

# Immunotherapy for cancers of the biliary tract

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## Abstract

Cancer of the biliary tract (CBT) are relatively uncommon types of tumors. They are frequently diagnosed at a late stage with weak response to conventional therapies such as chemotherapy and radiotherapy. Thus a totally new and different treatment approach is needed. There is growing evidence about its genomic pathophysiology which may alter our notions regarding this disease. Numerous molecular pathways have been implicated and many may be targeted by new agents with high hopes for our patients. We review here the molecular background and the genetic / epigenetic abnormalities of the two main types of cancer of the biliary tract, gall bladder cancer (GBC) and cholangiocarcinoma (CC). Immunotherapy has become the promising new approach for many neoplasm, including CBT. We will go over the available evidence behind the current and emerging immunotherapeutics in CBT. They are categorized as Food Drug Association (FDA) or not approved, as there is an abundant number of new agents under examination, as monotherapies or in combinations with conventional chemotherapy, in clinical trials. More research is required at the molecular basis of CBT tumorigenesis in order to reach an individualized targeted therapy.

**Abbreviations:** CBT: cancer of the biliary tract ; GBC: gall bladder cancer; CC: cholangiocarcinoma; FDA: food drug association; IHCC: intrahepatic cholangiocarcinoma; EHCC: extrahepatic cholangiocarcinoma; PSC: primary sclerosing cholangitis; HCV: Hepatitis C Virus; HBV: Hepatitis B virus; HER: human epidermal growth factor receptor; EGFR: epidermal growth factor receptor; KRAS: Kirsten rat sarcoma viral oncogene homolog; MAB: Monoclonal antibody; ARF: alternate reading frame protein; p16INK4a: multiple tumor suppressor 1; WAF-1: cyclin-dependent kinase inhibitor 1; mdm-2: Mouse double minute 2 homolog; NKG2D: Natural Killer Group 2D; AID: Activation-induced cytidine deaminase; SNP: single nucleotide polymorphisms; c-myc: myelocytomatosis cellular oncogene; IL: Interleukin; JAK: janus kinase; STAT: signal transducer and activator of transcription; TGFβ: transforming growth factor beta; Smad4: mothers against decapentaplegic homolog 4; HER-2: human epidermal growth factor receptor 2; COX-2: Cyclooxygenase-2; NO: nitric oxide; VEGF: vascular endothelial growth factor; MAPK or MEK: mitogen-activated protein kinase; WAF1/CIP1: wild-type activating fragment-1/cyclin-dependent kinase inhibitory protein-1; JNK: Jun N-terminal kinases; Bcl-2: B-cell lymphoma 2; TRAIL: TNF-related apoptosis-inducing ligand; Fas: first apoptosis signal; DNA: deoxyribonucleic acid; CDKN1A: cyclin-dependent kinase inhibitor 2A; MSI: microsatellite instability; FHIT: fragile histidine triad gene; HTERT: human Telomerase reverse transcriptase; EGFR: epidermal growth factor receptor; PDGFR: platelet derived growth factor receptor; RAF: rapidly accelerated fibrosarcoma; ERK: extracellular signal-regulated kinases; RET: rearranged during transfection; KIT: mast/stem cell growth factor; RTK: receptor tyrosine kinase; HGFR: hepatocyte growth factor receptor; FLT-3: FMS-like tyrosine kinase 3; TIE-2: TEK tyrosine kinase, endothelial; TRKB: tropomyosin-related kinase B; AXL tyrosine kinase: tyrosine-protein kinase receptor UFO; FGFR: fibroblast growth factor receptors; ALK: anaplastic lymphoma kinase; FKBP-12: FK binding protein-12; mTOR: mammalian target of rapamycin; CSC: cancer cell stemness; Wnt: wingless-type mouse mammary tumor virus integration site family member; DKK1:

Dickkopf-1; ATP: adenosine triphosphate; TK: tyrosine kinases; IDH: isocitrate dehydrogenase; PI3K: phosphatidylinositol 3-kinase; mRNA: messenger Ribonucleic acid; CEA: carcinoembryonic Antigen; ICAM-1: intercellular adhesion molecule 1; LFA-3: lymphocyte function-associated antigen 3.

