in the S phase, while LoVo cells accumulated in the G_2/M phase. Afrin *et al* (61) identified the molecular mechanisms involved in cell cycle arrest using 5-FU treatment. Notably, the mRNA levels of CDK2 and CDK4 and cell cycle regulatory proteins (cyclin D1 and cyclin E) decreased in HCT-116 cells, while the LoVo cells did not demonstrate any alteration in cyclin E, CDK2 or CDK4 mRNA

levels after 5-FU treatment, but cyclin D1 was suppressed. In both cell lines, the percentage of cells undergoing apoptosis significantly increased after 5-FU treatment. The apoptotic effects were confirmed by examining the mRNA expression of extrinsic and intrinsic apoptotic markers. In both cell lines, caspase 3, p53 and cleaved poly (ADP-ribose) polymerase levels increased after 5-FU treatment. The expression of the intrinsic apoptotic markers Bax/Bcl-2, cytochrome *c* and caspase 9 was increased in both cell lines. Furthermore, the expression of the extrinsic apoptotic markers FasL and caspase 8 increased after 5-FU treatment in both cell lines (61).

Summary of the safety, tolerability and disadvantages of 5-FU treatment. Oral administration of 5-FU is not clinically useful due to the large-scale metabolism by the DPD enzyme in the mucosa of the gastrointestinal tract and liver, which leads to extremely variable bioavailability (19). Therefore, this drug must be administered intravenously because of its rapid clearance, degradation and variable gastrointestinal absorption (20,22). This route is complicated by hospital visits and the risk of infection with IV administration (20). Moreover, this regimen is non-curative, and its effect on survival is unclear (23). Although 5-FU-based chemotherapy is the most widely used treatment for solid gastrointestinal tumors, it has various disadvantages, including toxicity, lack of selectivity (poor tissue specificity), lack of effectiveness and development of resistance (22). The systemic toxicities of this chemotherapeutic agent are neutropenia, stomatitis and diarrhea, which usually occur due to non-selective cytotoxicity (20). The principal side effects of continuous infusion of 5-FU were hand-foot syndrome (HFS), diarrhea, nausea, vomiting and mucositis (42).

The resistance of tumor cells to 5-FU chemotherapy is associated with the TS enzyme, which is one of the most important mechanisms of 5-FU resistance. Carrillo *et al* (22) summarized the proposed mechanisms of 5-FU resistance, including TS induction, decreased accumulation of activated metabolites, gene amplification and mutation, aberrant enzyme kinetics, imbalanced folate pools, imbalanced metabolite accumulation, imbalanced ATP: dTTP ratio, pharmacokinetic resistance, availability of the compound in the tumor, drug distribution, increased drug elimination, high DPD enzyme activity, increased transporter activity, ATP-binding cassette proteins and multidrug resistance proteins.

Overcoming 5-FU complications. The side effects of 5-FU treatment need to be overcome by i) improving selectivity toward cancer cells; ii) enhancing cell absorption and metabolic stability; iii) increasing specific tumor cell toxicity; and iv) increasing its bioavailability. Overcoming the development of 5-FU resistance, which is highly required due to the resistance of tumor cells to conventional chemotherapy, plays a key role in the fight against cancer. To fulfil these objectives, several oral fluoropyrimidines have been developed to achieve the need for more convenient therapy, and to enhance the safety and efficacy profile (22,40). These antimetabolites were designed to have a novel mechanism of action, bypass drug resistance, or be used in combination to enhance the effects of other drugs (2). Oral fluoropyrimidines are classified into three groups: 5-FU prodrugs, 5-FU combined with a DPD inhibitor, and 5-FU prodrugs combined with a DPD inhibitor (40). Efforts to identify more efficient chemotherapeutic agents for CRC have resulted in oral formulations and prodrugs of 5-FU with equivalent activity (23). Oral prodrugs of 5-FU were developed to reduce the problems of IV administration through oral dosing, and to ameliorate safety and efficacy by selectively delivering the active drug to the target cancer cell, thereby avoiding healthy tissue (20). In addition, the oral route may simulate continuous infusion schedules without the inconvenience, morbidity or cost associated with the IV route (23).

A prodrug is a pharmacologically inactive compound, that is, the precursor of a drug, which is converted into an active agent by a metabolic biotransformation process (25). These prodrugs are activated by biological mediators only when they enter specific tissues or cells. Moreover, these derivatives are created by modifying 5-FU, for example by coupling with peptides, amino acids, phospholipids and polymers (22). Among 5-FU prodrugs, capecitabine has been demonstrated to have clinical efficacy with low toxicity compared with 5-FU (19), as well as increased metabolic stability and selectivity toward tumor tissue (22).

4. Capecitabine

Capecitabine, a fluorouracil prodrug, is the only novel, universally approved and orally home-administered drug (19). Its brand name is Xeloda® (25,62). It was synthesized in the 1990s (25) and approved by the USA FDA in 2005 (20,24) for use as a first-line therapy in patients with metastatic CRC when single-agent fluoropyrimidine therapy is preferred (20). This oral-formulation prodrug is a nucleoside metabolic inhibitor used for the treatment of CRC in three settings: i) Monotherapy; ii) adjuvant treatment; iii) and combination therapy with other agents for metastatic or advanced disease (22,24). This drug has been studied in large-scale clinical trials of several solid tumors, including CRC, breast cancer and gastric cancer (19).

