

## REVIEW ARTICLE

# Wnt/ $\beta$ -catenin Pathway, PTEN/PI3K/AKT Pathway, RAS/Raf/Epidermal Growth Factor Receptor/Mitogen-activated Protein Kinases Pathway, and Nuclear Factor Kappa B in Colorectal Cancer Treatment and Management

Snigdha Thaman, Sankha Bhattacharya\*

Department of Pharmaceutics, School of Pharmacy and Technology Management, SVKM'S NMIMS Deemed-to-be University, Shirpur, Maharashtra 425405, India

**Received:** October 17, 2021  
**Accepted:** December 12, 2021  
**Published Online:** December 28, 2021

**\*CORRESPONDING AUTHOR**

Sankha Bhattacharya  
E-mail: sankhabhatt@gmail.com

**CITATION**

Thaman S, Bhattacharya S, 2021, Wnt/ $\beta$ -catenin Pathway, PTEN/PI3K/AKT Pathway, RAS/Raf/Epidermal Growth Factor Receptor/Mitogen-activated Protein Kinases Pathway and Nuclear Factor Kappa B in Colorectal Cancer Treatment and Management. *Cancer Plus*, 3(4):51–68. DOI: 10.18063/cp.v3i4.326

**Copyright:** © 2021 Thaman and Bhattacharya. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), permitting all noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Abstract:** Colorectal cancer (CRC) was once thought to be a rare disease, but it has now become a common, ongoing, and life-threatening disorder. The cells of the colon and rectum have been tainted by this cancerous growth. Colorectal disease is on the rise in agricultural countries as a result of a number of factors, including an aging population, unfavorable western dietary patterns, and an increase in risk factors such as alcohol consumption, lack of physical activity, and corpulence. For both primary and metastatic colorectal malignant growth, new methodologies have emerged. We examine the epidermal growth factor receptor (EGFR) pathway, new cytotoxic specialties such as capecitabine and Tegafur, irinotecan, oxaliplatin, angiogenesis inhibitors, and the EGFR mutation. FOLFOX (5-fluorouracil [5-FU]/leucovorin [LV] plus oxaliplatin) and FOLFIRI (5-FU/LV plus irinotecan) have both been shown to be effective cytotoxic medications for metastatic CRC, with typical survival rates of around 2 years. Natural agents such as Bevacizumab (a monoclonal antibody that targets vascular endothelial development factor, a key regulator of angiogenesis) and Cetuximab/Panitumumab (monoclonal antibodies coordinated against the EGFR) were thought to be helpful for cytotoxic treatment. Patients with CRC should keep receiving foundational chemotherapy, which includes 5-FU/LV infusions. In this article, we focused on various pathways, such as the Wnt/ $\beta$ -catenin pathway, PTEN/PI3K/AKT pathway, RAS/Raf/EGFR/mitogen-activated protein kinases pathway, and nuclear factor kappa B pathway and their importance's in CRC treatment and management.

**Keywords:** Colorectal cancer, Molecular basis of colorectal cancer, Wnt/ $\beta$ -catenin, PTEN/PI3K/AKT, RAS/Raf/epidermal growth factor receptor/mitogen-activated protein kinases, Nuclear factor kappa B

## 1. Introduction

According to Colorectal Cancer (CRC) Statistics 2020, CRC is the third most commonly diagnosed and researched disease and is the third leading cause of problem and increased mortality in the United States<sup>[1,2]</sup>. According to CRC Statistics 2020, the threat of CRC is increasingly falling on young people. Nonetheless, as a result of these clinical advancements, mortality rates arise from communicable diseases have increased globally, and malignant

growth mortality has increased<sup>[3]</sup>. The critical reasons for malignancy-related mortality have also expanded as a result of advancements in infection prevalence, the availability of screening administrations, and logical turns of events. In 1950, colorectal malignant growth was largely unknown, but it has since become a widespread disease in Western countries<sup>[4]</sup>. In non-industrial countries, CRC is on the rise, due to a combination of factors, including an aging population, unfavorable Western dietary patterns, and an increase in risk factors such as alcohol consumption, lack of physical activity, and weight increment. The rate of sickness has changed in some acquired disease conditions and inconsistent sickness rates<sup>[5]</sup>. New treatments have been developed for CRC that is both dominant and metastatic. This is beneficial because it gives patients more options, such as laparoscopic medical procedures for dominant illnesses, more aggressive metastatic infection resection (e.g. liver and pneumonic metastases), rectal malignant growth radiotherapy, and neoadjuvant and reassuring chemotherapies<sup>[5]</sup>. Treatment adequacy in the metastatic setting has improved since the introduction of specific substances. These include the 5-fluorouracil (5-FU) prodrugs capecitabine and Tegafur, the topoisomerase I inhibitor irinotecan, the platinum-containing specialist oxaliplatin; bevacizumab, a vascular endothelial growth factor-A (VEGF-A) counteracting agent; and cetuximab and panitumumab, epidermal growth factor receptor (EGFR) immune response antagonists. In the management of patients with metastatic CRC, a foundational organization of cytotoxic medications is required<sup>[6]</sup>.

## 2. Molecular basis of CRC

CRC is a malignant cancer that develops over time. It starts with an unpredictably developing tumor or tissue on the internal covering of the rectum or colon<sup>[7]</sup>. A polyp is the name for this strange growth. On the off chance that this polyp becomes destructive, a tumor may form on the colon or rectum mass<sup>[8]</sup>. This will spread into veins or lymph vessels, increasing the risk of metastasis to other parts of the body. Adenocarcinomas account for the vast majority of tumors that begin in the colorectal region (more than 95%)<sup>[9]</sup>. These start in the organs that deliver bodily fluids to the colon and rectum. Carcinoid tumors (starting in the intestinal chemical-delivering cells), gastrointestinal (GI) stromal tumors (framing in specific colonic cells known as Cajal interstitial cells), lymphomas (malignancies of the immune system that develop in the colon or rectum), and sarcomas are some of the more uncommon colorectal diseases (normally beginning in blood vessels, muscle layers however regularly shaping in colorectal walls)<sup>[10]</sup>. CRCs are a very diverse group of diseases on a molecular level. CRC progression is aided by genomic and epigenomic instability. The most fundamental type of instability is chromosomal instability (CIN)<sup>[11]</sup>. Up to 85% of colorectal diseases have CIN. This type of uncertainty is established

by the presence of aneuploid or polyploid DNA. These underlying changes in chromosomes can be investigated using techniques such as flow cytometry and complete exome sequencing. Microsatellites instability (MSI) in colorectal tumors, which accounts for nearly 15% of all CRCs<sup>[12]</sup>. The changes that occur in this type of colorectal malignant growth are different from those that occur in CIN colorectal diseases<sup>[13]</sup>. CRC can be caused by hypermethylation of gene loci containing CpG islands, just as it can be caused by worldwide DNA hypomethylation<sup>[14]</sup>. There is a subset of CRC that have a higher proportion of methylated CpG loci than other types of CRC. CRC is a disease caused by epithelial cells that line the colon or rectum of the GI tract and is most commonly caused by changes in the Wnt signaling pathway that improves signaling action<sup>[15,16]</sup>. The adenomatous polyposis coli (APC) gene, which produces the APC protein, is regularly altered in all colorectal diseases. The APC protein prevents  $\beta$ -catenin protein aggregation. Without APC,  $\beta$ -catenin accumulates to undeniable levels in the nucleus, joins DNA, and activates proto-oncogene transcription<sup>[17]</sup>. Despite the fact that APC is altered in most colon cancers, catenin has grown in some tumors due to  $\beta$ -catenin (CTNNB1) mutations that prevent it from being broken down or mutations in other APC-like genes such as axis inhibitor 1 (AXIN1), axis inhibitor 2 (AXIN2), transcription factor 7 like 2 (TCF7L2), or NKD1. Different changes must occur in the cell for it to become malignant, in addition to the deformities in the Wnt signaling pathway<sup>[18,19]</sup>. Normally, the p53 protein produced by the TP53 gene screens cell division and causes them to die in a modified manner if the Wnt pathway is disrupted<sup>[20]</sup>. A cell line acquires a TP53 gene mutation over time, transforming the tissue from a benign epithelial tumor cells to a potent epithelial malignancy. Rather than the p53 gene being altered on a regular basis, another cautious protein called BCL2 associated X (BAX) is altered<sup>[21]</sup>. Transforming growth factor- $\beta$  (TGF- $\beta$ ) and DCC (deleted in CRC) are two proteins that are frequently deactivated in colorectal tumors and are responsible for modified cell death. TGF- $\beta$  has a deactivating transformation in at least half of colorectal malignant growths<sup>[22]</sup>. TGF- $\beta$  is rarely deactivated, but suppressor of mothers against decapentaplegic, a downstream protein, is frequently deactivated. DCC usually has a chromosome that has been erased in colorectal disease<sup>[23]</sup>. Ding *et al.* (2019) discovered that microRNA-143-3p (miR-143-3p) suppresses tumorigenesis by targeting catenin- $\delta$ 1 in CRC<sup>[24]</sup>. Their findings revealed that miR-143-3p inhibited CRC tumor growth *in vitro* and *in vivo* by targeting catenin beta-1 (CTNND1), highlighting the importance of miR-143-3p and CTNND1 in CRC tumorigenesis and the potential value of miR-143-3p and CTNND1 in CRC prediction, diagnosis, and treatment<sup>[24,25]</sup>. According to Liu *et al.* (2018), the LIFR-AS1/miR-29a/TNFAIP3 hub plays a useful role in colorectal disease resistance to photodynamic therapy (PDT)<sup>[26]</sup>. They used microarrays

to find unregulated long non coding ribonucleic acid (lncRNAs), micro ribonucleic acid (miRNAs), and messenger ribonucleic acid (mRNAs) in PDT-treated HCT116 cells, as well as the downstream mRNA target and molecular mechanism, to figure out the lncRNA-miRNA cooperations linked to CRC resistance to PDT treatment<sup>[26,27]</sup>. LIFR-AS1 acts as a significant endogenous RNA (ceRNA) for micro ribonucleic acid-29a (miR-29a), repressing its expression and upregulating the expression of downstream target tumor necrosis factor alpha-induced protein 3 (TNFAIP3) and modulating CRC resistance to PDT<sup>[28]</sup>. Li *et al.* (2018) discovered the capability of collagen type VI alpha 3 chain (COL6A3) for colorectal malignant growth<sup>[29]</sup>. In CRC, COL6A3, which is expressed in cancer-related fibroblasts, is an independent prognostic factor. The ability of COL6A3 to expand and attacks CRC cells was confirmed through knockout experiments<sup>[30]</sup>. Eylem *et al.* (2020) subjected CRC-specific exosomes to an untargeted multi-omic analysis and demonstrated that in both clinical samples and cell culture, CRC pathways are linked together<sup>[31]</sup>. Zhu *et al.* (2018) discovered that chemokine receptor 6 (CCR6) promotes tumor angiogenesis through the AKT/nuclear factor kappa B (NF- $\kappa$ B)/VEGF pathway in CRC<sup>[32]</sup>. They discovered that inhibiting the multiplication and movement of human umbilical vein endothelial cells (HUVECs) silences CCR6, which can slow angiogenesis, whereas overexpression of CCR6 can speed up angiogenesis. They also looked into the molecular mechanisms, discovering that activation of the AKT/NF- $\kappa$ B pathway promotes the release of VEGF-A, which may play a role in CCR6-interceded tumor angiogenesis. They concluded that CCR6 contributes to tumor angiogenesis in CRC through the AKT/NF- $\kappa$ B/VEGF pathway. Jiang and his collaborators (2019) reported that O-GlcNAcylation, a unique posttranslational modification (PTM) involved in cancer metabolic reprogramming, increased the metastatic capability of CRC through the miR-101-O-GlcNAc/EZH2 regulatory feedback circuit<sup>[33]</sup>.

