

## Review

# Molecular Targets of Aptamers in Gastrointestinal Cancers: Cancer Detection, Therapeutic Applications, and Associated Mechanisms

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## Abstract

Gastrointestinal (GI) cancers are among the most common cancers that impact the global population, with high mortality and low survival rates after breast and lung cancers. Identifying useful molecular targets in GI cancers are crucial for improving diagnosis, prognosis, and treatment outcomes, however, limited by poor targeting and drug delivery system. Aptamers are often utilized in the field of biomarkers identification, targeting, and as a drug/inhibitor delivery cargo. Their natural and chemically modifiable binding capability, high affinity, and specificity are favored over antibodies and potential early diagnostic imaging and drug delivery applications. Studies have demonstrated the use of different aptamers as drug delivery agents and early molecular diagnostic and detection probes for treating cancers. This review aims to first describe aptamers' generation, characteristics, and classifications, also providing insights into their recent applications in the diagnosis and medical imaging, prognosis, and anticancer drug delivery system of GI cancers. Besides, it mainly discussed the relevant molecular targets and associated molecular mechanisms involved, as well as their applications for potential treatments for GI cancers. In addition, the current applications of aptamers in a clinical setting to treat GI cancers are deciphered. In conclusion, aptamers are multifunctional molecules that could be effectively used as an anticancer agent or drug delivery system for treating GI cancers and deserve further investigations for clinical applications.

Keywords: Alimentary and digestive tract cancer; single-stranded oligonucleotides; therapeutic targets; cancer diagnosis; cancer prognosis; drug delivery

## 1. Introduction

Gastrointestinal (GI) cancers can be broadly classified as esophageal, gastric or stomach, liver, colorectal, and pancreatic cancers. Globally, they contribute approximately 4.2 million new cases and 3.1 million deaths reported in 2020, accounting for approximately 22% of total cancer cases and 32% of total cancer-related deaths. Among these, colorectal cancer (CRC) is a principal malignant condition that contributes to approximately 1.1 million new cases, followed by 1,089,103 stomach/gastric cancer (GC), 905,677 liver cancer (LC), 604,100 esophageal cancer (EC), and 495,773 million cases for pancreatic cancer (PC) worldwide [1]. Although they share similar risk factors differ in etiology and epidemiological profiles [2]. It has been reported that the incidences of GI cancers are declining globally, but the treatment outcome of GI cancer patients is still unsatisfactory, primarily due to poor diagnosis.

Identifying biomarkers based on clinical features and gene analysis can significantly improve the diagnosis, prognosis, and cancer treatment outcome. Cancer biomarkers determine the significant molecular pathways associated with cancer recurrence and treatment efficiencies [3]. One of the major factors causing poor diagnosis in the early stages of GI cancers is they remain asymptomatic until they progress to an advanced stage [4]. Generally, biomarkers are characteristics that are objectively measured and evaluated during a disease progression or treatment, and they indicate a biological, pathological, or pharmacological response. Several biomarkers have been identified for GI cancers, including carbohydrate antigen CA72-4, CA12-5, BCA-225, human chorionic gonadotrophin (hCG), pepsinogen I/II, carcinoembryonic antigen (CEA), and CA19-9, with CEA and CA19-9 are the most widely used biomarkers in clinical diagnosis [5]. CEA is commonly associated with digestive tract cancer and is an independent risk factor for predictive liver metastasis relapse. Given that its levels are generally elevated in advanced stages, thus it is not a highly recommended screening biomarker for early-stage detection [6]. Nonetheless, its presence in the peritoneal fluid can also indicate GC relapse, such as a repetitive recurrence of cancer cells in patients [7]. On the other hand, CA19-9 is a glycolipid antigen used as a biomarker for CRC. It is a selective ligand for E-selectin found on endothelial cells [8] and has a 56% sensitivity for recurrence with 74% specificity. It has been reported that CA19-9-positive GI cancers have an antral location, prominent lymphatic, and venous invasion, with metastasis in lymph nodes, thus providing useful information on metastasis and recurrence. Intriguingly, the sensitivity of CA19-9

increases significantly to 87% when combined with CEA [9]. Besides, CA72-4, CA 125, and alpha-fetoprotein (AFP) are other biomarkers commonly used in diagnosing GI cancers. The limitations of molecular targets are their sensitivity and specificity. Some characteristics of the abovementioned biomarkers are yet to be discovered and evaluated for their sensitivity and specificity as a tool for diagnosis and prognosis.

Several biomarkers have been identified and developed in preclinical studies fail to get proper validation in clinical practice. For instance, HER2, a proto-oncogene encoded by human epidermal growth factor receptor 2 (ERBB2), is a cell membrane tyrosine receptor kinase and is the only biomarker available in clinical practice. Although its predictive value is debatable, it has emerged as an important diagnostic biomarker. HER2 gene amplification rate has shown to be 32% higher in intestinal-type cancer versus diffuse-type cancer. Trastuzumab, which mediates HER2 signaling, is perhaps the first small molecule inhibitor approved for GI cancer treatment [10]. Quantitative detection of conventional serum or tissue biomarkers is a challenge in GI cancer biomarker discovery because of their insufficient specificity and sensitivity, plus their detection threshold is low and cannot be detected in most cases in the early stages. Therefore, there is a pressing need to find a reliable GI cancer biomarker.

Aptamers are short single-stranded oligonucleotides (DNA, RNA, or modified nucleic acid sequences) or peptides that bind strongly to a specific target. They are generated by the cell SELEX (Systematic Evolution of Exponential enrichment) method with a range of detecting and therapeutic applications. Aptamers are chemical analogs of antibodies and confer several appreciable benefits over the antibodies or small ligands, owing to their high stability at a high temperature, strong affinity to their target, and desirable small size over antibodies. Unlike antibodies, they can be synthesized and modified chemically to specifically bind to molecular targets without causing toxicity and are non-immunogenic. They have greater scalability and desirable storage properties and no batch-to-batch variation as commonly seen with antibodies. Their tissue permeability, desirable pharmacokinetic profiles, and modifiability make them suitable for diagnostic and therapeutic applications over antibodies [11]. It has been proven that aptamers can be used to target molecules or cancer biomarkers and determine key downstream molecular signaling pathways to halt. This claim shows that aptamers can ultimately lead to cancer cell death and increase the impact and efficiency of treating cancer instead of

options such as radiation or chemotherapy. One of the major drawbacks of chemotherapy is the non-selective nature of anticancer drugs, which subsequently lowers their therapeutic efficacy. Given aptamers' unique and natural features, they can be very selective toward cancer cells and thus have multiple applications in cancer diagnostics, targeting, and therapeutics. Besides, they can be conjugated with drugs to reach cancer cells or with any molecules, compounds, nanoparticles (NPs), etc., to formulate a targeted drug delivery system, thus minimizing the toxicity and increasing the therapeutic efficacy of chemotherapy as compared to the individual entity. Due to their advantage over antibodies, aptamers can be very useful in cancer diagnostics as they can be used to detect circulating tumor cells and *in vivo* imaging. Detecting circulating tumor cells can be challenging due to their deficient presence requiring highly efficient capturing. The efficiency can be enhanced based on aptamer-based binding of circulating tumor antigen [12] by combining with radionuclides, fluorescent molecules, or another imaging molecule. Aptamers have become a vital tool in the imaging diagnosis of cancer cells. This review discusses the current comprehensive understanding of aptamers in GI cancer as tools for diagnosis, prognosis, and treatments, including their application in clinical settings, drug delivery systems, and identification of specific molecular targets or cancer biomarkers.

## 2. Generation Technologies and Different Types of Aptamers

### 2.1. Cell SELEX Process

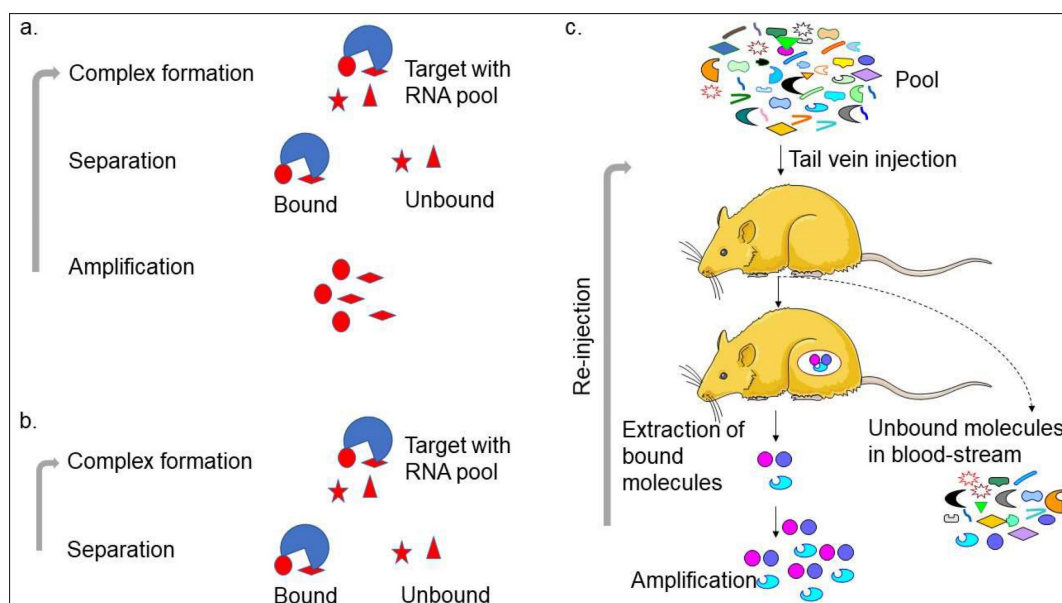
Aptamer or artificial antibody is the chemically synthesized oligonucleotide from the randomized library of molecules that is selected using the SELEX method involving binding, separation, and amplification (Figure 1a) [13]. In the first step, the target molecule can bind with pool molecules, separating bound molecules from unbound molecules to the target molecule. The unbound molecules are removed through washing. The initial pool is selected carefully, with the randomized molecules varying from 25 bases (N<sub>25</sub>). The general concept is that the pool molecules with higher numbers can make more versatile structures and yield high-affinity aptamers at possible levels. However, a wide range of aptamers can be selected using different pools [13-15]. The bound molecules are separated from the unbound molecules by several partition methods, such as titer plate, filter, magnetic bead, column matrix, etc. After eluting the bound molecules from the target, polymerase chain reaction (PCR)-amplification is

used to enrich the bound molecules and used for the next round of SELEX. Five to 12 rounds are required to get the specific aptamer in most cases. With the cell SELEX selection, two research teams successfully generated the first aptamers [16, 17]. After this, various aptamers were generated against a wide range of targets, such as toxins, organic molecules, peptides, proteins, carbohydrates, and antibiotics. Interestingly, it is also possible to generate aptamers against complex target structures and whole organisms [14, 15, 18, 19]. With the continuous selection of different types of aptamers, the understanding of the SELEX procedure is progressed well in different ways. Ultimately, several SELEX methods have been established that can generate aptamers in a short time and use less reagents. These SELEX methods are complex target SELEX, Photo-SELEX, *in vivo* SELEX, SELEX with Beacons, Cell-SELEX, Genomic SELEX, Spigelmers, MonoLEX, and *in silico* SELEX. Recently, researchers have used a single-step method as non-SELEX in which the aptamer is selected without the amplification process (Figure 1a-c). The microfluidic device-based aptamer selection is one of the advanced levels of the selection process, which includes all the selection steps on one chip [20]. The main benefit of the aptamer selection is that the SELEX process is carried out using *in vitro* and does not require used animals like antibody production. Besides, it is also possible to select aptamers against a wide range of non-immunogenic targets.

### 2.2. Types and Characteristics of Aptamers

Three major types of aptamers are DNA, RNA, and peptide aptamers, among which both DNA and RNA aptamers are widely used in many fields. Comparatively, DNA aptamer is more stable but less reactive than RNA aptamer, while RNA aptamer can make more secondary-dimensional structures than DNA aptamer. Additionally, it is easier to synthesize and stabilize RNA aptamer by simple chemical modification than DNA aptamer. Peptide aptamers are short amino acid sequences present within a scaffold protein. The short amino acids that are double-constrained within the protein are the target protein binding agent, and the protein aids in increasing the specificity and binding affinity [21].

DNA and RNA aptamers can create specific binding sites in their targets with adaptive conformational changes that alter the surface area of their targets. Contrarily, peptide aptamers create a smaller binding site that allows accurate target selection. DNA, RNA, and peptide aptamers exhibit different functional group variations, increasing the possibility of determining ideal targets [22]. The



**Figure 1.** Strategies with aptamer production. a. Conventional SELEX; b. Non-SELEX; and c. *In vivo*-SELEX are shown with the steps involved. Other strategies generated for SELEX are following predominantly the conventional SELEX and *in vivo*-SELEX.

comparison of highlighted properties between DNA, RNA, and peptide aptamers is presented in Table 1.

