Universidade de Lisboa

Faculdade de Farmácia

Benefits and drawbacks for the clinical use of 5-fluorouracil

Vera Filipa Silva Martins

Monografia orientada pela Doutora Maria Manuela Gaspar, Investigadora Auxiliar da FFUL

Mestrado Integrado em Ciências Farmacêuticas

2023

Universidade de Lisboa

Faculdade de Farmácia

Benefits and drawbacks for the clinical use of 5-fluorouracil

Vera Filipa Silva Martins

Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

Monografia orientada pela Doutora Maria Manuela Gaspar, Investigadora Auxiliar da FFUL

2023

Agradecimentos

Esta monografia encerra 5 anos que nunca irei esquecer, para além do que representa também uma grande conquista pessoal.

Começo por agradecer à minha orientadora, Doutora Maria Manuela Gaspar, por toda a ajuda e chamadas telefónicas.

Em seguida, quero agradecer aos meus pais e irmãs, por estarem sempre ao meu lado, por toda a paciência, por todas as palavras encorajadoras e por sempre acreditarem em mim. Obrigada por serem o meu pilar e por me apoiarem incondicionalmente.

Quero agradecer também ao meu namorado, que teve de ter muita paciência neste último período académico. Obrigada por melhorares tudo e por toda a alegria e carinho.

Um abraço caloroso aos amigos que ganhei nestes anos. Sem dúvida que tiveram um grande papel nas memórias que fiz e que virei a fazer. Um muito obrigada a vocês que fizeram todos os momentos, bons e maus, valerem a pena.

Table of contents

Index of Figures:

Index de Tables:

Resumo

O 5-Fluorouracil (5-FU) tem sido um importante fármaco anticancerígeno até à data. É um dos fármacos citotóxicos mais utilizados em vários tipos de cancro, como os gastrointestinais, da mama, da pele, da cabeça e do pescoço, e pode ser administrado por via intravenosa ou tópica, dependendo do tipo de cancro e da região a tratar. Após a sua ativação, os metabolitos do 5-FU podem exercer efeitos citotóxicos através da inibição da timidilato sintetase (TS), ou através da sua incorporação no RNA e DNA, eventos que acabarão por induzir citotoxicidade e morte celular. Apesar da sua elevada aplicabilidade, está comummente associada a efeitos secundários adversos que podem comprometer a sua utilização, como por exemplo cardiotoxicidade, mielossupressão, mucosite intestinal, diarreia grave e até o desenvolvimento de resistências à quimioterapia. Com o aumento do conhecimento do seu mecanismo de ação, foram desenvolvidas nas últimas décadas várias estratégias para aumentar a sua atividade anticancerígena e colmatar as suas desvantagens, que incluem a modificação química, o que deu origem a novas moléculas químicas, e a implementação de regimes baseados em análogos de 5-FU. Os pró-fármacos de 5-FU, por exemplo, capecitabina, tegafur, apresentam alterações químicas concebidas para serem melhor absorvidos através da mucosa gastrointestinal e aumentar a biodisponibilidade de 5-FU numa dose terapêutica mais baixa. Outra estratégia que está a receber atenção no tratamento do cancro é a sua associação a sistemas de veiculação de fármacos, com o objetivo de modular o seu perfil de biodistribuição. Alguns dos sistemas de veiculação de fármacos focados no presente trabalho foram os lipossomas, com alta afinidade por membranas biológicas; os niossomas, com alta estabilidade química; os transferssomas, com estrutura ultra-deformável; os etossomas, utilizando etanol como co-solvente; as microemulsões, altamente estáveis termodinamicamente; as microesponjas, com sua superfície altamente porosa; e as microagulhas. Outras formulações estão ainda a ser concebidas e testadas, como uma folha de duas camadas para a libertação controlada e local do fármaco por exemplo no cancro do pâncreas.

De um modo geral, as estratégias descritas no presente trabalho pretendem melhorar o índice terapêutico do importante fármaco anticancerígeno, o 5-FU.

Palavras-chave: 5-Fluoruracilo; Cancro; Sistemas de Veiculação de Fármacos

Abstract

5-Fluorouracil (5-FU) has been an important anti-cancer drug to date. It is one of the cytotoxic drugs most used in various types of cancer, such as gastrointestinal, breast, skin, head and neck, and can be administered intravenously or topically, depending on the type of cancer and region meant for treatment. After activation, 5-FU metabolites can exert cytotoxic effects via inhibition of thymidylate synthetase (TS), or through incorporation into RNA and DNA, events that will ultimately induce cytotoxicity and cell death. Despite having a high applicability, it is commonly associated to severe side effects that may compromise its use, for instance cardiotoxicity, myelosuppression, intestinal mucositis, severe diarrhoea and even the development of chemoresistance. With an increase in the knowledge of its mechanism of action, various treatment modalities have been developed over the past few decades to increase its anti-cancer activity while diminishing its disadvantages, which include the chemical modification being in the origin of new analogues of 5-FU. 5-FU prodrugs, such as capecitabine and tegafur, have a variety of chemical alterations designed to be better absorbed through the gastrointestinal mucosa and to increase the bioavailability of 5-FU at a lower therapeutic dose. Another strategy that is receiving attention in cancer treatment is its association to drug delivery systems (DDS), aiming to modulate its biodistribution profile. Some of the DDS focused in the present work were liposomes, with high affinity for biological membranes; niosomes, with high chemical stability; transfersomes, with an ultra-deformable structure; ethosomes, using ethanol as cosolvent; microemulsions, highly thermodynamically stable; microsponges, with its highly porous surface; and microneedles. Other formulations are still being designed and tested, such as a two-layered sheet for the controlled and local release of the drug in pancreatic cancer.

Overall, the described strategies in the present work intend to improve the therapeutic index of the important anticancer drug, the 5-FU.

Keywords: 5-Fluoruracil; Cancer; Drug Delivery Systems

Abbreviations

5-FU - 5-fluorouracil

TS - thymidylate synthase

CRC – colorectal cancer

UTP - uridine triphosphate

FUTP - fluorouridine triphosphate

FdUTP - 5-fluorodeoxyuridine triphosphate

dTTP - deoxythymidine triphosphate

DHFU - 5,6-dihydro-5-fluorouracil

FBAL - α-fluoro-β-alanine

FOLFOX - chemotherapy combination containing 5-FU, leucovorin and oxaliplatin

CAPOX – chemotherapy combination containing capecitabine and oxaliplatin

5-FU/LV - chemotherapy combination containing 5-FU and leucovorin

XELIRI - chemotherapy combination containing capecitabine and irinotecan

FA - folinic acid

FOLFIRINOX – chemotherapy combination containing 5-FU, FA, oxaliplatin and irinotecan

MTX - methotrexate

MVAC - chemotherapy combination containing MTX, vinblastine, doxorubicin and cisplatin

CMV - chemotherapy combination containing cisplatin, MTX and vinblastine

CMF - chemotherapy combination containing cyclophosphamide, MTX and 5-FU

CID - Chemotherapy-induced diarrhoea

ROS - reactive oxygen species

CSCs - cancer stem-like cells

DPD - dihydropyrimidine dehydrogenase

EGFR - epidermal growth factor receptor

CDHP - 5-chloro-2,4- dihydroxypyridine

OXO - potassium oxonate

RNR - ribonucleotide reductase

DDS - drug delivery system

SLNs – solid lipid nanoparticles

HPMC - hydroxypropyl methylcellulose

HCC - hepatocellular carcinoma

MFU - 1,3-bistertrahydrofuran-2yl-5FU

AKs - actinic keratosis

1. Introduction

5-Fluorouracil – overview

The 5-fluorouracil (5-FU) (Figure 1) is an antimetabolite drug used in chemotherapy. It is water-soluble, being administered either as intravenous bolus or as continuous intravenous infusion, with or without folinic acid as a cofactor, and either alone or with other chemotherapeutic agents and/or radiotherapy. Depending on the type of cancer, it can also be applied topically. It has a short initial half-life (5-20min), with significant hepatic, renal, and lung clearance. (Álvarez et al., 2012; Cassidy et al., 2015) It induces cytotoxicity either by interfering with essential biosynthetic activity through the inhibition of thymidylate synthase (TS) or by misincorporating its metabolites into RNA and DNA. Even though 5-FU has numerous advantages, its clinical application was limited due to the development of drug resistance after chemotherapy. (Sethy & Kundu, 2021)

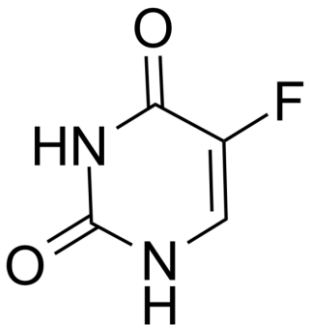

Figure 1. Chemical structure of 5-FU.