## 2.1. Wnt/ $\beta$ -catenin pathway

The Wnt signaling pathway influences cell progression, division, connection, and extremity<sup>[34]</sup>. Wnt refers to a group of hereditary secretory glycoprotein markers. It was first used in 1991<sup>[35]</sup> and it is the most troublesome sign in irregular CRC<sup>[36]</sup>. Until now, 11 receptors from the Frizzled (Fz) family have been discovered in humans<sup>[37]</sup>. The receptors Fz1 to Fz10, Smo, and the two LRP 5 and 6 co-receptors are all active in Wnt signaling<sup>[38]</sup>. For these receptors, 19 Wnt ligands that include Wnt1, 2, 2b, 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 8a, 8b, 9a, 9b, 10a, 10b, 11, and 16 have been discovered<sup>[39]</sup>. The most well-known Wnt signaling pathway is the Wnt standard signaling pathway. In the absence of Wnt ligands,  $\beta$ -catenin is abundant at cell intersections. Even after this, a small amount of  $\beta$ -catenin remains in the cytoplasm, where it is linked to a

complex that causes  $\beta$ -catenin proteasomal corruption<sup>[40]</sup>. During degradation, the Axin protein is responsible for obtaining essential components such as glycogen synthase kinase 3 (GSK3), casein kinase 1 (CK1), APC, and yes-associated protein 1/transcriptional coactivator with PDZ-binding motif (YAP/TAZ). GSK3 phosphorylates  $\beta$ -catenin at the Ser33, Ser37, and Thr41 deposits, whereas CK1 phosphorylates it at the Ser45 buildup. APC also prevents PP2A phosphatase-mediated-catenin dephosphorylation<sup>[41]</sup>. The YAP/TAZ complex then works with  $\beta$ -catenin ubiquitination and progressive proteasomal degradation using the E3 ubiquitin ligase beta-transducing repeats-containing proteins ( $\beta$ -TrCP), which detects Ser/Thr phosphorylation<sup>[42]</sup>. When a Wnt ligand binds to the Fz receptor and the LPR5/6 co-receptor,  $\beta$ -catenin delocalizes, causing it to collect in the cytoplasm and nucleus. When the Fz receptor dimers with the LRP5/6 co-receptor, Disheveled (Dvl) protein is activated, and CK1 phosphorylates LPR5/6 to enable Axin binding, causing the  $\beta$ -catenin degradation complex to be destroyed<sup>[43]</sup>. The accumulation and movement of  $\beta$ -catenin to the nucleus are taken into account in this system. Furthermore, the nuclear movement of FOXM1, a member of the Forkhead box (Fox) transcription factor family, is aided by its restriction to  $\beta$ -catenin.  $\beta$ -catenin binds to TCF/LEF transcription factors in the nucleus, causing co-repressors like Groucho/TLE to dissociate, allowing association with co-activators like cell cycle-related and expression elevated protein in tumor (CREPT), four and a half LIM domains protein 2 (FHL2), and cyclic AMP response element-binding protein (CBP/p300), as well as chromatin remodelers like brahma-related gene-1 (Brg-1)<sup>[44]</sup>. Wnt signaling inhibits tumor cell multiplication and differentiation, but it also plays an important role in endothelial function. In addition, the previous findings showed that piperine suppresses the Wnt/ $\beta$ -catenin pathway and has anti-cancer properties in CRC cells<sup>[45,46]</sup>. Zhang *et al.* (2018) used whole-genome sequencing to discover CREPT gene amplification in CRC<sup>[47]</sup>. According to the researchers, CREPT significantly increased CRC cell proliferation and metastasis *in vitro* and *in vivo*<sup>[47,48]</sup>. By increasing the relationship between p300 and  $\beta$ -catenin, CREPT promoted p300-mediated  $\beta$ -catenin acetylation and stabilization. Furthermore, CREPT promotes the Wnt/ $\beta$ -catenin pathway by cooperating with p300, which plays a key oncogenic role in colorectal carcinogenesis<sup>[49]</sup>. On the other hand, Sun *et al.* (2019) reported that Tre2 (USP6NL) promotes CRC cell proliferation through the Wnt/ $\beta$ -catenin pathway. The ubiquitin-specific protease 6 N-terminal-like protein (USP6NL), which is highly expressed in CRC tissues, regulates CRC cell proliferation through the Wnt/ $\beta$ -catenin pathway. The Wnt/ $\beta$ -catenin pathway was activated when USP6NL inhibited  $\beta$ -catenin ubiquitination. According to their findings, targeting USP6NL could be a novel therapeutic target in the treatment of CRC<sup>[50]</sup>. Neuronal pentraxin 2 (NPTX2), a

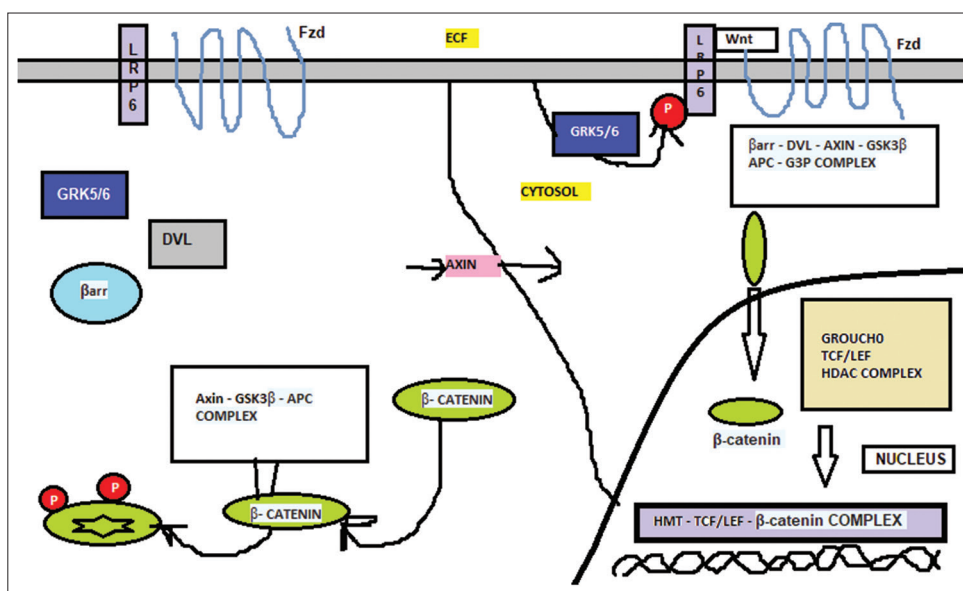


nervous system molecule, promotes CRC cell proliferation and metastasis by activating the Wnt/ $\beta$ -catenin pathway through direct interaction with Fz type receptor 6 FZD6<sup>[51]</sup>. Another study had demonstrated that activation of the Wnt/ $\beta$ -catenin pathway through ubiquitination of TLE3 RNF6 promotes CRC<sup>[52]</sup>. Zeng *et al.* (2020) discovered that thymol, a natural product derived from Chinese herbal medicine, inhibited CRC cell proliferation and metastasis while also inducing cell apoptosis and cell-cycle arrest. According to their findings, thymol has these effects by blocking the Wnt/ $\beta$ -catenin signaling pathway<sup>[53]</sup>. **Figure 1** depicts the Wnt signaling pathways in CRC.

## 2.2. PTEN/PI3K/AKT pathway

The PTEN/PI3K/AKT signaling pathway is involved in a number of natural processes, including cell death, digestion, multiplication, and development<sup>[54]</sup>. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a two-fold protein/lipid phosphatase with phosphatidylinositol, 3,4,5 triphosphate as its primary substrate (PIP3)<sup>[55]</sup>. AKT is activated by other PIP3-subordinate kinases once it reaches the membrane, triggered by an increase in PIP3. The PI3K/AKT/PTEN pathway promotes cell development and prevents apoptosis in response to a variety of extracellular sources such as growth factors, cytokines, chemicals, heat and oxidative pressure, hypoxia, and hypoglycemia<sup>[56]</sup>. The receptor undergoes self-phosphorylation and actuation when a developmental factor binds to its receptor. PI3K is then enlisted and activated at the plasma layer level. Initiated PI3K converts phosphatidylinositol (4,5) biphosphate (PIP2) to phosphatidylinositol. PIP3 binds to AKT and anchors it to the membrane, allowing phosphoinositide-subordinate kinase-1 to phosphorylate and activate it (PDK1)<sup>[57]</sup>. AKT

controls digestion, translation, apoptosis, and the cell cycle by phosphorylating a number of target proteins such as BAD (BCL-2 antagonist of cell death), caspase-9, mTOR (mammalian target of rapamycin), GSK3, and  $\beta$ -catenin. Ye *et al.* (2017) reported that inhibiting microRNA 19a (miR-19a) via the PTEN/PI3K/AKT pathway partially reversed CRC resistance to oxaliplatin<sup>[58]</sup>. Liu and his research teams (2019) showed that microRNA-543 (MiR-543) increased drug tolerance by suppressing the expression of phosphatase and tensin homolog (PTEN), a protein that inhibits protein kinase B (AKT) activation. MiR-543 could be a target for increasing CRC cell sensitivity to 5-FU via the PTEN/PI3K/AKT pathway<sup>[59]</sup>. Liu *et al.* (2019) reported that curcumin effectively inhibited CRC cell proliferation *in vitro* and *in vivo* by inhibiting the PTEN/PI3K/Akt pathways and decreasing microRNA 21 (miR-21) expression<sup>[60]</sup>. Alaaeddine *et al.* (2021) demonstrated that deletion or inhibition of cyclooxygenase 2 (COX-2) or arachidonate 5-lipoxygenase (5-LOX) increased PTEN development and inhibited cell and adenoma progression through the PI3K/AKT pathway in CRC<sup>[61]</sup>. A study by Ma *et al.* (2019) showed that C-X-C motif chemokine ligand 12 (CXCL12) and the tumor suppressor protein phosphatase and tensin homolog deleted on chromosome 10 (PTEN) possess the ability to control colon cancer metastatic and display an internal relationship between colon cancer and stromal cells<sup>[62]</sup>. Their findings show that the CXCL12/CXCR4/PI3K/Akt pathway is required for colon cancer cells to spread. Modifying the CXCR4, PTEN, or PI3K features may be promising alternative therapeutic pathways to prevent colon cancer from spreading aggressively<sup>[62,63]</sup>. Besides, naringin stimulated apoptosis in CRC cells and blocked the activation of the PI3K/AKT/mTOR signaling pathway in a dose-dependent manner. The findings also suggested that naringin could be a potential therapeutic



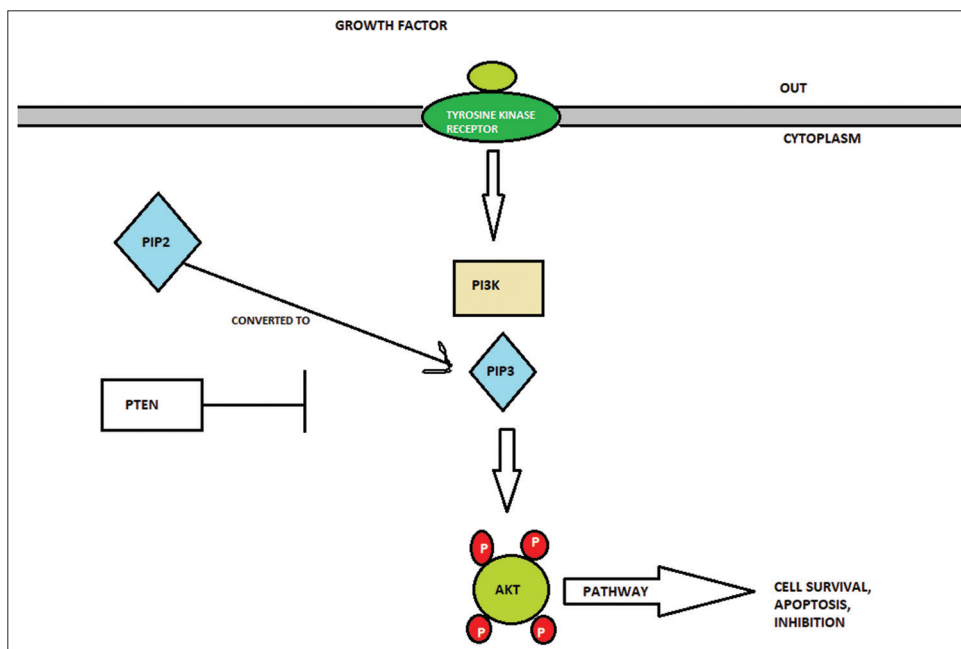
**Figure 1.** Wnt signaling pathways in colorectal cancer

agent for CRC therapy because it inhibits CRC cell proliferation and induces apoptosis by inhibiting the PI3K/AKT/mTOR signaling pathway<sup>[64]</sup>. The process of PTEN/PI3K/AKT pathway is discussed in details in **Figure 2**.