**Table 1.** Comparison among RNA, DNA, and peptide aptamers.

Properties	RNA Aptamers	DNA Aptamers	Peptide Aptamers
Structure	Limited due to chemical differences (O-H bond)	Limited due to chemical differences (C-H bonds)	Limited due to the presence of scaffold protein
Formation of aptamers	Able to form more secondary-dimensional structures than DNA aptamers	Able to form a limited type of secondary-dimensional structures than RNA aptamers	Able to form limited types of secondary-dimensional structures due to the scaffold protein
Target binding site	Binds to the loop region(s) and undergoes structural conformational changes	Binds to the loop region(s) and undergoes structural conformational changes	Binds to the target with the short peptide sequence, limited due to the sequence present in a scaffold protein

Aptamers are carefully selected after a certain round, and their binding affinity with the target is usually in the low-nanomolar to the low-femtomolar range. Moreover, aptamers are more specific to their targets; thus, they could discriminate among closely related molecules. This claim implies that they can distinguish the epitope on the target molecule, for instance, discriminate between the phosphorylated and dephosphorylated states. They can also differentiate the enantiomers of chiral molecules. Interestingly, because they are tiny, they can be delivered easier and can reach smaller areas in the biological system. Apart from that, aptamers have a versatile structure and can make various shapes, such as stems, loops, bulges, hairpins, pseudoknots, triplexes, and quadruplexes. Their secondary structures may fold into complex tertiary structures,

permitting them to make complementary shapes wrap around or part of the target or fit snugly into clefts and gaps within the surface of larger targets [23]. In most cases, aptamers bind with their targets in the loop region [14, 15]. Because of the structural variation, they can bind with their targets through electrostatic interaction or hydrogen bonding, as well as possible interaction with aromatic compounds and nucleobases. Besides, aptamer binding to a target can occur in three different ways, including aptamer-induced targets, targets-induced aptamers, or both. With these versatile structures, aptamers are widely utilized in several fields, particularly biosensing due to their high affinity and selectivity.

### 2.3. Aptamer Targets Highly Expressed Molecules for Cancer Diagnosis and Molecular Imaging

Aptamers are widely applied in many fields, such as biosensors, drug carriers, imaging, drug delivery, and medicine [24, 25]. Given that aptamers have high affinity and selectivity with the target molecules, their application focuses more on biosensor development (Figure 2) [26, 27]. It is essential to differentiate the closely related molecules to treat diseases and avoid spreading in medical diagnosis. Because only a few bases in the aptamer are involved in binding with the molecular targets; thus, they can differentiate from the closely related molecules. For instance, aptamers can differentiate the closely related strains within influenza virus types [15]. Due to the high selectivity of aptamers, they can be used to detect the analyte molecules in crude



samples. For instance, Lakshmi Priya et al. [28] found that aptamers could detect factor IX protein from human serum and reached the limit of detection (LOD) to the lower picomolar range, acknowledging that the LOD with aptamers can be as low as picomolar to femtomolar for diagnosing and in medical imaging [29-31]. Additionally, aptamers can also be arranged in the sandwich pattern using two aptamers with different binding sites.

Several studies have reported that aptamers can be useful tools in molecular imaging and diagnosis. Chen X et al. [32] proved that S3-2-3 aptamer was introduced to target esophageal squamous cell carcinoma (ESCC) KYSE150 cells for confocal tissue imaging, with fluorescent signals present and detected. This finding indicates that S3-2-3 aptamer can be used as a molecular imaging probe. Similarly, cy-apt 20 aptamer labeled with fluorescein isothiocyanate (FITC) successfully detected GC AGS cells with the emittance of strong fluorescence intensity [18, 33]. Next, Xu J et al. [34] indicated that a few selected aptamer sequences (i.e., JHIT1, JHIT2, JHIT3, JHIT4, JHIT5, JHIT6, and JHIT7) could differentiate between normal and LC cells through the binding analysis method, implying that aptamers can also be used for cancer diagnosis. Wan J et al. [35] also proved that selected DNA aptamers (i.e., yl19, yl19a, and yl23) could recognize and bind to the membrane proteins as potential molecular targets to assist in identifying LC cells in QBC-939, a type of human cholangiocarcinoma cell line, thus contributing to cancer diagnosis. Rong Y et al. demonstrated that LY-1 aptamer bound to the overexpressed Cytokeratin 19 (CK19) and vimentin in metastatic LC cells, indicating cancer cells in pulmonary cancer tissues and local LC tissues. This finding shows that LY-1 aptamer can be used as a diagnostic tool and in molecular imaging [36]. Moreover, the Apt-07S aptamer developed by Yu XX et al. recognized LC cells and differentiated them from normal cells by targeting Plk1 protein [37]. These studies have proved that aptamer can be used as a molecular imaging tool in GC and LC cancer cells by labelling with fluorescence and used as a diagnostic probe through their binding specificity and selectivity of highly expressed molecules in cancer cells that are lowly expressed in normal cells.

A study by Dong et al. discovered a truncated aptamer, AP613-1, specifically bound to Glypican-3 (GPC3) protein in GPC3-positive hepatocellular carcinoma (HCC) cells. The modified AP613-1 are aptamers with bases at both 5' and 3' terminals that are replaced by phosphorothioates, and these aptamers showed a higher binding affinity than the unmodified AP613-1 aptamer. Phosphorothioate-

modified AP613-1 binding to GPC3 showed potential in staining HCC cells for *in vivo* targeted imaging [38]. Next, the ApC1 aptamer also showed potential in molecular imaging and cancer diagnosis. It specifically targeted colon Caco-2 cancer cells with CD133 high expression as compared to HEK293 normal cells and other cancer type cells and emitted fluorescence signals in confocal tissue imaging, showing its potential to be a diagnosis tool on top of being a molecular imaging tool [39]. Collectively, truncated aptamer can improve the binding affinity of aptamer to highly expressed molecules like GPC3 in HCC, while ApC1 aptamer can be used as molecular imaging and diagnostic tools in colon cancer cells by targeting membrane-bound CD133 specifically.

Besides, targeted therapy and diagnostic tool for PC were developed using a single-stranded DNA (ssDNA) aptamer termed XQ-2d to target Cluster of Differentiation 71 (CD71), which was overexpressed in PC [40]. Interestingly, XQ-2d exhibited a high binding affinity against PC cells. Wu et al. (2019) further discovered that CD71 silencing prevented XQ-2d from attaching to PC cells, indicating that XQ-2d targets membrane-bound CD71 instead of total CD71. Additionally, it was found that XQ-2d and transferrin (Tf) shared the same binding site on CD71 but not the anti-CD71 antibody, as evidenced in a competitive examination. The distance between the binding sites of XQ-2d and anti-CD71 antibody was measured using a surface energy transfer (SET) nano-ruler, and it was roughly 15 nm. The spatial organization of XQ-2d and Tf competitively binding to CD71 was resolved using molecular dynamics modeling [40]. In another study, researchers tested the specificity of Cy3 coupling with P19/P1 aptamers to be employed as a diagnostic tool on archival human pancreatic duodenectomy tissue slices of pancreatic ductal adenocarcinoma (PDAC) patients. According to a scoring pattern from 72 patients, a favorable link was found between strong fluorescence signals in the high-mortality patient groups [41]. Matrix metalloproteinase 14 (MMP14) is overexpressed in various cancers and is linked to a bad PC prognosis. As for MMP-14 positive pancreatic cancer cell lines, aptamer M17 was identified to specifically probe and recognize PC cells for molecular imaging and diagnosis. Aptamer M17 demonstrated its capability to target MMP-14 and contributed to *in vivo* and *in vitro* tumor imaging [42]. It has been reported that modified DNA aptamers with amino acid-like side chains or drug-like ligands can provide unique benefits and improve specificity [43]. For instance, Thy-1 membrane glycoprotein (THY1 or CD90) has been discovered as an overexpressed neovasculature biomarker in PDAC. Thus, Wang et al. designed and

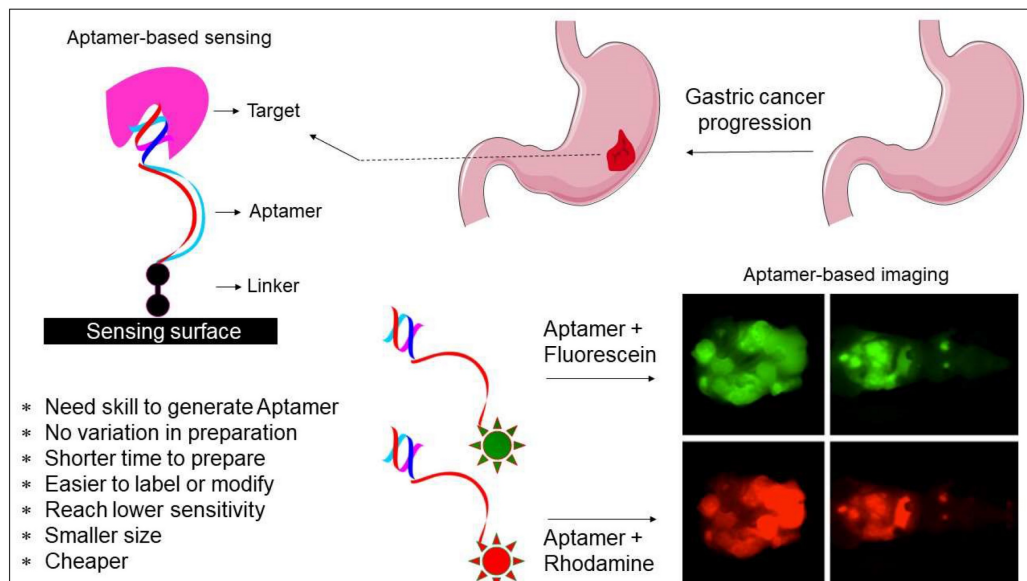
tested modified DNA X-aptamers targeting THY1. After library screening and affinity binding evaluation, three high-affinity THY1 X-aptamers (i.e., XA-B217, XA-B216, and XA-A9) were chosen. Using the THY1 antibody as a reference, these three X-aptamers displayed good binding affinity and specificity to THY1 protein and THY1-expressing PDAC cells. In the context of biomarker discovery and therapeutic applications, the development of these X aptamers provides highly selective and non-immunogenic affinity ligands for THY1 binding; thus, they could be used to aid molecular imaging of PDAC that targets THY1 [43]. Thus, aptamers like XQ-2D, P19/P1, THY1 X can be used as molecular imaging and diagnostic tools in PC by binding to membrane-bound CD71, MMP-14, and THY1.

### 3. Aptamer-based Recognition of GI Cancer and Applications

#### 3.1. Specific Detection for Different Cancer Types

Aptamers are mostly used in cancer diagnosis and have the advantage of recognizing cancer cells by binding to molecular targets. Therefore, they can determine the specific detection of GI cancer cells. For instance, Chen X et al. [32] utilized the cell-SELEX technology to successfully screen three different aptamers consisting of ssDNA sequences (i.e., S2, S3, and S8) with a strong affinity for ESCC KYSE150 cells. It was detected by flow cytometry assay that S2 aptamer showed specificity against KYSE150 cells, while S3 aptamer could significantly target KYSE150, KYSE30, KYSE140, EC109, TE-1, KYSE450, and KYSE70 cells. Next, the S8 aptamer showed a

significant fluorescence signal in KYSE150, KYSE30, KYSE140, and KYSE70 cells, implying that these aptamers are ESCC-specific recognition molecules. Besides, Chen et al. used several truncating techniques to produce specific aptamers with higher binding affinity against ESCC cells by designing the secondary structure of nucleic acid systems using the Nucleic Acid Package (NUPACK) software. S2-1 and S2-2, S3-1 and S3-2, and S8-1, S8-2, and S8-3 were successfully obtained from S2, S3, and S8 aptamers, respectively [44]. Following analysis by flow cytometry assay, S3-2-3 aptamer had the highest fluorescence intensity on truncated sequence optimization as evidenced by the flow cytometry analysis as compared to S3-2-1 and S3-2-2 aptamers, indicating that S3-2-3 aptamer is the best. Importantly, S3-2-3 aptamer also showed high binding affinity against KYSE30 cells, as indicated by the dissociation constant (Kd) in the nanomolar range ( $265.3 \pm 26.8$  nM). An ultrashort S3-2-3 aptamer with 18 bases was then identified and labeled with Cy5 for testing its potential to be applied for ESCC tissue imaging, as this method is cheap, simple, and selective. The confocal imaging revealed that ESCC tissues displayed significant red fluorescence signals, implying an important role of this labeled aptamer in clinical application for diagnosis and target therapy of ESCC. Further analysis by proteinase K and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that selected aptamers' targets were most likely membrane proteins, which may facilitate the discovery of biomarkers and targeted therapy of ESCC.



**Figure 2.** Potentials of aptamer in diagnosis. Both sensing and imaging-based identifications are shown. The advantages of aptamer and its suitability with different fluorescent compounds are displayed.

Alternatively, it was shown that epithelial cell adhesion molecule (EpCAM) protein could be targeted with aptamers. EpCAM is highly overexpressed in EC cell lines and has been reported to contribute to cancer metastasis to the adjacent organs and lead to poor prognosis [44]. Given this, Liu et al designed an EpCAM-specific aptamer, known as SYL3C, to differentiate EC cells from normal cells instead of using antibodies. SYL3C aptamer successfully showed potential to be a reliable biomarker for EC through aptamer-based staining targeting EpCAM expression. Therefore, EpCAM protein staining with SYL3C did not only identify severe dysplastic EC cells but also differentiated lesions and benign cells, showing the diagnostic and prognostic values of SYL3C aptamer by targeting EpCAM [44].