Capecitabine, as shown in Fig. 4, is a carbamate ester that is a cytidine nucleoside. A cytosine base is attached to a ribose sugar ring. The hydrogen atom at position 5 is replaced by a F atom, in which the amino group (NH_2) attached to position 4 is converted to its N-(pentyloxy) carbonyl derivative. It is also a carbamate ester (derived from carbamic acid, an organofluorine compound and a member of the cytidine class) (63,64). Moreover, it is a prodrug of fluoropyrimidine carbamate (20,65) and a carbamate derivative of 5'-deoxy-5-fluorouridine (5'-DFUR) (20). It is stable for ≥9 months in tablet form (20,66). The IUPAC name for capecitabine is 5'-deoxy-5-fluoro-*N*-[(pentyloxy) carbonyl] cytidine (64). The physical and chemical properties of capecitabine are listed in Table IV.

Compared with 5-FU therapy, the developed capecitabine is an alternative to 5-FU therapy and offers multiple advantages over the original fluorinated analog of uracil 5-FU, including reduced toxicity, convenience, tolerability, efficient agents and ease of administration (19,69). Patients can be free from hospital visits that do not require continuous infusion (unlike 5-FU) (18,19), which facilitates the adherence of patients to the treatment regimen and provides an improved clinical outcome (22). Therefore, the development of orally administered 5-FU that prevents its degradation in the gastrointestinal

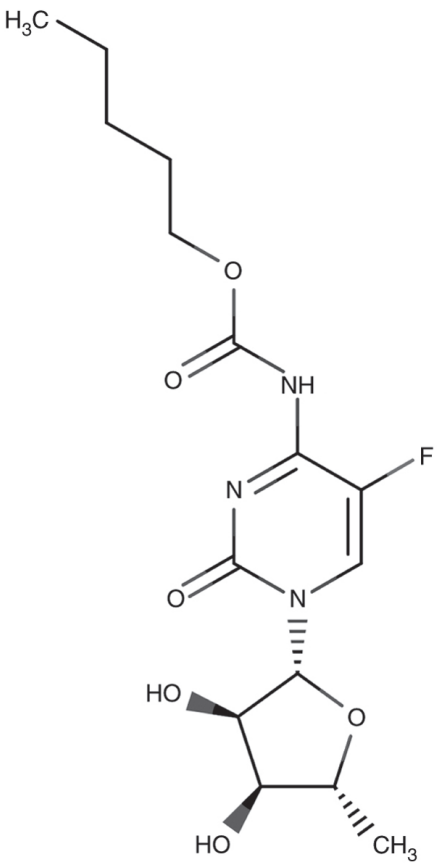

Figure 4. Chemical structure of capecitabine (63).

mucosa and other tissues, is expected to make the oral prodrug of 5-FU more bioavailable and increase the systemic exposure to the drug (23). Capecitabine has ~100% bioavailability in adults (20,40). The oral fluoropyrimidine carbamate mimics the serum concentration levels of 5-FU continuous infusion (24,69). Capecitabine is a tumor-selective cytotoxic agent that is selectively activated by the TP enzyme (42). This drug was designed as a prodrug to form 5-FU preferentially *in situ* or at the tumor site (69).

Metabolism of capecitabine. Capecitabine is a relatively non-cytotoxic drug *in vivo* and *in vitro* compared with 5-FU (19). Following oral administration (activation route), capecitabine was readily absorbed as an intact molecule in the prodrug form through the intestinal wall, and was then extensively metabolized enzymatically to its active compound (5-FU) through three metabolic activation steps (Fig. 5) (19,20,24). Once capecitabine was readily absorbed through the gastrointestinal tract, hepatic carboxylesterase (CES) hydrolyzed the majority of the compound to 5'-deoxy-5-fluorocytidine (5'-DFCR) (19,20,42). Cytidine deaminase (CDA), a ubiquitous enzyme detected in high concentrations in most tissues, including liver, plasma and tumor tissues, converted 5'-DFCR to 5'-DFUR (19,20,42). Finally, the TP enzyme hydrolyzed 5'-DFUR to the active drug 5-FU (19,20,42). This final metabolic step was considered to occur preferentially in tumor tissues because TP was overexpressed at consistently higher concentrations in various human solid tumors compared with normal adjacent tissues (19,20,24,42,69), and played a critical role in tumor growth, angiogenesis, invasion and metastasis (69). To evaluate this hypothesis, Schüller *et al* (70) conducted a study and observed that 5-FU was present at a concentration level 3.2-fold higher in CRC tissues than in adjacent healthy tissues. Moreover, the mean tissue/plasma 5-FU concentration ratios exceeded 20 for CRC, while they were 8-10 for other tissues. In brief, capecitabine reached higher levels of 5-FU in tumor tissues than in plasma or other tissues (71). This theoretically allows for the targeted intratumoral (*in situ*) release of 5-FU, thus enhancing the selective activation of capecitabine, and demonstrating improved tolerability of the drug and less systemic toxicity compared with IV 5-FU (19,20). Clinical evidence to support this were observed in a study of patients with CRC (19).