### 2.3. RAS/Raf/EGFR/mitogen-activated protein kinases (MAPK) pathway

The three primary subfamilies of MAPK are the extracellular-signal-controlled kinases (ERK MAPK), the c-jun N-terminal kinase or stress-activated protein kinases (JNK or SAPK), and MAPK14 (MAPK)<sup>[65]</sup>. The ERK MAPK pathway is probably the most important for cell proliferation. Several of these pathways are found downstream of growth factor receptors, including the epidermal growth factor pathway<sup>[66]</sup>. MAPK signaling is required for the maintenance of typical physiological cycles such as multiplication and differentiation<sup>[67-70]</sup>. The intracellular MAPK signaling network is complex, with many intermediates. The RAS signaling pathway is an important part of this system<sup>[71]</sup>. The MAPK is required for normal physiological processes such as proliferation and differentiation to continue<sup>[72]</sup>. The intracellular MAPK signaling network is a complex signaling pathway with numerous intermediates. The RAS signaling pathway is a crucial component of this system. For the standard RAS pathway, the main mediators are RAS and RAF proteins, as well as intracellular signal kinases, mitogen extracellular kinase (MEK), and extracellular signal-related kinase (ERK). RAS is usually found in an inactive guanosine diphosphate (GDP)-bound state that can be triggered by external stimuli such as mitogens, cytokines, and growth factors<sup>[73,74]</sup>. Ligands bind to the receptor tyrosine kinases

(RTKs), causing the receptor to dimerize. At that point, the triggered receptor binds to the growth factor receptor bound protein 2 (Grb2) protein, either directly or indirectly through other mediator proteins<sup>[75,76]</sup>. Following communication with Grb2, intracellular son of seven less (Sos) protein is selected for cell surface expression. Sos exhibits GEF (guanine nucleotide exchange factor) movement as part of the RTK-Grb2-Sos complex<sup>[77]</sup>. This complex then binds to RAS-GDP, causing GDP to be split. RAS that has been liberated from nucleotides prefers to attach to guanosine triphosphate (GTP) to activate a downstream signaling pathway. RAS-GAPs (RAS-GTPase-activating proteins) are proteins that deactivate the RAS signaling pathway by removing GTP from the activated RAS molecule<sup>[78]</sup>. When RAS is activated and GTP-bound, it forms a direct association with RAF, a kinase that belongs to the kinase family. RAF proteins, unlike those in the RAS family, have the ability to dimerize<sup>[79]</sup>. When RAS-GTP is active, RAF molecules are recruited to the cell surface. When RAF binds to the RAS-GTP molecule, a cascade of phosphorylation events take place, releasing RAF's 14-3-3 hindrance<sup>[80]</sup>. When RAF is activated, the isomers of the protein form heterodimers. The active RAF heterodimer then recruits, binds, and activates kinase suppressor of Ras-1 (KSR1), a platform protein<sup>[81]</sup>. The KSR1 protein is bound to 14-3-3 in the cytoplasm and then left latent. When RAS/RAF is activated, KSR1's restraint is broken, and it becomes activated to work with RAF heterodimers. MEK and ERK are RAS signaling pathway downstream substrates/activators that are both enlisted to connect with the KSR1 framework protein<sup>[82]</sup>. MEK is activated and phosphorylated by RAF, which then phosphorylates ERK<sup>[83]</sup>. After being phosphorylated, ERK is moved to



**Figure 2.** PTEN/PI3K/AKT pathway

the nucleus, where it initiates various proliferative and endurance signals<sup>[84]</sup>. The MAPK signaling pathway is responsible for maintaining cellular homeostasis. The signal mismatch is triggered by changes in pathway segments, which may lead to cancer development. Ubinuclein-2 (UBN2) is a nuclear protein that interacts with a number of transcription factors. Zhao *et al.* (2019) reported that UBN2 promotes tumor progression through the RAS/MAPK pathway and predicts a poor prognosis in CRC<sup>[85]</sup>. Through immunohistochemistry analysis, Tang *et al.* (2019) discovered that phosphoprotein enriched in astrocytes 15 kDa (PEA15) was highly expressed in CRC tissues and liver metastatic cancer tissues. The authors speculated that PEA15 could be a biomarker for CRC therapy-induced liver metastasis<sup>[86]</sup>. PEA15 also aided the growth of CRC liver metastasis through the ERK/MAPK signaling pathway<sup>[87]</sup>. Angius *et al.* (2019) reported that the activation of many downstream effectors, such as BRAF/RAS/MAPK, PI3K/AKT, RalGDS/p38MAPK, and others, is controlled by the KRAS oncogene, which will affect normal cell physiology, neoplastic cell biology, and therapeutic responses<sup>[88]</sup>. Ponsioen *et al.* (2021), through quantifying single-cell ERK dynamics in CRC organoids revealed that EGFR to be an amplifier of oncogenic MAPK pathway signaling<sup>[89]</sup>. **Figure 3** discusses the RAS/Raf/EGFR/MAPK pathway in details.

#### 2.4. NF- $\kappa$ B pathway

Two important pathways for the formation of homo- and heterodimeric complexes have been included<sup>[90]</sup>. The primary NF- $\kappa$ B actuation pathway, also known as the classical NF- $\kappa$ B actuation pathway, essentially influences RelA: p50 dimers, which are sequestered in the cytoplasm under non-invigorated conditions, thanks to the collaborations with inhibitory IB family proteins<sup>[91]</sup>. After stimulation with a variety of stimuli such as tumor necrosis factor-alpha (TNF- $\alpha$ ) or interleukin-1, infections, genotoxic agents, or ionizing radiation, IB molecules are phosphorylated by the IB kinase complex (IKK) at specific serine deposits, prompting their ubiquitination and degradation by the proteasome pathway. RelA: p50 dimers are then released and allowed to move to the nucleus, where they activate transcription of a number of target genes<sup>[92]</sup>. The regulation of innate resistance and aggravation depends on this pathway. Three IKKs, IKK $\alpha$ , IKK $\beta$ , and IKK $\gamma$ , as well as NEMO (NF- $\kappa$ B basic modulator) are included in the standard pathway<sup>[93]</sup>. Fewer TNF superfamily cytokines (such as B-cell activating factor receptor [BAFF], CD40L, and lymphotoxin [LT]) activate the subsequent pathway, known as the alternative NF- $\kappa$ B signaling pathway. The upstream kinase NF- $\kappa$ B -inciting kinase (NIK) activates IKK, causing p100, the key RelB inhibitor, to be phosphorylated and handled by the proteasome, allowing RelB: p52 and RelB: p50 to translocate and bind to DNA. The NF- $\kappa$ B pathway is activated by the IKK complex and its subunits

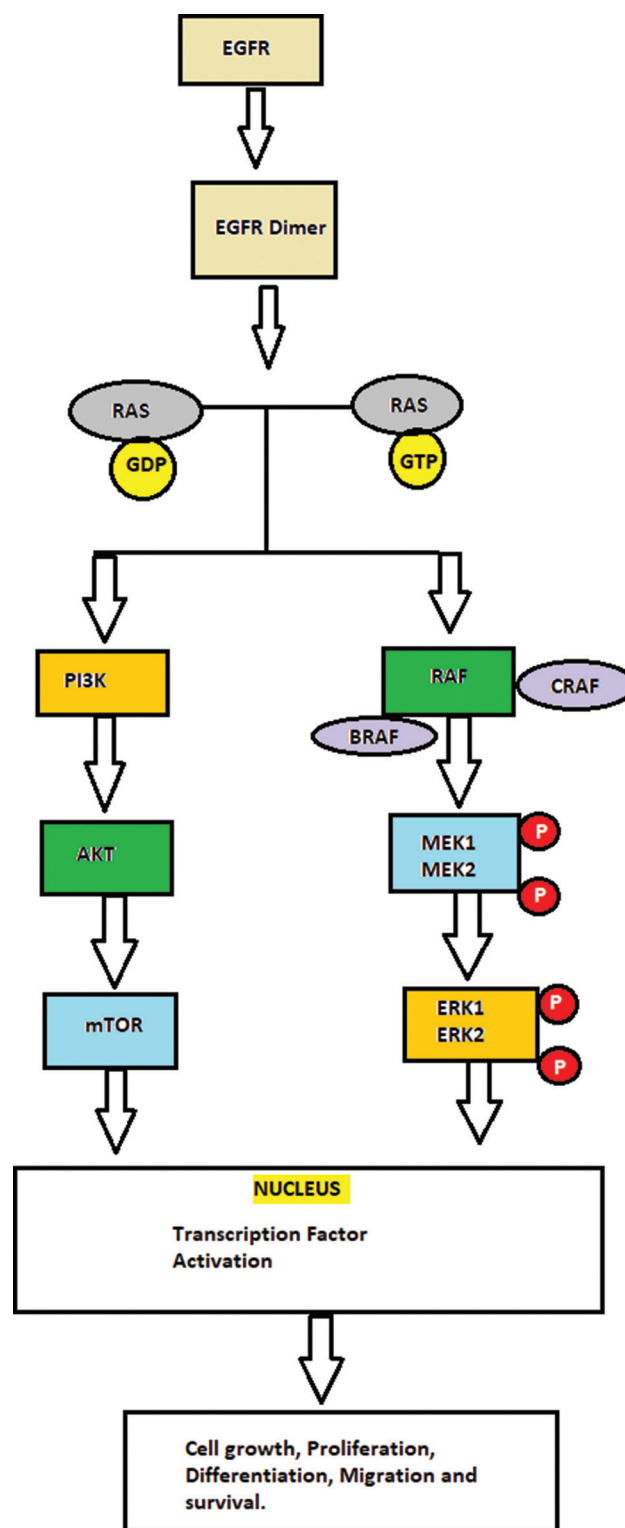
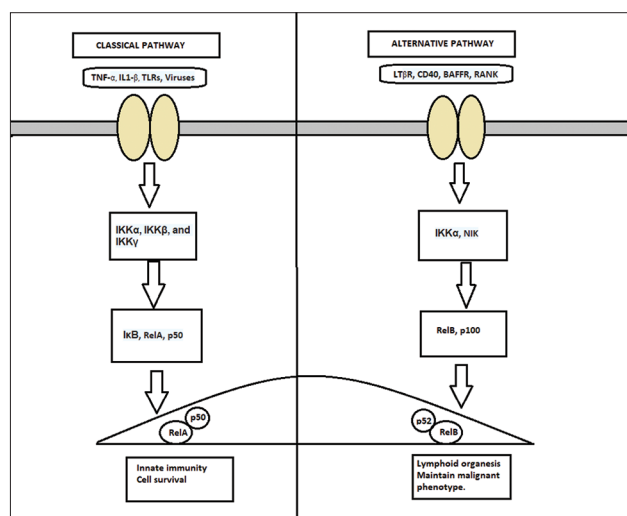


Figure 3. RAS/Raf/EGFR/MAPK pathway

indelibly phosphorylating IB inhibitory proteins (IB for the classical pathway and p100 for the alternative pathway)<sup>[94]</sup>. Kannathasan *et al.* (2020) reported that using miR-4454 as a microRNA-based therapeutic approach to silence guanine nucleotide-binding protein-like 3-like protein (GNL3L) could significantly reduce oncogenic cell survival that relies

on GNL3L/NF- $\kappa$ B signaling, potentially making miR-4454 a treatment for metastatic human CRC<sup>[95]</sup>. Liu *et al.* (2018) highlighted that double curtain like kinase 1 (DCLK1) plays a key role in epithelial-mesenchymal transition (EMT) in human CRC<sup>[96]</sup>. DCLK1 appears to act as a potent oncogene in CRC cells, driving their extremely malignant EMT character in an NF- $\kappa$ B -dependent manner<sup>[97]</sup>. They discovered that silencing DCLK1 expression stopped CRC cells from invading and spreading *in vivo*. Their findings revealed that DCLK1 regulates an EMT axis in CRC, suggesting that DCLK1 could be a therapeutic target for CRC metastasis<sup>[98]</sup>. Chun *et al.* (2019) found that activating the AKT/NF- $\kappa$ B/FOXD1/VEGF-A pathway in a CXCR2-dependent manner improved tube formation, proliferation, and migration in HUVECs using recombinant human CXCL5 (rhCXCL5)<sup>[99]</sup>. Inhibiting the AKT and NF- $\kappa$ B pathways or silencing CXCR2 and FOXD1 in rhCXCL5-stimulated HUVECs *in vitro*, on the other hand, could reduce tube forming capacity, proliferation, and migration<sup>[99]</sup>. CXCL5 promotes tumor angiogenesis in CRC by upregulating FOXD1 expression through the AKT/NF- $\kappa$ B pathway. Wang and colleagues (2018) discovered a rise in tripartite motif containing 31 protein coding (TRIM31) expression in CRC cells as well as a link between TRIM31 and CRC malignancy<sup>[100]</sup>. They concluded that TRIM31 promoted CRC invasion and metastasis by mediating chronic inflammation via the NF- $\kappa$ B pathway. Huang *et al.* (2015) showed that the circular RNA GLIS2, which may act as an oncogene, uses the miR-671 sponge system to maintain the abnormal activation state of the NF- $\kappa$ B signaling pathway in CRC cells<sup>[101]</sup>. Lin *et al.* (2012) discovered that after inhibiting AKT and activating GSK3, gilteritinib, a highly potent, and highly selective oral inhibitor of Fms-like tyrosine kinase 3 (FLT3)/Axl (tyrosine kinase TAM family) (FLT3/AXL), induces p53 upregulated modulator of apoptosis (PUMA) in CRC cells through the NF- $\kappa$ B pathway<sup>[102]</sup>. **Figure 4** illustrates the processes involved in NF- $\kappa$ B pathway.