Furthermore, Cao H-Y et al. [33] demonstrated the feasibility of using cy-apt 20 aptamer for detecting GC with high affinity and specificity, as indicated by fluorescence intensity emitted by bound GC AGS cells, which was seven times higher than LC HepG2 cells and SW620 CRC cells. This finding was confirmed in another study that the cy-apt 20 aptamers binding rate to AGS cells was >70%, while the binding affinity to non-GC cells was 30%, as measured by flow cytometry [18]. The *in vitro* efficacy of cy-apt 20 aptamer was also supported by an *in vivo* study in which the fluorescence signals were detectable in GC in a dose and time-changing manner, as visualized by the IVIS Spectrum Imaging System. Fluorescence signals became evident 10 minutes after delivery of 1 nM cy5-labeled cy-apt 20 aptamers, followed by gradually reaching a peak at 120 minutes. In another study by Cao H-Y et al, they reported that the fluorescence intensity of AGS cells steadily increased after 40 minutes with increasing concentrations of FITC-cy-apt 20 aptamer and peaking at 400 nM [18]. These findings show that targeted recognition can be established with a low dose of cy-apt 20 aptamer and can last sufficient for detection. Indirectly, it demonstrated that cy-apt 20 aptamer has acceptable specificity and sensitivity to target cells and is biostable *in vivo*.

According to Xu, J et al. [34], seven aptamer candidates (i.e., JHIT1, JHIT2, JHIT3, JHIT4, JHIT5, JHIT6, and JHIT7) showed apparent shifts in fluorescence intensity toward HepG2 HCC cells using flow cytometry, indicating that selected aptamers could distinguish HCC cells from normal liver cells. The Kd value further indicated that these aptamers were highly bound to HepG2 cells in the nanomolar range (64–349 nM). The absence of fluorescence shift after treating HepG2 cells with trypsin further confirmed that selected aptamers targeted membrane proteins. It was also noted that the seven mentioned

aptamers were exclusively for MCF-7 breast cancer cells instead of MDA-MB-231 cells. Histologically, MCF-7 cells belong to the Luminal A subtype and have high expression of estrogen receptors (ER) and progesterone receptors (PR), which do not express in MDA-MB-231 cells, suggesting that these aptamers may be hormone-dependent. Besides, Apt-07S aptamer could specifically detect HCC HepG2 and SMMC-7721 cells with low Kd, 194.7±69.8 nM and 224.2±60.4 nM, respectively, as well as at a particularly specific binding capacity to two additional HCC cells, HCC-LM3 and Huh7 cells [37]. According to the confocal imaging observation, Apt-07S aptamer only slightly bound to cells of other cancer types, such as MCF-7, H460, and SW480, as well as normal breast HBL-100 cells, indicating that Apt-07S aptamer has higher selectivity for HCC cells than normal cells. Furthermore, it was discovered that FAM-labeled Apt-07S aptamer was distributed both on the cell surface and in the cytoplasm of targeted cancer cells. This result implied that Apt-07S aptamer could precisely detect cancer cells and could be used as a possible carrier for transporting anticancer agents to cancer cells. Based on a cell growth inhibition rate study, Apt-07S aptamer was cytotoxic to HepG2 cells (>10 µM, p<0.05) and SMMC-7721 cells (>20 µM, p<0.05). Rong (2016) also discovered that Apt-07S aptamer integrated with ssDNA, Apt-07S-ASO-Plk1, was considerably more effective than antisense phosphorothioate oligodeoxynucleotide (ASODN)-polo-like kinase-1 (Plk1), at inhibiting HepG2 and SMMC-7721 cell growth (p<0.01), as well as less toxic to L02 normal liver cells [37].

Next, Wan, J et al. [35] showed that yl19 and yl23 aptamers exhibited a good binding capacity to QBC-939 cholangiocarcinoma cells, with equilibrium Kd values of 42.44±11.82 nM and 87.45±15.50 nM, respectively. Additionally, FAM-labeled yl19, yl19a, and yl23 aptamers demonstrated strong binding to QBC-939 cells while showing no significant recognition for HeLa cervical cancer cells; HepG2, Bel-7404, and SMMC-7721 HCC cells; CNE2 nasopharyngeal carcinoma cells, and A549 lung adenocarcinoma cells. When QBC-939 cells were treated with proteinase K, the fluorescence intensity on target cells containing aptamers yl19, yl19a, and yl23 was significantly reduced. This result indicated that the target molecules of these aptamers were most likely membrane proteins. The confocal imaging observation also showed that yl19 and yl23 aptamers could bind to the surface of target cells rather than control cells. Rong, Y et al [36] showed that LY-1 and LY-13 aptamers had high affinity against HCCLM9 HCC cells in which respective low Kds (167.3 ± 30.2 nM and 185.6 ± 28.3 nM) were measured.



Fluorescence imaging and flow cytometry analyses further indicated that LY-1 aptamer demonstrated significant specific binding to HCCLM9 cells as compared to other HCC cell lines. It was also found that the targets of LY-1 aptamer were mainly membrane proteins on HCCLM9 cells after pretreatment with proteinase K. Besides, a study involving a xenograft model of HCC with pulmonary metastasis discovered that QD605-labeled LY-1 aptamer could detect both local LC tissues and metastatic lung tissues. The Western blotting analysis further confirmed the result that QD605-labeled LY-1 aptamer was capable of binding to a subpopulation of HCCLM9 cells, with a higher amount of CK19 and Vimentin presented, which are the protein markers associated with cancer migratory and invasiveness. Interestingly, QD605-labeled LY-1 aptamer treatment regulated metastatic HCC behaviors, as evidenced by a significant inhibition of cell migration ( $29.3 \pm 3.06\%$  of LY-1 treated cells vs  $97.1 \pm 2.11\%$  of control cells;  $p < 0.05$ ) and cell invasion ( $53.8 \pm 4.36\%$  of LY-1 treated cells vs  $96.83 \pm 3.14\%$  of control cells;  $p < 0.05$ ) in both non-coated and basal membrane coated (Matrig) HCC cells. Similarly, the xenograft mouse model transplanted with HCCLM9 cells also showed that LY-1 aptamer inhibited tumor growth after intraperitoneal injection ( $757.4 \pm 70.68 \text{ mm}^3$ ) as compared to Phosphate-buffered saline, PBS, control ( $1962 \pm 139.8 \text{ mm}^3$ ) and NK8-treated mice ( $1569 \pm 308.2 \text{ mm}^3$ ). More importantly, the aptamers were non-toxic because mice did not show abnormal behavioral or body weight changes after treatment [36].

PC is usually not discovered until it has progressed to the point where surgery is no longer an option. In this regard, Ray et al. [45] designed an aptamer that could detect PC in the early stage. An *in vitro* positive or negative selection technique was established to identify RNA ligands (aptamers) that could detect structural variations between the secretomes of PC and non-cancerous cells to identify PC biomarkers. The molecular recognition technique discovered an aptamer (M9-5) that variably binds to conditioned media of malignant and non-cancerous human pancreatic cell lines. With great sensitivity and specificity, M9-5 aptamer could distinguish between the serum of PC patients and healthy individuals. Cyclophilin B (CypB) was identified as the target of M9-5 aptamer using biochemical purification procedures and mass spectrometric analysis. This molecular recognition-based approach discovered CypB as a serum biomarker while also developing a novel reagent to detect it in body fluids [45]. In addition to cancer cells, Kim YJ et al. [46] also demonstrated that aptamers 1 and 146 could bind to

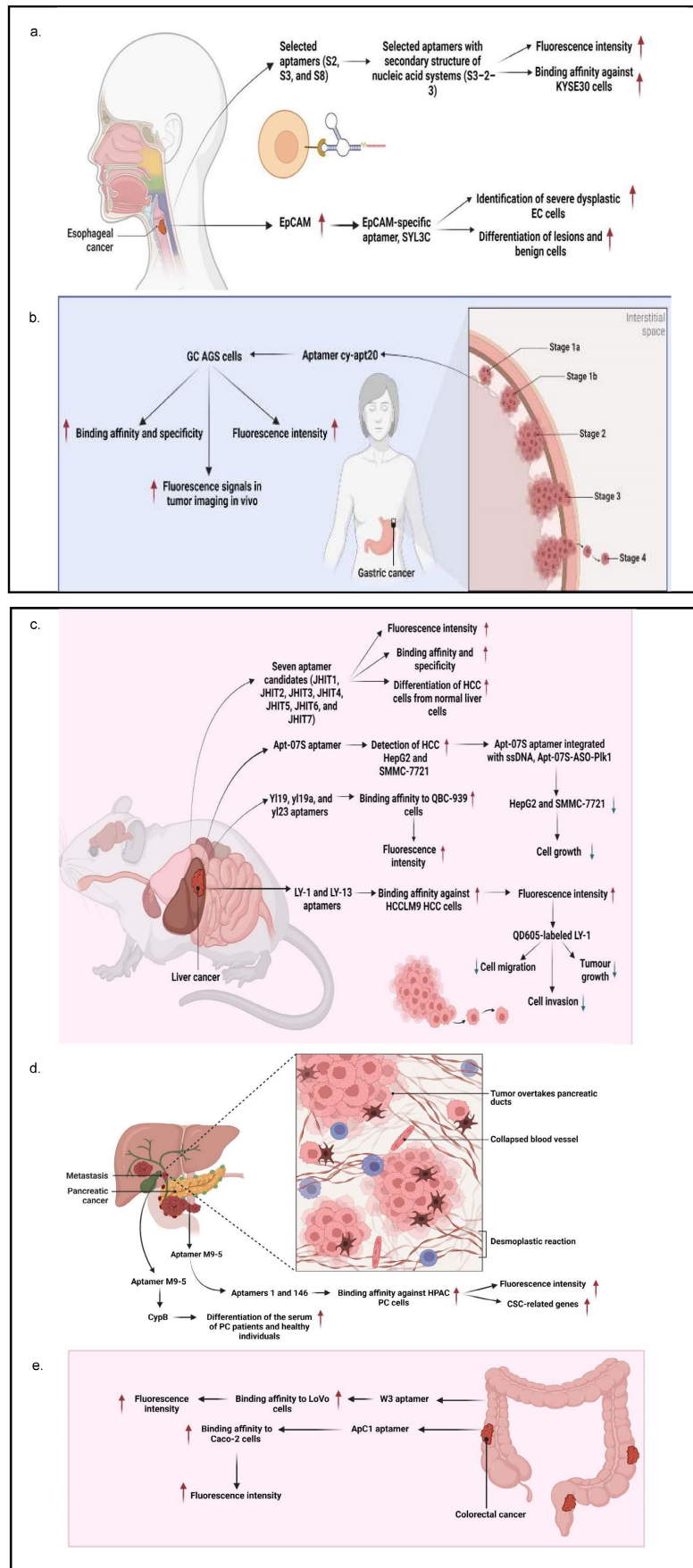
cancer stem cells (CSCs) of the HPAC PC cells. They found that the binding affinity of aptamers 1 and 146 showed  $K_d$  values of 22.18 and 22.62 nM, respectively. Using Fluorescence-activated cell sorting (FACS) analysis, it was revealed that aptamer 1-positive cells had elevated levels of CSC-related genes (i.e., *CD133*, *CD24*, and *ALDH1*). On the other hand, aptamer 146-positive cells exhibited higher expression levels of CSC-associated genes, such as *CD133*, *CD24*, *IHH*, and *nanog*. Further analysis using a confocal microscope confirmed the colocalization of *CD44*, *CD24*, *ESA*, and *CD133* with both aptamers, indicating they bound to CSCs successfully.

In a study by Li et al. [47], they studied a CRC-specific aptamer that specifically targeted ephrin type-A receptor 2 (EphA2), a protein involved in cell migration and invasion, in EphA2-expressing CRC cell line, LoVo cells. The binding affinity was reported to be  $12.43 \pm 0.97 \text{ nM}$   $k_D$  proving a corresponding strong interaction between W3 and EphA2. This aptamer W3 successfully detected EphA2-expressing cells even though the presence of the target cells was as low as 1% and also emits strong fluorescence signals in CRC-positive cells when compared with adjacent CRC-negative cells. Using UNAFold software, Maimaitiyiming Y et al. [39] predicted that both forward and reverse primer binding sites participated in the important formation of hairpins for efficient binding of ApC1 aptamer to Caco-2 cells by truncation experiment. This finding also suggests that aptamers' fixed primer binding areas are critical for efficient target binding and that truncation is irrelevant to all aptamers. Both confocal microscopy and flow cytometry demonstrated that ApC1 aptamer only adhered to the Caco-2 cell membrane. Furthermore, aptamer ApC1 could rapidly and specifically internalize into Caco-2 cells because a strong fluorescence signal measured by flow cytometer was detected in the cytoplasm of Caco-2 cells as compared to other cancer cell lines, such as 293T leukemia, HeLa, MCF-7, NB4, and HL-60 cells. Lastly, pretreatment of aptamer ApC1 with trypsin indicated its target could be a Caco2 cell membrane protein. The above-mentioned studies have clearly indicated that aptamer-based detections and applications have exponentially increased over the years and has shown distinguished potential in GI cancers. Other than aptamers that contribute to diagnosis and prognosis, aptamers also have the potential to act as a drug delivery agent assisting in the process of chemotherapeutic drugs transport for cancer treatments. The summarization of aptamers as diagnostic or prognostic tools in GI cancers is mentioned in Table 2 and is summarized in Figure 3.