As presented in Fig. 6, after the transformation of capecitabine into the active form 5-FU, it is degraded (inactivation route) into fewer toxic metabolites. This is mediated by the DPD enzyme, followed by urinary elimination, as aforementioned. Briefly, when capecitabine is absorbed unchanged (inactive form) through the intestinal wall (20), it is selectively activated in tumors by the TP enzyme into its only active metabolite, 5-FU (20,22,65). Once 5-FU is activated, it exerts cytotoxic effects.

The present review used DrugBank 5.1.8 to identify the DTs of capecitabine. Capecitabine was output as DB01101 from DrugBank 5.1.8, and the DTs of capecitabine, including DNA, RNA, TS, liver CES 1, CDA, TP and DPD were selected (32) (Table V). When the SwissADME and DrugBank 5.1.8 databases were used to represent pharmacokinetic properties, it was revealed that capecitabine was readily absorbed from the intestine and did not cross the BBB (32,45), thus not causing toxicity to the CNS, similar to its parental 5-FU.

Effect of capecitabine treatment on the apoptotic pathway and the cell cycle phases of CRC cell lines. When the human colon cancer cell line Caco-2 was exposed to capecitabine treatment (2 μM), the intrinsic apoptotic mechanism was induced through the activation of caspase-9. This drug induced the apoptotic-oxidative stress pathway by increasing intracellular ROS levels (57). A previous study reported that capecitabine treatment of the human colon cancer cell line HCT-15 caused DNA condensation and triggered apoptosis in a dose-dependent manner. Additionally, capecitabine (5 μM) increased the population of HCT-15 cells in the G_0/G_1 phase, decreased the population of cells in S phase and caused a significant increase in the production of ROS compared with untreated cells (72). Capecitabine (300 μM) was evaluated on human CRC cell lines (LS174T and TP-transfected LS174T-c2) to identify the role of the Fas system in the apoptotic mechanism of these cell lines. Early and late apoptosis were induced by capecitabine in TP-transfected LS174T-c2 cells, while late apoptosis was induced in LS174T cells. This sensitivity to capecitabine treatment was accompanied by a strong overexpression of the CD95-Fas receptor (apoptosis receptor) on the cell surface of the treated cells (73). Capecitabine was applied as a single agent to the human colon cancer cell lines HCT-116, HT-29 and Caco-2, and it significantly stimulated apoptosis in HT-29 and Caco-2 cells, but no apoptotic effect was observed in HCT-116 cells (74). In Caco-2 cells, 100 μM capecitabine significantly increased the number of apoptotic cells compared with the control group (75).

Table IV. Physical and chemical properties of capecitabine.

Property name	Description	(Refs.)
Form (Appearance)	Crystalline powder	(67)
Color	White to off-white	(67)
Solubility	In water 26 mg/ml at 20˚C	(68)
Molecular weight	359.35 g/mol	(30)
Molecular formula	$C_{15}H_{22}FN_3O_6$	(63)
Formal charge	0	(30)
Rotatable bonds count	8	(45)
Hydrogen bond donor count	3	(30)
Hydrogen bond acceptor count	8	(45)
Number of rings	2	(31,32)
Acid/Base property	An extremely weak basic compound	(63)
pK_a	1.9	(63)