**Figure 4.** NF- $\kappa$ B pathway

### 3. Treatment of CRC

#### 3.1. Fluoropyrimidines

##### 3.1.1. Intravenous fluorouracil

CRC, also known as colon cancer, is one of the most commonly studied malignancies in the world<sup>[103]</sup>. Over 1,000,000 new cases of colorectal malignant growth are analyzed every year around the world, with a large portion of approximately 1,000,000 people succumbed to the disease<sup>[104]</sup>. According to American Cancer Society, CRC is one of the leading causes of cancer-related death in the United States. Several factors contribute to the development of colon cancer. Almost all colon tumors begin in the organs of the colon's coating, and vast majority begin as noncancerous (benign) polyps that eventually progress to cancer. Early detection, on the other hand, will result in a complete recovery through a combination of medical procedures, medication regimens (chemotherapy), and radiation therapy<sup>[105]</sup>. Heidelberger was fascinated by tumor biosynthesis of nucleic acids and the development of drugs that could prevent cancer from spreading as a side effect of chemotherapy. He hypothesized that by tinkering with the combination of DNA and RNA in a series of mouse trials, he could prevent the development of specific tumors by incorporating a fluorine molecule into the design of a pyrimidine known as uracil<sup>[106]</sup>. Heidelberger sought the help of Robert Duschinsky at Hoffman-LaRoche, a pharmaceutical company, to complete the synthesis of the new substance known as 5-FU so that tests on its tumor-fighting properties could be expanded<sup>[107]</sup>. After clinical preliminaries at the McArdle Laboratory that revealed the new medication's solid guarantee, fluorouracil was approved for chemotherapeutic therapy of a few human malignancies, particularly colon disease. 5-FU therapy has been shown to prolong survival rate in a variety of cancers. The drug most notable effect has been in the treatment of CRC. Dynamic metabolites of 5-FU disrupt DNA and RNA amalgamation through a cycle that includes the folate metabolic pathway. During the S phase of the cell cycle, 5-FU inhibits DNA synthesis by limiting the supply of thymidylate<sup>[108]</sup>. Thymidine phosphorylase (TP) is also necessary for the development of the dynamic metabolite 5-FU. 5-FU inhibits thymidylate synthetase through its metabolite 5-fluorodeoxyuridine monophosphate (FdUMP). FdUMP forms a covalent ternary complex with thymidylate synthetase and 5,10-methylenetetrahydrofolate. The complex relationship with folinic acid improves its stability. 5-FU has the ability to suppress RNA amalgamation, production, and capacity<sup>[109]</sup>. The specific tumor gathering of fluorouracil-based medications inside tumor tissues improves resiliency, but side effects can occur. Symptoms of 5-FU treatment include leukopenia, loose bowels, stomatitis, and queasiness. The main site of action appears to be thymidylate synthase when 5-FU is combined with 5-formyltetrahydrofolate



(folinic acid and leucovorin [LV]), resulting in a marked and sustained inhibition of DNA synthesis. N5, N10-methylenetetrahydrofolate, a reduced folate cofactor, stabilizes FdUMP binding to thymidylate synthase, resulting in this reaction<sup>[110]</sup>. As a result, LV boosts 5-FU's anti-cancer properties. In another study, an investigation on how dysbiosis in the gut microbiota affected the efficacy of 5-FU therapy for CRC had been carried out. ABX (an antibiotic contains mixture of antibiotics) administration reduced the antitumor potency of 5-FU in mice, which were discovered using the CRC mouse model and high-throughput sequencing<sup>[111]</sup>. Supplementation of 5-FU with probiotics did not significantly improve the efficacy of 5-FU therapy. Ndreshkjana *et al.* (2019) examined the effects of novel 5-FU/Thymoquinone (TQ) hybrids in CRC cells *in vitro* and *in vivo*. They discovered new targets for single-drug therapy that go beyond the established mechanisms of action of 5-FU and TQ. Both hybrid strategies were found to be highly effective against CD133+ cancer stem cells (CSC) populations in CRC, simultaneously inhibiting the WNT/ $\beta$ -catenin and PI3K/AKT signaling pathways<sup>[112]</sup>. As a result, their findings support the hypothesis of combining the clinical drug 5-FU with the plant-derived compound TQ, and suggested the usage of hybridization concept to develop new CRC drug candidates against CRC.

### 3.1.2. Oral fluoropyrimidines

Fluoropyrimidines were initially ineffective when taken orally. Intravenous fluorouracil was found superior to oral fluorouracil in terms of tumor reaction rate and mean length of tumor reaction in a randomized study of patients with metastatic CRC<sup>[113]</sup>. The rate-limiting catalyst dihydropyrimidine dehydrogenase (DPD), which is mostly concentrated in the liver, catabolized 5-FU almost immediately after application. Oral fluoropyrimidines come in two varieties: those that contain a DPD inhibitor and those that do not<sup>[114]</sup>. Capecitabine (Xeloda<sup>®</sup>) is a non-DPD 5-FU oral prodrug that passes through the GI mucosa undamaged until it is converted to fluorouracil in three-step enzymatic processes. It only becomes cytotoxic after being transformed to 5-FU<sup>[115]</sup>. Following oral administration, capecitabine is metabolized in the liver by carboxylesterase into 5-deoxy-5-fluorocytidine and cytidine deaminase into 5-deoxy-5-fluorouridine. The final phase of transformation is carried out by pyrimidine phosphorylase, which has been shown to be present in higher concentrations in tumors than in normal tissue, implying that it may have greater tumor selectivity. The most common side effect of this medication is hand-foot disorder, which is similar to that of fluorouracil infusion. Capecitabine was shown to be therapeutically equivalent to bolus fluorouracil and LV in metastatic CRC preliminaries, with no significant differences in median time to tumor progression or median overall endurance<sup>[116]</sup>. Tegafur is a prodrug that is converted to 5-FU by hepatic cytochrome P450 enzymes and are fundamental dissolvable compounds. As a byproduct

of this reaction, 5-FU is produced. Uracil is added at a molar concentration of 1:4. (5-FU: uracil) to increase the concentration of 5-FU and to prevent it from being degraded by DPD<sup>[116]</sup>. In preclinical models, this molar combination has been shown to be the most effective. Chan *et al.* (2018) investigated the link between serum folate levels and capecitabine toxicity in CRC patients receiving capecitabine treatment<sup>[117]</sup>. They concluded that serum folate levels, but not red cell folate, were associated with a higher risk of Grade 2 toxicity during capecitabine treatment. Excessive folate intake should be avoided before and after capecitabine-based chemotherapy.

### 3.2. Irinotecan

Irinotecan hydrochloride is a camptothecin analog that has a higher aqueous solubility than camptothecin, which is derived from Chinese tree, *Camptotheca acuminata*<sup>[118]</sup>. Irinotecan is a prodrug that is converted to 7-ethyl-10-hydroxycamptothecin in the body (SN-38). Irinotecan has a broad range of antitumor activity *in vitro* and *in vivo*, and its toxicity is more consistent and clinically acceptable than the isolated structure. After clinical trials, irinotecan was cost-effective for treating breast, cervical, and ovarian malignant growths in Japan in 1994. Irinotecan was first approved in the United States in 1996 for the treatment of metastatic CRC resistant to 5-FU, and later as the mainline treatment of metastatic CRC in combination with 5-FU/LV. Through carboxylesterases, it is converted to SN-38. Irinotecan inhibits topoisomerase I, a nuclear enzyme involved in unwinding DNA during transcription. Irinotecan has shown promising antitumor activity against metastatic colorectal malignant growth when used alone as a first-line treatment or as a second-line therapy after fluorouracil treatment failure<sup>[119]</sup>. By inhibiting topoisomerase I, an enzyme that catalyzes the breakage and rejoining of DNA strands during DNA replication, SN-38 causes DNA discontinuity, and modified cell death. SN-38 is primarily used in the liver, where it is glucuronidated and then excreted through the biliary system. A polymorphism in the uridine diphosphate glucuronosyltransferase isoform 1A1 (UGT1A1) gene, which is responsible for glucuronidation of SN-38, has been discovered and it allows for a reduction in SN-38 inactivation, resulting in increased treatment-related toxicity<sup>[120]</sup>. Raised serum bilirubin levels have also been linked to an abundance of irinotecan-interceded toxicity, so this medication is usually avoided in patients with hyperbilirubinemia. The most common side effects of irinotecan are diarrhea, myelosuppression, and alopecia. The levels of soluble human leukocyte antigen G (HLA-G) expression and the HLA-G/irinotecan relationship in metastatic CRC patients treated with an irinotecan-based approach were investigated by Scarabel *et al.* (2020)<sup>[121]</sup>. The findings revealed a link between HLA-G levels and irinotecan (CPT-11) pharmacokinetics, implying that sHLA-G and camptothecin-11 (CPT-11) may have a molecular relationship. The relationship between



HLA-G polymorphs and CPT-11 was investigated using computational modeling, which supported the hypothesis that CPT-11 could attack the peptide-binding cleft of the most common HLA-G polymorphs.

### 3.3. Oxaliplatin

Oxaliplatin is a platinum compound used to treat cancer that has a DACH (1,2-diaminocyclohexane) moiety<sup>[122]</sup>. Oxaliplatin is a drug that binds to plasma proteins and spreads throughout the body. It is a platinum analog that has been used in France as a first-line therapy for metastatic CRC since 1997. The MOSAIQ® Plaza for Medical Oncology” preliminary findings led to the development of an oxaliplatin-based adjuvant treatment. Because of the oxaliplatin-based protocols, FOLFOX4 and FOLFOX6 protocols were developed, followed by FOLFOX7 protocol. Oxaliplatin forms DNA adducts, preventing DNA replication and resulting in cell death. Single-agent oxaliplatin has been shown to reduce viability in patients with metastatic colon cancer. Because of the synergistic collaboration with 5-FU, which is likely due to oxaliplatin-induced “down-regulation” of thymidylate synthetase, clinical benefit has been linked to the combination of oxaliplatin and 5-FU and LV. In two randomized clinical trials in patients with metastatic CRC, the addition of oxaliplatin to infusional fluorouracil and LV (FOLFOX) improved tumor response rates and disease-free survival, with a trend toward an improvement in overall survival (OS). Acute neuropathy (muscle spasms, difficulty breathing, or swallowing) as well as chronic, cumulative peripheral sensory neurotoxicity (paresthesias and/or dysesthesias of the hand, foot, and mouth) are possible side effects of oxaliplatin. The cumulative dose of oxaliplatin is linked to chronic neurotoxicity, with symptoms typically appear after a dose of >600 – 800 mg/m<sup>2</sup>. Despite the fact that paraneoplastic neurological syndromes is thought to be reversible, it will persist for a long time once oxaliplatin is stopped. Asadzadeh *et al.* (2019) investigated the effects of using oxaliplatin to suppress prominin1 (CD133) in CRC treatment<sup>[123]</sup>. They concluded that suppression of CD133 in combination with oxaliplatin therapy could be a promising treatment option for CRC. Meanwhile, Wang *et al.* (2020) suggested that increasing tri-methylation of lysine 27 on histone H3 protein (H3K27me3) levels in CRC patients could improve oxaliplatin sensitivity<sup>[124]</sup>. Madigan *et al.* (2020) suggested a new cellular target for improving chemotherapeutic drug efficacy in CRC by revealing a critical cellular function for glucosylceramide synthase, a ceramide-metabolizing enzyme, in oxaliplatin chemosensitivity<sup>[125]</sup>. The authors discovered that CD4 T cells in CRC target antigens were upregulated by oxaliplatin<sup>[125]</sup>.

### 3.4. Angiogenesis inhibitors

Angiogenesis is the process by which new blood vessels are formed<sup>[126]</sup>. During this cycle, endothelial cells, which line the inside walls of vessels, relocate, extend,

and differentiate. Angiogenesis plays a crucial role in cancer development because strong tumors require a blood supply to grow larger than a few millimeters<sup>[127]</sup>. Tumors may be able to stimulate the growth of this blood supply by emitting signals that promote angiogenesis. Tumors can also cause angiogenesis signaling molecules to develop in surrounding normal cells. The new blood vessels fuel growing tumors with oxygen and nutrients, causing the tumor to spread and malignant growth cells to penetrate surrounding tissue and travel throughout the body, and form new cancer cell settlements known as metastases. Angiogenesis inhibitors have been developed to prevent tumors from growing beyond a certain size or proliferating without a blood supply. The growth factor VEGF aids in the proliferation, survival, and migration of endothelial cells. Because it is possibly one of the main proteins that tumor cells frequently communicate with, VEGF is a successful target of anticancer therapy<sup>[128,129]</sup>. Bevacizumab (Avastin®) is an anti-VEGF monoclonal counter-acting agent that is antagonistic to VEGF monoclonal IgG1 immunizer<sup>[130]</sup>. It has been approved for use in the treatment of advanced colorectal disease (CRC), non-small cell lung cancer, metastatic breast cancer, and progressed renal cell disease when combined with chemotherapy. It has been approved by the US Food and Drug Administration as a single agent for the treatment of advanced glioblastoma multiforme. Bevacizumab prevents VEGF from binding to its cell surface receptors as it travels through the body. This restriction prevents tumor vessels from becoming microvascular, limiting blood flow to tumor tissues. These effects also reduce tissue interstitial pressing factor, increase vascular penetrability and chemotherapeutic drug delivery, and promote tumor endothelial cell apoptosis. Bevacizumab can cause hypertension, asymptomatic proteinuria, thromboembolic events, GI perforation, and wound healing complications. At higher bevacizumab doses (10 – 15 mg/kg), some side effects, such as thromboembolism, are more common. Depending on the severity of the side effects, bevacizumab should be discontinued temporarily or permanently. Shen *et al.* (2020) discovered that reducing liver metastasis stiffness improves bevacizumab response in metastatic CRC<sup>[131]</sup>. Artaç, *et al.* (2020) discovered that the efficacy of bevacizumab may be reduced in obese patients<sup>[132]</sup>. Patients who are obese and have Kras wild-type left-sided tumors treated with bevacizumab-based regimens may have a worse prognosis than non-obese patients.