5-FU is a heterocyclic aromatic organic compound with a structure similar to that of the pyrimidine molecules of DNA and RNA; it is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen. Due to its structure, 5-FU interferes with nucleoside metabolism and can be incorporated into RNA and DNA, leading to cytotoxicity and cell death. (N. Zhang et al., 2008)

Despite the improvement in response rate and disease-free survival in many cancers, the highest impact was shown in the treatment of colorectal cancer (CRC). (Sethy & Kundu, 2021)

However, for patients with localized and locoregional CRC, as well as for those with resectable distant metastases, surgery remains the only curative option. (Vodenkova et al., 2020)

It has been demonstrated that 80% to 85% of 5-FU is catabolized into inactive metabolites by dihydropyrimidine dehydrogenase (DPD), and only 1 to 3% of the original dose of 5-FU mediates the cytotoxic effects on tumor cells and normal tissues through anabolic actions, thereby inhibiting DNA synthesis and RNA processing and function.

a) Anabolic route:

5-FU is an uracil analogue with a fluorine atom at the C-5 position. After intravenous administration of 5-FU, it enters the cells using the same transport mechanism as uracil - facilitated transport. Then, 5-FU is converted into the following active metabolites: 1) fluorouridine triphosphate (FUTP), which is incorporated into RNA instead of uridine triphosphate (UTP); 2) fluorodeoxyuridine triphosphate (FdUTP), which is incorporated into DNA instead of deoxythymidine triphosphate (dTTP); and 3) fluorodeoxyuridine monophosphate (FdUMP), which inhibits the activity of TS in the ternary complex. FUTP causes modifications in RNA processing and function, and FdUTP and FdUMP cause DNA damage; both of which lead to cell death.

b) Catabolic route:

DPD is an enzyme present in the liver, intestinal mucosa and other tissues. DPD catabolizes 5-FU to 5,6-dihydro-5-fluorouracil (DHFU), finally leading to the formation of α-fluoro-β-ureido-propionic acid and α-fluoro-β-alanine (FBAL). After investigating the kinetics of 5-FU and 5-FU metabolites in cancer patients following intravenous bolus administration of radio-labeled 5-FU, it was revealed that approximately 60–90% of the administered 5-FU was excreted in urine as FBAL within 24 hours. Although the majority of patients tolerate 5-FU quite well, some cancer patients with DPD deficiency have been found to be more susceptible to severe side effects after receiving 5-FU at recommended levels, including diarrhoea, mucositis, and neurotoxicity.(Miura et al., 2010) (Vodenkova et al., 2020)

Attending various factors such as age, gender, disease state and organ function, the pharmacokinetics of 5-FU may vary. In this sense, it is important to determine individualized doses in order to improve the efficacy of 5-FU and decrease the likelihood of developing severe

toxicity, controlling the exposure to 5-FU. (van Kuilenburg & Maring, 2013) Studies investigating the drug's excretion have revealed that it is influenced by hepatic metastases and glomerular filtration rate as determined by creatinine clearance. Females and older age groups were shown to have poorer 5-FU clearance. (Arshad et al., 2020)

2. Clinical applications of 5-FU

5-FU is used in a variety of cancers, both as a single agent and in combination with other drugs. It can be administered both intravenous and topically, depending on the type of cancer. In Table 1 are depicted some clinical applications for 5-FU.

Table 1. Types of cancer using 5-FU in its regimen.

Cancer	Treatment	Side effects	References
Colorectal	5-FU alone, FOLFOX, CAPOX	Nausea and vomiting, oral mucositis, diarrhoea, red painful palms and soles (hand–foot syndrome), peripheral neuropathy (when oxaliplatin is included)	Cassidy et al., 2015; Tytherleigh et al., 2008 Colorectal Cancer NICE Guideline, 2020
Cervix	5-FU, Cisplatin, MTX, Bleomycin, Paclitaxel, Topotecan.		
Oesophageal	5-FU/LV, XELIRI, Capecitabine, Ipilimumab.		
Head and neck	5-FU, Taxanes, MTX, Pemetrexed, Cetuximab.		
Gastric	5-FU, Epirubicin, Cisplatin, Capecitabine.		
Pancreatic	5-FU/FA, Gemcitabine, Capecitabine, Erlotinib, FOLFIRINOX.		
Small intestine	5-FU, Paclitaxel, Streptozotocin, Doxorubicin Carboplatin, Topotecan, Bevacizumab.		
Renal	5-FU, Gemcitabine (though citotoxic drugs have little use due to high chemoresistance)		
Blader/ ureter	Gemcitabine, Cisplatin, MVAC, CMV, 5-FU, Mitomycin.		
Penile	5-FU (topical), MTX, Bleomycin, Cisplatin.		
Breast	Tamoxifen, Epirubicin-CMF, Trastuzumab, Vinorelbine.		
Skin	5-FU (topical), Bevacizumab, Vemurafenib, Trametinib.	Erythema, xerosis, and burning (the above can also occur)	Cassidy et al., 2015; Yen Moore, 2009

5-FU – 5-fluoruracil; FOLFOX – combination of 5-FU, leucovorin and oxaliplatin; CAPOX – combination of capecitabine and oxaliplatin; MTX – methotrexate; 5-FU/LV – combination of 5-FU and leucovorin; XELIRI – combination of capecitabine and irinotecan; 5-FU/FA – combination of 5-FU and folinic acid; FOLFIRINOX – combination of 5-FU, FA, oxaliplatin and irinotecan; MVAC – combination of MTX, vinblastine, doxorubicin and cisplatin; CMV – combination of cisplatin, MTX and vinblastine; CMF - cyclophosphamide, MTX and 5-FU.

3. Drawbacks associated to 5-FU administration

All medicines can cause unwanted side effects, and 5-FU is no exception.

One limitation to chemotherapy is the fact that cytotoxic drugs are non-selective, meaning that they are able to kill both cell populations - normal and cancer cells. Therefore, this lack of selectivity originates major toxic issues. Some of the acute complications include damage to the most susceptible organs, such as bone marrow, gastrointestinal mucosa, hair follicles and gonads, due to their rapidly dividing cells. Thus, myelosuppression, nausea, vomiting, hair loss, and reduced fertility are some of the early side effects of chemotherapy.

Oogenesis and spermatogenesis are both vulnerable to cytotoxic damage. Recovery of fertility after chemotherapy is possible, but it seems to depend on the patient's age, the type and dose of medications received, and the duration of treatment. Many men and women are subsequently able to conceive by natural means, but it is common practice to provide young patients at high risk of chemotherapy-induced infertility semen or ovarian tissue storage before starting treatment. The majority of cytotoxic substances are teratogenic and can be hazardous to the fetus. As a result, chemotherapy patients are advised to utilize effective contraception while undergoing treatment. (Corrie, 2008)

In human clinical studies, unintended exposure to 5-FU or therapy with FOLFOX, a chemotherapy combination containing 5-FU, leucovorin, and oxaliplatin, during the second and third trimesters of pregnancy, showed no damaging impacts on the health of the fetus. The evaluation of the reproductive toxicity of 5-FU, however, cannot be fully tested in humans and is instead evaluated in human-induced pluripotent stem cells, which has been suggested to achieve comparable results. (Aikawa, 2020) In these cells, 5-FU inhibited neural differentiation by downregulating the expression of the mitochondrial fusion proteins Mfn1/2 and lowering intracellular ATP levels, suggesting that 5-FU-induced mitochondrial dysfunction may underlie the developmental and reproductive toxicities. (Naren et al., 2022)

Myelosuppression, which results from cell cycle arrest, is the main side effect of 5-FU and it is dose-limiting in chemotherapy. Moreover, myelosuppression leads to a decrease in white blood cells, which is a risk factor for infection and febrile neutropenia. (Ishibashi et al., 2021) Nevertheless, studies revealed that the typical patient treated with 5-FU combination

medication (FOLFIRINOX) experienced greater myelosuppression than the patient receiving 5-FU monotherapy. (Arshad et al., 2020)

Approximately 50 to 80% of patients receiving 5-FU develop intestinal mucositis with severe diarrhoea, nausea, vomiting and anorexia. (A. B. Benson et al., 2004) Treatments are frequently stopped or de-escalated as a result. There are currently no effective therapeutics strategies for 5-FU-induced intestinal mucositis aside from the prescription of antidiarrheal medications, antibiotics, and mucosal protective agents. It is thought that 5-FU-induced intestinal mucositis progresses over five stages: initiation, primary damage response, signal amplification, ulceration and healing.

Reactive oxygen species cause DNA and non-DNA damage during the initiation phase. Primary mucosal damage causes apoptosis and the release of inflammatory cytokines in the second phase, which ultimately result in more mucosal damage in the third phase. In the fourth phase, loss of mucosal integrity causes severely painful lesions that are vulnerable to infection. However, these lesions recover in the final phase through epithelial cell proliferation and differentiation as well as the re-establishment of the local microbiota. Therefore, apoptosis, inflammatory cytokines, intestinal microbiota, direct cytotoxicity and reactive oxygen species are considered to be key pathogenic determinants for recovery. (Hamouda et al., 2017)

Although being quite rare, neurotoxicity has also been observed. It may involve either the central or the peripheral nervous system, is dose-limiting and may lead to treatment discontinuation. (Taillibert et al., 2016) Fluorocitrate, a by-product of the metabolism of 5-FU, is one potential factor in neurotoxicity. This by-product interferes with the Krebs cycle, impairing the adenosine triphosphate-dependent urea cycle and preventing the conversion of ammonia to urea. Ammonia build-up in the brain is converted to glutamine, which has been connected with cerebral oedema and an increase in intracranial pressure. (S. A. Thomas et al., 2015) Some cases of overexposure have led to acute confusion plus dysarthria. Seizure, confusion and signs of metabolic encephalopathy were observed in some patients under 5-FU-based treatment. (Cordier et al., 2011) In the peripheral nervous system, chemotherapy-induced peripheral neuropathy and myopathy are the two main complications of chemotherapy, hence intestinal mucositis may also be associated with intestinal motility alterations maybe due to an effect on the enteric nervous system. (Costa et al., 2019)

After anthracyclines, 5-FU is the chemotherapeutic drug most frequently associated with cardiotoxicity. The most common symptoms of fluoropyrimidine-related cardiotoxicity are chest pain, which can also emerge as atypical chest pain, angina, and acute coronary syndromes, including myocardial infarction. Patients with pre-existing cardiovascular disease receiving continuous infusions of 5-FU, as opposed to a bolus-based regimen, may be at higher risk, and as so, it is recommended prophylactic antianginal therapy. (Sara et al., 2018)

Other side effects of cytotoxic chemotherapy can develop sometimes over many months and in order to avoid such problems, limitations to the amount of drug that can be prescribed over time have been established. Some examples are hand-foot syndrome with nail dystrophy and cardiac ischaemic events. (Corrie, 2008)

Fluoropyrimidines have also been associated with severe diarrhoea. When 5-FU is administered with leucovorin (5-FU/LV), both the therapeutic effectiveness and frequency of diarrhoea are increased. Up to 50% of patients receiving weekly 5-FU/LV combination treatment reported experiencing diarrhoea. Moreover, the severity of the diarrhoea can increase when 5-FU is administered by bolus injection rather than intravenous infusion. Clinical factors predictive for fluoropyrimidine-induced diarrhoea are female sex, Caucasian race and presence of diabetes. Usually it is a dose-related adverse effect and may be associated with other features of toxicity.