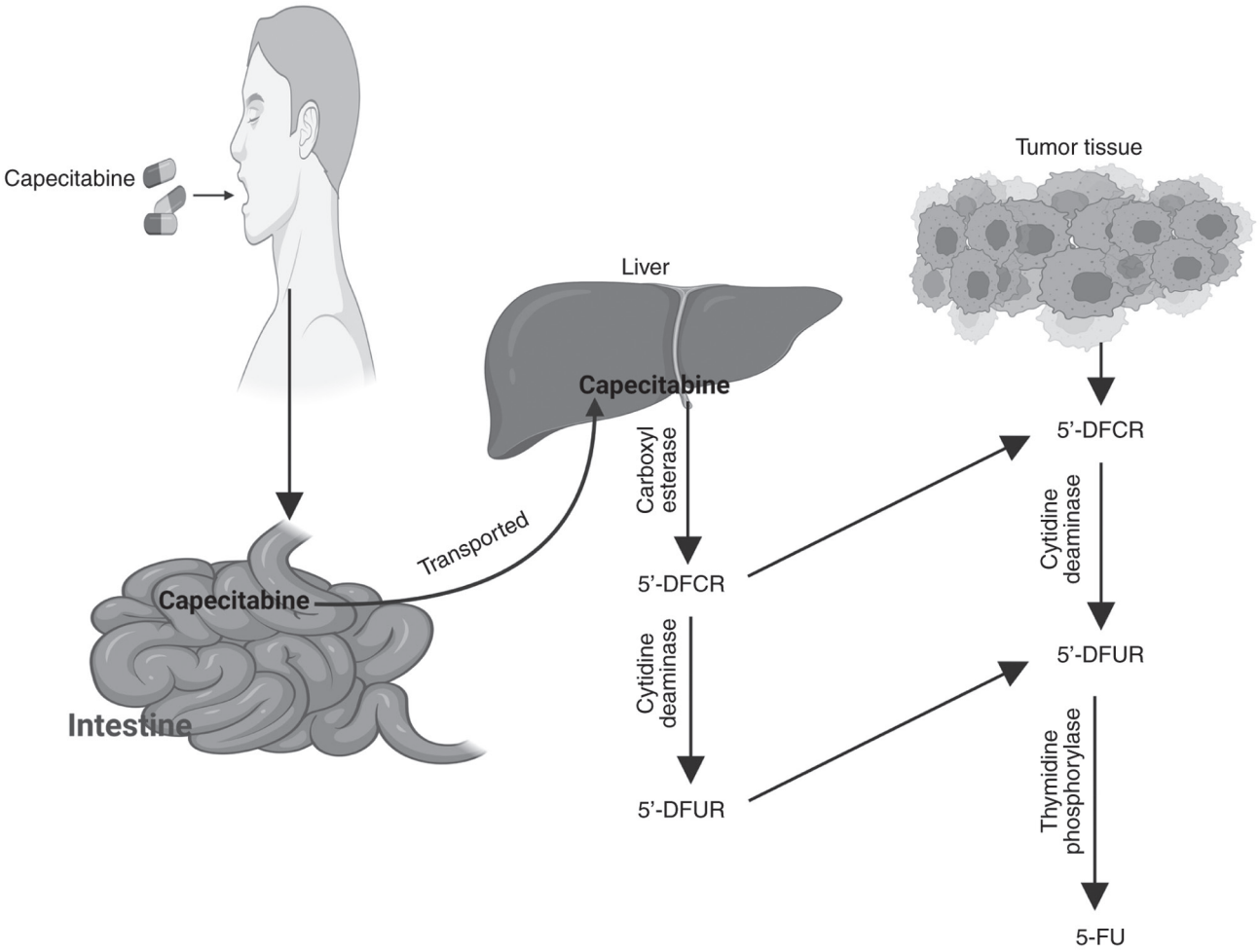

Figure 5. Three-step metabolic activation of the capecitabine prodrug. Capecitabine is readily absorbed through the intestinal wall and converted into 5'-DFCR by the carboxylesterase enzyme, and then into 5'-DFUR by the cytidine deaminase enzyme. Both steps take place in the liver. Finally, the thymidine phosphorylase enzyme converts 5'-DFUR into the active drug 5-FU. The final step occurs in both cancer and normal healthy tissues. The illustration was created with BioRender (www.biorender.com). 5-FU, 5-fluorouracil; 5'-DFCR, 5'-deoxy-5-fluorocytidine; 5'-DFUR, 5'-deoxy-5-fluorouridine.

Clinical outcomes from using capecitabine therapy over 5-FU therapy. In patients with metastatic CRC, the clinical outcomes of capecitabine as monotherapy were evaluated in comparison to IV 5-FU/leucovorin (LV) combination regimen, and the results revealed a higher response rate (RR) than 5-FU/LV, and an equivalency to 5-FU/LV in terms of overall and progression-free survival (19). Although the beneficial outcome of capecitabine in the increasing of RR was determined, the lack