### 3.5. EGFR inhibitors

The EGFR is a transmembrane glycoprotein that affects cell development, expansion, and modified cell death through signaling pathways<sup>[133]</sup>. It can be found in a variety of cancers, including those of the colon, lung, breast, and head and neck. The expression of EGFR on the surface of tumor cells has been found in up to 80% of CRC tumors,

and tumors that express EGFR have a worse prognosis<sup>[134]</sup>. Antibodies targeting the extracellular space of EGFR and small molecular inhibitors of the intracellular tyrosine kinase domain have been developed to limit the capacity of this transmembrane receptor. Until now, only anti-EGFR monoclonal antibodies cetuximab (Erbix<sup>®</sup>) and panitumumab (Vectibix<sup>®</sup>) have been shown to be effective in colorectal disease<sup>[135]</sup>. In patients with CRC, small molecule inhibitors of EGFR's intracellular tyrosine kinase domain, such as erlotinib (Tarceva<sup>®</sup>), appear to be insufficient<sup>[136]</sup>. Cetuximab is a monoclonal anti-EGFR IgG1 antibody<sup>[137]</sup>. Cetuximab monotherapy is effective in irinotecan-resistant patients. The most common side effects of cetuximab treatment are acneiform rash, hypomagnesemia, and infusion reactions, with approximately 3% of patients experiencing serious hypersensitivity reactions to cetuximab infusion<sup>[138]</sup>. When cetuximab is added to oxaliplatin-fluoropyrimidine combinations, it improves grade 3/4 toxicities such as GI toxicity, skin rash, and lethargy in general. Capecitabine and other drugs cause more GI toxicity but less neutropenia when used together. Fortunately, FOLFOX has shown no signs of hypersensitivity so far (with or without cetuximab)<sup>[139]</sup>. Panitumumab is a monoclonal antibody made entirely of human IgG2 that targets the EGFR. Many trials have looked into its efficacy in pretreated metastatic CRC. Sabra, *et al.* (2019) successfully developed cetuximab-conjugated engineered citrus pectin-chitosan nanoparticles for selective delivery of curcumin (Cet-MCPCNPs) for the treatment of CRC<sup>[140]</sup>. According to the Kopetz *et al.* (2019), the combination of encorafenib, cetuximab, and binimetinib resulted in slightly longer average survival and a higher response rate than conventional treatment in patients with metastatic CRC with the v-raf murine sarcoma viral oncogene homolog B1 (BRAF V600E) mutation<sup>[141]</sup>. Price *et al.* (2014) showed that patients with baseline circulating tumor DNA (ctDNA) RAS mutations had worse outcomes than those who were wild type (WT) Ras proteins before starting therapy, but the emergence ctDNA RAS mutations were not linked to worse patient outcomes in panitumumab-treated patients<sup>[142]</sup>.

#### 4. Adjuvant treatment of CRC

Rectal and colon cancer are sensitive to radiation therapy, chemotherapeutic agents, and target-oriented medications<sup>[143]</sup>. Because of the anatomic structure of the pelvis and the close proximity of the organs, the circumferential margin distance is limited when a rectal tumor is surgically excised<sup>[144]</sup>. To reduce the risk of local recurrence, current treatment guidelines recommended complete mesorectal excision as the best surgical approach and chemoradiation therapy in patients with Stages II and III tumors. The depths of tumor extension across the bowel wall, as well as the presence or absence of nodal involvement, all affect the chances of local recurrence.

Patients with Stage III cancer require adjuvant therapy (lymph node involvement). Patients with Stage II, T3N0 disease require more evidence on which subgroup of patients will likely benefit from adjuvant care, with the exception of those with unfavorable factors already listed for colon cancer. Patients with stage II T4N0 rectal tumors are good candidates for neoadjuvant chemoradiation.

#### 5. Stage III colon cancer

A large number of patients with Stage III colon cancer and a small number of patients with Stage II disease can benefit from adjuvant chemotherapy. According to the findings of the Multicenter International Study of Oxaliplatin/5-FU/LV in the Adjuvant Treatment of Colon Cancer (MOSAIC) and the National Surgical Adjuvant Breast and Bowel Project C-07 trials, combination regimens that include fluoropyrimidine and oxaliplatin are the current standard of care<sup>[143]</sup>. Adjuvant care approaches for patients with rectal cancer (Stages II and III) now require preoperative chemoradiotherapy, according to a Phase III comparison of pre-operative versus post-operative chemoradiotherapy performed in Germany. For many years, fluorouracil was thought to be ineffective as a colon cancer adjuvant therapy with a meta-analysis of randomized trials conducted before 1987 found only a slight, statistically insignificant benefit. In retrospect, these clinical studies were hampered by heterogeneous patient samples, small sample sizes, and poor treatment adherence<sup>[145]</sup>. Bolus 5-FU and LV post-operative adjuvant chemotherapy were given to patients with high-risk colon cancer, and it improves patient outcomes and became the standard of care early 1990s. Capecitabine is an oral fluoropyrimidine preferentially converted to 5-FU by TP at the tumor site, where TP activity is significantly higher than in healthy tissue, in which it could replace infusional 5-FU in colon cancer adjuvant therapy. Capecitabine was approved in the United States and Europe in 2005 for the adjuvant treatment of Stage III colon cancer based on the results of the Xeloda in Adjuvant Colon Cancer Therapy trial. According to the findings, capecitabine was found to be at least as effective as IV bolus 5-FU/LV with a lower toxicity profile. Another drug that can be used instead of 5-FU in colon cancer adjuvant therapy is tegafur and uracil (UFT). Despite the fact that the majority of colon cancer patients are 65 or older, they are underrepresented in clinical trials and less likely to receive adjuvant treatment. According to pooled data comparisons and population-based trials, adjuvant therapy has consistently demonstrated a consistent and comparable survival advantage in all age groups, with no increase in treatment-related toxicity in older patients. African-American patients have a higher CRC -specific mortality rate than Caucasian patients, as disease rates are measured by race. Differences in comorbid disease, socio-demographic causes, stage at diagnosis, tumor biology, and drug reception have all been investigated as

causes of variation in outcomes. 5-FU-based combination regimens containing irinotecan or oxaliplatin have been shown to benefit patients with metastatic disease. These combinations have been studied as adjuvants as well. The safety and efficacy of 5-FU plus irinotecan in the adjuvant treatment of colon cancer were investigated in two major trials. Adding irinotecan to weekly bolus 5-FU/LV did not improve disease free survival (DFS) or OS in Stage III disease, but it did raise the risk of lethal and nonlethal toxicities. A French Phase III trial found no significant DFS benefit for FOLFIRI when compared to bolus plus CI 5-FU/LV. As a result of these findings, irinotecan is no longer used in the adjuvant treatment of patients with CRC. Combinations of oxaliplatin have shown to be more effective<sup>[143]</sup>.

## 6. Stage II colon cancer

Adjuvant fluorouracil-based therapy has a lesser effect in patients with Stage II colon cancer<sup>[146]</sup>. On several occasions, subset reviews of studies involving patients with Stages II and III illness have failed to show a clinically significant survival advantage for Stage II patients receiving adjuvant treatment. According to a pooled analysis of seven trials, patients who received fluorouracil-based adjuvant treatment had an average 5-year survival rate of 81%, compared to 80 percent in patients who underwent surgery alone ( $P = 0.11$ ). In trials of adjuvant treatment for colon cancer, patients with Stage IIIA disease had slightly better survival rates than patients with Stage IIB disease. Although growing evidence suggests that adjuvant therapy may improve the prognosis of a subset of patients with high-risk Stage II disease, such as medically fit patients with T4 tumors, poorly differentiated histology, bowel perforation at presentation, or  $<12$  lymph nodes sampled, adjuvant therapy in Stage II disease remains a complicated and daunting issue. In surveys, patients' willingness to undergo adjuvant treatment in exchange for these minor benefits was confirmed. Other prognostic indicators for Stages II and III colon cancer that have not yet been identified for clinical practice decisions include allelic loss at chromosome 18q and MSI, the latter of which confers a better prognosis. The availability of monoclonal antibodies that target the EGFR, such as cetuximab, or the VEGF, such as bevacizumab, has increased<sup>[147,148]</sup>. Anti-VEGF agents can prevent tumor growth and metastasis by inhibiting angiogenesis and improving anticancer therapy delivery to the tumor, resulting in increased tumor cell death<sup>[149]</sup>. Finally, anti-VEGF therapy can disrupt the tumor's established blood supply, resulting in even more tumor cell death. In addition to promoting tumor cell survival and development, EGFR has been shown to affect tumor-associated angiogenesis. Capecitabine is now being used in combination with selective agents, either with or without oxaliplatin, in the treatment of cancer.

## 7. Stage II and stage III rectal cancer

According to some clinical trials conducted in the 1980s, adding systemic chemotherapy to post-operative radiation reduced the risk of local recurrence and increased OS after resection of Stage II and Stage III rectal cancers<sup>[150]</sup>. In a subsequent study, infusional fluorouracil with radiotherapy was found to be more effective than equivalent radiotherapy with parallel bolus fluorouracil. Pre-operative chemoradiotherapy using a bolus 5-FU/LV protocol increased patient compliance, reduced toxicity, and improved local control when compared to a post-operative procedure. There were no differences in disease-free or OS between the pre-operative and post-operative therapy arms. As a result, for Stages II and III rectal cancer, pre-operative combination modality treatment with radiation and chemotherapy, followed by surgical resection with TME, is usually considered standard of care<sup>[151]</sup>.

## 8. Conclusion

Long-haul endurance for people with rectal disease was once uncommon, even after undergoing a complex medical procedure. CRC-targeted drugs are currently being updated for improved medicine safety, fewer adverse events, and more individualized administration plans, following a long period of research and development. The discovery of 5-FU, the development of decreased folate LV as a helpful potentiator of 5-FU cytotoxicity, and the introduction of new cytotoxic and natural agents have all aided chemotherapy progress for CRC patients. As we move into a time of altered malignant growth medication, essential chemotherapy using infusional 5-FU/LV remains the standard of care for patients with CRC, but there is a need for observational preliminaries that examine how existing treatment regimens can be customized for individual patients. The fact is that today's CRC patients have a wide range of medications to choose from, all of which have the potential to be beneficial in the long run. More individualized medicines with a significantly longer lifespan and fewer side effects are also possible.

## Acknowledgments

We would like to acknowledge Dr. R.S. Gaud; Director, SVKM's NMIMS, Shirpur Campus, and Dr. Ambikanandan Misra; Director-Pharmaceutical Research, Shobhaben Pratapbhai School of Pharmacy & Technology Management, SVKM's NMIMS, for providing necessary facilities and given me profound motivation while perusing this project.

## Funding

This compilation has received no funding from any authorities.

## Conflict of interest

The authors declare that they have no competing interests.



## Author contributions

Snigdha Thaman and Sankha Bhattacharya contributed to the study's conception and design, material preparation, data collection, and analysis. Snigdha Thaman wrote the first draft of the manuscript. The final manuscript was read and approved by Sankha Bhattacharya.