## Introduction/Epidemiology

Cancer of the Biliary Tract (CBT) is not a common form of cancer. It mainly includes gall bladder cancer (GBC), in about 2/3<sup>rd</sup> of the cases, and Cholangiocarcinoma (CC). These are often very aggressive malignancies as they are discovered at late stages of the disease. They carry a poor prognosis with little response to chemotherapy and radiation therapy. According to GLOBOCAN 2012, it was estimated that the incidence of GBC among males and females in the developed countries was 2,3/100,000 and 2,0/100,000 compared to 2.0/100,000 and 2,4/100,000 in developing countries, respectively. On the other hand, the mortality rates were slightly higher in the developing countries with 1,6/100,000 for males and 2,0/100,000 for females when compared to their counterparts in the developed world with 1,5/100,000 and 1,4/100,000, respectively [1]. In the Unites States, it is expected that 11,420 new cases will be diagnosed with GBC and 3,710 patients will die from it in 2016 [2].

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## Signal Transduction and Autoimmunity

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Then again, there are imprecise numbers for the worldwide and US incidence and mortality of CC. The reason is the lack of a single international classification of CC. It is divided into intrahepatic CC (IHCC) and extrahepatic CC (EHCC) which in turn is subdivided into perihilar CC and distal CC. Often, IHCC is included under primary liver cancers and sometimes there is confusion between intra and extrahepatic disease. In addition, there are major differences in its geographical distribution. Between 1997 and 2007, it had a high incidence in the East part of the globe, mainly Thailand, China and Korea, with the highest incidence is in the northeast of Thailand about 85 cases /100,000. While in the western parts of the world, it is much less with the highest recorded cases in Italy, UK and the US with 3,36/100,000, 2,27/100,000 and 1,67/100,000, respectively [3]. In the US, the incidence of IHCC and EHCC are almost similar, 0,88/100,000 and 0,72/100,000 from the period of 1998 to 2009, with a peak rate occurred at the age of 75 years and above [4]. It is worth noting that the incidence of IHCC is increasing worldwide, while the EHCC incidence is dropping [5-6].

### Risk factors

In most patients, CBT develops without a clear etiology; however, multiple risk factors for CBT have been established. The exact mechanism by which many of these risk factors lead to CBT is unidentified, but it may be that they are directly related to chronic inflammation in the biliary system [7]. This inflammation will cause dysplasia followed by genetic abnormalities [8-9].

**1. Hepatic infections with liver flukes:** parasitic infections with liver flukes, such as *Clonorchis sinensis* and *Opisthorchis viverrini*, are associated with a high incidence of IHCC and EHCC, especially in the Asian countries [10-11].

**2. Gall stones:** it is the most important risk factor for GBC. It causes long term inflammation leading to Porcelain gallbladder [8-9].

**3. Pathologic conditions affecting the biliary tree:** choledochal cysts, cholangitis/primary sclerosing cholangitis (PSC), secondary biliary cirrhosis, choledocholithiasis and liver flukes, are associated with both IHCC and EHCC development [10]. CC is found in 10-30% of adults with bile duct cysts [3]. The prevalence of cholangiocarcinoma in patients who have primary sclerosing cholangitis is 5% to 10%. [12]. Cholelithiasis and cholecystectomy are associated exclusively with EHCC other than gallbladder cancer. In contrast, HCV, HBV, hepatic schistosomiasis, hepatolithiasis and liver cirrhosis are associated exclusively with IH-CCA [3].

**4. Pancreaticobiliary maljunction:** it is a congenital anomaly of the union of the pancreatic and biliary ducts that is located outside the duodenal wall associated sphincter system. It is associated exclusively with EHCC and GBC. EHCC was diagnosed in 2% to 27% of the patients according to different case series. The incidence of PBM is thought to be 1.5-2.0% [13-15].

**5. Inflammatory bowel diseases:** Both ulcerative colitis and Crohn's disease increase the risk of cholangiocarcinoma [7, 16].

**6. Older age:** Older people are more prone as compared to younger people to develop bile duct cancer and gall bladder cancer [3].

**7. Obesity:** It increases the risk of gall bladder cancer and cholangiocarcinoma. [17, 18].

**8. Diabetes:** Some studies show that people with diabetes have a higher risk of bile duct cancer [7, 16].

**9. Alcohol and tobacco:** both Alcohol consumption and smoking increase the risk of getting Cholangiocarcinoma, like many other malignancies [7, 16].

**10. Industrial and environmental chemicals:** Workers in the rubber and textile industries are more likely to have gallbladder cancer than the general public [19].