**Table 2.** The diagnostic or prognostic roles of aptamers in GI cancers.

Type of Cancer	Aptamer	Diagnostic/Prognostic Tool	Kd values	Ref
Esophageal	S2, S3, and S8	<ul style="list-style-type: none"> <li>High affinities of S2, S3, and S8 for targeting KYSE150 cells with Kds in the nanomolar range</li> <li>S2 aptamer was only bound to KYSE150 cells as its target, but S3 and S8 aptamers were capable of targeting a series of ESCC cells.</li> <li>Useful targeted membrane proteins were captured by S3–2–3 aptamer.</li> </ul>	S2: 9.37±1.46 nM, S3: 19.77±4.1 9 nM, S8: 7.90±5.68 nM	[32]
Esophageal	SYL3C	<ul style="list-style-type: none"> <li>differentiate EC cells from normal cells instead of using antibodies</li> <li>identify severe dysplastic EC cells but also differentiated lesions and benign cells</li> </ul>	Not available	[41]
Gastric	cy-apt 20	<ul style="list-style-type: none"> <li>78% maximal binding rate to GC cells as accessed by flow cytometry</li> <li>Cell fluorescence imaging or <i>in vivo</i> tumor imaging found strong fluorescence signals that lasted for 120 minutes and peaked at 1.25 nM</li> </ul>	Not available	[19]
Gastric	cy-apt 20	<ul style="list-style-type: none"> <li>Higher binding affinity to AGS cells than non-AGS cells</li> <li>Strong fluorescence at 40 minutes and peaked at 400 nM</li> </ul>	Not available	[37]
Liver	JHIT1 JHIT2 JHIT3 JHIT4 JHIT5 JHIT6 JHIT7	<ul style="list-style-type: none"> <li>Aptamer candidates demonstrated a significant change in fluorescence intensity when tested with HepG2 cells.</li> <li>Aptamers' binding affinity to HepG2 cells was in the nanomolar range (64–349 nM).</li> <li>Aptamers also bound to cell lines from other cancer types expressing proteins that were comparable to those expressed by HepG2 cells.</li> <li>Suggests that these aptamers may be hormone-dependent</li> </ul>	Not available	[48]
Liver	Apt-07S	<ul style="list-style-type: none"> <li>Apt-07S aptamer targeted HepG2 and SMMC-7721 cells with low Kd values.</li> <li>Apt-07S aptamer did not bind to normal breast cancer cells.</li> <li>Apt-07S was toxic to HepG2 and SMMC-7721 cells</li> <li>Apt-07S-ASO-Plk1 significantly inhibited HepG2 and SMMC-7721 cell growth as compared to ASODN-Plk1.</li> </ul>	HepG2: 194.7 ± 69.8 nM, SMMC-7721: 224.2 ± 60.4 nM	[49]
Liver	y119, y119a, y123	<ul style="list-style-type: none"> <li>y119 and y123 aptamers specifically bound to QBC-939 cells.</li> <li>y119, y119a, and y123 aptamers bound to QBC-939 cells' membrane protein.</li> </ul>	y119: 42.44±11.82 nM y123:	[50]
Liver	LY-1	<ul style="list-style-type: none"> <li>HCCLM9 cells were recognized by LY-1 and LY-13 aptamers with low Kd values.</li> <li>LY-1 aptamer targeted membrane proteins on the surface of HCCLM9 cells.</li> <li>LY-1 aptamer bound to HCCLM9 cells with higher expression levels of CK19 and Vimentin and aptamer treatments were non-toxic <i>in vivo</i>.</li> </ul>	1: 167.3 ±30.2 nM 13: 185.6 ±28.3 nM	[51]
Pancreatic	M9-5	<ul style="list-style-type: none"> <li>It distinguished between the serum of PC patients and healthy individuals.</li> <li>It bound to CypB and identified it as a serum biomarker while detecting it in body fluids.</li> </ul>	Not available	[52]
Pancreatic	Aptamer-1 &146	<ul style="list-style-type: none"> <li>Aptamers 1 and 146 showed high specificity to HPAC, pancreatic adenocarcinoma epithelial cell line, and sphere cells.</li> </ul>	22.18– 22.62 nM	[53]
Colorectal	Aptamer W3	<ul style="list-style-type: none"> <li>Aptamer W3 targeted EphA2 in LoVo CRC cells even if the abundance was as low as 1% and emits strong fluorescence signals in CRC-positive cells.</li> </ul>	12.43 ± 0.97 nM	[47]
Colorectal	ApC1	<ul style="list-style-type: none"> <li>ApC1 aptamer showed excellent specificity to Caco-2 cells as compared with other cell lines, such as 293T, HeLa, MCF-7, NB4, and HL-60.</li> <li>Membrane proteins were the target of ApC1 aptamer on Caco-2 cells.</li> </ul>		[39]



**Figure 3.** Aptamer-based specific detection of GI cancers. a. Specific detection in esophageal cancer; b. specific detection in gastric cancer; c. specific detection in liver cancer; d. specific detection in pancreatic cancer; and e. specific detection in colorectal cancer. This figure is created with biorender.com.

### 3.2. Aptamers as an Effective Drug Delivery System for Chemotherapy

Chemotherapy is one of the traditional clinical treatment methods for GI cancers, and it has many concerning side effects. Therefore, there is a need for an alternative method to reduce the side effects and improve the effectiveness of chemotherapy. Aptamers can be a useful tool to act as a drug delivery agent in transporting chemotherapeutic drugs to the target sites for higher treatment efficacy. For instance, a study by Ramezanzpour M et al. [54] on chimera consisted of nucleolin-specific aptamer (aptNCL) and microRNA (miRNA) let-7d demonstrated potential therapeutic effects on GC cells using either covalent conjugation with SM (PEG)<sub>2</sub> as a bifunctional crosslinker (product known as conjugate-1) or non-covalent conjugation with 19bp complementary sticky end sequences (product known as conjugate-2). Nucleolin is a protein that acts as a regulator of ribosomal DNA (rDNA) transcription and as a binding site for TNF- $\alpha$  inducing protein (Tip $\alpha$ ), a recently discovered carcinogenic factor transcribed from *Helicobacter pylori* (*H. pylori*) strain from mouse GC cell line. It found that synergistic effects of the chimera on Januse kinase-2 (JAK-2) expression and activity, as measured by real-time PCR and enzyme-linked immunosorbent assay (ELISA) methods, could have a therapeutic effect on GC cells. Specifically, the chimera (i.e., conjugate-1, conjugate-2, aptNCL mixture of aptNCL and let-7d, or transfected let-7d) caused a substantial decrease in JAK-2 expression in MKN-45 GC cells after 24 hours treatment as compared to aptamer alone or untreated control condition ( $p=0.0001$ ). Both conjugate-1 and transfected miRNA let-7d induced a considerable reduction in JAK-2 expression but not by conjugate-2 after 24 hours ( $p<0.01$  and  $p=0.0001$ , respectively) and 48 hours ( $p=0.0001$  and  $p<0.01$ , respectively), most likely due to enhanced stability. JAK-2 expression was unaffected by the aptNCL as compared to aptamer alone or untreated control condition. In contrast, under each of the chimera, the JAK-2 expression level of nucleolin-negative human derma fibroblast (HDF) cells (negative control) remained unchanged as compared to the control condition. Surprisingly, the transfected miRNA let-7d resulted in a significant reduction in JAK-2 expression in HDF cells ( $p=0.0001$ ), demonstrating the non-specific effect of let-7d (lipofectamine) on both cancer and normal cells.

By means of internalization assay, Dua et al. [48] showed that Tetramethylrhodamine (TAMRA)-labeled SQ2 aptamer internalized into alkaline phosphatase placental-like 2 (ALPPL-2) expressing PC cells (i.e., Capan-1 and PANC-1 positive cells)

within 30 minutes of incubation. This assay was carried out with RiboShredder, a harsh cocktail of ribonucleases, RNases, (to remove surface-bound aptamers) and 0.1% sodium azide (to inhibit internalization of any free TAMRA fluorochrome generated upon degradation of the RNA oligonucleotide). Intriguingly, treatment with endosomal inhibitors (M $\beta$ CD) resulted in a 40% reduction in SQ2 binding to PANC-1 positive cells and nearly full loss of aptamer internalization, demonstrating that ALPPL-2-mediated cellular uptake of SQ2 is an energy-dependent mechanism. ALPPL-2 was mostly found in the cholesterol-rich fraction, and when cholesterol was removed from the plasma membrane, a portion of this glycosylphosphatidylinositol-anchored protein (GPI-AP) shed and the rest became incapable of caveolae-mediated endocytosis [48] suggesting that caveolae-mediated endocytosis is the most common mechanism for ALPPL-2 internalization in PANC-1 positive cells. Besides, the result further showed that SQ2 aptamer bound to ALPPL-2 with a K<sub>d</sub> of 20 nM, which is low enough for targeted drug delivery to ALPPL-2 positive cells. The removal of the surface-bound aptamers revealed a significant amount of internalized SQ2 in both PANC-1 positive cells (26%) and Capan-1 positive cells (20%). Furthermore, the confocal microscopy observation identified the presence of streptavidin magnetic beads (2.8  $\mu$ m in diameter) inside PANC-1 positive cells, indicating that SQ2 aptamer might effectively act as a cargo carrying NPs and other large molecules. The study also found that SQ2 carrying a 5-fluoro-2'-deoxyuridine (5FdU) pentamer (SQ2-5FdU) could inhibit Capan-1 cell growth by 40% and 52% at concentrations of 5 mM and 10 mM, respectively. Thus, SQ2 aptamer could successfully deliver the anticancer nucleoside drug, 5FdU to ALPPL-2-expressing PC cells without imposing toxicity on normal cells.

Gemcitabine, the gold standard drug for PC, has shown unsatisfactory therapeutic effects due to chemoresistance and low targeting of cancer cells. Ray P et al. [49] established an epidermal growth factor receptor-conjugated aptamer to deliver gemcitabine (E07-Gem polymer). It was purposely designed for binding to PC MiaPaCa-2 cells expressing EGFR. The results demonstrated that E07-GEM aptamer specifically recognized and bound to EGFR protein expressed on the cell surface of MiaPaCa-2 cells, indicating that this might be a viable approach to delivering gemcitabine to EGFR-expressing cells. Based on the internalization assay (RiboShredder assay), E07-Gem polymer was successfully internalized into MiaPaCa-2 cells. The process of endocytosis performed better at the physiological

temperature of 37°C and was inhibited at 4°C. The percentage of E07-GEM internalized was higher at 100 nM (17%) versus 400 nM (6%). Upon treatment with E07-Gem polymer, MiaPaCa-2 cell viability was greatly inhibited as compared to mE07-Gem polymer or with the unescorted Gem polymer. Doxorubicin (Dox) is one of the most commonly used chemotherapy drugs for many cancers. A study by Wu et al. [40] determined that aptamer XQ-2d binds specifically to CD71, an overexpressed protein in PC. An XQ-2d-based complex for loading Dox was used to develop an XQ-2d-mediated targeted treatment for pancreatic cancer. A chimera was generated with this aptamer, XQ-2d-sd3-DOX, to investigate the inhibitory effect of this chimera sequence in PC. The chimera did exhibit significant suppression of proliferation in PL45 cells, pancreatic adenocarcinoma epithelial cell line, showing the potentiality of molecular targeted therapy.

According to a study by Yao F, et al. [50], fluorescent-labeled Apt-HJ aptamer exhibited a high affinity toward colon cancer cells (nucleolin-positive CT26 cells), as evidenced by considerably higher fluorescence signals in flow cytometry detection. This study evaluated the drug loading capacity of Apt-HJ after mixing with Dox at increasing molar ratios due to Dox intercalated into double-stranded DNA (dsDNA) structures to form a Dox-DNA complex while free Dox emitted a red fluorescence that will be quenched when Dox intercalated into dsDNA. The findings show that the fluorescence was almost quenched when the molar ratio of Apt-HJ to Dox increased to 1:17, indicating that most Dox molecules intercalated into the DNA structure of Apt-HJ. After treating with Apt-HJ-Dox, red fluorescence was observed in CT26 cells but slightly in Chinese hamster ovary (CHO) cells as observed under confocal microscopy, directly indicating that Apt-HJ-Dox was delivered into CT26 cells but not CHO control cells. Interestingly, the result also showed that Apt-HJ-Dox killed CT26 cancer cells (55.14% ± 4.34%) efficiently while also reducing damage to normal CHO control cells (80.72% ± 5.71%,) as compared to free Dox. Apt-HJ-Dox exhibited a substantially larger half maximal inhibitory concentration, IC<sub>50</sub>, for CHO cells than free Dox (8.481 M vs. 2.579 M), demonstrating that Apt-HJ-Dox induced preferential cytotoxicity against CT26 cells while reducing toxicity toward control cells. Furthermore, a similar result was also observed in the *in vivo* study in which Apt-HJ-Dox demonstrated pronounced antitumor activity in mice carrying CT26 tumors as compared to free Dox and Apt-HJ. The average tumor volume of mice treated with Apt-HJ-Dox was five times smaller than that of

the control group, with no additional weight loss observed in the Apt-HJ-Dox group.