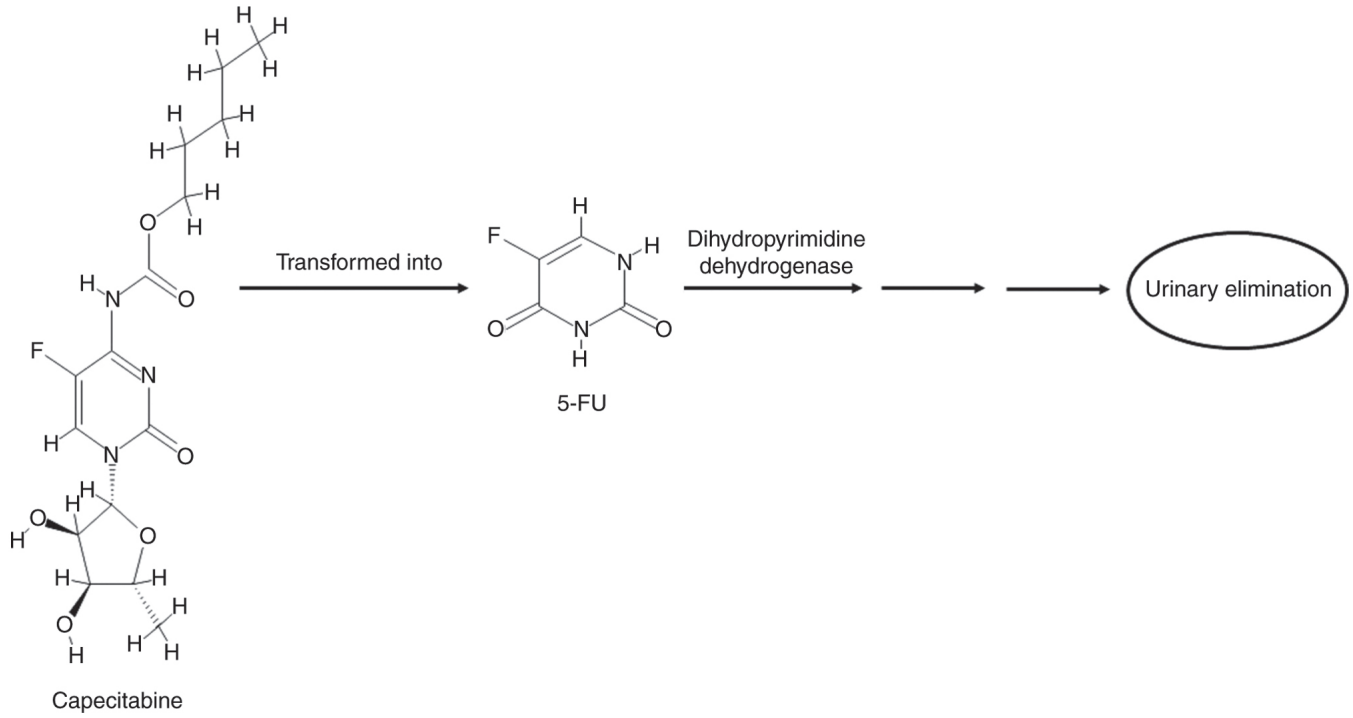

Figure 6. Degradation and elimination of capecitabine from the body. Primarily, capecitabine is converted to 5-FU and then undergoes degradation into lesser toxic metabolites as its parental 5-FU, which is mediated by the dihydropyrimidine dehydrogenase enzyme, followed by urinary elimination. 5-FU, 5-fluorouracil.

of differences in other variables indicated that the advantages of such combination in improving outcomes were similar to those of treatment with capecitabine as a single agent.

Summary of the safety and tolerability of capecitabine treatment. Since capecitabine is not a cytotoxic drug (42), its toxicity profile reflects that of its major active metabolite 5-FU (19,42). This prodrug has a well-established safety profile and can be provided safely to patients with advanced age, and renal and hepatic dysfunction (40). However, capecitabine should be administered with caution in these critical cases. Moreover, this oral therapy demonstrated a favorable tolerability profile (76). The most reported adverse effects associated with capecitabine therapy were HFS, diarrhea (19,40,42), nausea, vomiting, stomatitis (19,40) and hyperbilirubinemia (20). Previous adverse effects, including diarrhea, stomatitis and nausea, occurred significantly less frequently with continuous infusion of 5-FU (77). These toxic effects of capecitabine are considered secondary to 5-FU phosphorylation in the gastrointestinal tract (40). Myelosuppression with low incidence, weakness, fatigue, abdominal pain (20), myocardial infarction, angina and anemia (77) have also been reported as adverse effects of capecitabine therapy (20). Capecitabine treatment is less expensive than other chemotherapies for the treatment of toxic side effects (65). HFS may be supported with the use of pyridoxine (vitamin B6), while nausea and vomiting are easily controlled with antiemetics (19). However, a previous study reported that combined treatment of pyridoxine with capecitabine was not effective against HFS (78).

Metabolizing enzymes required for the activation of capecitabine to 5-FU. Capecitabine prodrug (inactive form) can be converted into 5-FU (active form) using three ordered metabolic activation steps, which involve CESs, CDA and TP (20,24).

CESs. Mammalian CESs are key enzymes belonging to the superfamily of serine hydrolase enzymes (79-83). Three CES isoenzymes are detected in the human body: CES1, CES2 and CES3. They are classic xenobiotic-metabolizing enzymes that play critical roles in the metabolism of a wide variety of endogenous esters, ester-containing drugs and environmental toxicants (83). Two human carboxylesterase genes (*CES1* and *CES2*) code for the mature 533-amino acid enzyme (84). Structurally, CESs are members of the superfamily of α/β-fold proteins, which comprises alternate α-helices and β-sheets joined together by loops of different lengths (85). The three-dimensional structure of CES1 consists of a central catalytic domain, an αβ domain and an adjacent regulatory domain that contains a low-affinity surface ligand-binding Z-site (86,87). As their name implies (serine hydrolases), these enzymes are characterized by the presence of the amino acid serine at the enzyme active site (83). CESs can be found as monomers, trimers or hexamers (83), with a molecular weight of 60 kDa (80,83). The majority of mammalian CESs are intracellular proteins located within the lumen of the endoplasmic reticulum in numerous tissues (79,81,82,83). The elementary physiological function of CES appears to be xenobiotic metabolism (83). CES enzymes exhibit broad substrate specificity, which efficiently catalyzes the ester cleavage of a large number of endogenous and xenobiotic substrates with ester, thioester, carbamate and amide bonds into the corresponding carboxylic acid, alcohols, thiols and amines (80,83,88). Therefore, they play major roles in the endobiotic metabolism and activation and/or detoxification

Table V. Identification of selected direct cellular targets of capecitabine in humans using DrugBank 5.1.8.