**11. Typhoid:** Infection with salmonella increases the risk of gallbladder cancer [20].

### Pathophysiology and molecular basis of cholangiocarcinoma

During the development of CC, there are mutations in the genes such as K-ras, p53, p14ARF, p16INK4a and  $\beta$ -catenin. The incidence of p53 gene mutation is high and occurs in 20 to 80% of cases [21]; and in some cases, the p53 protein forms complexes with other molecules such as WAF-1 and mdm-2, which favors its inactivation [22].

NKG2D and AID (Activation-induced cytidine deaminase) genes are also found to play an important role in the development of CC. NKG2D is also known as a natural killer group 2, member D cell receptor and have a vital role in the tumor surveillance by cell-mediated cytotoxicity. In a recent study by Melum et al., it was noticed that two single nucleotide polymorphisms (SNPs) of the NKG2D gene were associated with an increased risk of CC in PSC-affected patients [23].

In CC patients, there was an increased production of AID and it results in the generation of somatic mutations in tumor-related genes such as p53, c-myc and the promoter region of the INK4A/p16 sequences [24].

The molecular pathways, which are involved in CC are: IL-6, JAK/STAT, TGF $\beta$ , Smad4, HER-2, COX-2, NO, apoptosis, VEGF, estrogens, neuropeptides and hormones. Check if there is others.

#### IL-6

There is an increased level of IL-6 in the bile and serum of CC affected patients. This cytokine stimulates cell proliferation by an autocrine/paracrine mechanism and specifically promotes the activation of p44/p42 and p38 mitogen-activated protein kinase (MAPK) pathways, which in turn, decrease the expression of p21 (WAF1/CIP1), a cell cycle controller protein [25].

#### JAK/STAT and TGF $\beta$

JAK/STAT pathway is one of the key signaling mechanisms in CC cells, mediating their resistance to apoptosis [26]. The mutations of TGF $\beta$  receptor and the alterations of intracellular signaling mediators (e.g., Smad4), together with the intracellular overexpression of cyclin D1 in CC cells, induce a resistance to the inhibitory effect of TGF $\beta$  cells [27].

#### ErbB-2

Cyclooxygenase-2 (COX-2) production is increased by ErbB-2, which forms a complex with a subunit of the IL-6 receptor. Additionally, it also implicates a close link between signaling of IL-6 and ErbB-2 [28].

#### COX-2 and NO

Inducible nitric oxide synthase enhances COX-2 expression through activation of p38 MAPK and JNK1/2. The link between these two proteins are implicated in the development and growth of CC [29].

## Apoptosis

Bcl-2 is an anti-apoptotic protein and is expressed in cholangiocarcinoma cells in a higher amount. Notch-1 and COX-2 reduce TRAIL-mediated apoptosis and high levels of COX-2 inhibit Fas-induced apoptosis in CC cells [30].

## VEGF

Biliary tumors proliferate as they are surrounded by a rich vascular network, which provides an adequate support of oxygen and metabolites to malignant cholangiocytes in order to enhance tumor development and growth [31].

## Pathophysiology and molecular basis of gallbladder cancer

GBC results from the accumulation of multiple genetic alterations, including oncogenes, tumour suppressor genes, DNA repair genes as well as microsatellite instability and important epigenetic alterations, represented mainly by methylation of the gene promoter areas. [32,33]

### Kras

Patients with an anomalous arrangement of the pancreaticobiliary duct have a higher frequency of mutation of the K-ras gene than subjects without this condition [34].

### p53

Mutations of the TP53 gene, have been found in 27 to 70% of GBC cases [35].

### p21/ CDKN1A

The expression of cyclin-dependent kinase inhibitor p21 has been observed in 28% of GBCs. Patients with no p21 expression and with p53 mutations survive longer than those with p21 expression and p53 mutation. On the other hand, patients without the expression of p21, but with the expression of p27 have a better survival rate than those positive for both p21 and p27 [36].

### p16/ CDKN2/INK4

Deletion of this gene has been observed in region 9p21 in half of all the GBCs. Inactivation of gene p16 in 41%, or by loss of heterozygosity in 11% or by methylation in 24% was also observed [37].

### COX-2

In adenocarcinoma, the expression varies from 59.2% to 71.9%, suggesting that COX-2 is involved early in gallbladder carcinogenesis [38].

There is a high degree of microsatellite instability (MSI) in 10% of GBC. The considerable reduction or loss of immunohistochemical reactivity of fragile histidine triad gene (FHIT) and the loss of its alleles is almost universal in GBC [39].

HTERT expression was found in 73 and 66% of gallbladder adenocarcinoma and the oncogene c-erb-B2 is associated with neoplastic progression in gallbladder carcinoma. Hypermethylation in gene promoter areas is a common epigenetic mechanism, inactivating these tumor suppressor genes [40].