Apart from this, Nejabat M et al. [51] showed that covalent conjugation of DOX-encapsulated hollow mesoporous silica NPs (HMSNs) coated with acetylated carboxymethyl cellulose NPs to AS1411 aptamer (Apt-NP-DOX) led to a specific delivery of Dox to nucleolin-overexpressed MCF-7 cells. Based on flow cytometry analysis, the cellular uptake efficiency of Apt-NP-DOX was higher in nucleolin-overexpressed MCF-7 cells than conventional Dox, HMNS/AcCMC-DOX (NP-DOX). Additionally, no significant difference was observed between the cellular uptake of targeted and non-targeted formulations in nucleolin-negative CHO cells. However, nucleolin positive cell lines (i.e., MCF-7 cells and C26 colon cancer cells) demonstrated superior toxicity when treated with Apt-NP-DOX than NP-DOX. However, there no significant difference in cytotoxicity was observed between Apt-NP-DOX and NP-DOX in CHO cells. Conventional DOX showed higher cytotoxicity in all cell lines tested, as it has a small size and can freely enter cells via simple diffusion. Similarly, a single dosage of Apt-NP-DOX dramatically reduced tumor growth in BALB/C mice transplanted with C26 cells as compared to free Dox- and NP-Dox-treated groups. Interestingly, mice administered with Apt-NP-DOX did not cause body weight loss and death when compared to mice administered with NP-DOX and free Dox. The types of chemotherapy treatments and their drug delivery methods for GC, PC and CRCs are summarized in Table 3.

#### 4. Aptamers Bind to Different Molecular Targets as a Potential Cancer Treatment

Aptamers specifically bind to molecular targets in a cell, and they vary between surface proteins, peptides, toxins, and small molecules. They are selectively identified to distinguish cancer-related proteins and cells involved in tumorigenesis [52]. Identifying cancer biomarkers or molecular targets is crucial for improving cancer treatments, such as chemotherapeutic drug delivery, molecular imaging, and targeted molecular therapy. Many studies have identified various types of molecular targets for different types of GI cancer to be targeted by aptamers as potential cancer therapy. In this section, the different types of molecular targets targeted by aptamers as potential cancer treatments are listed for several types of GI cancers, including esophageal, gastric, liver, pancreatic, and colorectal.



**Table 3.** The types of chemotherapy cancer treatment and its drug delivery system for these different types of GI cancers.

Type of Cancer	Delivery System	Type of Chemotherapy	Main finding	Ref
Gastric	aptNCL	miRNA let-7d	<ul style="list-style-type: none"> <li>In MKN-45 cells, chimera containing aptNCL and miRNA let-7d resulted in a reduction in JAK-2 expression after treatment for 24 and 48 hours.</li> <li>Conjugates showed a more potent anti-proliferative effect against GC cells as compared with aptNCL alone (<math>p = 0.0001</math>).</li> <li>aptNCL-let-7d conjugate efficiently carried let-7d into cancer cells, leading to an inhibitory effect on MKN-45 GC cells.</li> </ul>	[54]
Pancreatic	ALPPL-2	5-fluoro-2'-deoxyuridine	<ul style="list-style-type: none"> <li>SQ2 aptamer was successfully internalized into ALPPL-2-expressing PC cells (i.e., Capan-1 and PANC-1 positive cells).</li> <li>The cellular absorption of SQ2 aptamer by ALPPL-2 is an energy-dependent process.</li> <li>SQ2 aptamer bound to ALPPL-2 with a low Kd of 20 nM.</li> <li>SQ2 aptamer could deliver the anticancer nucleoside, 5FdU, to ALPPL-2-expressing PC cells without inducing toxicity to normal ..</li> </ul>	[48]
Pancreatic	epidermal growth factor receptor (EGFR)	Gemcitabine-containing polymers	<ul style="list-style-type: none"> <li>E07 aptamer identified and bound to EGFR protein present on the surface of MiaPaCa-2 cells.</li> <li>Gemcitabine was delivered to MiaPaCa-2 cells via the E07 aptamer and led to cell death.</li> </ul>	[49]
Pancreatic	CD71 protein	Dox	<ul style="list-style-type: none"> <li>XQ-2d-sd3-DOX chimera identified to bound to CD71 protein in PL45 cells</li> <li>Inhibited proliferation in PL45 cells, a type of PC cell line.</li> </ul>	[40]
Colorectal	Apt-HJ	Dox	<ul style="list-style-type: none"> <li>Apt-HJ aptamer bound to nucleolin-positive CT26 cells with a high affinity.</li> <li>Dox-loaded Apt-HJ complex (Apt-HJ-Dox) could load roughly 17 Dox molecules into CT26 cells.</li> <li>Apt-HJ-Dox killed CT26 cells in a targeted manner.</li> </ul>	[50]
Colorectal	acetylated carboxymethyl cellulose-coated HMSNs NPs (Ac-CMC/HMSNs)	Dox	<ul style="list-style-type: none"> <li>Apt-NP-DOX was toxic to MCF-7 cells.</li> <li>Apt-NP-DOX delivered Dox to MCF-7 cells in a targeted manner.</li> <li>A single dosage of Apt-NP-DOX substantially reduced tumor development in BALB/C mice transplanted with C26 cells.</li> </ul>	[51]

#### 4.1. Esophageal Cancer

EC is listed as one of the most common types of GI cancer, and it is ranked the ninth most prevalent cancer worldwide. It consists of two subtypes, namely esophageal adenocarcinoma (EAC) and ESCC [32]. Although ESCC contributes to about 90% of total EC cases, EAC affiliates with higher mortality when compared to ESCC [53]. The lack of efficient

treatments due to poor diagnosis and prognosis in EC causes this dismaying outcome on a global scale [55]. EC often pervades and metastasizes into the adjacent organs prior to early detection, and this causes the existing cancer treatments to be less responsive to cancer cells [44]. Currently, the most common conventional treatments for EC are chemotherapy and curative surgery [32]. Recently, targeted therapy has been reflecting tremendous progress in improving the

diagnosis and prognosis of EC by applying aptamers targeting molecular targets [44].

Chen et al. [32] established three aptamers (i.e., S2, S3, and S8) and an ultrashort aptamer S3-2-3 using the cell-SELEX technology. These aptamers successfully bound to membrane proteins in ESCC tissues. The same study confirmed that the aptamers bound to membrane proteins in their selected KYSE150 cells but did not mention any specific membrane proteins. Because the target of the aptamers were membrane proteins, thus a decline in fluorescence signals of the aptamers was observed after the proteins were degraded with trypsin and proteinase K as compared to the untreated control. Apart from these, SOX2 protein, also known as sex-determining region Y-box 2 (SRY), is also a molecular target of ESCC cells, which has been shown to regulate the growth and maintenance of esophagus stem cells and overexpressed in different types of cancer malignancies contributing to the initiation, invasion, and migration. Liu et al. generated a peptide aptamer, P42, and a synthetic peptide aptamer, sP42, to specifically target SOX2 protein and suppress the malignant tendencies by inhibiting proliferation, migration, and invasion in ESCC cells. Treatment of both P42 and sP42 aptamers successfully repressed tumor growth and proliferation, as shown in xenograft mouse and zebrafish models. The ectopic expression of P42 aptamers significantly reduced about 86% of the tumor mass weight and inhibited the metastasis of ESCC cells in zebrafishes [55]. A more recent study by Zhang et al. [56] developed a few aptamers to detect ESCC-positive cells with cell-SELEX technology. The most successful aptamer to detect the ESCC-positive cells was an aptamer labelled A2. This study reports that the molecular target for aptamer A2 is a cell surface receptor protein, integrin beta-1 (integrin  $\beta$ 1), also commonly known as the CD29 protein. This aptamer contributed to the clinical imaging by emitting strong fluorescence signals when detecting ESCC-positive cells with adjacent cells. The aptamer A2 also showed potential to be a great drug delivery agent, it suppressed tumor growth in xenograft mouse models.

#### 4.2. Gastric Cancer

GC was ranked the third most common by World Health Organization in 2015 [19]. It is known as one of the aggressive malignancies because of its 5-year survival rate of less than 25% and is only identified in patients once it has developed to an advanced stage [33]. The most prevalent treatments for GC are curative and adjuvant treatments. Although many improvements have been made in these treatments, the lack of early detection and

vigorous advancement of tumors hinder their significant development [19, 33]. Therefore, much research has been done to identify molecular targets in targeted therapy and determine the best aptamer as a detection tool using *in vitro* screening [19]. Watanabe et al. [57] and Daei et al. [58] discovered that nucleolin could be a molecular target for GC-positive cells. They found that nucleolin was overexpressed in GC cells; thus, they generated aptamers specifically bound to nucleolin-positive cells instead of nucleolin-negative cells [57, 58]. Watanabe et al. used a well-known anticancer aptamer, AS1411, which surpassed the Phase I clinical trial to treat acute myeloid leukemia and kidney renal cell carcinoma. AS1411 aptamer specifically bound to nucleolin and triggered inhibition of GC cell growth and induced apoptosis. GC cell growth inhibition was determined with a dose-dependent addition of aptamers. MKN-1, a GC cell line with a low presence of nucleolin, showed a similar rate of cell growth inhibition as compared to MKN-74, a GC cell line with a high presence of nucleolin [57]. On the other hand, Daei et al. generated a conjugate of the AS1411 aptamer, known as an aptNCL-let-7d conjugate, bound to nucleolin to release let-7d, a tumor suppressor, into GC cells. Both aptNCL and aptNCL-let-7d conjugate significantly inhibited GC cell proliferation as compared to their counterparts for both 24 hours and 48 hours of treatment. In addition, aptNCL-let-7d triggered high production of let-7d in GC cells and significantly reduced tumorigenesis. It has been proved that let-7d has a role in anticancer activity through targeting oncogenes, such as JAK2/STAT3, RAS, high mobility group A2 (HMGA2), and cellular Myc (c-MYC), which are crucial for cancer progression [58].

JAK-2 protein plays a prominent role in regulating growth, invasion, and apoptosis in GC cells. It is also associated with the signal transducer and activator of the transcription (STAT) pathway contributing to cancer cell growth and proliferation. Ramezani et al. [54] attempted to use a nucleolin-specific aptamer with miRNA let-7d chimera to evaluate the effect on the expression of JAK-2. After treating JAK-2-positive GC cells with NCL-Apt-miRNA let-7d chimera, it was found that JAK-2 expression levels were significantly downregulated. This finding indicates that JAK-2 can be used as a molecular target in GC cells, and this specific chimera has the potential as a targeted therapy for GC. Furthermore, both control counterparts, NCL-Apt and miRNA let-7d aptamer, did not show a promising effect on JAK-2 expression in GC cells, implying that the combination of NCL-Apt and miRNA let-7d showed a great response in inhibiting JAK-2 activity [54].

Besides, several RNA aptamers have been designed to specifically bind to other identified molecular targets in GC, including CEA A01 and A02; cancer antigen 50 (CA50), A01, and A02; and CA72 A01 and A02. CEA is a surface protein that contributes to cell adhesion and intracellular signaling, while CA50 is a monoclonal antibody that has a prominent presence in GI cancers. CA72-4 is a tumor-associated antigen that is more sensitive than CEA. The combination of aptamers binding to their respective type of six antigens reduced GC cell viability and proliferation [19]. Lastly, aptamers have targeted another molecular target: ERBB-2/HER2. ERBB-2/HER2 is a receptor protein that has a crucial role in epitheliogenesis and has been shown to be overexpressed in GC cells. Mahlknecht et al. selected a trimeric aptamer, an aptamer with the trimeric version of the identical 14 selected nucleotides, to treat ERBB-2 positive GC cells. The treatment of trimeric aptamer targeting ERBB-2 significantly reduced cell growth and proliferation *in vitro* and *in vivo*. The study further showed that when the trimeric aptamer was introduced to ERBB-2-positive cells, the cell viability was suppressed successfully. As for the tumor growth in mice, the aptamer inhibited the growth of tumor mass as compared to the antibody treatment [59].

### 4.3. Liver cancer

LC is the fifth most common cancer and does have one of the highest mortality rates [37]. HCC is the most common type of LC; it has invasive and metastatic properties that fail many common treatment options [36]. Due to these properties, HCC is often unable to be diagnosed early in patients and is most likely to reoccur quickly. Despite having a low survival rate and high metastasis rate, the common treatments used for LC were curative surgery, chemotherapy, or locoregional therapies [37]. Therefore, there is an urgent need to search for a treatment that does not cause high toxicity and specifically targets LC cells [60].

AFP is the first tumor marker identified in LC and possesses an important role in diagnosing and treating LC. It is a major serum glycoprotein and is highly expressed in HCC cells. In a study by Lee and Lee [61], a specific aptamer was created to target AFP in HCC cells to regress the AFP overexpression and to activate cell growth inhibition to prevent HCC proliferation. Thus, they developed group I RNA aptamer, and it specifically bound to AFP and successfully inhibited AFP-mediated cell proliferation and overexpression of oncogene mRNA [61]. Similarly, Dong et al. also developed AP273 aptamer binding to AFP in AFP-expressing HCC cells. It was

shown that AP273 aptamer could inhibit HCC cell migration and invasion after the cells were transfected with AP273 [62]. Both studies have proved that AFP is an ideal molecular target for LC. It has been reported that only 30-40% of LC are AFP-negative; thus, a different molecular target is required for HCC. Heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) has been reported to contribute to mutant p53 expression that influences cell proliferation and oncogenic properties. Cell proliferation and migration regressed when hnRNP A1 are downregulated with siRNA-specific for hnRNP A1, siRNPA1, in HCC cells. Besides, a new aptamer targeting hnRNP A1 was also developed by Li et al. [63]. In this study, high levels of hnRNP A1 expression were detected in HCC cells using hnRNP A1-specific ssDNA aptamer, BC15, because it was proven to target hnRNP A1 specifically. This aptamer also inhibited tumor growth in a xenograft model transplanted with HepG2 cells stably expressing an enhanced green fluorescent protein, HepG2-EGFP [63].