Chemotherapy-induced diarrhoea (CID) appears to be a multifactorial process in which there is an imbalance between absorption and secretion in the small bowel due to acute damage to the intestinal mucosa, including loss of intestinal epithelium, superficial necrosis, and bowel wall inflammation.

CID can be debilitating and, in some cases, life threatening. Findings in such patients include volume depletion, renal failure, and electrolyte disorders such as metabolic acidosis and, depending upon water intake, hyponatremia or hypernatremia. (Stein et al., 2010)

Despite the encouraging progress in chemotherapy, the patients' response rates remain low and the benefit from 5-FU-based therapy is often compromised by the development of resistance, which may be innate or acquired. (Vodenkova et al., 2020)

Different mechanisms can explain this 5-FU resistance. Some are disease-specific, whereas others, such as drug efflux, are evolutionarily conserved. These are complex mechanisms that can occur simultaneously in cells exposed to 5-FU.

On the one hand, there may be alterations in enzymes involved in 5-FU metabolism and activation. High levels of TS prior to 5-FU-based treatments are associated with disturbed folate pools and lead to intrinsic resistance. In contrast, TS gene amplification and mutations are linked to acquired resistance. These findings point to new and improved approaches to treatment: 5-FU should not be administered to patients with tumors displaying TS amplification.

On the other hand, cancer cells have the capacity to create defenses against cytotoxic drugs. It has been described that certain CRC cells develop resistance to 5-FU as a result of altered cytoplasmic p53 binding, associated with apoptosis. Similarly, P53 regulators, such as caspase-9 and its cofactor, apoptotic protease activating factor 1, can be inactivated, also leading to drug resistance.

Adding to this, 5-FU has the potential to produce reactive oxygen species (ROS), which cause oxidative damage and ultimately the death of cancer cells. However, a small subpopulation of cancer cells called cancer stem-like cells (CSCs) are able to develop cellular adaptive responses to ROS to survive. (Blondy et al., 2020)

The presence of CSCs in a cancer cell population was contemplated to have a major contribution in causing chemoresistance and cancer relapse. Its properties can be attributed to self-renewal, cell differentiation capacities, high tumorigenicity, and aggressive behaviour along with its withstanding capacity to chemotherapy. (Sethy & Kundu, 2021)

The susceptibility of tumour cells to chemotherapy is significantly influenced by their effect in the cell cycle and their capacity to undergo apoptosis in response to drug treatment. Some authors suggest that abnormal regulation of nucleotide metabolism, amino acid metabolism, cytoskeleton organization, transport, and oxygen metabolism may underlie the differential resistance to 5-FU. (De Angelis et al., 2006)

4. Strategies to overcome 5-FU disadvantages

Extensive research has been performed, focused on the biomodulation of 5-FU to improve its anti-cancer activity. These include decreasing 5-FU degradation, increasing TS inhibition, and inducing 5-FU activation, which can be addressed by using 5-FU analogues or prodrugs, a combination of 5-FU or its prodrugs and enzyme inhibitors. (Sethy & Kundu, 2021)

Chemical modification of 5-FU

5-FU prodrugs have a variety of chemical alterations designed to be well absorbed through the gastrointestinal mucosa and enzymatically converted afterwards into 5-FU in the liver or within the tumor itself. 5-FU prodrugs may increase the bioavailability of 5-FU at a lower therapeutic dose thus minimizing the toxic side effects. Also, their administration is more convenient by reducing the time spent in an infusion. (Vodenkova et al., 2020)

As so, to enhance the physicochemical, pharmacological, and pharmacokinetic characteristics of 5-FU, many derivatives have been created. Some of these have found a place in clinical practice, particularly for oral administration. (Álvarez et al., 2012)

- Capecitabine

Capecitabine (Figure 2) is an orally active prodrug of 5-FU, preferentially activated in tumour and liver tissue, and has the potential to replace the prolonged or continuous infusion of 5-FU. It has been shown to be active in a wide range of cancers such as breast and GI cancers. (Cassidy et al., 2015) TS inhibition and incorporation into RNA and DNA are the most important mechanisms of action of capecitabine. In phase III clinical trials, patients with CRC who received capecitabine as monotherapy demonstrated a considerably reduced incidence of toxic side effects than those who received 5-FU in combination with Leucovorin (5-FU/LV). (García-Alfonso et al., 2021; Vodenkova et al., 2020)

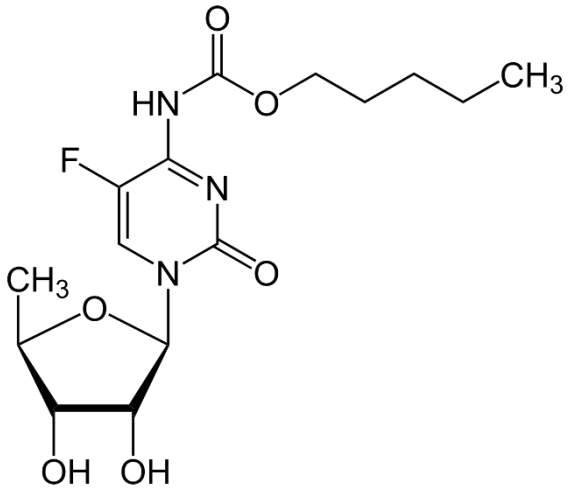

Figure 2. Chemical structure of Capecitabine.

- Tegafur

Also known as FTO or 1-[2-tetrahydrofuryl], Tegafur (Figure 3), this 5-FU prodrug, is activated through two separate metabolic pathways: (i) via hepatic microsomes by cytochrome P450 isoenzyme CYP2A6 and (ii) by systemic soluble enzymes. The principal adverse effects are gastrointestinal (i.e. nausea, vomiting, diarrhoea, and mucositis) and neurological toxicities (i.e. change in mental status, cerebellar ataxia, and coma). Therefore, Tegafur is not well tolerated by patients and has limited application as a single agent. The combination of Tegafur and uracil came with the purpose of overcoming these drawbacks, increasing the therapeutic index of Tegafur by blocking the DPD-mediated degradation of fluorouracil´s pyrimidine base. There is also the association of Tegafur and two enzyme inhibitors: 5-chloro-2,4- dihydroxypyridine (CDHP), a DPD inhibitor that maintains the plasma concentration of 5-FU; and potassium oxonate (OXO), which inhibits the phosphorylation of 5-FU in the gastrointestinal tract decreasing the potential serious gastrointestinal toxicities. (García-Alfonso et al., 2021; Vodenkova et al., 2020)

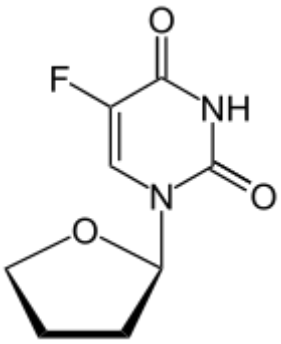

Figure 3. Chemical structure of Tegafur.

- Doxifluridine

The purpose of doxifluridine (Figure 4), a fluoropyrimidine derivative, is to avoid the rapid degradation of 5-FU by DPD in the gut wall. This prodrug is converted to 5-FU by uridine phosphorylase, which is abundantly expressed in malignant cells. (Álvarez et al., 2012) Doxifluridine was also developed to improve the therapeutic activity of 5-FU while reducing its toxicity, including the immunosuppressive, myelosuppressive, and cardiotoxic effects. In addition, the pharmaceutical formulation of doxifluridine has been developed to enhance cellular permeability, so it was shown, in dogs, to have a higher plasma concentration compared to 5-FU. (Baek et al., 2013)

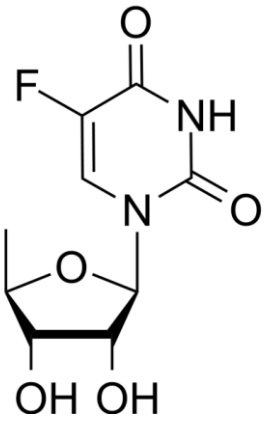

Figure 4. Chemical structure of Doxifluridine.

- Eniluracil

Eniluracil, or 5-ethynyluracil (Figure 5), was developed as a novel modulator of 5-FU, not being cytotoxic by itself. It is an uracil analogue with an ethynyl substituent at the C-5 position which irreversibly inhibits DPD. *In vivo* studies performed in rats demonstrated that this analogue resulted in a higher oral bioavailability than 5-FU, facilitating uniform absorption and predictable toxicity allowing the replacement of intravenous bolus and continuously infused dose. However, neutropenia and diarrhoea were two of Eniluracil's primary adverse effects. Eniluracil's further development was suspended in 2000 as a result of the unfavourable findings from clinical trials on CRC patients. (García-Alfonso et al., 2021)

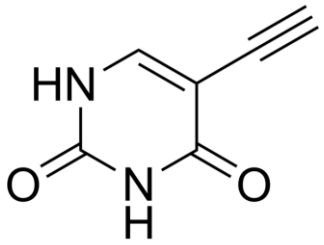

Figure 5. Chemical structure of Eniluracil.

- Gemcitabine

Gemcitabine (Figure 6) is a derivative of 5-FU for the treatment of non-small cell lung and pancreatic cancers. The triphosphate analogue of gemcitabine, like 5-FU and other pyrimidine analogues, replaces one of the nucleic acid building components, in this case cytidine, during DNA replication, causing apoptosis. The ribonucleotide reductase (RNR) enzyme is another target of gemcitabine. The diphosphate analogue inactivates the enzyme irreversibly by attaching to the RNR active site. RNR inhibition results in cell death because the cell is unable to synthesize the deoxyribonucleotides needed for DNA replication and repair. (Álvarez et al., 2012)

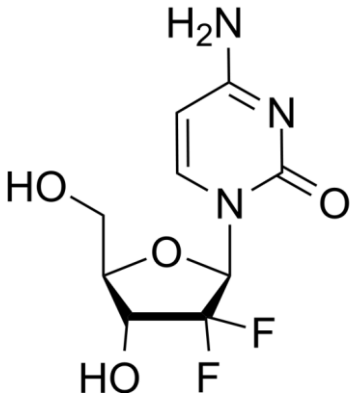

Figure 6. Chemical structure of Gemcitabine.