Drug name	DrugBank Identity (DB-ID)	Target name	Type of target	Gene name	Uniprot ID
Capecitabine	DB01101	DNA	Nucleotide	-	-
		RNA	Nucleotide	-	-
		Thymidylate synthase	Protein	*TYMS*	P04818
		Liver carboxylesterase 1	Protein	*CES1*	P23141
		Cytidine deaminase	Protein	*CDA*	P32320
		Thymidine phosphorylase	Protein	*TYMP*	P19971
		Dihydropyrimidine dehydrogenase [NADP (+)]	Protein	*DPYD*	Q12882

of xenobiotics (83). Furthermore, CESs are considered classic xenobiotic-metabolic enzymes responsible for the biotransformation of a wide range of ester-containing drugs, prodrugs and environmental toxins. CESs can be readily hydrolyzed by numerous clinical drugs with ester moieties, such as anticancer prodrugs (irinotecan and capecitabine) (83,88). Typically, both CES1 and CES2 are highly expressed in the epithelia of most metabolic organs, including liver, kidney and intestine, indicating that these two isoenzymes play protective roles against xenobiotics (82,83,89). Notably, the expression levels of CES1 and CES2 in tumor tissues and cancer cell lines are markedly different from those in normal healthy tissues and cells. For example, the human colon carcinoma cell line Caco-2 mainly expresses CES1, whereas the expression level of CES1 in the normal human intestine is markedly low (89). CES2 is overexpressed in various cancer cell lines (90). Both CES1A1 and CES2 isoenzymes are expressed in colon adenocarcinoma (91).

CDA. CDA is an ubiquitous enzyme that is a member of the cytidine and deoxycytidylate deaminase families (92). Human CDA is involved in the pyrimidine salvage pathway, which metabolizes numerous cytidine analogs used as prodrugs in chemotherapy (93). The human *CDA* gene codes a 146-amino-acid protein that relies on zinc (Zn^{2+}) binding. Human CDA is a 52-kDa homotetrameric enzyme with a molecular weight of 15 kDa of each subunit, with all four subunits requiring an essential zinc atom at their active site, which plays a crucial role in its catalytic activity (92-96). CDA is a cytoplasmic protein that may also be located in the nucleus (92). Alternatively, CDA may be referred to as cytidine amino hydrolase, in which the molecular role of CDA is the hydrolytic deamination function, which plays a major role in the recycling of free pyrimidines (92). CDA is an enzyme of the pyrimidine salvage pathway that catalyzes the biotransformation of cytidines and deoxycytidines into uridines and deoxyuridines, respectively, by hydrolyzing the amine moiety into ketones with the release of free ammonia (92,93,96). The pyrimidine salvage pathway contributes to two different aims: i) The recycling of pyrimidines for the synthesis of other nucleotides, which will be integrated into the synthesis of DNA and RNA molecules; and ii) the catabolism of pyrimidines to ensure a constant source of carbon and nitrogen, leading to the formation of β-alanine. Of note, this hydrolase enzyme plays a vital role in both pathways (92). The clinical relevance of human CDA is its ability to deaminate its natural substrate and several chemotherapeutic agents, including prodrugs (93). CDA is commonly expressed in the liver, spleen and bone marrow, and moderately expressed in other tissues, including kidney, lung, large intestinal mucosa and colon mucosa (92,93,97). This enzyme is mainly located in liver and tumor tissues (98). A previous study reported that pancreatic cancer tissues mainly expressed high levels of CDA in epithelial tumor cells, not in the stroma (99). CDA overexpression facilitated the metabolism of synthetic cytidine analogs for integration into DNA molecules, leading to cell cycle arrest and cell death (92). In healthy normal cells, the metabolites of these synthetic cytidine analogs are not inserted into the DNA molecule due to the presence of cytidine monophosphate kinase 1 enzyme, which is involved in nucleoside recycling and metabolic pathways, and acts as a barrier to protect the genome. These observations provide a therapeutic opportunity to treat CDA-overexpressing tumors (92).