Anticancer drugs are often used in treating LC, particularly Dox. Due to the fact that Dox exerts significant side effects on the heart and liver, thus a more efficient anticancer drug delivery method is required to reduce toxicity and achieve active targeting in the tumor simultaneously. Zhou et al. discovered an aptamer-based drug delivery agent called CD133-apt-Dox. This aptamer was designed to target CD133. CD133 is highly expressed in LC, and it showed that CD133-apt is ideally bound to CD133-positive cells. The cellular uptake of CD133-apt-Dox was higher and more efficient than free Dox. Furthermore, CD133-apt-Dox also demonstrated stronger cytotoxicity against HCC cells and organoids, affected and impaired stem-likeness causing drug resistance in mice, and blocked CD133-mediated oncogenic pathway by reducing tumor growth and proliferation. These findings indicate that CD133 can be used as a molecular target for LC, and CD133-apt can be used as a drug delivery agent for Dox [64]. Similarly, in the most recent study by Yin et al. [65] it was reported that CD133 was the molecular target for CD133 aptamer-DOX conjugate as a drug delivery agent for a HCC cell line. This aptamer specifically bound to CD133-positive expressing cell line when compared to a CD133-negative expressing cell line and increased the uptake and retention of Dox in the HCC cell line. CD133 aptamer-DOX conjugate was proven to promote apoptosis and suppressed autophagy to enhance the efficiency of Dox treatment. A study done by Liu et al. [60] successfully designed an aptamer-mediated gene delivery to LC cells by using highly expressed EpCAM as the LC cell target. Ad5-PTEN is a recombinant adenovirus expressing

the tumor-suppressor gene of PTEN that has anticancer properties in reducing tumor proliferation, invasion, and migration. This study evaluated the delivery efficiency of the Ad5-PTEN gene to EpCAM-conjugated aptamers, EpDT3-mediated Ad5-PTEN (EpDT3-PEG-Ad5-PTEN, EPAP). EPAP treatment inhibited the migration capacity of EpCAM-positive LC cells and repressed their metastasis. Therefore, EPAP can be a good alternative cancer therapy for LC [60]. Dong et al. identified that GPC3, a cellular membrane proteoglycan that has been shown to overexpress in HCC, is another molecular target that has been conjugated with an aptamer for treating HCC. In fact, GPC3 is highly expressed in many HCC cells via antibody-based histopathological systems and has been identified as a tumor biomarker [38]. Another study on HCC evaluated the potential of an aptamer in targeting HCC cells and being a drug delivery agent. The result showed that the aptamer with an integrated ssDNA (Apt-07S-ASO-Plk1) bound to a *Plk1*. *Plk1* is a gene that is involved in cell proliferation and is highly expressed in HCC cells. *Plk1* gene silencing using ASODN in this selected aptamer could enhance HCC cell growth inhibition and decrease *Plk1* expression [37].

#### 4.4. Pancreatic cancer

Many researchers have utilized different targets of aptamers to develop agents that can be used for the targeted treatment in PC. A secretory protein termed pancreatic adenocarcinoma up-regulated factor (PAUF) is widely produced in PC, which has been implicated in PC cell proliferation and metastasis. In view of this, Kim et al. developed and tested a 2'-fluoropyrimidine modified RNA aptamer (P12FR2) to target PAUF. P12FR2 bound to PAUF with an apparent K<sub>d</sub> of 77 nM. In a wound-healing experiment, P12FR2 aptamer suppressed PAUF-induced PANC-1 cell migration. Furthermore, in an *in vivo* xenograft model transplanted with CFPAC-1 PC cells, intraperitoneal injection of P12FR2 significantly reduced tumor growth by up to 60% without inducing weight loss in the treated mice. They found that the aptamer P12FR2 had the potential to be useful in the treatment of PC [66]. Despite current chemotherapeutic agents and molecular target inhibitors, the 5-year survival rate of PDAC is still depressing. More appropriate multi-drug approaches are thus mandatory to flatten the rising prevalence rate of PDAC. Yoon et al. [41] proposed a novel aptamer-based strategy for delivering targeted molecular therapy in advanced PDAC patients who failed to respond to other treatments. The upregulation of the transcriptional factor CCAAT/enhancer-binding protein (C/EBP), known for its

anti-proliferative properties, was reported as a novel means of providing anti-tumorigenic therapy in PDAC. For the creation of a cell type-specific delivery vehicle, small activating RNA (saRNA) duplexes were designed to promote C/EBP expression, followed by attaching to PDAC specific 2'-Fluoropyrimidine RNA aptamers (2'F-RNA) - P19 and P1. Intriguingly, P19- and P1-C/EBP-saRNA conjugates drastically reduced PDAC cell proliferation while increasing C/EBP expression. Similarly, it was found that injections of saRNA/aptamer conjugates into the tail veins of xenograft mice, either transplanted with PANC-1 or gemcitabine-resistant AsPC-1 cells, reduced tumor size with no significant side effects [41].

Dua et al. [67] identified an aptamer SQ-2 that could recognize PC cells with a high specificity using a selection strategy that could generate aptamers for two PC cell lines in one selection scheme. ALPPL-2, an oncofetal protein, was identified as the target of SQ-2. ALPPL-2 gene silencing via RNA interference revealed new tumor-associated roles of this protein in PC cell proliferation and invasion. Furthermore, in a different study, the target of aptamer P15 was identified as the intermediate filament vimentin, a biomarker of epithelial-mesenchymal transition (EMT), which is an intracellular protein but is specifically expressed on the plasma membrane of cancer cells, according to the results of an unbiased proteomic mass spectrometry approach. Tumor cell metastasis assays were performed *in vitro* because EMT plays a critical role in the transition of cancer cells to invasive cells. The tumor metastasis of P15-treated PC cells was significantly reduced. EMT-related gene expression analysis was used to identify differentially expressed genes to investigate the downstream effects of P15 (DEG). The expression of matrix metalloproteinase 3 (MMP3), which is involved in cancer invasion, was downregulated in P15-treated cells among five DEGs. P15 binding to cell surface vimentin inhibited tumor cell invasion and was associated with lower MMP3 expression, implying that P15 could be used as an anti-metastatic therapy in PC [68].

G-protein-coupled cholecystokinin B receptor (CCKBR) is expressed constitutively in PDAC, which plays a crucial role in cell proliferation and apoptosis. It has been proven that when CCKBR is downregulated, cell proliferation decreases, and cell apoptosis increases [69]. Clawson et al. [70] demonstrated DNA aptamers' characterization and targeting efficiency binding to CCKBR. A pool of DNA aptamers was discovered using dual SELEX selection against "exposed" CCKBR peptides and CCKBR-expressing PDAC cells. Eight DNA aptamers were chosen for initial characterization after selection



based on projected structures and features. The DNA aptamers bound exclusively to CCKBR and found that they suppressed cancer cell proliferation and reduced PDAC cell growth. Only one aptamer, dubbed AP1153, was chosen for further binding and localization experiments because it possessed the most reliable predicted secondary structure. Clawson et al. discovered that AP1153 aptamer did not activate CCKBR signaling pathways, and AP1153 aptamer was internalized by PDAC cells in a receptor-mediated manner, as visualized using three-dimensional confocal microscopy. The binding affinity of AP1153 aptamer was found to be 15 pM. The bioconjugation of AP1153 aptamer to the surface of fluorescent NPs made NP delivery to PDAC tumors much easier. This AP1153-targeted NP delivery system's selectivity improved the early detection of PDAC lesions and enhanced chemotherapeutic treatments for PDAC patients [70]. In PC cells, increased expression of high mobility group A (HMGA) protein is linked to resistance to gemcitabine [71]. The use of HMGA-targeted AT-rich phosphorothioate DNA (AT-sDNA) aptamers to reduce HMGA carcinogenic activity was established by Watanabe et al. [71]. After treatment with gemcitabine, AsPC-1 and MiaPaca-2 cell transfected with AT-sDNA was evaluated. Both cells showed a significant increase in cell death when AT-sDNA-transfected cells were compared to non-AT-rich sDNA-treated cells. The findings suggested that HMGA targeted DNA aptamers could be used to improve chemotherapy efficacy in PC treatment [71]. Lastly, a different study showed that PC cells also highly expresses CD71 providing potential as a molecular target for combating PC. In this study, they generate an aptamer that is specific to CD71, paclitaxel (PTX)-modified polydopamine (PDA) nanospheres with the conjugation of peptidomimetic AV3 called Apt-PDA@PTX/AV3 bioconjugates. *In vitro*, this aptamer specifically inhibited cell survival and promoted cell apoptosis within PC-expressing cells. *In vivo*, this aptamer successfully showed results in inhibiting desmoplastic stroma, a result that corresponds with a poor prognosis, showing great potential in efficiently suppressing PC cells [72].

#### 4.5. Colorectal Cancer

CRC is one of the most frequent cancers, with rates of diagnosis and incidence steadily rising [73]. CEA is overexpressed in CRC patients and is linked to cell adhesion, anoikic resistance, and metastasis to the liver [74]. 5-fluorouracil, 5-FU, is one of the most frequently used chemotherapeutic agents to treat CRC [75]. However, chemoresistance is a severe issue with 5-FU therapy because it causes CEA overexpression,

subsequently causing 5-FU resistance. In a mouse model, a CEA-specific RNA aptamer was found to suppress CRC cells' hepatic metastasis. In chemoresistant LS174T CRC cells, Lee and Lee [76] investigated if shielding CEA with CEA aptamer could improve 5-FU sensitivity. When compared to cells treated with 5-FU alone (IC<sub>50</sub> 31.46 M), CEA aptamer sensitized 5-FU-resistant LS174T cells to 5-FU by more than 5-fold (IC<sub>50</sub> of 5.995 M). Furthermore, in a xenograft mouse model, CEA aptamer coupled with 5-FU synergistically reduced the growth of chemoresistant tumors. In the 5-FU-resistant LS174T and xenograft tumors, a combination of 5-FU and CEA aptamer increased caspase-8 activity via aptamer-mediated disruption of CEA interaction with death receptor 5. CEA-specific aptamer increased 5-FU sensitivity in both *in vitro* and *in vivo* studies, thus constituting a novel 5-FU adjuvant to overcome chemoresistance in CRC patients [76].

One of the most significant drawbacks in cancer treatment is the development of chemoresistance and the failure to eliminate CSCs [77]. For future effective management of cancer, novel molecular therapeutic methods to overcome these constraints are urgently needed. AlShamaileh et al. [78] demonstrated that EpCAM-aptamer-guided survivin RNAi successfully downregulated survivin in CRC cells and a xenograft mouse model. Besides, the aptamer-guided survivin RNAi improved the sensitivity to 5-FU or oxaliplatin in CRC stem cells, increased apoptosis, inhibited tumor growth, and improved the overall survival of mice bearing xenograft CRC when used in combination with conventional chemotherapeutic agents. The findings suggest that survivin is one of the essential factors in CRC stem cells' innate chemoresistance. As a result, aptamer-mediated survivin targeting in CSCs combined with chemotherapeutic agents could offer a novel way to improve treatment outcomes in oncology clinics [78]. MiRNAs, a tumor suppressor gene, are overexpressed in CRC and have been reported to prevent or delay cancer progression [79]. However, using miRNAs as therapeutic agents needs to resolve several challenges, including a rapid breakdown in plasma, low absorption, and off-target effects [80]. For the targeted therapy of CRC, Zhao et al. [80] established a cationic liposome-based nanoparticle loaded with miR-139-5p (miR-139-5p-HSPC/DOTAP/Chol/DSPE-PEG2000-COOH NPs) known as MNPs and miR-19-5p surface-decorated with EpCAM aptamer (miR-139-5p-EpCAM Apt-HSPC/DOTAP/Chol/DSPE-PEG2000-COOH) known as MANPs, which were  $136.1 \pm 4.8$  nm and  $150.3 \pm 8.8$  nm in size, with a round form and functional dispersion capabilities. MANPs signifi-

cantly reduced CRC cell proliferation, migration, and invasion. The study also showed that MANPs could be taken up and targeted *in vitro* and *in vivo*. It also found that MANPs reduced the development of HCT8 cells and displayed a strong tumor-suppressive effect in subcutaneous HCT8 colorectal carcinoma mice [80]. The findings show that MANPs is a good carrier for delivering therapeutic miRNAs to CRC patients. MANPs prove to be an efficient targeted therapy by inducing anticancer activities. Another recent study [81] reported an aptamer with magnetic mesoporous silica nanoparticles with EpCAM showed efficient delivery of 5-FU to CRC cancer cells. This aptamer, Apt-PEG-Au-NPs@5-FU, targets EpCAM in EpCAM-expressing CRC cell line, induces cell apoptosis *in vitro* and inhibits tumor growth *in vivo*. Moreover, this aptamer increases uptake of the chemotherapeutic drug showing great potential is efficient drug delivery of 5-FU in CRC.