## References

1. Siegel RL, Miller KD, Sauer AG, *et al.*, 2020, Colorectal Cancer Statistics, 2020. *CA Cancer J Clin*, 70:145–64. DOI: 10.3322/caac.21601
2. Kaidanovich-Beilin O, Woodgett JR, 2011, GSK-3: Functional Insights From Cell Biology and Animal Models. *Front Mol Neurosci*. 4:40. DOI: 10.3389/fnmol.2011.00040
3. Mathers CD, Loncar D, 2006, Projections of Global Mortality and Burden of Disease from 2002 to 2030. *PLoS Med*, 3:e442. DOI: 10.1371/journal.pmed.0030442
4. Altchek A, 2003, Clues to Tumors: New Concepts of Symptoms, Signs, Syndromes, Paraneoplastic Syndromes, and Predisposition to Ovarian Cancer. In: *Diagnosis and Management of Ovarian Disorders*. Cambridge, Massachusetts: Academic Press. pp231–269. DOI: 10.1016/B978-012053642-9/50024-7
5. Brenner H, Chen C, 2018, The Colorectal Cancer Epidemic: Challenges and Opportunities for Primary, Secondary and Tertiary Prevention. *Br J Cancer*, 119:785–92. DOI: 10.1038/s41416-018-0264-x
6. Yasui H, Tsurita G, Imai K, 2014, DNA Synthesis Inhibitors for the Treatment of Gastrointestinal Cancer. *Expert Opin Pharmacother*, 15:2361–72. DOI: 10.1517/14656566.2014.958074.
7. Papillon J, 2012, Rectal and Anal Cancers: Conservative Treatment by Irradiation an Alternative to Radical Surgery. Berlin: Springer Science and Business Media. DOI: 10.1007/978-3-642-68613-9
8. Jasperson KW, Patel SG, Ahnen DJ, 2017, APC-Associated Polyposis Conditions. GeneReviews®. Seattle, WA: University of Washington, Seattle.
9. Goldstein NS, Sanford W, Coffey M, *et al.*, 1996, Lymph Node Recovery from Colorectal Resection Specimens Removed for Adenocarcinoma: Trends over Time and a Recommendation for a Minimum Number of Lymph Nodes to be Recovered. *Am J Clin Pathol*, 106:209–16. DOI: 10.1093/ajcp/106.2.209
10. Robinson TL, Sircar K, Hewlett BR, *et al.*, 200, Gastrointestinal Stromal Tumors may Originate from a Subset of CD34-Positive Interstitial Cells of Cajal. *Am J Pathol*, 156:1157–63. DOI: 10.1016/S0002-9440(10)64984-X
11. Lee JK, Choi YL, Kwon M, *et al.*, 2016, Mechanisms and Consequences of Cancer Genome Instability: Lessons From Genome Sequencing Studies. *Annu Rev Pathol*, 11:283–312. DOI: 10.1146/annurev-pathol-012615-044446
12. Gollapudi P, 2019, Studies of Chromosome Damage Induced by Topoisomerase II Inhibitors in Human Cells (Doctoral Dissertation, UC Riverside).
13. Grady WM, Carethers JM, 2008, Genomic and Epigenetic Instability in Colorectal Cancer Pathogenesis. *Gastroenterology*, 135:1079–99. DOI: 10.1053/j.gastro.2008.07.076
14. Richardson B, Yung R, 1999, Role of DNA methylation in the regulation of cell function. *J Lab Clin Med*, 134:333–40. DOI: 10.1093/jn/132.8.2401S
15. Hawkins N, Norrie M, Cheong K, *et al.*, 2002, CpG Island Methylation in Sporadic Colorectal Cancers and its Relationship to Microsatellite Instability. *Gastroenterology*, 122:1376–87. DOI: 10.1053/gast.2002.32997
16. Walsh MD, Clendenning M, Williamson E, *et al.*, 2013, Expression of MUC2, MUC5AC, MUC5B, and MUC6 Mucins in Colorectal Cancers and their Association with the CpG Island Methylator Phenotype. *Mod Pathol*, 26:1642–56. DOI: 10.1038/modpathol.2013.101
17. Papas TS, Kan NC, Watson DK, *et al.*, 1985, Myc, a Genetic Element that is Shared by a Cellular Gene (proto-myc) and by Viruses with one (MC29) or Two (MH2) Onc Genes. In: *RNA Tumor Viruses, Oncogenes, Human Cancer and AIDS: On the Frontiers of Understanding*. Springer, Boston, MA. pp1–13. DOI: 10.1007/978-1-4613-2583-3\_1
18. Ilyas M, 2005, Wnt Signalling and the Mechanistic Basis of Tumour Development. *J Pathol*, 205:130–44. DOI: 10.1002/path.1692
19. Fodde R, Smits R, Clevers H, 2001, APC, Signal Transduction and Genetic Instability in Colorectal Cancer. *Nat Rev Cancer*, 1:55–67. DOI: 10.1038/35094067
20. Hientz K, Mohr A, Bhakta-Guha D, *et al.*, 2017, The Role of p53 in Cancer Drug Resistance and Targeted Chemotherapy. *Oncotarget*, 8:8921. DOI: 10.18632/oncotarget.13475
21. Olumi AF, Grossfeld GD, Hayward SW, *et al.*, 2000, Carcinoma-Associated Fibroblasts Stimulate Tumor Progression of Initiated Human Epithelium. *Breast Cancer Res*, 2:1. DOI: 10.1186/bcr138
22. Nasrallah A, El-Sibai M, 2014, Colorectal Cancer Causes and Treatments: A Minireview. *Open Colorectal Cancer J*, 7: 1–14. DOI: 10.2174/1876820201407010001
23. de Caestecker MP, Piek E, Roberts AB, 2000, Role of Transforming Growth Factor- $\beta$  Signaling in Cancer. *J Natl*



- Cancer Inst*, 92:1388–402. DOI: 10.1093/jnci/92.17.1388
24. Ding X, Du J, Mao K, *et al.*, 2019, MicroRNA-143-3p Suppresses Tumorigenesis by Targeting Catenin- $\delta 1$  in Colorectal Cancer. *OncoTargets Ther*, 12:3255. DOI: 10.2147/OTT.S184118
  25. Qiu Z, Guo W, Wang Q, *et al.*, 2015, MicroRNA-124 Reduces the Pentose Phosphate Pathway and Proliferation by Targeting PRPS1 and RPIA mRNAs in Human Colorectal Cancer Cells. *Gastroenterology*, 149:1587–98. DOI: 10.1053/j.gastro.2015.07.050
  26. Liu K, Yao H, Wen Y, *et al.*, 2018, Functional Role of a Long Non-Coding RNA LIFR-AS1/miR-29a/TNFAIP3 Axis in Colorectal Cancer Resistance to Photodynamic Therapy. *Biochim Biophys Acta*, 1864:2871–80. DOI: 10.1016/j.bbadis.2018.05.020
  27. Chen SH, Lin F, Zhu JM, *et al.*, 2021, An Immune-related lncRNA Prognostic Model in Papillary Renal Cell Carcinoma: A lncRNA Expression Analysis. *Genomics*, 113:531–40. DOI: 10.1016/j.ygeno.2020.09.046
  28. Cheng Z, Wang G, Zhu W, *et al.*, 2020, LEF1-AS1 Accelerates Tumorigenesis in Glioma by Sponging miR-489-3p to Enhance HIGD1A. *Cell Death Dis*, 11:1–1. DOI: 10.1038/s41419-020-02823-0
  29. Liu W, Li L, Ye H, *et al.*, 2018, Role of COL6A3 in Colorectal Cancer. *Oncol Rep*, 39:2527–36. DOI: 10.3892/or.2018.6331
  30. Le CC, Bennisroune A, Langlois B, *et al.*, 2020, Functional Interplay Between Collagen Network and Cell Behavior within Tumor Microenvironment in Colorectal Cancer. *Front Oncol*, 10:527. DOI: 10.3389/fonc.2020.00527
  31. Eylem CC, Yilmaz M, Derkus B, *et al.*, 2002, Untargeted Multi-omic Analysis of Colorectal Cancer-specific Exosomes Reveals Joint Pathways of Colorectal Cancer in Both Clinical Samples and Cell Culture. *Cancer Lett*, 469:186–94. DOI: 10.1016/j.canlet.2019.10.038
  32. Zhu CC, Chen C, Xu ZQ, *et al.*, 2018, CCR6 Promotes Tumor Angiogenesis via the AKT/NF- $\kappa$ B/VEGF Pathway in Colorectal Cancer. *Biochim Biophys Acta Mol Basis Dis*, 1864:387–97. DOI: 10.1016/j.bbadis.2017.10.033
  33. Jiang M, Xu B, Li X, *et al.*, 2019, Correction: O-GlcNAcylation Promotes Colorectal Cancer Metastasis via the miR-101-O-GlcNAc/EZH2 Regulatory Feedback Circuit. *Oncogene*, 38:5744–5. DOI: 10.1038/s41388-019-0834-2
  34. Taipale J, Beachy PA, 2001, The Hedgehog and Wnt Signalling Pathways in Cancer. *Nature*, 411:349–54. DOI: 10.1038/s41388-019-0834-2
  35. Carraway KL, Hull SR, 19991, Cell Surface Mucin-type Glycoproteins and Mucin-like Domains. *Glycobiology*, 1:131–8. DOI: 10.1093/glycob/1.2.131
  36. DuRand GE, Seta N, 2000, Protein Glycosylation and Diseases: Blood and Urinary Oligosaccharides as Markers for Diagnosis and Therapeutic Monitoring. *Clin Chem*, 46:795–805. DOI: 10.1093/clinchem/46.6.795
  37. Huang HC, Klein PS, 2004, The Frizzled Family: Receptors for Multiple Signal Transduction Pathways. *Genome Biol*, 5:1–7. DOI: 10.1186/gb-2004-5-7-234
  38. Takebe N, Miele L, Harris PJ, *et al.*, 2015, Targeting Notch, Hedgehog, and Wnt Pathways in Cancer Stem Cells: Clinical Update. *Nat Rev Clin Oncol*, 12:445–64. DOI: 10.1038/nrclinonc.2015.61
  39. Sonderegger S, Husslein H, Leisser C, *et al.*, 2007, Complex Expression Pattern of Wnt Ligands and Frizzled Receptors in Human Placenta and its Trophoblast Subtypes. *Placenta*, 28:S97–102. DOI: 10.1016/j.placenta.2006.11.003
  40. Hawkins AG, Pedersen EA, Treichel S, *et al.*, 2020, Wnt/ $\beta$ -catenin-Activated Ewing Sarcoma Cells Promote the Angiogenic Switch. *JCI Insight*, 5:e135188. DOI: 10.1172/jci.insight.135188
  41. Van Es JH, Barker N, Clevers H, 2002, You Wnt some, you lose some: Oncogenes in the Wnt Signaling Pathway. *Curr Opin Genet Dev*, 13:28–33. DOI: 10.1016/S0959-437X(02)00012-6
  42. Mandati V, 2013, Le Rôle de L'extrémité C-terminale de la Protéine Merline dans sa Fonction Anti-tumorale (Doctoral Dissertation, Paris 11).
  43. Chang TH, Hsieh FL, Zebisch M, *et al.*, 2015, Structure and Functional Properties of Norrin Mimic Wnt for Signalling with Frizzled4, Lrp5/6, and Proteoglycan. *Elife*, 4:e06554. DOI: 10.7554/eLife.06554.028
  44. Wang IC, Chen YJ, Hughes D, Petrovic V, *et al.*, 2005, Forkhead box M1 Regulates the Transcriptional Network of Genes Essential for Mitotic Progression and Genes Encoding the SCF (Skp2-Cks1) Ubiquitin Ligase. *Mol Cell Biol*, 25:10875–94. DOI: 10.1128/MCB.25.24.10875-10894.2005
  45. Haeghele L, Ingold B, Naumann H, *et al.*, 2003, Wnt Signalling Inhibits Neural Differentiation of Embryonic Stem Cells by Controlling Bone Morphogenetic Protein Expression. *Mol Cell Neurosci*, 24:696–708. DOI: 10.1016/S1044-7431(03)00232-X
  46. de Almeida GC, Oliveira LF, Predes D, *et al.*, 2002, Piperine Suppresses the Wnt/ $\beta$ -catenin Pathway and has Anti-cancer Effects on Colorectal Cancer Cells. *Sci Rep*, 10:1–2. DOI: 10.1038/s41598-020-68574-2
  47. Zhang Y, Wang S, Kang W, *et al.*, 2018, CREPT Facilitates Colorectal Cancer Growth through Inducing Wnt/ $\beta$ -