Combination therapy

Furthermore, a number of therapeutic agents have been combined with 5-FU in order to increase its therapeutic index either by improving the therapeutic effect or reducing toxicity.

Adding to this, some combinations may even avoid certain side effects, for example, the anthracyclines cardiotoxicity can be reduced using regimens such as carboplatin/docetaxel/trastuzumab. (Cassidy et al., 2015)

Combinations of 5-FU and folinic acid (FA) are the cornerstone of colon cancer therapy. (Carnaghi et al., 2000) FA is administered through infusion before or simultaneously with 5-FU, thus potentiating the interaction between TS and 5-FU. Compared to the single-agent 5-FU, it has a greater response rate in advanced CRC despite being more toxic. (Cassidy et al., 2015)

For instance, in many situations, 5-FU is used with Leucovorin since it was reported that this combination showed improved response rates compared with single-agent 5-FU. In addition to this combination, its concomitant use with oxaliplatin or irinotecan has shown promising results in the cytotoxic regimens for the CRC treatment. Another effective treatment with significant clinical outcomes is 5-FU in combination with methotrexate, an antifolate and purine biosynthesis inhibitor. (Sethy & Kundu, 2021)

Also, in metastatic colon cancer conditions, agents targeting angiogenesis such as bevacizumab, ramucirumab, and aflibercept or epidermal growth factor receptor (EGFR)-directed treatments including panitumumab and cetuximab are commonly used along with fluoropyrimidine-based chemotherapy. (Vodenkova et al., 2020)

With modern technology, thousands of genes can be quantified in tiny fragments of tumor tissue. These gene expression profiles might one day allow treatment approaches to be planned according to their probability of success in individual patients. For instance, point mutations in the p53 gene seem to be associated with resistance to chemotherapy in many solid tumors. Similarly, ErbB2, a member of the epidermal growth factor receptor family, is overexpressed in some breast cancers and is generally recognized as a sign of poor prognosis. (Corrie, 2008)

5-FU treatment has been found to activate autophagy in human colon cancer in order to shield cancer cells from apoptosis. Consequently, autophagy inhibition may offer promising therapeutic potential as 5-FU-based adjuvant chemotherapy against colon cancer. 3-MA is an autophagy inhibitor that blocks the activity of type I PI3K, a kinase that is essential for vesicle nucleation, the first phase of autophagosome formation. In previous reports, it was discovered that 3-MA could enhance 5-FU–induced cell death and, later on, it was proposed that it could be a potential adjuvant agent in the treatment of primary colon cancer. (Li et al., 2010)

The diverse population of malignant cells in the tumor microenvironment does, in fact, confer resistance to anti-cancer medications, and as a result, tumor relapse is highly expected to occur when individual therapeutic agents are used. Ergo, combination therapy is more effective, killing the cells resistant to either drug when used as single agents, for treating cancer progression and metastasis. However, this is not applied to cells with mutations since it may originate cross-resistance to both drugs. (Sethy & Kundu, 2021)

4-acetylantroquinonol B (4-AAQB) is a novel bioactive compound that has a dynamic anti-tumor effect through deregulating major oncogenic and stemness signal transduction pathways. Additionally, it appears that 4-AAQB and 5-FU work well together to deplete CRC spheroids. Therefore, this combination therapy can be a novel approach for the treatment of metastatic or recurrent CRC rather than the conventional combination therapy using 5-FU, Leucovorin, and Oxaliplatin. (Sethy & Kundu, 2021)

Moreover, it was discovered that the triple combination of tamoxifen, 5-FU, and a fusicoccin derivative (ISIR-042) was successful in overcoming therapeutic resistance against pancreatic cancer. (Miyake et al., 2015)

A combination of 5-FU and OXPHOS inhibitors such as metformin and antimycin A, significantly decreased the survival of resistant cells whereas such effect was attenuated in the case of untreated resistant cells.(Denise et al., 2015)

In 2016, Jiang and co-workers synthesized a hybrid drug known as BC-01, which combines an aminopeptidase N (CD13) inhibitor, ubenimex, and 5-FU into a single molecule. Having more effective anti-angiogenic, anti-metastatic, and tumor growth inhibitory actions than other

licensed pro-drugs, BC-01 demonstrated better *in vitro* and *in vivo* anticancer efficacy. (Jiang et al., 2016)

FDA approved the medicine called macitentan, which was found to enhance sensitivity to oxaliplatin and 5-FU, therefore being able to target colorectal CSCs as well as interfering with components of the tumour microenvironment such as vascular, fibroblast, inflammatory, and lymphatic cells by interfering with both endothelin A and endothelin B receptors. Because of this, a combination of macitentan and other cytotoxic medications, including 5-FU and oxaliplatin, may target the most aggressive colorectal CSCs or inhibit the growth of tumours by inactivating the aforementioned receptors. (Cianfrocca et al., 2017)

The endogenous hormone melatonin is known to reduce angiogenesis, elevate ROS, and control the epithelial-to-mesenchymal transition while acting as an anti-metastatic agent. It has been demonstrated that combining 5-FU and melatonin reduces the stemness of cancer cells by cellular prion protein downregulation, a ubiquitous glycoprotein strongly related to medication resistance and cancer metastasis. (Lee et al., 2018)

An efficient therapeutic target for the treatment of CRC is mitogen-activated protein kinase 1 (MEK1 or MAP2K1), which is a downstream and an upstream target of Raf-1 and ERK pathways, respectively. According to a recent study, a small molecule MEK inhibitor (U0126) makes CRC more sensitive to the effects of oxaliplatin and 5-FU treatment by increasing the production of H2AX and decreasing the expression of XRCC1 and TS. (Jing et al., 2018)

Overexpression of COX-2 has been associated with resistance to conventional therapies and overall poor prognosis in several types of cancer. COX-2-selective inhibitors have been shown to induce apoptosis in solid tumour cell lines, including glioma, head and neck, oesophageal, and colon cancer. Etodolac, a selective inhibitor of COX-2, is widely used to treat patients with inflammatory pain as a non-steroidal anti-inflammatory drug (NSAID), and also may have potential clinical applications as a preventative and therapeutic cancer drug. Some studies have suggested that etodolac causes down-regulation not only of COX-2 but also TS, which leads to an increased sensitivity to 5-FU. (Murata et al., 2011)

Association of 5-FU to Drug Delivery Systems

Aiming to increase the bioavailability of 5-FU while reducing its side effects, another strategy has been used namely the association of 5-FU to drug delivery systems (DDS). (Ewert de Oliveira et al., 2021) A DDS refers to the method of associating an active pharmaceutical ingredient to a delivery system. In addition, a properly designed drug delivery system can modulate the journey of the loaded drug when entering the biological system of an organism. The drug delivery process can be conventional, such as pills, solutions, and semi-solid preparations, or advanced, such as targeted delivery, transdermal delivery, and stimuli-responsive delivery. (Kandula et al., 2023) Regarding 5-FU, in the following section several examples of delivery systems incorporating this cytotoxic drug will be presented.

Liposomes

Liposomes (Figure 7) are synthetic vesicles composed of one or more lipid bilayers separated by aqueous compartments with high similarity to biological membranes. (Luiz et al., 2023) These lipid-based systems may incorporate hydrophobic or hydrophilic molecules in the lipid bilayer or in the aqueous compartment, respectively. Their main constituents are phospholipids, amphiphilic molecules, that are able to increase the solubility of hydrophobic molecules while improving the affinity for biological membranes. (Singh et al., 2017) The delivery can occur passively, being only based on the increase of permeability and retention, or actively, by including specific ligands at liposome surface allowing a preferential targeting to receptors overexpressed at pathological sites. (Bozzuto & Molinari, 2015)

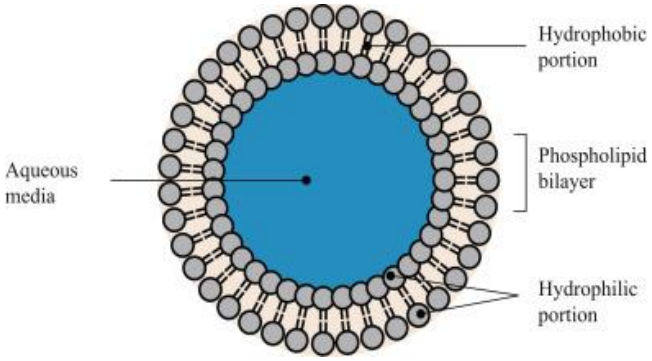

Figure 7. Schematic representation of a Liposome

For example, Glavas-Dodov et al. (2003) developed Phospholipon 90H liposomes and cholesterol for 5-FU encapsulation. *In vitro*, the 5-FU liposomal formulation presented high stability, allowing a continuous drug release, thus favouring the prolonged release of 5-FU. (Glavas-Dodov et al., 2003)

This formulation was also tested, in mice, via parenteral administration, for CRC. Although 5-FU liposomes exhibited a higher 5-FU plasma concentration, in comparison to the compound in the free form, further improvements are still needed to reduce their elimination rate. (A. M. Thomas et al., 2011)

