Gastroenterology

# Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | GASTRO-D-16-01679R1 |
| **Full Title:** | Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis |
| **Article Type:** | Solicited Reviews in Basic Clin. Gastro. |
| **Section/Category:** | Translational |
| **Keywords:** | Keywords: liver cancer, cell of origin, hepatocytes, stem cells, molecular classification, prognosis. |
| **Corresponding Author:** | Josep M Llovet, MD<br>Liver Cancer Translational Research Laboratory<br>Barcelona, SPAIN |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author's Institution:** | Liver Cancer Translational Research Laboratory |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Daniela Sia |
| **First Author Secondary Information:** | |
| **Order of Authors:** | Daniela Sia |
| | Augusto Villanueva |
| | Scott L. Friedman |
| | Josep M Llovet, MD |
| **Order of Authors Secondary Information:** | |
| **Manuscript Region of Origin:** | UNITED STATES |
| **Abstract:** | Primary liver cancer is the second leading cause of cancer-related death worldwide and therefore a major public health challenge. We review hypotheses of the cell of origin of liver tumorigenesis, and clarify the classes of liver cancer, based on molecular features and how these affect patient prognosis. Primary liver cancer comprises hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (iCCA), and other rare tumors, notably fibrolamellar carcinoma and hepatoblastoma. The molecular and clinical features of HCC vs iCCA are distinct, although they have overlapping risk factors and pathways of oncogenesis. A better understanding of the cell types originating liver cancer can aid in exploring molecular mechanisms of carcinogenesis and therapeutic options. Molecular studies have identified adult hepatocytes as the cell of origin. These cells have been proposed to transform directly into HCC cells (via a sequence of genetic alterations), to de-differentiate into hepatocyte precursor cells (which then become HCC cells that express progenitor cell markers), or to trans-differentiate into biliary-like cells (which give rise to iCCA). Alternatively, progenitor cells also give rise to HCCs and iCCAs with markers of progenitor cells. Advances in genome profiling and next-generation sequencing have led to the classification of HCCs based on molecular features, and assigned them to categories such as proliferation-progenitor, proliferation-transforming growth factor beta, and Wnt-catenin beta 1. iCCAs have been assigned to categories of proliferation and inflammation. Overall, proliferation subclasses are associated with a more aggressive phenotype and poor outcome of patients, although more specific signatures have refined our prognostic abilities. Analyses of genetic alterations have identified those that might be targeted therapeutically, such as fusions in the FGFR2 gene and mutations in genes encoding isocitrate dehydrogenases (in approximately 60% of iCCAs) or amplifications at 11q13 and 6p21 (in approximately 30% of HCCs). Further studies of these alterations are needed before these can be used as biomarkers in clinical decision |

| | |
|---|---|
| | making. |

**Title:** Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis

Daniela Sia[1], Augusto Villanueva[1], Scott L. Friedman[1] and Josep M. Llovet[1,2,3]

[1] Mount Sinai Liver Cancer Program (Divisions of Liver Diseases, Hematology and Medical Oncology, Department of Medicine), Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, USA.

[2] Liver Cancer Translational Research Laboratory, BCLC, Liver Unit, CIBEREHD, IDIBAPS, Hospital Clinic, University of Barcelona, Catalonia, Spain.

[3] Institució Catalana de Recerca i Estudis Avançats, Barcelona, Catalonia, Spain.

**Grant support:** DS is the recipient of the Andrea Marie Fuquay Memorial Research Fellowship (Cholangiocarcinoma Foundation). AV is the recipient of the American Association for the Study of Liver Diseases Foundation Alan Hofmann Clinical and Translational Award. SLF is supported by NIH grants DKRO156621 and AA. 020709. J.M.L. has grants from the US National Cancer Institute (NCI) (P30CA165979), the European Commission Horizon 2020 (HEP-CAR 667273–2), the Samuel Waxman Cancer Research Foundation, the Grant I+D Program (SAF2013-41027) and the Asociación Española Contra el Cáncer (AECC).

**Disclosures:** AV and JML are co-inventors of a patent: “Compositions, Kits, and methods for identification, assessment, prevention and therapy of hepatic disorders”.