- Catenin Pathway by Enhancing p300-mediated  $\beta$ -catenin Acetylation. *Oncogene*, 37:3485–500. DOI: 10.1038/s41388-018-0161-z
48. Zheng G, Li W, Zuo B, *et al.*, 2016, High Expression of CREPT Promotes Tumor Growth and is Correlated with Poor Prognosis in Colorectal Cancer. *Biochem Biophys Res Commun*, 480:436–42. DOI: 10.1016/j.bbrc.2016.10.067
  49. Qin J, Wen B, Liang Y, *et al.*, 2019, Histone Modifications and their Role in Colorectal Cancer. *Pathol Oncol Res*, 4:1–1. DOI: 10.1007/s12253-019-00663-8
  50. Sun K, He SB, Yao YZ, *et al.*, 2019, Tre2 (USP6NL) Promotes Colorectal Cancer Cell Proliferation via Wnt/ $\beta$ -catenin Pathway. *Cancer Cell Int*, 19:1–2. DOI: 10.1186/s12935-019-0823-0
  51. Xu C, Tian G, Jiang C, *et al.*, 2019, NPTX2 Promotes Colorectal Cancer Growth and Liver Metastasis by the Activation of the Canonical Wnt/ $\beta$ -Catenin Pathway via FZD6. *Cell Death Dis*, 10:1–2. DOI: 10.1038/s41419-019-1467-7
  52. Liu L, Zhang Y, Wong CC, *et al.*, 2018, RNF6 Promotes Colorectal Cancer by Activating the Wnt/ $\beta$ -catenin Pathway via Ubiquitination of TLE3. *Cancer Res*, 78:1958–71. DOI: 10.1158/0008-5472.CAN-17-2683
  53. Zeng Q, Che Y, Zhang Y, *et al.*, 2020, Thymol Isolated from *Thymus vulgaris* L. Inhibits Colorectal Cancer Cell Growth and Metastasis by Suppressing the Wnt/ $\beta$ -catenin Pathway. *Drug Des Dev Ther*, 14:2535. DOI: 10.2147/DDDT.S254218
  54. Zhou F, Xue M, Qin D, *et al.*, HIV-1 Tat Promotes Kaposi's Sarcoma-Associated Herpesvirus (KSHV) vIL-6-induced Angiogenesis and Tumorigenesis by Regulating PI3K/PTEN/AKT/GSK-3 $\beta$  Signaling Pathway. *PLoS One*, 8:e53145. DOI: 10.1371/journal.pone.0053145
  55. Poon HY, Stone JC, 2009, Functional Links between Diacylglycerol and Phosphatidylinositol Signaling Systems in Human Leukocyte-derived Cell Lines. *Biochem Biophys Res Commun*, 390:1395–401. DOI: 10.1016/j.bbrc.2009.11.004
  56. Mattson MP, Culmsee C, Yu ZF, 2000, Apoptotic and Antiapoptotic Mechanisms in Stroke. *Cell Tissue Res*, 301:173–87. DOI: 10.1007/s004419900154
  57. Ganeshan TK, 2019, Investigating the Protective Role of the Natural Hormone Melatonin, in Reducing Drug-induced Cardiotoxicity in the Therapy of Chronic Diseases (Doctoral Dissertation, University of Westminster).
  58. Ye M, Zhang Y, Zhang X, *et al.*, 2017, Targeting FBW7 as a Strategy to Overcome Resistance to Targeted Therapy in Non-Small Cell Lung Cancer. *Cancer Res*, 77:3527–39. DOI: 10.1158/0008-5472.CAN-16-3470
  59. Liu G, Zhou J, Dong M, 2019, Down-regulation of miR-543 Expression Increases the Sensitivity of Colorectal Cancer Cells to 5-Fluorouracil through the PTEN/PI3K/AKT Pathway. *Biosci Rep*, 39:BSR20190249. DOI: 10.1042/BSR20190249
  60. Liu H, Wang J, Tao Y, *et al.*, 2019, Curcumin Inhibits Colorectal Cancer Proliferation by Targeting miR-21 and Modulated PTEN/PI3K/Akt Pathways. *Life Sci*, 221:354–61. DOI: 10.1016/j.lfs.2019.02.049
  61. Alaaeddine RA, Elzahhar PA, AlZaim I, *et al.*, 2021, The Emerging Role of COX-2, 15-LOX and PPAR $\gamma$  in Metabolic Diseases and Cancer: An Introduction to Novel Multi-target Directed Ligands (MTDLs). *Curr Med Chem*, 28:2260–300. DOI: 10.2174/0929867327999200820173853
  62. Ma J, Sun X, Wang Y, *et al.*, 2019, Fibroblast-derived CXCL12 Regulates PTEN Expression and is Associated with the Proliferation and Invasion of Colon Cancer Cells via PI3k/Akt Signaling. *Cell Commun Signaling*, 2019 Dec;17(1):1–2. DOI: 10.1186/s12964-019-0432-5
  63. Yin X, Liu Z, Zhu P, *et al.*, 2019, CXCL12/CXCR4 Promotes Proliferation, Migration, and Invasion of Adamantinomatous Craniopharyngiomas via PI3K/AKT Signal Pathway. *J Cell Biochem*, 120:9724–36. DOI: 10.1002/jcb.28253
  64. Cheng H, Jiang X, Zhang Q, *et al.*, 2020, Naringin Inhibits Colorectal Cancer Cell Growth by Repressing the PI3K/AKT/mTOR Signaling Pathway. *Exp Ther Med*, 19:3798–804. DOI: 10.3892/etm.2020.8649
  65. Sui X, Kong N, Ye L, *et al.*, 2014, p38 and JNK MAPK Pathways Control the Balance of Apoptosis and Autophagy in Response to Chemotherapeutic Agents. *Cancer Lett*, 344:174–9. DOI: 10.1016/j.canlet.2013.11.019
  66. Hackel PO, Zwick E, Prenzel N, *et al.*, 1999, Epidermal Growth Factor Receptors: Critical Mediators of Multiple Receptor Pathways. *Curr Opin Cell Biol*, 11:184–9. DOI: 10.1016/S0955-0674(99)80024-6
  67. Segmüller N, Ellendorf U, Tudzynski B, *et al.*, 2007, BcSAK1, a Stress-Activated Mitogen-activated Protein Kinase, is Involved in Vegetative Differentiation and Pathogenicity in *Botrytis cinerea*. *Eukaryotic Cell*, 6:211–21. DOI: 10.1128/EC.00153-06
  68. Widmann C, Gibson S, Jarpe MB, *et al.*, 1999, Mitogen-activated Protein Kinase: Conservation of a Three-kinase Module from Yeast to Human. *Physiol Rev*, 79:143–80. DOI: 10.1152/physrev.1999.79.1.143
  69. Rose BA, Force T, Wang Y, 2010, Mitogen-activated Protein Kinase Signaling in the Heart: Angels Versus Demons in a Heart-breaking Tale. *Physiol Rev*, 90:1507–46. DOI: 10.1152/physrev.00054.2009

70. Keyse SM, 2000, Protein Phosphatases and the Regulation of Mitogen-Activated Protein Kinase Signalling. *Curr Opin Cell Biol*, 12:186–92. DOI: 10.1016/S0955-0674(99)00075-7
71. Orban PC, Chapman PF, Brambilla R, 1999, Is the Ras-MAPK Signalling Pathway Necessary for Long-term Memory Formation? *Trends Neurosci*, 22:38–44. DOI: 10.1016/S0166-2236(98)01306-X
72. Severin S, Ghevaert C, Mazharian A, 2010, The Mitogen-activated Protein Kinase Signaling Pathways: Role in Megakaryocyte Differentiation. *J Thromb Haemost*, 8:17–26. DOI: 10.1111/j.1538-7836.2009.03658.x
73. Morandell S, Stasyk T, Skvortsov S, *et al.*, 2008, Quantitative Proteomics and Phosphoproteomics Reveal Novel Insights into Complexity and Dynamics of the EGFR Signaling Network. *Proteomics*, 8:4383–401. DOI: 10.1002/pmic.200800204
74. Preisinger C, Von Kriegsheim A, Matallanas D, *et al.*, 2008, Proteomics and Phosphoproteomics for the Mapping of Cellular Signalling Networks. *Proteomics*, 8:4402–15. DOI: 10.1002/pmic.200800136
75. Peschard P, Fournier TM, Lamorte L, *et al.*, 2001, Mutation of the c-Cbl TKB Domain Binding Site on the Met Receptor Tyrosine Kinase Converts it into a Transforming Protein. *Mol Cell*, 8:995–1004. DOI: 10.1016/S1097-2765(01)00378-1
76. Marmor MD, Yarden Y, 2004, Role of Protein Ubiquitylation in Regulating Endocytosis of Receptor Tyrosine Kinases. *Oncogene*, 23:2057–70. DOI: 10.1038/sj.onc.1207390
77. Feller SM, Lewitzky M, 2006, Potential Disease Targets for Drugs that Disrupt Protein-protein Interactions of Grb2 and Crk Family Adaptors. *Curr Pharm Des*, 12:529–48. DOI: 10.2174/138161206775474369
78. Schneekloth AR, 2009, Development of Proteolysis Targeting Chimeric Molecules (PROTACs) and Studies on the Biological Activity of Tyroscherin. Yale University.
79. Claperon A, Therrien M, 2007, KSR and CNK: Two Scaffolds Regulating RAS-mediated RAF Activation. *Oncogene*, 26:3143–58. DOI: 10.1038/sj.onc.1210408
80. Kolch W, 2000, Meaningful Relationships: The Regulation of the Ras/Raf/MEK/ERK Pathway by Protein Interactions. *Biochem J*, 351:289–305. DOI: 10.1042/bj3510289
81. Cato AR, 2013, The Effects of Hexabromocyclododecane (HBCD) and Tetrabromobisphenol A (TBBPA) on Mitogen-activated Protein Kinases in Human Natural Killer Cells (Doctoral Dissertation, Tennessee State University).
82. Downward J, 20003, Targeting RAS Signalling Pathways in Cancer Therapy. *Nat Rev Cancer*, 3:11–22. DOI: 10.1038/nrc969
83. Hilger RA, Scheulen ME, Strumberg D, 2002, The Ras-Raf-MEK-ERK Pathway in the Treatment of Cancer. *Oncol Res Treatment*, 25:511–8. DOI: 10.1159/000068621
84. Attar-Schneider O, Drucker L, Zismanov V, *et al.*, 2021, Bevacizumab Attenuates Major Signaling Cascades and eIF4E Translation Initiation Factor in Multiple Myeloma Cells. *Lab Investig*, 92:178–90. DOI: 10.1038/labinvest.2011.162
85. Zhao YL, Zhong SR, Zhang SH, *et al.*, 2019, UBN2 Promotes Tumor Progression via the Ras/MAPK Pathway and Predicts Poor Prognosis in Colorectal Cancer. *Cancer Cell Int*, 19:126. DOI: 10.1186/s12935-019-0848-4
86. Tang B, Liang W, Liao Y, *et al.*, 2019, PEA15 Promotes Liver Metastasis of Colorectal Cancer by Upregulating the ERK/MAPK Signaling Pathway. *Oncol Rep*, 41:43–56. DOI: 10.3892/or.2018.6825
87. Hindupur SK, Balaji SA, Saxena M, *et al.*, 2014, Identification of a Novel AMPK-PEA15 Axis in the Anoikis-resistant Growth of Mammary Cells. *Breast Cancer Res*, 16:1–6. DOI: 10.1186/s13058-014-0420-z
88. Angius A, Pira G, Scanu AM, *et al.*, 2019, MicroRNA-425-5p Expression Affects BRAF/RAS/MAPK Pathways in Colorectal Cancers. *Int J Med Sci*, 16:1480–91. DOI: 10.7150/ijms.35269
89. Ponsioen B, Post JB, des Amorie JR, *et al.*, 2021, Quantifying Single-cell ERK Dynamics in Colorectal Cancer Organoids Reveals EGFR as an Amplifier of Oncogenic MAPK Pathway Signalling. *Nat Cell Biol*, 23:377–90. DOI: 10.1038/s41556-021-00654-5
90. Mellado M, Rodríguez-Frade JM, Vila-Coro AJ, *et al.*, 2001, Chemokine Receptor Homo- or Heterodimerization Activates Distinct Signaling Pathways. *EMBO J*, 20:2497–507. DOI: 10.1093/emboj/20.10.2497
91. Goel A, Singh S, 2020, Emerging Approaches for the Treatment of Alzheimer Disease: Targeting NF- $\kappa$ B Pathway. DOI: 10.22541/au.159493419.94920936
92. Bu Y, 2014, Phosphorylation of NF- $\kappa$ B RelA/p65 on Ser536 Signals Cancer Cells TO Death and Enhances Chemosensitivity. Southern Illinois University at Carbondale.
93. Majdalawich AF, Zhang L, Fuki IV, 2006, Adipocyte Enhancer-binding Protein-1 (AEBP1) is a Novel Player in Macrophage Cholesterol Homeostasis, Inflammation, and Atherogenesis. *Proc Natl Acad Sci U S A*, 103:2346–51. DOI: 10.1073/pnas.0508139103
94. Bose D, 2002, Human Herpesvirus K1 Open Reading Frame Activates NF $\kappa$ B and Contributes to the Inflammatory Phenotype. University of Maryland, Baltimore.