## Immunotherapy for cholangiocarcinoma

Still, there is no FDA approved immunotherapeutic drug for

cholangiocarcinoma and gallbladder cancer.

Newer immunotherapeutic drugs are currently in clinical trials. These drugs target specific parts of cancer cells or their surrounding environments. Sorafenib, bevacizumab, pazopanib, and regorafenib are examples of the drugs that target blood vessel growth and are being studied as a therapy for CC.

Cetuximab and panitumumab are in the clinical trials as the therapy for CC, usually in combination with chemotherapy or other targeted drugs. Trametinib, an MEK inhibitor is also in clinical trial for CC.

### A. Monoclonal antibodies (MABs)

**a. Non FDA approved Monoclonal antibodies:** There is no FDA approved MAB available as the therapy for cholangiocarcinoma. The non-FDA approved MABs have been mentioned in the Table-1 below.

**1. Cetuximab:** A recombinant, chimeric monoclonal antibody directed against the epidermal growth factor (EGFR) with antineoplastic activity. Cetuximab binds to the extracellular domain of the EGFR, thereby preventing the activation and subsequent dimerization of the receptor; the decrease in receptor activation and dimerization may result in an inhibition in signal transduction and anti-proliferative effects. This agent may inhibit EGFR-dependent primary tumor growth and metastasis. EGFR is overexpressed on the cell surfaces of various solid tumors.

**2. Bevacizumab:** A recombinant humanized monoclonal antibody directed against the VEGF, a pro-angiogenic cytokine. Bevacizumab binds to VEGF and inhibits VEGF receptor binding, thereby preventing the growth and maintenance of tumor blood vessels.

**3. Panitumumab:** A human monoclonal antibody produced in transgenic mice that attaches to the transmembrane EGFR. Panitumumab may inhibit autocrine EGF stimulation of tumor cells that express the EGFR, thereby inhibiting tumor cell proliferation.

### B. Kinase inhibitors

**a. Non FDA approved Kinase inhibitors:** There is no FDA approved kinase inhibitor available as the therapy for cholangiocarcinoma. The kinase inhibitors that are under clinical trials have been mentioned in the Table-2 below.

**1. Sorafenib:** It is a synthetic compound targeting growth signaling and angiogenesis. Sorafenib blocks the enzyme RAF kinase, a critical component of the RAF/MEK/ERK signaling pathway that controls cell division and proliferation; in addition, sorafenib inhibits the VEGFR-2/PDGFR-beta signaling cascade, thereby blocking tumor angiogenesis.

**2. Regorafenib:** An orally bioavailable small molecule with potential antiangiogenic and antineoplastic activities. Regorafenib binds to and inhibits VEGF receptors 2 and 3, and Ret, Kit, PDGFR and Raf kinases, which may result in the inhibition of tumor angiogenesis and tumor cell proliferation. VEGFRs are receptor tyrosine kinases

**Table 1.** Non FDA approved Monoclonal antibodies [41-43].

Monoclonal Antibodies	Clinical trial identifier no.	Phase	Study Design	Target
Cetuximab	NCT01267344	Phase II	Randomized, Efficacy Study, double blind	EGFR
Bevacizumab	NCT01007552	Phase II	Safety/Efficacy Study, open label	VEGF
Panitumumab	NCT00779454	Phase II	Non-Randomized, Safety/Efficacy Study, open label	EGF

**Table 2.** Non FDA approved Kinase inhibitors [44-50].

Kinase Inhibitors	Clinical trial identifier no.	Phase	Study Design	Target
Sorafenib	NCT00955721	Phase I, Phase II	Non-Randomized, Safety/Efficacy Study, open label	VEGF
Pazopanib	NCT01438554	Phase I	Safety Study, open label	VEGFR-1,2,3, c-kit and PDGF-R
Regorafenib	NCT02053376	Phase II	Safety Study, open label	VEGF
Trametinib	NCT02042443	Phase II	Randomized, Efficacy Study, double blind	MEK
Cabozantinib	NCT01954745	Phase II	Efficacy Study, open label	RTK
BGJ398	NCT02150967	Phase II	Safety/Efficacy Study, open label	FGFRs
LDK378	NCT02374489	Phase II	Safety Study, open label	ALK

**Table 3.** Non FDA approved mTOR inhibitors [51].

mTOR inhibitors	Clinical trial identifier no.	Phase	Study Design	Target
RAD001	NCT00973713	Phase II	Randomized, Efficacy Study, open label	FKBP-12

**Table 4.** Non FDA approved Cancer Cell Stemness Inhibitors (CCSIs) [52].

CCSIs	Clinical trial identifier no.	Phase	Study Design	Target
BBI503	NCT02232633	Phase II	Safety/Efficacy Study, open label	Cancer stem cell

that play important roles in tumor angiogenesis; the receptor tyrosine kinases RET, KIT, and PDGFR, and the serine/threonine-specific Raf kinase are involved in tumor cell signaling.