TP. TP is a key enzyme in nucleoside metabolism and plays a critical role in the pyrimidine salvage pathway (100). Human TP is a tumor-associated angiogenic growth factor, the sole endothelial mitogen (101), and a member of the pyrimidine nucleoside phosphorylase family (101,102). TP is also known as platelet-derived endothelial cell growth factor (PD-ECGF) (100,103). The human *TP* gene encodes a protein of 482 amino acids with a molecular weight of 51 kDa. Human TP is a homodimeric protein composed of two identical subunits. Each subunit consists of a large α/β domain that contains a phosphate-binding site and a small α-helical domain that comprises a thymidine-binding site (100,102,104,105). TP is located intracellularly (102) in the cytosol and nucleus (106). In the cytoplasm, it presents enzymatic activity, while in the nucleus, it adjusts the pyrimidine nucleoside pool for DNA synthesis (100). TP is abundant in macrophages, platelets, stromal cells and cancer cells (107). The enzyme plays a dual role in the body by serving a metabolic function and a vital role in angiogenesis (100). The metabolic function of TP is to drive the pyrimidine nucleoside salvage pathway as a key enzyme (100). In the presence of inorganic phosphate (P_i), TP catalyzes the reversible conversion of thymidine to T and 2-deoxy-α-D-ribose-1-phosphate (2DDRP) (100,102,103), and the phosphorolysis of deoxyuridine to U and 2DDRP (100). The latter is further degraded to 2-D-deoxyribose (102). This reaction occurs through the cleavage of the glycosidic bond of pyrimidine 2-deoxynucleotides, most likely through an SN2-like transition state involving the nucleobase 2'-deoxyribose and P_i (101). Moreover, TP has deoxyribosyl transferase

activity, which involves the transfer of a deoxyribosyl moiety from a pyrimidine nucleoside to another pyrimidine base, resulting in a new pyrimidine nucleoside (102). This enzyme maintains a sufficient pool of pyrimidine nucleotides available for DNA repair and replication processes (100,101). In addition to its metabolic function, TP plays a key role in angiogenesis because it is identical to the angiogenic factor PD-ECGF (100). The TP enzyme utilizes thymidine and uridine nucleoside as substrates due to the overlapping substrate specificity for both the TP and uridine phosphorylase enzymes, as reported in a previous study on patients with colon cancer (100,108). In cancer, TP plays a complex role in cancer progression through its role in angiogenesis and determines the response to tumor treatments (100). TP catalyzes the conversion of the oral fluoropyrimidine prodrug capecitabine to the active form of 5-FU, which interferes with DNA synthesis (100,101). Furthermore, this enzyme is considered a predictive marker of the response to fluoropyrimidine (100). TP is expressed by various cells (102) and is frequently co-expressed with the vital angiogenic factor vascular endothelial growth factor (109). A previous study has shown that TP is highly expressed in a wide range of solid tumors compared with normal healthy tissues (103). Moreover, this enzyme is overexpressed in lung, gastric, breast, colorectal, bladder, cervical, esophageal cancer and oral squamous carcinoma (100). Overexpression of TP in cell cultures and xenograft models has been revealed to increase the sensitivity to 5-FU anticancer agents (100).

Opinion on capecitabine combined chemotherapy. Capecitabine represents a major advance in cancer therapeutics and has demonstrated promising anticancer activity as monotherapy or in combination with other chemotherapeutic drugs in colorectal, breast, pancreatic and head and neck cancer (40). Specific drug combinations in cancer treatment may provide additional positive effects that may be absent in a single drug, such as enhancement of tumor therapy efficiency (110). Therefore, the purpose of multidrug regimens (combined therapy) that usually involve drugs referred to different classes is to increase efficacy, decrease toxicity, bypass drug resistance and increase survival, at least when classical chemotherapy is concerned (12,13,111). One of the most widely studied chemotherapies in neoadjuvant and adjuvant settings is capecitabine treatment (111). Neoadjuvant and adjuvant chemotherapy combined with capecitabine has been used clinically, resulting in a significant improvement in patient survival and safety in different tumors, including CRC and breast cancer (111,112). An example of such combination therapy is XELOX (CAP + oxaliplatin) combined with camrelizumab (immune checkpoint inhibitor) plus bevacizumab or regorafenib (anti-angiogenic agents), which increases the rate of responses in patients with mCRC with microsatellite stability (113).

Drug resistance to 5-FU-based chemotherapy is one of the greatest challenges in CRC management. It can be acquired or intrinsic during treatment, and is considered to occur in ~50% of patients with metastatic CRC. Therefore, one of the emerging fields of precision medicine is determining the biological mechanisms associated with 5-FU-based chemotherapy response. This approach is expected to have a significant role in identifying patients likely to benefit from 5-FU-based chemotherapy in the future. Therefore, resistance to treatment may be predicted or overcame (114).

Factors that affect the response to capecitabine therapy. Chemoresistance to capecitabine may be due to various conditions that are relevant to the molecular features (genetic or/and epigenetic) and metabolic characteristic of patients with CRC, including: i) High MSI CRC, which is a CRC tumor with high MSI that arises from a deficiency in mismatch repair (MMR) genes (115); ii) single-nucleotide polymorphisms (SNPs), which are genetic variants of genes that are included in capecitabine activation steps (*CES*, *CDA* and *TYMP*), as well as the *TYMS* and *DPYD* genes, which are included in 5-FU metabolism (116); iii) *TP53* mutation (117); and iv) epigenetic alterations, including DNA methylation, histone modifications and abnormal changes of non-coding RNAs as regulators of the gene expression (118).

The majority of genetic (such as MSI and *TP53* mutation) and epigenetic modifications were mainly involved in the molecular pathogenicity of CRC, and these altered mechanisms accounted for a significant proportion of the molecular classification in CRC cases (4).