Zhubech, a new 5-FU analogue liposomal formulation, is being tested against pancreatic cancer. It consists of 1,3-bistertrahydrofuran-2yl-5FU (MFU)-loaded liposomes and the entrapment efficiency of MFU was about 97%. The *in vitro* drug release tests showed that free MFU was released 80, 90 and 95% after 2, 4 and 6 h, respectively. In comparison MFU release from Zhubech, (the liposomal formulation) was about 50% within the first 2h, followed by 70% release within 6h, reaching steady state by 8h. In the *in vivo* efficacy studies, performed in a murine model, a significant tumour growth suppression was observed for mice treated with Zhubech compared to the untreated animals or those that received 5-FU. Tumour progression was observed for two weeks, after which the 5-FU group observed a geometric increase whereas a decrease in tumour volume was observed for the animals treated with Zhubech. These findings provide strong evidence in favour of the potential therapeutic use of Zhubech as a lipid-based nanoformulation that is able to improve the stability in blood circulation of MFU, increasing its half-life and accumulation at tumour sites. (Ndemazie et al., 2023)

Niosomes

Niosomes (Figure 8) are also colloidal spheres, but nonionic surfactant-based, being less likely to suffer oxidation as it happens with natural phospholipids; making niosomes chemically more stable but less flexible than liposomes. (Baillie et al., 1985)Paolino et al. (2008) developed a niosome system using a new surfactant, which was able to encapsulate 45% of 5-FU (in contrast to the 25% in liposomes). According to *in vitro* studies, the authors showed that the developed niosomes were eight times more able of penetrating skin than 5-FU in the free form. (Paolino et al., 2008)

The combination of curcumin loaded-niosomal nanoparticles with heat-killed *Saccharomyces cerevisiae* (a yeast probiotic reported as cancer cell death inducer) showed, *in vitro*, significant improvement in the treatment efficiency of colon cancer, demonstrating a higher decrease in the expression of genes involved in metastasis. (Jadid et al., 2023)

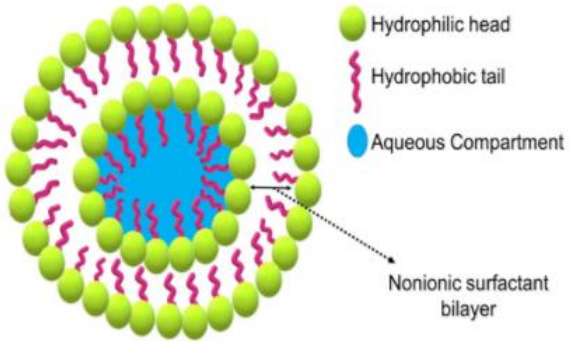

Figure 8. Schematic representation of a Niosome

Transfersomes

Transfersomes (Figure 9), also known as ultra-deformable liposomes, are highly flexible, enabling them to squeeze through the pores of the stratum corneum. Its preferred form is a highly deformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. (H. A. Benson, 2006) Alvi et al. (2011) produced three different vesicular systems for topical 5-FU: transfersomes, liposomes, and niosomes. According to the *in vitro* results, the three drug vehicles presented better results of absorption, skin retention, and lower minimum inhibitory concentration in comparison with free 5-FU. Additionally, the analysis of encapsulation efficiency demonstrated that transfersomes were able to entrap 5-FU at a higher extent than liposomes and niosomes (up to 80%). (Alvi et al., 2011)

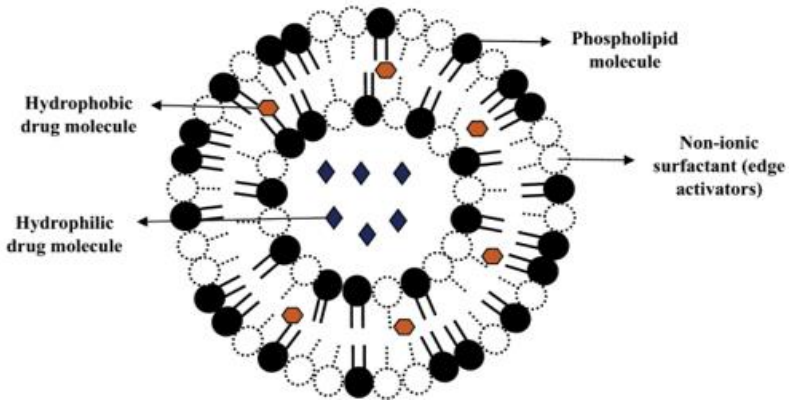

Figure 9. Schematic representation of a Transfersome

Ethosomes

Ethosomes (Figure 10) are nanovesicle carriers composed of phospholipids and ethanol, an efficient permeation enhancer. (Verma & Pathak, 2010) Puri and Jain (2012) developed an ethosome-based topical gel formulation (ethogel) for skin targeting of 5-FU. The formulation efficiency was compared with a commercial 5-FU cream in an *in vitro* skin study, concluding that the developed ethogel exhibited better permeation, higher anticancer activity, and less side effects. (Puri & Jain, 2012)

Microwaves have been being used recently to facilitate polar drug penetration into skin for transdermal drug delivery system. Pre-treating the skin with microwave at 2450 MHz for 2.5 minutes and the applying ethosomes topically confirmed, through *in vitro* experiments, that skin drug and/or ethosome penetration as well as retention via dermal protein fluidization was increased, when compared with the use of ethosomes alone. (Khan & Wong, 2018)

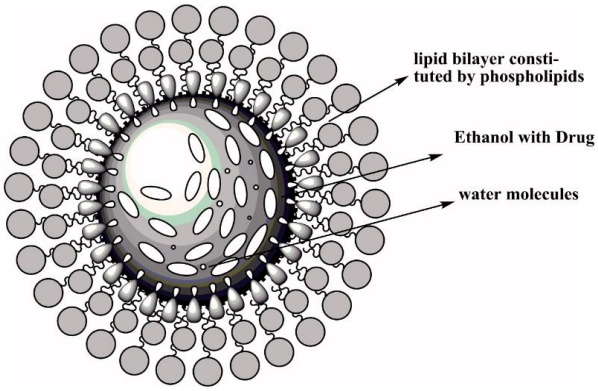

Figure 10. Schematic representation of an Ethosome

Microemulsions

Microemulsions (Figure 11) are optically transparent and thermodynamically stable. It is a single phase formed from two immiscible liquids (i.e., oil and water), with the addition of a suitable surfactant or a surfactant mixture. (Jeirani et al., 2014)

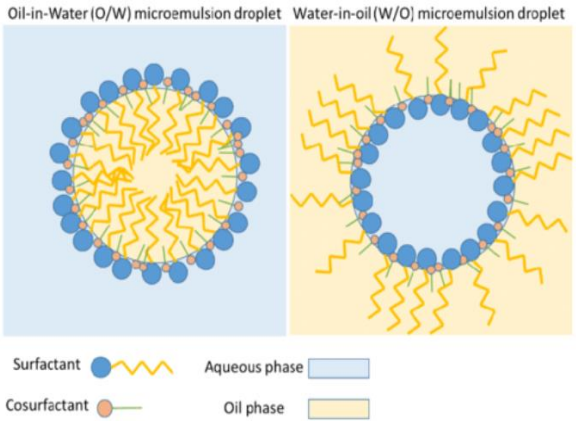

Figure 11. Schematic representation of oil in water (o/w) and water in oil (w/o) Microemulsions

They can increase drug loading, enhance penetration through biological membranes, and change drug pharmacokinetics. (Muzaffar et al.) Goindi et al. (2014) developed ionic water-in-oil microemulsions to deliver 5-FU topically. Comparing the designed drug vehicle with several controls, all developed microemulsions, including the control, showed better skin permeation and retention *in vitro* as compared to aqueous solutions. *In vivo*, using excised abdominal skin of mice, all microemulsions were effective in reducing tumor damage, which was not observed in the commercial formulation (Flonida®). Regarding side effects, the control microemulsions and the commercial formulation originated erythema and skin irritation, whereas in the groups treated with ionic microemulsions did not. Moreover, after 4 weeks of treatment, a total disappearance of skin lesions for the animals that received ionic microemulsions at 0.2 and 0.4% 5-FU was observed. Moreover, no side effects in the epidermis were registered. (Goindi et al., 2014)

Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) (Figure 12) are composed of solid lipids at room temperature instead of liquid oil phases found in emulsions. Lipid nanoparticles can enhance topical penetration and the permeation of encapsulated substances, being used for transdermal application and epidermal targeting. (Souto et al., 2011) Khallaf et al. (2016) developed SLNs based on lecithin and Poloxamer 188, were able to load up to 47% of 5-FU. *In vitro* studies on absorption showed that SLNs containing 5-FU increased the penetration into lipophilic barriers. Then, the same hydrogel was topically applied to mice with Ehrlich Ascites Carcinoma. According to histological results, and comparing with placebo and free 5-FU, the SLNs loaded with 5-FU showed greater penetration capacity and decreased the inflation and haemorrhage in the tumour area, when compared with the negative control and the free drug. (Khallaf et al., 2016)

To enhance 5-FU cytotoxic efficacy against CRC, Safwat et al. developed 5-FU loaded gold NPs. *In vitro* studies showed a slower release rate and substantially better anticancer effect when compared to the free drug, suggesting that it is possible to reduce 5-FU doses and, consequently, its side effects. (Safwat et al., 2016)

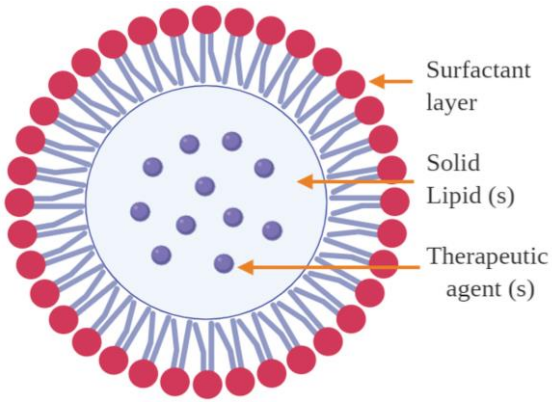

Figure 12. Schematic representation of a Solid Lipid Nanoparticle

Microneedles

Microneedles (Figure 13) are medical devices used for transdermal drug delivery through the opening of micro channels in the corneum extract.