**3. Trametinib:** An orally bioavailable inhibitor of mitogen-activated protein kinase kinase (MEK MAPK/ERK kinase) with potential antineoplastic activity. Trametinib specifically binds to and inhibits MEK 1 and 2, resulting in an inhibition of growth factor-mediated cell signaling and cellular proliferation in various cancers. MEK 1 and 2, dual specificity threonine/tyrosine kinases often upregulated in various cancer cell types, play a key role in the activation of the RAS/RAF/MEK/ERK signaling pathway that regulates cell growth.

**4. Pazopanib:** A small molecule inhibitor of multiple protein tyrosine kinases with potential antineoplastic activity. Pazopanib selectively inhibits VEGFR-1, -2 and -3, c-kit and PDGFR, which may result in inhibition of angiogenesis in tumors in which these receptors are upregulated.

**5. Cabozantinib:** An orally bioavailable, small molecule receptor tyrosine kinase (RTK) inhibitor with potential antineoplastic activity. Cabozantinib strongly binds to and inhibits several RTKs, which are often overexpressed in a variety of cancer cell types, including hepatocyte growth factor receptor (MET), RET (rearranged during transfection), VEGFR-1/ 2/ 3, KIT, FMS-like tyrosine kinase 3 (FLT-3), TIE-2 (TEK tyrosine kinase, endothelial), tropomyosin-related kinase B (TRKB) and AXL tyrosine kinase. This may result in an inhibition of both tumor growth and angiogenesis, and eventually lead to tumor regression.

**6. BGJ398:** An orally bioavailable pan inhibitor of human fibroblast growth factor receptors (FGFRs) with potential antiangiogenic and antineoplastic activities. Pan-FGFR kinase inhibitor BGJ398 selectively binds to and inhibits the activities of FGFRs, which may result in the inhibition of tumor angiogenesis and tumor cell proliferation, and the induction of tumor cell death. FGFRs are a family of receptor tyrosine kinases, which may be upregulated in various tumor cell types and may be involved in tumor cell differentiation and proliferation, tumor angiogenesis, and tumor cell survival.

**8. LDK378:** An orally available inhibitor of the receptor tyrosine kinase activity of anaplastic lymphoma kinase (ALK) with antineoplastic activity. Upon administration, ceritinib binds to and inhibits wild-type

ALK kinase, ALK fusion proteins and ALK point mutation variants. Inhibition of ALK leads to both the disruption of ALK-mediated signaling and the inhibition of cell growth in ALK-overexpressing tumor cells. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development. ALK dysregulation and gene rearrangements are associated with a variety of tumor cell types.

### C. mTOR inhibitors

#### a. Non-FDA approved mTOR inhibitors

**1. Everolimus:** A derivative of the natural macrocyclic lactone sirolimus with immunosuppressant and anti-angiogenic properties. In cells, everolimus binds to the immunophilin FK Binding Protein-12 (FKBP-12) to generate an immunosuppressive complex that binds to and inhibits the activation of the mammalian Target of Rapamycin (mTOR), a key regulatory kinase. Inhibition of mTOR activation results in the inhibition of T lymphocyte activation and proliferation associated with antigen and cytokine (IL-2, IL-4, and IL-15) stimulation and the inhibition of antibody production.

### D. Cancer Cell stemness inhibitor

#### a.Non-FDA approved cancer cell stemness inhibitor

**1. BBI503:** An orally available cancer cell stemness (CSC) kinase inhibitor with potential antineoplastic activity. Although the exact target has not been fully elucidated, but BBI503 targets and inhibits one or more pathways involved in cancer stem cell survival. As a result, CSC growth, as well as heterogeneous cancer cell growth is inhibited. CSCs, self-replicating cells able to differentiate into heterogeneous cancer cells, appear to be responsible for both tumor relapse and metastasis.