95. Kannathasan T, Kuo WW, Chen MC, *et al.*, 2020, Chemoresistance-Associated Silencing of miR-4454 Promotes Colorectal Cancer Aggression through the GNL3L and NF- $\kappa$ B Pathway. *Cancers*, 12:1231. DOI: 10.3390/cancers12051231
96. Liu W, Wang S, Sun Q, *et al.*, 2018, DCLK1 Promotes Epithelial-Mesenchymal Transition via the PI3K/Akt/NF- $\kappa$ B Pathway in Colorectal Cancer. *Int J Cancer*, 142:2068–79. DOI: 10.1002/ijc.31232
97. Westphalen CB, Quante M, Wang TC, 2017, Functional Implication of Dclk1 and Dclk1-expressing Cells in Cancer. *Small GTPases*, 8:164–71. DOI: 10.1080/21541248.2016.1208792
98. Makino S, Takahashi H, Okuzaki D, *et al.*, 2020, DCLK1 Integrates Induction of TRIB3, EMT, Drug Resistance and Poor Prognosis in Colorectal Cancer. *Carcinogenesis*, 41:303–12. DOI: 10.1093/carcin/bgz157
99. Chen C, Xu ZQ, Zong YP, *et al.*, 2019, CXCL5 Induces Tumor Angiogenesis via Enhancing the Expression of FOXD1 Mediated by the AKT/NF- $\kappa$ B Pathway in Colorectal Cancer. *Cell Death Dis*, 10:1–5. DOI: 10.1038/s41419-019-1431-6
100. Wang H, Yao L, Gong Y, *et al.*, 2018, TRIM31 Regulates Chronic Inflammation via NF- $\kappa$ B Signal Pathway to Promote Invasion and Metastasis in Colorectal Cancer. *Am J Transl Res*, 10:1247.
101. Huang L, Wang X, Wen C, *et al.*, 2015, Hsa-miR-19a is Associated with Lymph Metastasis and Mediates the TNF- $\alpha$  Induced Epithelial-to-Mesenchymal Transition in Colorectal Cancer. *Sci Rep*, 5:1–2. DOI: 10.1038/srep13350
102. Lin SY, Li TY, Liu Q, *et al.*, 2012, GSK3-TIP60-ULK1 Signaling Pathway Links Growth Factor Deprivation to Autophagy. *Science*, 336:477–81. DOI: 10.1126/science.1217032
103. Toyota M, Ahuja N, Ohe-Toyota M, *et al.*, 1999, CpG Island Methylator Phenotype in Colorectal Cancer. *Proc Natl Acad Sci*, 96:8681–6. DOI: 10.1073/pnas.96.15.8681
104. Retsky M, Demicheli R, Hrushesky W, *et al.*, 2010, Surgery Triggers Outgrowth of Latent Distant Disease in Breast Cancer: An Inconvenient Truth? *Cancers*, 2:305–37. DOI: 10.3390/cancers2020305
105. Ahmed FE, 20003, Colon Cancer: Prevalence, Screening, Gene Expression and Mutation, and Risk Factors and Assessment. *J Environ Sci Health Part C*, 21:65–131. DOI: 10.1081/GNC-120026233
106. Miller EC, Miller JA, 1983, Charles Heidelberger 1920-1983. *Cancer*, 43, 155.
107. Heidelberger C, Chaudhuri NK, Danneberg P, *et al.*, 1957, Fluorinated Pyrimidines, a New Class of Tumour-inhibitory Compounds. *Nature*, 179:663–6. DOI: 10.1038/179663a0
108. Sethy C, Kundu CN, 2021, 5-Fluorouracil (5-FU) Resistance and the New Strategy to Enhance the Sensitivity against Cancer: Implication of DNA Repair Inhibition. *Biomed Pharmacother*, 137:111285. DOI: 10.1016/j.biopha.2021.111285
109. Plà Solans H, 2014, Design, Synthesis and Biological Evaluation of New Polymer-Drug Conjugates Based on Polyglutamic Acid and 5-Fluorouracil for the Treatment of Advanced Colorectal Cancer (Doctoral Dissertation, Universitat de Barcelona).
110. Schaffer S, 2018, Exploiting the Overexpression of CYP2A6 in Colon Cancer with the 5-Fluorouracil Prodrug, Tegafur (Doctoral Dissertation, University of the Sciences in Philadelphia).
111. Vodenkova S, Buchler T, Cervena K, *et al.*, 2020, 5-fluorouracil and other Fluoropyrimidines in Colorectal Cancer: Past, Present and Future. *Pharmacol Ther*, 206:107447. DOI: 10.1016/j.pharmthera.2019.107447
112. Ndreshkjana B, Çapci A, Klein V, *et al.*, 2019, Combination of 5-Fluorouracil and Thymoquinone Targets Stem Cell Gene Signature in Colorectal Cancer Cells. *Cell Death Dis*, 10:1–6. DOI: 10.1038/s41419-019-1611-4
113. Malet-Martino M, Martino R, 2002, Oncologist Clinical Studies of three Oral Prodrugs of 5-fluorouracil. *Oncologist*, 7:288–323. DOI: 10.1634/theoncologist.7-4-288
114. Petty RD, Cassidy J, 2004, Novel Fluoropyrimidines: Improving the Efficacy and Tolerability of Cytotoxic Therapy. *Curr Cancer Drug Targets*, 4:191–204. DOI: 10.2174/1568009043481533
115. Perrain V, Bihan K, Bompair F, *et al.*, 2021, Leukoencephalopathy with Transient Splenic Lesions Related to 5-Fluorouracil or Capecitabine. *Eur J Neurol*, 28:2396–402. DOI: 10.1111/ene.14857
116. Twelves CJ, 2006, X-ACT Investigators. Xeloda® in Adjuvant Colon Cancer Therapy (X-ACT) Trial: Overview of Efficacy, Safety, and Cost-effectiveness. *Clin Colorectal Cancer*, 6:278–87. DOI: 10.3816/CCC.2006.n.046
117. Chan SL, Chan AW, Mo F, *et al.*, 2018, Association between Serum Folate Level and Toxicity of Capecitabine During Treatment for Colorectal Cancer. *Oncologist*, 23:1436. DOI: 10.1634/theoncologist.2017-0637
118. Sriram D, Yogeewari P, Thirumurugan R, *et al.*, 2005, Camptothecin and its Analogues: A Review on their Chemotherapeutic Potential. *Nat Prod Res*, 19:393–412. DOI: 10.1080/14786410412331299005
119. Feun L, Savaraj N, 2008, Topoisomerase I Inhibitors for the Treatment of Brain Tumors. *Expert Rev Anticancer Ther*, 8:707–16. DOI: 10.1586/14737140.8.5.707



120. Tanabe K, Ikegami Y, Ishida R, *et al.*, 1991, Inhibition of Topoisomerase II by Antitumor Agents bis (2, 6-dioxopiperazine) Derivatives. *Cancer Res*, 51:4903–8.
121. Scarabel L, Garziera M, Fortuna S, *et al.*, 2020, Soluble HLA-G Expression Levels and HLA-G/irinotecan Association in Metastatic Colorectal Cancer Treated with Irinotecan-based Strategy. *Sci Rep*, 10:1–1. DOI: 10.1038/s41598-020-65424-z
122. Pendyala L, Creaven PJ, 1993, *In Vitro* Cytotoxicity, Protein Binding, Red Blood Cell Partitioning, and Biotransformation of Oxaliplatin. *Cancer Res*, 53:5970–6.
123. Asadzadeh Z, Mansoori B, Mohammadi A, *et al.*, 2019, microRNAs in Cancer Stem Cells: Biology, Pathways, and Therapeutic Opportunities. *J Cell Physiol*, 234:10002–17. DOI: 10.1002/jcp.27885
124. Wang Q, Chen X, Jiang Y, *et al.*, 2020, Elevating H3K27me3 Level Sensitizes Colorectal Cancer to Oxaliplatin. *J Mol Cell Biol*, 12:125–37. DOI: 10.1093/jmcb/mjz032
125. Madigan JP, Robey RW, Poprawski JE, *et al.*, 2020, A Role for Ceramide Glycosylation in Resistance to Oxaliplatin in Colorectal Cancer. *Exp Cell Res*, 388:111860. DOI: 10.1016/j.yexcr.2020.111860
126. Leahy KM, Koki AT, Masferrer JL, 200, Role of Cyclooxygenases in Angiogenesis. *Curr Med Chem*, 7:1163–70. DOI: 10.2174/0929867003374336
127. Chaplain MA, 1996, Avascular Growth, Angiogenesis and Vascular Growth in Solid Tumours: The Mathematical Modelling of the Stages of Tumour Development. *Math Comput Model*, 23:47–87. DOI: 10.1016/0895-7177(96)00019-2
128. Neufeld G, Cohen T, Gengrinovitch S, *et al.*, 1999, Vascular Endothelial Growth Factor (VEGF) and its Receptors. *FASEB J*, 13:9–22. DOI: 10.1096/fasebj.13.1.9
129. Shinkaruk S, Bayle M, Lain G, *et al.*, 2003, Vascular Endothelial Cell Growth Factor (VEGF), an Emerging Target for Cancer Chemotherapy. *Curr Med Chem Anticancer Agents*, 3:95–117. DOI: 10.2174/1568011033353452
130. Ferrara N, Hillan KJ, Novotny W, 2005, Bevacizumab (Avastin), a Humanized Anti-VEGF Monoclonal Antibody for Cancer Therapy. *Biochem Biophys Res Commun*, 333:328–35. DOI: 10.1016/j.bbrc.2005.05.132
131. Shen Y, Wang X, Lu J, *et al.*, 2020, Reduction of Liver Metastasis Stiffness Improves Response to Bevacizumab in Metastatic Colorectal Cancer. *Cancer Cell*, 37:800–17. DOI: 10.1016/j.ccell.2020.05.005
132. Artaç M, Korkmaz L, Coşkun HŞ, *et al.*, 2019, Bevacuzimab may be Less Effective in Obese Metastatic Colorectal Cancer Patients. *J Gastrointestinal Cancer*, 50:214–20. DOI: 10.1007/s12029-017-0047-2
133. Dent P, Reardon DB, Park JS, *et al.*, 1999, Radiation-induced Release of Transforming Growth Factor  $\alpha$  Activates the Epidermal Growth Factor Receptor and Mitogen-activated Protein Kinase Pathway in Carcinoma Cells, Leading to Increased Proliferation and Protection from Radiation-induced Cell Death. *Mol Biol Cell*, 10:2493–506. DOI: 10.1091/mbc.10.8.2493
134. Tinhofer I, Klinghammer K, Weichert W, *et al.*, 2011, Expression of Amphiregulin and EGFRvIII affect Outcome of Patients with Squamous Cell Carcinoma of the Head and Neck Receiving Cetuximab-Docetaxel Treatment. *Clin Cancer Res*, 17:5197–204. DOI: 10.1158/1078-0432.CCR-10-3338
135. Dokala A, Thakur SS, 2017, Extracellular Region of Epidermal Growth Factor Receptor: A Potential Target for Anti-EGFR Drug Discovery. *Oncogene*, 36:2337–44. DOI: 10.1038/onc.2016.393
136. Roskoski R Jr., 2019, Small Molecule Inhibitors Targeting the EGFR/ErbB Family of Protein-tyrosine Kinases in Human Cancers. *Pharmacol Res*, 139:395–411. DOI: 10.1016/j.phrs.2018.11.014
137. Humblet Y, 2004, Cetuximab: An IgG1 Monoclonal Antibody for the Treatment of Epidermal Growth Factor Receptor-expressing Tumours. *Expert Opin Pharmacother*, 5:1621–33. DOI: 10.1517/14656566.5.7.1621
138. Fakih M, Vincent M, 2010, Adverse Events Associated with Anti-EGFR Therapies for the Treatment of Metastatic Colorectal Cancer. *Curr Oncol*, 17 Suppl 1:S18. DOI: 10.3747/co.v17is1.615
139. Mao C, Zeng X, Zhang C, *et al.*, 2021, Mechanisms of Pharmaceutical Therapy and Drug Resistance in Esophageal Cancer. *Front Cell Dev Biol*, 9:22. DOI: 10.3389/fcell.2021.612451
140. Sabra R, Billa N, Roberts CJ, 2019, Cetuximab-conjugated Chitosan-pectinate (Modified) Composite Nanoparticles for Targeting Colon Cancer. *Int J Pharm*, 572:118775. DOI: 10.1016/j.ijpharm.2019.118775
141. Kopetz S, Chang GJ, Overman MJ, *et al.*, 2009, Improved Survival in Metastatic Colorectal Cancer is Associated with Adoption of Hepatic Resection and Improved Chemotherapy. *J Clin Oncol*, 27:3677. DOI: 10.1200/JCO.2008.20.5278
142. Price TJ, Peeters M, Kim TW, *et al.*, 2014, Panitumumab Versus Cetuximab in Patients with Chemotherapy-refractory Wild-type KRAS Exon 2 Metastatic Colorectal Cancer (ASPECCT): A Randomised, Multicentre, Open-Label, Non-Inferiority Phase 3 Study. *Lancet Oncol*, 15:569–79. DOI: 10.1016/S1470-2045(14)70118-4

143. Carrato A, 2008, Adjuvant Treatment of Colorectal Cancer. *Gastrointest Cancer Res*, 2 4 Suppl 2:S42.
144. Beets-Tan RG, Beets GL, Vliegen RF, *et al.*, 2001, Accuracy of Magnetic Resonance Imaging in Prediction of Tumour-free Resection Margin in Rectal Cancer Surgery. *Lancet*, 357:497–504. DOI: 10.1016/S0140-6736(00)04040-X
145. Rödel C, Graeven U, Fietkau R, *et al.*, 2015, Oxaliplatin Added to Fluorouracil-based Preoperative Chemoradiotherapy and Postoperative Chemotherapy of Locally Advanced Rectal Cancer (the German CAO/ARO/AIO-04 study): Final Results of the Multicentre, Open-label, Randomised, Phase 3 Trial. *Lancet Oncol*, 16:979–89. DOI: 10.1016/S1470-2045(15)00159-X
146. Sobrero A, Köhne CH, 2006, Should Adjuvant Chemotherapy become Standard Treatment for Patients with Stage II Colon Cancer? *Lancet Oncol*, 7:515–7. DOI: 10.1016/S1470-2045(06)70727-6
147. Evrard C, Tachon G, Randrian V, *et al.*, 2019, Microsatellite Instability: Diagnosis, Heterogeneity, Discordance, and Clinical Impact in Colorectal Cancer. *Cancers*, 11:1567. DOI: 10.3390/cancers11101567
148. Bond CE, Nancarrow DJ, Wockner LF, *et al.*, 2014, Microsatellite Stable Colorectal Cancers Stratified by the BRAF V600E Mutation Show Distinct Patterns of Chromosomal Instability. *PLoS One*, 9:e91739. DOI: 10.1371/journal.pone.0091739
149. Jain RK, 2002, Tumor Angiogenesis and Accessibility: Role of Vascular Endothelial Growth Factor. *Semin Oncol*, 29:3–9. DOI: 10.1016/S0093-7754(02)70063-8
150. Bosset JF, Collette L, Calais G, *et al.*, 2006, Chemotherapy with Preoperative Radiotherapy in Rectal Cancer. *N Engl J Med*, 355:1114–23. DOI: 10.1056/NEJMoa060829
151. Bosset JF, Bosset M, Nguyen F, *et al.*, 2008, Defining Preoperative Treatment Strategies in t3 Rectal Cancer. *Gastrointest Cancer Res*, 2 4 Suppl: S54–7.