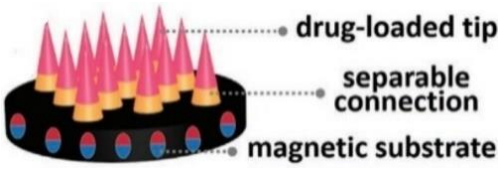

Figure 13. Schematic representation of Microneedles

It has been proved that the use of needles is able to increase the penetration of topical 5-FU, either through systems in which the drug is incorporated into the needles or through the use of microneedling before the application of 5-FU cream to open channels of penetration for the topical drug applied next. (Hao et al., 2020) Naguib et al. (2014) observed that mice treated with microneedles before the application of the 5-FU cream had complete disappearance of the melanoma lesions. These results were significantly better in comparison to mice treated only with 5-FU cream. The penetration potential of 5-FU was also shown to be 4.5 higher when using microneedling before the application of the drug in porcine skin. (Naguib et al., 2014) Hao et al. (2020) recently developed 5-FU and indocyanine loaded nanoparticles, which were integrated into near-infrared responsive dissolvable microneedles for skin cancer therapy. The system presented a good drug release control and promising antitumor activity in a murine model with melanoma. (Hao et al., 2020)

Zhang et al. developed magneto-responsive microneedle robots for intestinal macromolecule delivery. These robots are constituted by three components - the magnetic substrate (controlled externally), the separable connection, and tips (drug-loaded). Using a commercial enteric capsule encapsulation, they can be orally administered being only released in the small intestine. Benefitting from their polarized magnetic substrate, the tips of the microneedle robots can be oriented to the small intestinal wall, overcoming the barriers reaching the affected tissue, and delivering encapsulated actives under specific magnetic fields. Besides, after the separable connection degrades, the tips can be left inside the tissue for continuous active release, and the magnetic substrate can be excreted safely. *In vivo* studies, in minipigs, the microneedle robots were able to penetrate the intestinal mucosa. . In addition, *ex vivo* studies using pig small intestine and human small intestine tissues were also tested. The penetration depths reached 500 μm in *in vivo* pig small intestine tissue and almost 600 μm in *ex vivo* human small intestine tissue. (X. Zhang et al., 2021) The same strategy could be applied to 5-FU treatment in CRC.

Microsponges

Microsponges (MS) (Figure 14) are micro-sized particles with high porosity used to target and modify drug release, enhance stability and decrease side effects.

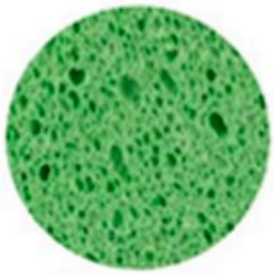

Figure 14. Schematic representation of a Microsponge

MS can entrap up to 90% of the drug, where bacteria cannot penetrate, and may efficiently be taken up by the macrophages in the colon, making them very advantageous. Othman and collaborators developed 5-FU-loaded MS compressed in enteric-coated, using pectin and hydroxypropyl methylcellulose (HPMC) tablets as a novel colon-targeted drug delivery model. This system combines two strategies that are pH-sensitive delivery and biodegradation by microbial enzymes (pectinase) in the colon environment. The *in vivo* findings have shown that the pectin-HPMC tablets were able to reach the colon without degradation in the upper GI system. Thus, it proved to be more patient compatible by providing a better treatment route over the current chemotherapy via injection or infusion. (Othman et al., 2020) The release of 5-FU from MS was characterized by an immediate burst release, due to the dissolution of drug adsorbed in microsponge's surfaces, followed by a moderately slow release, thus ensuring a controlled drug release. (Othman et al., 2017)

Other examples

Recently, a two-layered sheet for the controlled release and local disposition of 5-FU in the liver surface, for hepatocellular carcinoma (HCC), without leakage into the peritoneal cavity was developed. The sheet was produced using poly(lactic-co-glycolic acid) (PLGA) and hydroxypropyl cellulose (HPC), having 5-FU on the side to be attached to the organ.

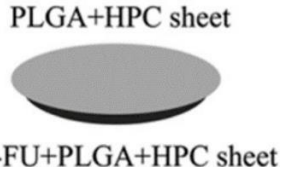

Figure 15. Schematic representation of a Two-layer sheet

The abdominal domain of the rats was cut open, followed by the application of the two-layered sheet on the liver surface, which was then removed at day 2, 7, 14, and 28 after application, followed by collection of blood and liver samples. The *in vivo* tests showed a relatively high 5-FU concentration in the liver with no significant increase in transaminases, whereas 5-FU could not be detected in the plasma. This new two-layered sheet formulation is thought to be promising, although more research is needed. (Akagi et al., 2023)

A new one-pot formulation composed of methacrylate derivatives (hydroxyethyl methacrylate copolymerized with methacrylic acid) was designed for the delivery of 5-FU in order to treat colorectal carcinoma. This system is able to retain the drug and prolong its release allowing a site-specific drug delivery to the colon, by using an effective time- and pH-dependent approach. The drug entrapment efficiency reached about 91%. The *in vitro* release studies, showed a cumulative release lower than 27% for all pellets after 5 h. The developed pellets demonstrated potential in protecting 5-FU from being released in the upper gastrointestinal tract as well as promoting a prolonged release afterwards. This therapeutic strategy presents high potential for the delivery of 5-FU to the colon. (Bayan et al., 2022)

5. New formulations in clinical trials

A novel formulation of 4% 5-FU cream is a topical therapeutic option recently approved for the treatment of multiple actinic keratosis (AKs) that increases patient compliance and clinical outcome. In a pilot study, thirty patients with a diagnosis of multiple AKs were treated with the cream once a day for 30 consecutive days, for 9 months, to assess its efficacy. At 12-week follow-up visit, 25 patients (83%) achieved complete clinical and dermatoscopic clearance. Treatment was generally well tolerated. Only three patients (10%) discontinued therapy due to adverse events onset (pain and burning); thirteen patients (43%) did not report any adverse reaction; sixteen subjects (53%) experienced erythema, thirteen (43%) scabs, eleven (37%) skin erosions, six (20%) bleeding, and two (7%) desquamations. The study concluded the 5-FU 4% cream was associated with a clinical improvement and a reduced incidence of local reaction. (Toffoli et al., 2023)

6. Conclusions and perspectives

5-Fluoruracil (5-FU) is a chemotherapy drug that works by interfering with DNA synthesis, leading to cell death and preventing cancer cells from proliferating. While 5-FU is considered a practical and preferred treatment option for many patients, its toxicity profile has been a concern. Its side effects may cause discomfort and other adverse events such as mucositis, diarrhoea, nausea, cardiotoxicity and even reduced fertility. Another disadvantage associated with 5-FU is the development of chemoresistance. Several mechanisms can contribute to chemoresistance. One of the most common mechanisms is the increased expression of enzymes metabolizing 5-FU. For example, when dihydropyrimidine dehydrogenase (DPD) levels are elevated, more 5-FU is metabolized, reducing the drug concentration available to kill cancer cells.

Consequently, researchers are exploring different strategies to improve the safety and efficacy of 5-FU. These strategies include combined therapies, design of delivery systems such as liposomes, microemulsions, microneedles, or solid lipid nanoparticles incorporating 5-FU, or developing 5-FU analogues and prodrugs. These pro-drugs are designed to improve the effectiveness and reduce the toxicity of 5-FU chemotherapy and effectively treat several types of cancer, including colorectal, breast, and gastric cancers.

In addition to the strategies mentioned, there are other ways in which researchers are trying to enhance the effectiveness of 5-FU while reducing its side effects. One such approach is the use of genetic testing to identify patients who are more likely to benefit from 5-FU treatment. Patients with specific genetic mutations, such as those in the thymidylate synthase gene, may respond better to 5-FU treatment and be more likely to experience side effects. Targeting these patients for 5-FU treatment could improve treatment outcomes while minimizing toxicity.

Despite its limitations, 5-FU continues to be an essential chemotherapeutic drug used to treat several malignancies. Further research into its mechanisms of action and optimization of regimens will provide new insights into this drug and shape the future of cancer treatment.

7. References

Aikawa, N. (2020). A novel screening test to predict the developmental toxicity of drugs using human induced pluripotent stem cells. *The Journal of Toxicological Sciences*, *45*(4), 187–199. https://doi.org/10.2131/jts.45.187

Akagi, T., Kariya, Y., Hamasaki, H., Sakaguchi, M., Miyamoto, H., Fumoto, S., & Nishida, K. (2023). Development of a Two-Layered Sheet Formulation of 5-Fluorouracil for Application to Rat's Livers to Ensure Controlled Release and Local Drug Disposition. *Chemical and Pharmaceutical Bulletin*, *71*(4), c22-00775. https://doi.org/10.1248/cpb.c22-00775

Álvarez, P., Marchal, J. A., Boulaiz, H., Carrillo, E., Vélez, C., Rodríguez-Serrano, F., Melguizo, C., Prados, J., Madeddu, R., & Aranega, A. (2012). 5-Fluorouracil derivatives: a patent review. *Expert Opinion on Therapeutic Patents*, *22*(2), 107–123. https://doi.org/10.1517/13543776.2012.661413

Alvi, I. A., Madan, J., Kaushik, D., Sardana, S., Pandey, R. S., & Ali, A. (2011). Comparative study of transfersomes, liposomes, and niosomes for topical delivery of 5-fluorouracil to skin cancer cells. *Anti-Cancer Drugs*, *22*(8), 774–782. https://doi.org/10.1097/CAD.0b013e328346c7d6

Arshad, U., Ploylearmsaeng, S., Karlsson, M. O., Doroshyenko, O., Langer, D., Schömig, E., Kunze, S., Güner, S. A., Skripnichenko, R., Ullah, S., Jaehde, U., Fuhr, U., Jetter, A., & Taubert, M. (2020). Prediction of exposure-driven myelotoxicity of continuous infusion 5-fluorouracil by a semi-physiological pharmacokinetic–pharmacodynamic model in gastrointestinal cancer patients. *Cancer Chemotherapy and Pharmacology*, *85*(4), 711–722. https://doi.org/10.1007/s00280-019-04028-5