## Cell Stemness Inhibitors (CCSIs) [52]

### Immunotherapy for gall bladder cancer

Currently, the drugs which are under clinical trials are as follows:

#### A. Monoclonal antibodies

**a. Non FDA approved Monoclonal antibodies:** There is no FDA approved MAB available as the treatment for gallbladder cancer. The agents that are under clinical trials have been mentioned in Table-5 below.



**1. Bevacizumab:** A recombinant humanized monoclonal antibody directed against the VEGF, a pro-angiogenic cytokine. Bevacizumab binds to VEGF and inhibits VEGF receptor binding, thereby preventing the growth and maintenance of tumor blood vessels.

**2. Panitumumab:** A human monoclonal antibody produced in transgenic mice that attaches to the transmembrane EGFR. Panitumumab may inhibit autocrine EGF stimulation of tumor cells that express the EGFR, thereby inhibiting tumor cell proliferation.

**3. Trastuzumab:** A recombinant humanized monoclonal antibody directed against the human epidermal growth factor receptor 2 (HER2). After binding to HER2 on the tumor cell surface, trastuzumab induces an antibody-dependent cell-mediated cytotoxicity against tumor cells that overexpress HER2. HER2 is overexpressed by many adenocarcinomas, particularly breast adenocarcinomas.

**4. DKN-01:** A humanized monoclonal antibody directed against Wnt antagonist Dickkopf-1 (DKK1) with potential anti-osteolytic activity. DKK1-neutralizing monoclonal antibody DKN-01, binds to and inhibits DKK1, which restores Wnt pathway signaling. Reactivation of the Wnt signaling pathway may result in the differentiation and activation of osteoblasts within the bone matrix and the reversal of tumor-induced osteolytic disease. Elevated levels of circulating DKK1, a potent Wnt signaling pathway antagonist, is associated with a number of neoplastic diseases.

**5. Ramucirumab:** It is a recombinant, fully human monoclonal antibody directed against human VEGFR-2 with antiangiogenesis activity. Ramucirumab specifically binds to and inhibits VEGFR-2, which may result in an inhibition of tumor angiogenesis and a decrease in tumor nutrient supply. VEGFR-2 is a pro-angiogenic growth factor receptor tyrosine kinase expressed by endothelial cells.

**B. Kinase inhibitors**

**a. Non FDA approved Kinase inhibitors:** There is no FDA approved kinase inhibitor available as the therapy for gall bladder cancer. The kinase inhibitors that are under clinical trials have been

mentioned in the Table-6 below.

**1. Sorafenib:** It is a synthetic compound targeting growth signaling and angiogenesis. Sorafenib blocks the enzyme RAF kinase, a critical component of the RAF/MEK/ERK signaling pathway that controls cell division and proliferation; in addition, sorafenib inhibits the VEGFR-2/PDGFR-beta signaling cascade, thereby blocking tumor angiogenesis.

**2. Trametinib:** An orally bioavailable inhibitor of mitogen-activated protein kinase kinase (MEK MAPK/ERK kinase) with potential antineoplastic activity. Trametinib specifically binds to and inhibits MEK 1 and 2, resulting in an inhibition of growth factor-mediated cell signaling and cellular proliferation in various cancers. MEK 1 and 2, dual specificity threonine/tyrosine kinases often upregulated in various cancer cell types, play a key role in the activation of the RAS/RAF/MEK/ERK signaling pathway that regulates cell growth.

**3. Erlotinib:** A quinazoline derivative with antineoplastic properties. Competing with adenosine triphosphate, erlotinib reversibly binds to the intracellular catalytic domain of EGFR tyrosine kinase, thereby reversibly inhibiting EGFR phosphorylation and blocking the signal transduction events and tumorigenic effects associated with EGFR activation.

**4. Selumetinib:** An orally active, small molecule with potential antineoplastic activity. Selumetinib is an ATP-independent inhibitor of mitogen-activated protein kinase kinase (MEK or MAPK/ERK kinase) 1 and 2. MEK 1 and 2 are dual-specificity kinases that are essential mediators in the activation of the RAS/RAF/MEK/ERK pathway, are often upregulated in various cancer cells, and are drivers of diverse cellular responses, including proliferation. Inhibition of both MEK1 and 2 by selumetinib prevents the activation of MEK1/2 dependent effector proteins and transcription factors, thereby leading to an inhibition of cellular proliferation in various cancers.