Multiple cellular and molecular changes have been reported to play crucial roles in the lack of CRC response to capecitabine treatment (119). These changes can be classified into four categories: i) Intracellular factors; ii) extracellular factors; iii) cell surface factors; and iv) cell-phenotype state. These factors can affect the response to capecitabine drug or/and its parental 5-FU.

Changes in intracellular factors. Various intracellular factors alter the response to therapy. Mutation of tumor-suppressor genes including *TP53* (117) and *APC* mutations were reported to lower the sensitivity to therapy (119). Changes in drug metabolism enzymes could affect the response to treatment as aforementioned (SNPs in *CES*, *CDA*, *TYMP* and *DPYD* genes) (116). Moreover, changes in drug targets such as SNPs in the *TYMS* gene cause a worse clinical outcome (116). Irregularities of non-coding RNA including microRNA-520g participation in resistance to 5-FU therapy (120). In addition, changes in DNA repairing mechanisms may affect the response to the treatment, which involves three different mechanisms, including: i) Changes in nucleotide excision repair mechanism as SNPs in the *ERCC1* and *ERCC2* genes (121); ii) defects in the MMR mechanism, which are accompanied by deficient MMR members in CRC, which account for developing a MSI phenotype in CRC tumors (115); and iii) changes in the base excision repair (BER) mechanism, which is accompanied by the high expression of BER proteins, and is correlated with more aggressive tumor characteristics and poor clinical outcomes in CRC cases (122). Alterations in the first two aforementioned mechanisms lead to resistance of capecitabine and 5-FU therapies, and reduced the overall survival of patients with mCRC (119). In addition, aberrant survival signaling pathways in CRC that involved hyperactivation of the Wnt/β-catenin, Notch and NF-κB signaling pathways could further enhance chemoresistance, cell proliferation and survival (119). The final variable is metabolic reprogramming such as the Warburg effect (119,123) which is associated with tumor aggressiveness and poor clinical outcome in CRC (123).

Changes in cell surface factors. The first variable of cell surface factors is changes in drug export pumps, such as SNPs in the *ABCC4* gene that affect multidrug resistance protein 4 function and lead to a decrease in intracellular drug concentration and therefore a lower clinical response (124). The second variable is the aberrant activation of the survival signaling pathway, which is represented by hyperactivation of the Wnt/β-catenin and Notch signaling pathways in CRC, which are relevant to resistance (119). Notably, the signal cascades of these pathways begin with the stimulation of their cell surface receptors, and then are transmitted to the intracellular space and maybe to the nucleus, such as the Wnt/β-catenin signaling pathway.

Changes in extracellular factors. The extracellular factors are known as the adaptation to the tumor microenvironment, which can affect the response to such therapy, and involve hypoxic condition, gut microbiota, impairment of pro- and anti-inflammatory cytokines, and the extracellular matrix surrounding CRC cells (119).

Cell-phenotype state. The first cellular phenotype is the epithelial-to-mesenchymal transition (EMT), which contributes to enhance the cell migratory capacity and thus promotes tumor metastasis. The EMT process is also associated with the malignancy of CRC such as drug resistance (125). The second one is the quiescent state of cancer stem cells (CSCs), which increases the chance of CSCs to become resistant. Moreover, as the majority of conventional chemotherapeutic drugs target proliferating cancer cells, a number of CSCs often survive and promote cancer relapse (119).

5. Conclusion

Chemotherapeutic agents include several drugs with different mechanisms of action, including antimetabolites that target DNA and RNA molecules. Although 5-FU, an antimetabolite drug, is the main treatment for several solid tumors, including CRC and breast cancer, IV 5-FU is associated with certain health issues, such as systemic toxicity and development of resistance. Capecitabine is a prodrug of 5-FU that was developed to eliminate the local (GI) toxicity of 5-FU. Capecitabine has been demonstrated to be effective in the treatment of adenocarcinoma of colorectal and breast origin. Moreover, the expression of three metabolizing activation enzymes (CES, CDA and TP) that catalyze the conversion of the capecitabine prodrug into 5-FU in CRC tissues indicates that capecitabine is a preferred, effective and targeted therapy for the treatment of CRC. The cellular mechanism of action of capecitabine is the same as that of its parental 5-FU when it is activated and enters cancer cells. Blocking DNA and RNA synthesis is the predominant cellular mechanism of action of 5-FU and capecitabine. Moreover, they alter the cell cycle phases and cause apoptosis in several CRC cell lines. Capecitabine is a promising anticancer agent, either as a monotherapy or in combination with other drugs. Combined chemotherapy with capecitabine plays a significant role in efficacy, resistance and survival. The chemoresistance to capecitabine treatment could be according to changes that are categorized as intracellular factors, extracellular factors and cell surface factors, or cell-phenotype state. Future research on CRC should expand the study of the effect of capecitabine with novel and safe approved FDA drugs that target cellular pathways other than those that target nucleic acids, such as the β-adrenergic pathway (also called the stress pathway), which are implicated in carcinogenesis, tumor progression and metastasis.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

SA and HA designed the present review, collected information, and wrote the manuscript. AA and PP wrote and reviewed the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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