Baek, I., Lee, B., Kim, M.-S., & Kwon, K. (2013). Pharmacokinetic analysis of doxifluridine and its metabolites, 5-fluorouracil and 5-fluorouridine, after oral administration in beagle dogs. *European Journal of Drug Metabolism and Pharmacokinetics*, *38*(4), 295–299. https://doi.org/10.1007/s13318-013-0130-4

Baillie, A. J., Florence, A. T., Hume, L. R., Muirhead, G. T., & Rogerson, A. (1985). The preparation and properties of niosomes—non-ionic surfactant vesicles. *Journal of Pharmacy and Pharmacology*, *37*(12), 863–868. https://doi.org/10.1111/j.2042-7158.1985.tb04990.x

Bayan, M. F., Jaradat, A., Alyami, M. H., & Naser, A. Y. (2022). Smart Pellets for Controlled Delivery of 5-Fluorouracil. *Molecules*, *28*(1), 306. https://doi.org/10.3390/molecules28010306

Benson, A. B., Ajani, J. A., Catalano, R. B., Engelking, C., Kornblau, S. M., Martenson, J. A., McCallum, R., Mitchell, E. P., O'Dorisio, T. M., Vokes, E. E., & Wadler, S. (2004). Recommended Guidelines for the Treatment of Cancer Treatment-Induced Diarrhea. *Journal of Clinical Oncology*, *22*(14), 2918–2926. https://doi.org/10.1200/JCO.2004.04.132

Benson, H. A. (2006). Transfersomes for transdermal drug delivery. *Expert Opinion on Drug Delivery*, *3*(6), 727–737. https://doi.org/10.1517/17425247.3.6.727

Blondy, S., David, V., Verdier, M., Mathonnet, M., Perraud, A., & Christou, N. (2020). 5-Fluorouracil resistance mechanisms in colorectal cancer: From classical pathways to promising processes. *Cancer Science*, *111*(9), 3142–3154. https://doi.org/10.1111/cas.14532

Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. *International Journal of Nanomedicine*, 975. https://doi.org/10.2147/IJN.S68861

Carnaghi, C., Zucali, P., & Garassino, I. (2000). [Combination of 5-Fluorouracil and folinic acid--is it still the standard therapy for advanced colorectal carcinoma?]. *Tumori*, *86*(5 Suppl 2), S19-25.

Cassidy, J., Bissett, D., Spence OBE, R. A. J., Payne, M., & Morris-Stiff, G. (Eds.). (2015). *Oxford Handbook of Oncology* (Vol. 1). Oxford University Press. https://doi.org/10.1093/med/9780199689842.001.0001

Cianfrocca, R., Rosanò, L., Tocci, P., Sestito, R., Caprara, V., Di Castro, V., De Maria, R., & Bagnato, A. (2017). Blocking endothelin-1-receptor/β-catenin circuit sensitizes to chemotherapy in colorectal cancer. *Cell Death & Differentiation*, *24*(10), 1811–1820. https://doi.org/10.1038/cdd.2017.121

Colorectal cancer NICE guideline. (2020). www.nice.org.uk/guidance/ng151

Cordier, P.-Y., Nau, A., Ciccolini, J., Oliver, M., Mercier, C., Lacarelle, B., & Peytel, E. (2011). 5-FU-induced neurotoxicity in cancer patients with profound DPD deficiency syndrome:

a report of two cases. *Cancer Chemotherapy and Pharmacology*, *68*(3), 823–826. https://doi.org/10.1007/s00280-011-1666-0

Corrie, P. G. (2008). Cytotoxic chemotherapy: clinical aspects. *Medicine*, *36*(1), 24–28. https://doi.org/10.1016/j.mpmed.2007.10.012

Costa, D. V. S., Bon-Frauches, A. C., Silva, A. M. H. P., Lima-Júnior, R. C. P., Martins, C. S., Leitão, R. F. C., Freitas, G. B., Castelucci, P., Bolick, D. T., Guerrant, R. L., Warren, C. A., Moura-Neto, V., & Brito, G. A. C. (2019). 5-Fluorouracil Induces Enteric Neuron Death and Glial Activation During Intestinal Mucositis via a S100B-RAGE-NFκB-Dependent Pathway. *Scientific Reports*, *9*(1), 665. https://doi.org/10.1038/s41598-018-36878-z

De Angelis, P. M., Svendsrud, D. H., Kravik, K. L., & Stokke, T. (2006). Cellular response to 5-fluorouracil (5-FU) in 5-FU-resistant colon cancer cell lines during treatment and recovery. *Molecular Cancer*, *5*(1), 20. https://doi.org/10.1186/1476-4598-5-20

Denise, C., Paoli, P., Calvani, M., Taddei, M. L., Giannoni, E., Kopetz, S., Kazmi, S. M. A., Pia, M. M., Pettazzoni, P., Sacco, E., Caselli, A., Vanoni, M., Landriscina, M., Cirri, P., & Chiarugi, P. (2015). 5-Fluorouracil resistant colon cancer cells are addicted to OXPHOS to survive and enhance stem-like traits. *Oncotarget*, *6*(39), 41706–41721. https://doi.org/10.18632/oncotarget.5991

Ewert de Oliveira, B., Junqueira Amorim, O. H., Lima, L. L., Rezende, R. A., Mestnik, N. C., Bagatin, E., & Leonardi, G. R. (2021). 5-Fluorouracil, innovative drug delivery systems to enhance bioavailability for topical use. *Journal of Drug Delivery Science and Technology*, *61*, 102155. https://doi.org/10.1016/j.jddst.2020.102155

García-Alfonso, P., Muñoz Martín, A. J., Ortega Morán, L., Soto Alsar, J., Torres Pérez-Solero, G., Blanco Codesido, M., Calvo Ferrandiz, P. A., & Grasso Cicala, S. (2021). Oral drugs in the treatment of metastatic colorectal cancer. *Therapeutic Advances in Medical Oncology*, *13*, 175883592110090. https://doi.org/10.1177/17588359211009001

Glavas-Dodov, M., Fredro-Kumbaradzi, E., Goracinova, K., Calis, S., Simonoska, M., & Hincal, A. A. (2003). 5-Fluorouracil in topical liposome gels for anticancer treatment--formulation and evaluation. *Acta Pharmaceutica*, *53*(4), 241–250.

Goindi, S., Arora, P., Kumar, N., & Puri, A. (2014). Development of Novel Ionic Liquid-Based Microemulsion Formulation for Dermal Delivery of 5-Fluorouracil. *AAPS PharmSciTech*, *15*(4), 810–821. https://doi.org/10.1208/s12249-014-0103-1

Hamouda, N., Sano, T., Oikawa, Y., Ozaki, T., Shimakawa, M., Matsumoto, K., Amagase, K., Higuchi, K., & Kato, S. (2017). Apoptosis, Dysbiosis and Expression of Inflammatory Cytokines are Sequential Events in the Development of 5-Fluorouracil-Induced Intestinal Mucositis in Mice. *Basic & Clinical Pharmacology & Toxicology*, *121*(3), 159–168. https://doi.org/10.1111/bcpt.12793

Hao, Y., Chen, Y., He, X., Yang, F., Han, R., Yang, C., Li, W., & Qian, Z. (2020). Near-infrared responsive 5-fluorouracil and indocyanine green loaded MPEG-PCL nanoparticle integrated with dissolvable microneedle for skin cancer therapy. *Bioactive Materials*, *5*(3), 542–552. https://doi.org/10.1016/j.bioactmat.2020.04.002

Ishibashi, M., Ishii, M., Yamamoto, S., Mori, Y., & Shimizu, S. (2021). Possible involvement of TRPM2 activation in 5-fluorouracil-induced myelosuppression in mice. *European Journal of Pharmacology*, *891*, 173671. https://doi.org/10.1016/j.ejphar.2020.173671

Jadid, M. F. S., Jafari-Gharabaghlou, D., Bahrami, M. K., Bonabi, E., & Zarghami, N. (2023). Enhanced anti-cancer effect of curcumin loaded-niosomal nanoparticles in combination with heat-killed Saccharomyces cerevisiae against human colon cancer cells. *Journal of Drug Delivery Science and Technology*, *80*, 104167. https://doi.org/10.1016/j.jddst.2023.104167

Jeirani, Z., Jan, B. M., Ali, B. S., See, C. H., & Saphanuchart, W. (2014). Pre-prepared Microemulsion Flooding in Enhanced Oil Recovery: A Review. *Petroleum Science and Technology*, *32*(2), 180–193. https://doi.org/10.1080/10916466.2011.586968

Jiang, Y., Li, X., Hou, J., Huang, Y., Jia, Y., Zou, M., Zhang, J., Wang, X., Xu, W., & Zhang, Y. (2016). Discovery of BC-01, a novel mutual prodrug (hybrid drug) of ubenimex and fluorouracil as anticancer agent. *European Journal of Medicinal Chemistry*, *121*, 649–657. https://doi.org/10.1016/j.ejmech.2016.05.068

Jing, C., Li, H., Du, Y., Cao, H., Liu, S., Wang, Z., Ma, R., Feng, J., & Wu, J. (2018). MEK inhibitor enhanced the antitumor effect of oxaliplatin and 5-fluorouracil in MEK1 Q56P-mutant colorectal cancer cells. *Molecular Medicine Reports*. https://doi.org/10.3892/mmr.2018.9730

Kandula, S., Singh, P. K., Kaur, G. A., & Tiwari, A. (2023). Trends in smart drug delivery systems for targeting cancer cells. *Materials Science and Engineering: B*, *297*, 116816. https://doi.org/10.1016/j.mseb.2023.116816

Khallaf, R. A., Salem, H. F., & Abdelbary, A. (2016). 5-Fluorouracil shell-enriched solid lipid nanoparticles (SLN) for effective skin carcinoma treatment. *Drug Delivery*, *23*(9), 3452–3460. https://doi.org/10.1080/10717544.2016.1194498