**5. Imatinib:** A tyrosine kinase inhibitor with antineoplastic activity. Imatinib binds to an intracellular pocket located within tyrosine kinases (TK), thereby inhibiting ATP binding and preventing phosphorylation and the subsequent activation of growth receptors

**Table 5.** Non FDA approved Monoclonal antibodies [53-57].

Monoclonal antibodies	Clinical trial identifier no.	Phase	Study Design	Target
Panitumumab	NCT01308840	Phase II	Non-Randomized, Safety/Efficacy Study, open label	EGFR
Bevacizumab	NCT00361231	Phase II	Safety/Efficacy Study, open label	VEGF
Trastuzumab	NCT00478140	Phase II	Efficacy Study, open label	HER2
DKN-01	NCT02375880	Phase I	Non-Randomized, Safety/Efficacy Study, open label	DKK1
Ramucirumab	NCT02520141	Phase II	Non-Randomized, Safety/Efficacy Study, open label	VEGF2

**Table 6.** Non FDA approved Kinase inhibitors [47,58-69].

Kinase Inhibitors	Clinical trial identifier no.	Phase	Study Design	Target
Erlotinib	NCT00033462	Phase II	Efficacy Study, open label	EGFR
Sorafenib	NCT01093222	Phase II	Safety/Efficacy Study, open label	VEGF
Trametinib	NCT02042443	Phase II	Randomized, Efficacy Study, double blind	MEK
Selumetinib	NCT01859182	Phase II	Efficacy Study, open label	MAPK/ERK kinase
Imatinib	NCT01153750	Phase II	Non-Randomized, Efficacy Study, open label	TK
Pazopanib	NCT01855724	Phase II	Efficacy Study, open label	VEGFR-1,2,3, c-kit and PDGF-R
MEK 162	NCT02105350	Phase I	Safety/Efficacy Study, open label	MEK1/2
MEK 162	NCT01828034	Phase I/II	Safety/Efficacy Study, open label	MEK1/2
Ponatinib Hydrochloride	NCT02265341	Phase II	Non-Randomized, Efficacy Study, open label	FGFR2 Fusions
Dasatinib	NCT02428855	Phase II	Non-Randomized, Efficacy Study, open label	IDH Mutation
BKM120	NCT01501604	Phase II	Safety/Efficacy Study, open label	PIK3CA Mutations
SPL-1620	NCT01773785	Phase II	Safety/Efficacy Study, open label	endothelin-B receptor
BB1608	NCT01325441	Phase I/II	Safety/Efficacy Study, open label	CSC

and their downstream signal transduction pathways. This agent inhibits TK encoded by the bcr-abl oncogene as well as receptor TKs encoded by the c-kit and PDGFR oncogenes. Inhibition of the bcr-abl TK results in decreased proliferation and enhanced apoptosis in malignant cells of Philadelphia-positive hematological malignancies such as CML and ALL; effects on c-kit TK activity inhibit mast-cell and cellular proliferation in those diseases overexpressing c-kit, such as mastocytosis and gastrointestinal stromal tumor.

**6. Pazopanib:** A small molecule inhibitor of multiple protein tyrosine kinases with potential antineoplastic activity. Pazopanib selectively inhibits VEGFR-1/2/3, c-kit and PDGF-R, which may result in inhibition of angiogenesis in tumors in which these receptors are upregulated.

**7. MEK 162:** An orally available inhibitor of MEK1/2 with potential antineoplastic activity. Binimetinib, noncompetitive with ATP, binds to and inhibits the activity of MEK1/2. Inhibition of MEK1/2 prevents the activation of MEK1/2-dependent effector proteins and transcription factors, which may result in the inhibition of growth factor-mediated cell signaling. This may eventually lead to an inhibition of tumor cell proliferation and an inhibition in production of various inflammatory cytokines, including IL-1, -6 and tumor necrosis factor. MEK1/2 are dual-specificity threonine/tyrosine kinases that play key roles in the activation of the RAS/RAF/MEK/ERK pathway and are often upregulated in a variety of tumor cell types.

**8. Ponatinib Hydrochloride:** It is a multi-targeted tyrosine-kinase inhibitor targeting pan-FGFR 1 to 4. Ponatinib has been designed to be effective against these types of tumors. An orally bioavailable multitargeted RTK inhibitor with potential antiangiogenic and antineoplastic activities. This agent inhibits other tyrosine kinases including those associated with VEGFRs and FGFRs; in addition, it inhibits the tyrosine kinase receptor TIE2 and FMS-related tyrosine kinase receptor-3 (Flt3). RTK inhibition by ponatinib hydrochloride may result in the inhibition of cellular proliferation and angiogenesis and may induce cell death.