Lastly, S100P functions as a  $\text{Ca}^{2+}$  binding protein in calcium-dependent signal transduction pathways involved in various biological activities. It has been shown to play a crucial role in promoting cell proliferation, invasion, and metastasis in CRC tissues and is overexpressed in CRC [82]. As a result, S100P could be a promising molecular target for CRC treatment. For instance, Sun et al. [82] used the SELEX technique and high-throughput sequencing to create a new DNA aptamer targeting S100P (AptS100P-1). AptS100P-1 had a significant affinity for S100P protein, as evidenced in the binding experiment. AptS100P-1 was also relatively stable in a cell culture system and could be utilized to detect S100P via flow cytometry, dot blot assays, and fluorescence microscopy. Besides, it also attached and inhibited CRC cell growth both *in vitro* and *in vivo*. AptS100P-1 also prevented S100P-expressing CRC cells from migrating and transitioning from epithelial to mesenchymal cells by suppressing the expression of EMT protein and overturning the EMT process. The findings indicate that using AptS100P-1 to target S100P could be a viable treatment strategy for CRC [82]. Figure 4 below mentions the different types of molecular targets targeted by aptamers as potential cancer treatments in GI cancers and Table 4 below mentions all the relevant functions and molecular mechanisms of aptamers associated with molecular targets in GI cancers.

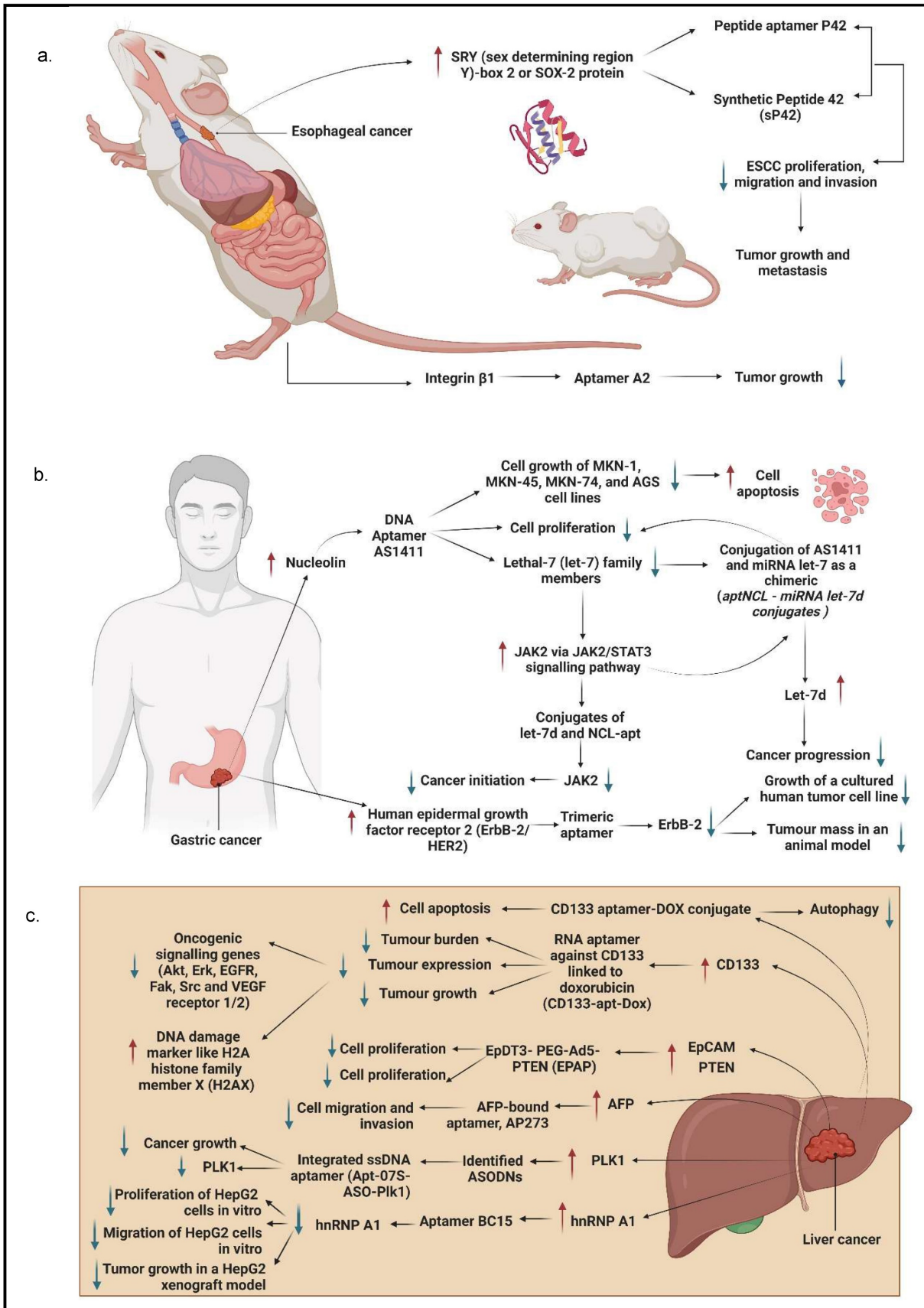
## 5. Current Applications of Aptamers in Clinical Settings for GI Cancer Treatment

The number of clinical trials evaluating aptamer-based drugs is increasing due to their great potential as prognostic, diagnostic, and therapeutic tools. Therapeutic aptamers are promising agents for

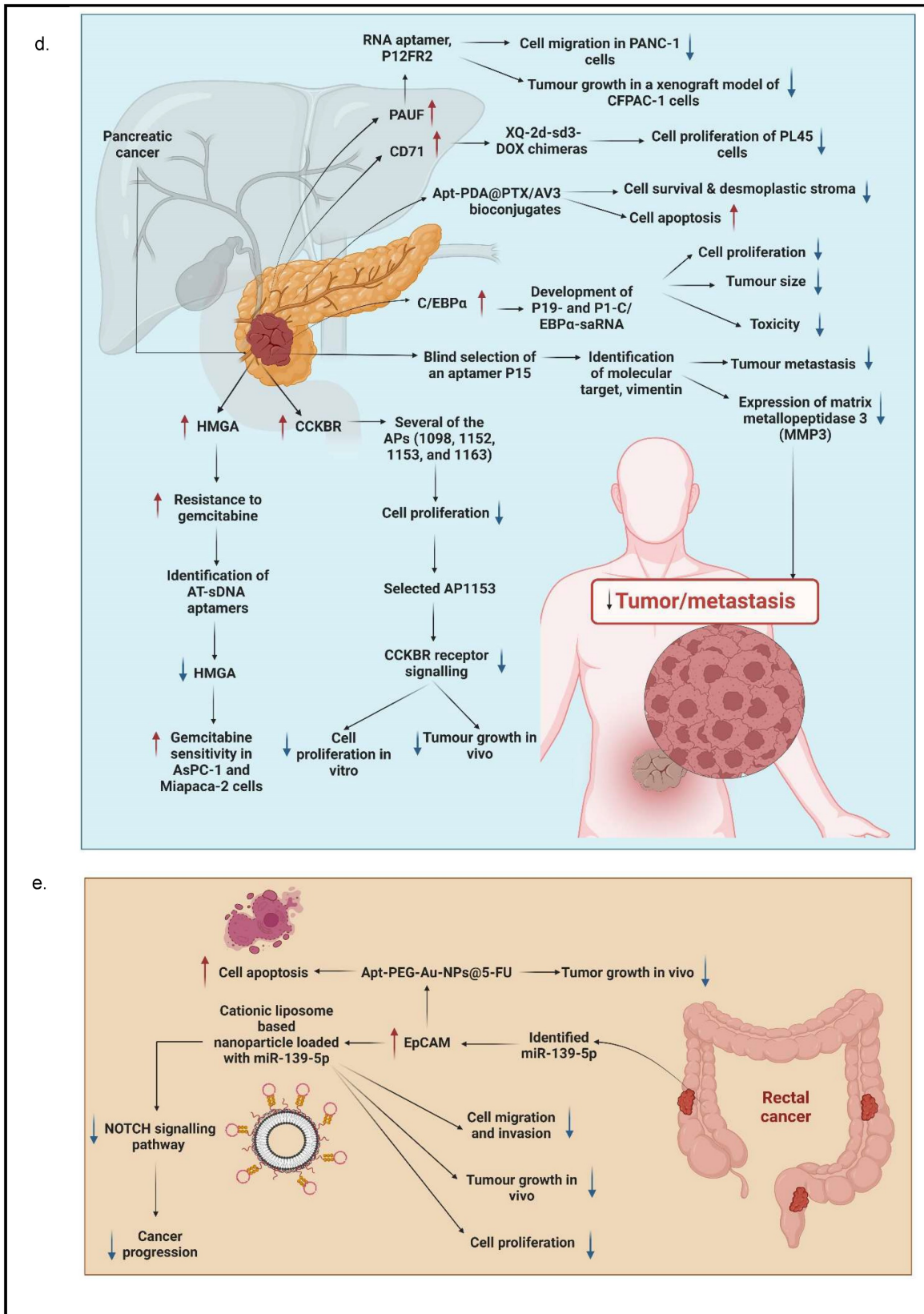
treating GI cancers in the clinical setting because they can efficiently differentiate between normal and tumor tissues, as well as between different tumor types and can exert better tumor-killing effects [83]. In preclinical trials, few therapeutic aptamers with antagonistic functions have shown high affinity, specificity, and inhibitory effects against GI cancers. Additionally, preclinical studies also validate their efficacies and safety profiles [84]. Up to now, mounting evidence has reported that two anticancer aptamers have been successfully tested in clinical trials, namely AS1411 and NOX-A12 aptamers. AS1411, a nucleolin-targeting DNA aptamer, is the first aptamer that has made a successful transition to clinical trials for cancer treatment. It shows an effective inhibition against nucleolin function in cancer cells and displays an immense anti-proliferative effect in solid tumors [85] and renal cell carcinoma [86, 87]. While NOX-A12 is a second 45-nt L-ribose-based RNA therapeutic aptamer, which is an antagonist for chemokine CXCL12 (or SDF1). It is also known as a Spiegelmer because of its mirror-image oligonucleotides that have high resistance to nucleases. In preclinical trials, NOX-A12 effectively prevented chemotaxis of hematologic malignancies [88] and is currently in clinical trials testing on CRC or PC [89]. On the other hand, a clinical trial study with 40 participants for PC has completed its trial for a cancer stem cell specific aptamer to study the prognostic role detecting the cancer stem cells but no results or articles has been published based on this study [90]. To date, the literature shows that the research on RNA and DNA aptamers has expeditiously increased, but the development of clinically useful aptamer therapeutics is growing slowly. This section highlights the current advances and applications of RNA, DNA, and conjugation of aptamers in research and clinical trials.

### 5.1. RNA aptamers

RNA aptamers have unique advantages for therapeutic and diagnostic applications because of their high binding affinity and specificity for cancer treatment. To date, there are still few RNA aptamers under preclinical and clinical trials for cancer treatment. As mentioned above, NOX-A12 is a well-established RNA aptamer in clinical trials, and, currently, clinicians are testing its anti-proliferative effectiveness on CRC or PC [91]. The above findings imply that research on RNA aptamers is still in its infancy, where it requires further understanding of nucleic acid chemistry target interaction, tissue distribution, and pharmacokinetics. Therefore, only a few RNA aptamers have reached the market after several challenges.







**Figure 4.** Different types of molecular targets targeted by aptamers as potential cancer treatments. a. Potential aptamer-based treatments in esophageal cancer; b. potential aptamer-based treatments in gastric cancer; c. potential aptamer-based treatments in liver cancer; d. potential aptamer-based treatments in pancreatic cancer; and the potential aptamer-based treatments in colorectal cancer. This figure is created with biorender.com.



**Table 4.** Molecular targets and aptamers for specific type of GI cancers by literature search. The respective functions and molecular mechanisms of aptamers are mentioned below.