Khan, N. R., & Wong, T. W. (2018). 5-Fluorouracil ethosomes – skin deposition and melanoma permeation synergism with microwave. *Artificial Cells, Nanomedicine, and Biotechnology*, *46*(sup1), 568–577. https://doi.org/10.1080/21691401.2018.1431650

Lee, J. H., Yun, C. W., Han, Y.-S., Kim, S., Jeong, D., Kwon, H. Y., Kim, H., Baek, M.-J., & Lee, S. H. (2018). Melatonin and 5-fluorouracil co-suppress colon cancer stem cells by regulating cellular prion protein-Oct4 axis. *Journal of Pineal Research*, *65*(4), e12519. https://doi.org/10.1111/jpi.12519

Li, J., Hou, N., Faried, A., Tsutsumi, S., & Kuwano, H. (2010). Inhibition of autophagy augments 5-fluorouracil chemotherapy in human colon cancer in vitro and in vivo model. *European Journal of Cancer*, *46*(10), 1900–1909. https://doi.org/10.1016/j.ejca.2010.02.021

Luiz, H., Oliveira Pinho, J., & Gaspar, M. M. (2023). Advancing Medicine with Lipid-Based Nanosystems—The Successful Case of Liposomes. *Biomedicines*, *11*(2), 435. https://doi.org/10.3390/biomedicines11020435

Miura, K., Kinouchi, M., Ishida, K., Fujibuchi, W., Naitoh, T., Ogawa, H., Ando, T., Yazaki, N., Watanabe, K., Haneda, S., Shibata, C., & Sasaki, I. (2010). 5-FU Metabolism in Cancer and Orally-Administrable 5-FU Drugs. *Cancers*, *2*(3), 1717–1730. https://doi.org/10.3390/cancers2031717

Miyake, T., Honma, Y., Urano, T., Kato, N., & Suzumiya, J. (2015). Combined treatment with tamoxifen and a fusicoccin derivative (ISIR-042) to overcome resistance to therapy and to enhance the antitumor activity of 5-fluorouracil and gemcitabine in pancreatic cancer cells. *International Journal of Oncology*, *47*(1), 315–324. https://doi.org/10.3892/ijo.2015.2979

Murata, S., Adachi, M., Kioi, M., Torigoe, S., Ijichi, K., Hasegawa, Y., Ogawa, T., Bhayani, M. K., Lai, S. Y., Mitsudo, K., & Tohnai, I. (2011). Etodolac improves 5-FU sensitivity

of head and neck cancer cells through inhibition of thymidylate synthase. *Anticancer Research*, *31*(9), 2893–2898.

Muzaffar, F., Singh, U. K., & Chauhan, L. (n.d.). *REVIEW ON MICROEMULSION AS FUTURISTIC DRUG DELIVERY*.

Naguib, Y. W., Kumar, A., & Cui, Z. (2014). The effect of microneedles on the skin permeability and antitumor activity of topical 5-fluorouracil. *Acta Pharmaceutica Sinica B*, *4*(1), 94–99. https://doi.org/10.1016/j.apsb.2013.12.013

Naren, G., Guo, J., Bai, Q., Fan, N., & Nashun, B. (2022). Reproductive and developmental toxicities of 5-fluorouracil in model organisms and humans. *Expert Reviews in Molecular Medicine*, *24*, e9. https://doi.org/10.1017/erm.2022.3

Ndemazie, N. B., Bulusu, R., Zhu, X. Y., Frimpong, E. K., Inkoom, A., Okoro, J., Ebesoh, D., Rogers, S., Han, B., & Agyare, E. (2023). Evaluation of Anticancer Activity of Zhubech, a New 5-FU Analog Liposomal Formulation, against Pancreatic Cancer. *International Journal of Molecular Sciences*, *24*(5), 4288. https://doi.org/10.3390/ijms24054288

Othman, M. H., Zayed, G. M., Ali, U. F., & Abdellatif, A. A. H. (2020). Colon-specific tablets containing 5-fluorouracil microsponges for colon cancer targeting. *Drug Development and Industrial Pharmacy*, *46*(12), 2081–2088. https://doi.org/10.1080/03639045.2020.1844730

Othman, M. H., Zayed, G. M., El Sokkary, G. H., F Ali, U., & Abdellatif, A. A. (2017). Preparation and Evaluation of 5-Fluorouracil Loaded Microsponges for Treatment of Colon Cancer. *Journal of Cancer Science & Therapy*, *09*(01). https://doi.org/10.4172/1948-5956.1000433

Paolino, D., Cosco, D., Muzzalupo, R., Trapasso, E., Picci, N., & Fresta, M. (2008). Innovative bola-surfactant niosomes as topical delivery systems of 5-fluorouracil for the treatment of skin cancer. *International Journal of Pharmaceutics*, *353*(1–2), 233–242. https://doi.org/10.1016/j.ijpharm.2007.11.037

Puri, R., & Jain, S. (2012). Ethogel topical formulation for increasing the local bioavailability of 5-fluorouracil. *Anti-Cancer Drugs*, *23*(9), 923–934. https://doi.org/10.1097/CAD.0b013e3283534051

Safwat, M. A., Soliman, G. M., Sayed, D., & Attia, M. A. (2016). Gold nanoparticles enhance 5-fluorouracil anticancer efficacy against colorectal cancer cells. *International Journal of Pharmaceutics*, *513*(1–2), 648–658. https://doi.org/10.1016/j.ijpharm.2016.09.076

Sara, J. D., Kaur, J., Khodadadi, R., Rehman, M., Lobo, R., Chakrabarti, S., Herrmann, J., Lerman, A., & Grothey, A. (2018). 5-fluorouracil and cardiotoxicity: a review. *Therapeutic Advances in Medical Oncology*, *10*, 175883591878014. https://doi.org/10.1177/1758835918780140

Sethy, C., & Kundu, C. N. (2021). 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: Implication of DNA repair inhibition. *Biomedicine & Pharmacotherapy*, *137*, 111285. https://doi.org/10.1016/j.biopha.2021.111285

Singh, R. P., Gangadharappa, H. V., & Mruthunjaya, K. (2017). Phospholipids: Unique carriers for drug delivery systems. *Journal of Drug Delivery Science and Technology*, *39*, 166–179. https://doi.org/10.1016/j.jddst.2017.03.027

Souto, E. B., Doktorovova, S., & Boonme, P. (2011). Lipid-based colloidal systems (nanoparticles, microemulsions) for drug delivery to the skin: materials and end-product formulations. *Journal of Drug Delivery Science and Technology*, *21*(1), 43–54. https://doi.org/10.1016/S1773-2247(11)50005-X

Stein, A., Voigt, W., & Jordan, K. (2010). Review: Chemotherapy-induced diarrhea: pathophysiology, frequency and guideline-based management. *Therapeutic Advances in Medical Oncology*, *2*(1), 51–63. https://doi.org/10.1177/1758834009355164

Taillibert, S., Le Rhun, E., & Chamberlain, M. C. (2016). Chemotherapy-Related Neurotoxicity. *Current Neurology and Neuroscience Reports*, *16*(9), 81. https://doi.org/10.1007/s11910-016-0686-x

Thomas, A. M., Kapanen, A. I., Hare, J. I., Ramsay, E., Edwards, K., Karlsson, G., & Bally, M. B. (2011). Development of a liposomal nanoparticle formulation of 5-Fluorouracil for parenteral administration: Formulation design, pharmacokinetics and efficacy. *Journal of Controlled Release*, *150*(2), 212–219. https://doi.org/10.1016/j.jconrel.2010.11.018

Thomas, S. A., Tomeh, N., & Theard, S. (2015). Fluorouracil-induced Hyperammonemia in a Patient with Colorectal Cancer. *Anticancer Research*, *35*(12), 6761–6763.

Toffoli, L., Dianzani, C., Bonin, S., Guarneri, C., Guarneri, F., Giuffrida, R., Zalaudek, I., & Conforti, C. (2023). Actinic Keratoses: A Prospective Pilot Study on a Novel Formulation of 4% 5-Fluorouracil Cream and a Review of Other Current Topical Treatment Options. *Cancers*, *15*(11), 2956. https://doi.org/10.3390/cancers15112956

Tytherleigh, M. G., Warren, B. F., & McC. Mortensen, N. J. (2008). Management of early rectal cancer. *British Journal of Surgery*, *95*(4), 409–423. https://doi.org/10.1002/bjs.6127

van Kuilenburg, A. B., & Maring, J. G. (2013). Evaluation of 5-fluorouracil pharmacokinetic models and therapeutic drug monitoring in cancer patients. *Pharmacogenomics*, *14*(7), 799–811. https://doi.org/10.2217/pgs.13.54

Verma, P., & Pathak, K. (2010). Therapeutic and cosmeceutical potential of ethosomes: An overview. *Journal of Advanced Pharmaceutical Technology & Research*, *1*(3), 274. https://doi.org/10.4103/0110-5558.72415

Vodenkova, S., Buchler, T., Cervena, K., Veskrnova, V., Vodicka, P., & Vymetalkova, V. (2020). 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. *Pharmacology & Therapeutics*, *206*, 107447. https://doi.org/10.1016/j.pharmthera.2019.107447

Yen Moore, A. (2009). Clinical applications for topical 5-fluorouracil in the treatment of dermatological disorders. *Journal of Dermatological Treatment*, *20*(6), 328–335. https://doi.org/10.3109/09546630902789326

Zhang, N., Yin, Y., Xu, S.-J., & Chen, W.-S. (2008). 5-Fluorouracil: Mechanisms of Resistance and Reversal Strategies. *Molecules*, *13*(8), 1551–1569. https://doi.org/10.3390/molecules13081551

Zhang, X., Chen, G., Fu, X., Wang, Y., & Zhao, Y. (2021). Magneto-Responsive Microneedle Robots for Intestinal Macromolecule Delivery. *Advanced Materials*, *33*(44), 2104932. https://doi.org/10.1002/adma.202104932