**9. Dasatinib:** It is an orally bioavailable synthetic small molecule-inhibitor of SRC-family protein-tyrosine kinases. Dasatinib binds to and inhibits the growth-promoting activities of these kinases. SRC-family protein-tyrosine kinases interact with a variety of cell-surface receptors and participate in intracellular signal transduction pathways; tumorigenic forms can occur through altered regulation or expression of the endogenous protein and by way of virally-encoded kinase genes. isocitrate dehydrogenase (IDH1/IDH2) mutations define a distinct subtype of ICC. IDH mutated IHCC cells are hypersensitive to dasatinib and critically dependent on SRC activity for survival and proliferation.

**BKM120 (buparlisib):** It is an orally bioavailable specific oral inhibitor of the pan-class I phosphatidylinositol 3-kinase (PI3K) family of lipid kinases with potential antineoplastic activity. Buparlisib specifically inhibits class I PIK3 in the PI3K/AKT kinase (or protein kinase B) signaling pathway in an ATP-competitive manner, thereby inhibiting the production of the secondary messenger phosphatidylinositol-3,4,5-trisphosphate and activation of the PI3K

signaling pathway. This may result in inhibition of tumor cell growth and survival in susceptible tumor cell populations. Activation of the PI3K signaling pathway is frequently associated with tumorigenesis. Dysregulated PI3K signaling may contribute to tumor resistance to a variety of antineoplastic agents.

**SPI-1620:** It is a highly selective peptide agonist of the endothelin-B receptor. Endothelin B receptor agonist SPI-1620 binds to endothelin-B receptors on endothelial cells in tumor blood vessels, which, unlike the angioarchitecture of normal blood vessels, are relatively devoid of smooth muscle. This agent may induce a transient, selective increase in blood flow to a tumor, which may result in an increase in the delivery of anticancer agents to the tumor and, so, an increase in anticancer agent efficacy

**BBI608 (napabucasin):** It is an orally available cancer cell stemness inhibitor with potential antineoplastic activity. Even though the exact target has yet to be fully elucidated, napabucasin appears to target and inhibit multiple pathways involved in cancer cell stemness. This may ultimately inhibit cancer stemness cell growth as well as heterogeneous cancer cell growth. CSCs, self-replicating cells that are able to differentiate into heterogeneous cancer cells, appear to be responsible for the malignant growth, recurrence and resistance to conventional chemotherapies.

### C. Vaccine therapy

**a. Non FDA approved Vaccine Therapy:** There is no FDA approved vaccine therapy available for gall bladder cancer. The non-FDA approved biological has been mentioned in the Table-7 below.

**1. TRICOM-CEA (6D):** An active Immunotherapy with Autologous Dendritic Cells Infected with CEA-6D Expressing Fowl pox -Tricom in Patients with gallbladder cancer is in ongoing clinical trial.

**2. CEA RNA-pulsed DC cancer vaccine:** A vaccine comprising autologous dendritic cells pulsed with mRNA-encoded Carcinoembryonic Antigen (CEA) that targets tumor cells expressing CEA.

**3. Recombinant fowl pox-CEA (6D)/TRICOM vaccine:** A cancer vaccine comprised of a recombinant fowl pox virus vector encoding the carcinoembryonic antigen (CEA) and a TRIad of CO stimulatory Molecules (B7-1, ICAM-1 and LFA-3).

### Conclusion

We are making great advances in treating cholangiocarcinoma and gall bladder cancer as our knowledge of the function of the immune system continues to grow. Immunotherapy has shown a promising development in the past few years. The recent activities have increased our understanding of the tumor microenvironment, various immunotherapeutic modalities or combination therapy (like chemotherapy with immunotherapy). Additionally, the effects of such modalities as monotherapy or in combination of conventional therapies with immunotherapy in cancer patients are still exploratory phase. The complete perspective of immunotherapy treatment has not

**Table 7.** Non FDA approved Vaccines [70-72].

Vaccines	Clinical trial identifier no.	Phase	Study Design	Target
TRICOM-CEA (6D)	NCT00027534	Phase I	Safety Study, open label	Tumor cells
CEA RNA-pulsed DC cancer vaccine	NCT00004604	Phase I	Safety Study, open label	CEA
Recombinant fowl pox-CEA(6D)/TRICOM vaccine	NCT00028496	Phase I	Safety/Efficacy Study, open label	CEA

been realized and/or utilized. Proper preclinical and clinical designs are the important pillars in understanding the future of immunotherapy in treating cancer patients.

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