Type of cancer	Molecular targets	Aptamers	Function of aptamer	Molecular Mechanism	Ref
Esophageal	SRY (sex determining region Y)-box 2 or SOX-2 protein	several peptide aptamers including P42, Synthetic Peptide 42 (sP42)	Treatment tool	The P42 aptamer can inhibit cell proliferation, cell migration and cell invasion when overexpressed and tagged to SOX2 protein in ESCC cell lines. Treatment using this aptamer significantly reduced tumor growth and metastasis.	[55]
Esophageal	integrin $\beta$ 1	A2 aptamer	Treatment tool, drug delivery agent	The A2 aptamer targets ESCC-positive cells that expresses integrin $\beta$ 1 and suppresses tumor growth <i>in-vivo</i> .	[56]
Gastric	nucleolin	DNA aptamer AS1411	Treatment – anti-cancer therapy	AS1411 aptamer bound to nucleolin inhibits GC cell lines via S-phase cell cycle arrest.	[57]
Gastric	nucleolin	AS1411 aptamer (aptNCL) and let-7d	Targeted therapy	This aptamer inhibits cell proliferation in nucleolin-overexpressing cells.	[58]
Gastric	JAK-2	the conjugate of NCL-Apt and miRNA let-7d	Targeted therapy	In JAK-2 overexpressed cells, this nucleolin-specific aptamer–miRNA let-7d chimera showed anti-tumor effects by inhibiting JAK-2 expression and activities.	[54]
Gastric	ERBB2/HER2	trimeric aptamer	Treatment tool	This aptamer inhibits cell growth in ERBB2/HER2-expressing cells <i>in vivo</i> and <i>in vitro</i> .	[59]
Liver	CD133 protein	CD133 aptamer-DOX conjugate	Drug delivery agent	CD133 aptamer-DOX binds to CD133-expressing cells and promotes apoptosis and inhibits autophagy.	[65]
Liver	EpCAM	RNA aptamer EpDT3 and PEG-Ad5-PTEN modified	Treatment tool	EPAP inhibited cell growth and motility in EpCAM-expressing cells <i>in vitro</i> . The aptamer alone expressed similar characteristics <i>in vivo</i> .	[60]
Liver	AFP	AP273	Targeted therapy, diagnostic/prognostic tool	This aptamer specifically binds to AFP-expressing cells and inhibits cell motility <i>in vitro</i> .	[62]
Liver	Protein Plk1	integrated ssDNA (Apt-07S-ASOPlk1)	Drug delivery agent	This aptamer inhibited cell growth <i>in vitro</i> and showed characteristics of being potentially used for anti-cancer drug delivery because it preferably binds to protein PLK1-expressing	[37]
Liver	heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1)	hnRNP A1-specific ssDNA aptamer, BC15	Targeted therapy, treatment tool	This aptamer can inhibit cell proliferation and migration <i>in vivo</i> and tumor growth <i>in vitro</i> .	[63]

Type of cancer	Molecular targets	Aptamers	Function of aptamer	Molecular Mechanism	Ref
Pancreatic	pancreatic adenocarcinoma up-regulated factor	2'-fluoropyrimidine modified RNA aptamer (P12FR2)	Treatment tool	This aptamer suppresses cell migration in PAUF-expressing cells <i>in vitro</i> and reduces tumor growth <i>in vivo</i> .	[66]
Pancreatic	CD71 protein	ssDNA aptamer, XQ-2d	Targeted therapy	This aptamer specifically binds to CD71 and has potential use in targeted therapy and drug delivery. XQ-2d-sd3-DOX chimeras have abilities to deliver Doxorubicin drugs to CD71-expressing cells to	[40]
Pancreatic	transcriptional factor CCAAT/enhancer-binding protein	P19- and P1-C/EBP-saRNA aptamer conjugates	Targeted therapy	C/EBPa-conjugated P19 and P1 aptamers exhibited inhibition of cell proliferation <i>in vitro</i> and reduction of tumor growth <i>in vivo</i> .	[41]
Pancreatic	cell surface Vimentin (CSV)	Aptamer P15	Treatment - anti-metastatic therapy	This aptamer suppresses cell migration and invasion in vimentin-expressing pancreatic cell lines.	[68]
Pancreatic	G-protein-coupled cholecystokinin B receptor (CCKBR)	DNA aptamer AP1153	Treatment - Chemotherapeutic	This aptamer disabled the CCKBR receptor signaling and inhibited cell proliferation <i>in vitro</i> and tumor growth <i>in vivo</i> .	[70]
Pancreatic	high mobility group A (HMGA) protein	AT-rich phosphorothioate DNA (AT-sDNA) aptamers	Treatment - Chemotherapeutic	This aptamer preferably binds to HMGA-overexpressing cells and inhibits cell growth. This aptamer restored the chemosensitivity of gemcitabine.	[71]
Pancreatic	CD71 protein	Apt-PDA@PTX/A V3 bioconjugates	Treatment - Chemotherapeutic	This aptamer specifically inhibits cell survival and promotes cell apoptosis within PC-expressing cells <i>in vitro</i> and successfully showed results in inhibiting desmoplastic stroma <i>in vivo</i> .	[72]
Colorectal	EpCAM	epithelial cell adhesion molecule (EpCAM) aptamer (Apt) (miR-139-5p-EpCAM Apt-	Targeted therapy	This aptamer specifically delivers miRNAs to EpCAM-positive cells and inhibits cell growth and motility. It also has anti-tumor properties <i>in vivo</i> .	[80]
Colorectal	EpCAM	Apt-PEG-Au-NPs@5-FU	Targeted therapy, drug-delivery agent	This aptamer induces cell apoptosis <i>in vitro</i> and inhibits tumor growth <i>in vivo</i> .	[81]

## 5.2. DNA aptamers

DNA aptamers are well explored as compared to RNA aptamers because of their stability which allows them to be used as a diagnostic tool. As discussed earlier, AS1411 is a well-known first nucleolin-targeting DNA aptamer and is used for cancer treatment in clinical trials. It shows promising anti-proliferative activity against different cancer types, such as colon, liver, lung, breast, and cervical [92]. A clinical trial in its early phase is studying Sgc8, a DNA aptamer, that is being used to measure the radiation exposure via radiation therapy of  $^{68}\text{Ga}$ -Sgc8, a protein, in healthy participants and to study its diagnostic value in colorectal cancer patients but no results are available [93]. These preclinical and clinical results suggest that DNA aptamers are highly in demand and have the potential to be used in GI cancer identification and treatment.

## 5.3. Conjugation of Aptamers

Aptamers are broadly used in cancer research due to their specificity, and flexibility, and can be structurally modified in order to conjugate with various molecules (e.g., fluorescent agents, NPs, quantum dots, etc.) to increase their targeting efficiency for drug delivery to cancerous tissues. Even though aptamer drug conjugates are well applied in cancer research, it has not progressed to clinical trials [94]. Although there are several promising research investigating on potential conjugation of aptamers with various molecules, such as fluorescent agents, NPs, quantum dots, etc., for cancer therapy, much work needs to be done prior to translating into clinical practice, including reducing the toxicity of the conjugate and improving the binding specificity and sensitivity to target cells.

## 6. Conclusions and Future Perspectives

Aptamers are discovered via cell SELEX technology and are currently and widely used in cancer diagnosis, as a prognostic tool, and as potential biomarker discoveries. They are better alternatives to antibodies for their low toxicity, easy production, and no limitations against their targets' size. Cell SELEX technology is currently the most efficient procedure for selecting specific aptamers that bind to molecular targets or membrane proteins in GI cancers. However, GI cancers are more prevalent in Asia, primarily due to the lack of diagnostic and prognostic tools. Aptamers bind to cancer biomarkers, potentially improving the diagnosis, prognosis, and treatments for GI cancers. They are often known for their versatility because they can be easily modified specifically to their aimed targets. Besides, they can be

generated specifically to target biomarkers in various secondary or tertiary structures. Their application can detect and differentiate cancer cells with specific affinity and act as a drug delivery system for chemotherapy treatments. There are a few types of aptamers, including RNA, DNA, and conjugate aptamers. DNA aptamers are widely studied for their stability in cancer treatments when compared to RNA aptamers, although RNA aptamers are discovered to have high specificity and binding affinity in cancer-related studies. On the other hand, conjugated aptamers are favored in chemotherapeutic treatments for their increased target efficiency as drug delivery agents. RNA aptamers are easily synthesized but have the possibility of breaking down easily in our system, and conjugated aptamers need more studies done to determine their toxicity. In this review, we have briefly discussed the generation of aptamers, the Cell SELEX process, types of aptamers, several different GI cancers associated molecular targets, the application of aptamers as a cancer biomarker, diagnostic or prognostic tool, and chemotherapeutic drug delivery agents. The GI cancers we reviewed were esophageal, gastric, liver, pancreatic and CRCs. In summary, the identified molecular targets for EC it was SOX-2 protein, for GC they were nucleolin, JAK-2 protein and ERBB2/HER2 protein, for LC they were CD133, EpCAM, AFP, Protein Plk1 and hnRNP A1 proteins, for pancreatic cancer they were (PAUF), CD71, C/EBP, CSV, CCKBR, HMGA proteins and lastly for CRC it was EpCAM protein. As for applications of aptamers in diagnosis or prognosis and chemotherapy, we also identified the key aptamers in esophageal, gastric, liver, pancreatic, and CRCs. In EC, the aptamers S2, S3, and S8 demonstrated characteristics to be effective diagnosis and prognosis tools. In GC, the aptamers cy-apt-20 and aptNCL demonstrated roles as cancer biomarkers and as drug delivery agents, respectively. In LC, aptamers that contributed as cancer biomarkers and diagnostic or prognostic tools were identified, and they were JHIT1 to JHIT7, Apt-07S, y119, y119a, y123, and LY-1. In pancreatic cancer, M9-5, Aptamer 1 & 146 were identified as diagnostic markers, and the chemotherapeutic drug delivery methods used were through the determined molecular targets such as ALPPL-2, EGFR, and CD71 proteins. As for CRC, there was only one aptamer identified as a diagnostic or prognostic tool, ApC1 and the drug delivery method was through aptamers loaded with Dox, Apt-HJ-Dix, and Apt-NP-DOX. Aptamers-based cancer treatments, diagnosis, or prognosis have and are still facing many obstacles prior to reaching the end user markets due to their inadequate sensitivity and specificity as cancer diagnostic and prognostic tools. Although

aptamers have drawbacks, there is still a crucial need for aptamer-associated cancer biomarkers and molecular targets to diagnose, diagnose, and treat GI cancers. Additionally, current studies on the application of aptamers are limited to common GI cancers, such as esophageal, gastric, liver, pancreatic, and colorectal. Thus, it is necessary to also investigate their applicability in the less common GI cancers like anal cancer. Further studies need to be done to determine aptamers with high specificity and affinity to GI cancers for overcoming insufficient sensitivity, effective molecular targets as potential cancer biomarkers, the functionality of relevant targets, and aptamers in GI cancer prognosis, diagnosis, and treatment. Currently, the application of aptamers in pre-clinical phases is limited to several types of GI cancers, and aptamers should be widened out with their potential applications and tested for their function as diagnostic, prognostic, bio-sensing, or imaging tools as well as therapeutic agents, targeting molecular targets, and as effective drug delivery systems.

## Abbreviations

GI: Gastrointestinal cancer; SELEX: Systematic evolution of ligands by exponential enrichment; CRC: colorectal cancer; GC: stomach/gastric cancer; LC: liver cancer; EC: esophageal cancer; PC: pancreatic cancer; hCG: human chorionic gonadotrophin; CEA: carcinoembryonic antigen; AFP: alpha-fetoprotein; ERBB2/HER2: human epidermal growth factor receptor 2; DNA: deoxyribonucleic acid; RNA: ribonucleic acid; NPs: nanoparticles; PCR: polymerase chain reaction; LOD: limit of detection; ESCC: esophageal squamous cell carcinoma; FITC: fluorescein isothiocyanate; CK19: Cytokeratin 19; GPC3: Glypican-3; HCC: Hepatocellular carcinoma; ssDNA: single-stranded DNA; CD71: transferring receptor-1; Tf: transferrin; SET: surface energy transfer; PDAC: pancreatic ductal adenocarcinoma; MMP14: Matrix Metalloproteinase 14; THY1/CD90: Thy-1 membrane glycoprotein; NUPACK: Nucleic Acid Package; Kd: dissociation constant; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EpCAM: epithelial cell adhesion molecule; ER: estrogen receptors; PR: progesterone receptors; ASODN: antisense phosphorothioate oligodeoxynucleotide; Plk1: polo-like kinase-1; PBS: Phosphate-buffered saline; CypB: Cyclophilin B; CSC: cancer stem cells; FACS: Fluorescence-activated cell sorting; EphA2: ephrin type-A receptor 2; miRNA: microRNA; rDNA: ribosomal DNA; Tip $\alpha$ : TNF- $\alpha$  inducing protein; H.pylori: *Helicobacter pylori*; JAK-2: Janus kinase 2; ELISA: enzyme-linked immunosorbent assay; HDF: human derma fibroblast;

TAMRA: Tetramethylrhodamine; ALPPL-2: alkaline phosphatase placental-like 2; GPI-AP: glycosylphosphatidylinositol-anchored proteins; 5FdU: 5-fluoro-2'-deoxyuridine; EGFR: epidermal growth factor receptor; Dox: Doxorubicin; dsDNA: double stranded DNA; CHO: Chinese hamster ovary; IC50: half maximal inhibitory concentration; HMSNs: hollow mesoporous silica NPs; EAC: esophageal adenocarcinoma; SOX2/SRY: sex-determining region Y-box 2; integrin  $\beta$ 1: integrin beta 1; STAT: Signal transducer and activator of transcription; HMGA2: high mobility group A2; c-MYC: cellular MYC; mRNA: messenger RNA; hnRNP A1: heterogeneous nuclear ribonucleoprotein A1; siRNPA1: siRNA-specific for hnRNP A1; PAUF: pancreatic adenocarcinoma up-regulated factor; C/EBP: CCAAT/enhancer binding protein; saRNA: small activating RNA; EMT: epithelial mesenchymal transition; MMP3: matrix metalloproteinase-3; CCKBR: cholecystokinin B receptor; HMGA: high mobility group A; AT-sDNA: AT-rich phosphorothioate DNA; PDA: paclitaxel (PTX)-modified polydopamine; 5-FU: 5-fluorouracil; S100P: S100 Calcium Binding Protein P; CXCL12/SDF1: chemokine CXCL12.

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## Author Contributions

Y.S.W. designed the flow of the review, planned the manuscript as well as supervised the entire work. K.W.G. and A.V. wrote and co-supervised the entire work. A.S., M.S.S., K.B., S.G., R.M.C., A.K., M.S., V.S., N.K.F., S.F., and M.M.R.F. collected the data and wrote the manuscript. MS made the table and figure for the subsections of the manuscript. AV revised the format of the manuscript. All the co-authors revised the final draft. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy. All authors have read and agreed to the published



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## Competing Interests

The authors have declared that no competing interest exists.

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