**Total Word counts:** current 6543

**References:** current 176

**Tables:** 2

**Figures:** 5

**Address for correspondence:**

Josep M. Llovet, MD
Professor of Medicine
Mount Sinai Liver Cancer Program
Division of Liver Diseases
Icahn School of Medicine at Mount Sinai
1425 Madison Avenue, Box 11-23
New York 10029, NY
Phone: 1-2126599503
Fax: 212-849-2574
Email: Josep.Llovet@mssm.edu

**ABSTRACT**

Primary liver cancer is the second leading cause of cancer-related death worldwide and therefore a major public health challenge. We review hypotheses of the cell of origin of liver tumorigenesis, and clarify the classes of liver cancer, based on molecular features and how these affect patient prognosis. Primary liver cancer comprises hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (iCCA), and other rare tumors, notably fibrolamellar carcinoma and hepatoblastoma. The molecular and clinical features of HCC vs iCCA are distinct, although they have overlapping risk factors and pathways of oncogenesis. A better understanding of the cell types originating liver cancer can aid in exploring molecular mechanisms of carcinogenesis and therapeutic options. Molecular studies have identified adult hepatocytes as the cell of origin. These cells have been proposed to transform directly into HCC cells (via a sequence of genetic alterations), to de-differentiate into hepatocyte precursor cells (which then become HCC cells that express progenitor cell markers), or to trans-differentiate into biliary-like cells (which give rise to iCCA). Alternatively, progenitor cells also give rise to HCCs and iCCAs with markers of progenitor cells. Advances in genome profiling and next-generation sequencing have led to the classification of HCCs based on molecular features, and assigned them to categories such as proliferation–progenitor, proliferation–transforming growth factor beta, and Wnt–catenin beta 1. iCCAs have been assigned to categories of proliferation and inflammation. Overall, proliferation subclasses are associated with a more aggressive phenotype and poor outcome of patients, although more specific signatures have refined our prognostic abilities. Analyses of genetic alterations have identified those that might be targeted therapeutically, such as fusions in the *FGFR2* gene and mutations in genes encoding isocitrate dehydrogenases (in approximately 60% of iCCAs) or amplifications at 11q13 and 6p21 (in approximately 30% of HCCs). Further studies of these alterations are needed before these can be used as biomarkers in clinical decision making.

Liver cancer is the second most common cause of cancer-related death worldwide and one of the few neoplasms whose incidence and mortality have been steadily increasing[1, 2], with the highest increase in mortality in the United States (US) during the last 2 decades (**Figure 1**[3]). Liver cancer comprises a heterogeneous group of malignant tumors with different histologic features and unfavorable prognosis that range from hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) to mixed hepatocellular-cholangiocarcinoma (HCC-CCA), fibrolamellar HCC, and the pediatric neoplasm hepatoblastoma[4, 5]. Among these, HCC and iCCA are the most common primary liver cancers, whereas the other neoplasms, including mixed HCC-CCA tumors[5], account for less than 1% of cases. The burden of liver cancer is increasing globally—there could be 1 million cases by 2030[6]. It is not clear how direct-acting antiviral (DAA) agents, which can cure hepatitis C virus (HCV) infection, will affect the burden of HCC. It has been estimate that curing more than 90% of patients with HCV infection would eliminate 15% of HCC cases in the US[7]. However, there is debate over the effects of DAA agents on progression of HCC[8-11].

HCC alone accounts for 90% of all primary liver cancer cases with nearly 800,000 new annual cases[2]. Its highest incidence is in Asia and Sub-Saharan Africa due to the high prevalence of hepatitis B virus (HBV) infection[6]. Unlike other cancers, the main risk factors associated with HCC are well defined and include viral hepatitis (B and/or C), alcohol abuse and non-alcoholic fatty liver disease in patients with metabolic syndrome and diabetes. Other co-factors of HCC development, such as aflatoxin B1 and tobacco, increase the incidence of the disease if other common risk factors are present[12].

iCCA is the second most common liver cancer, with the highest incidence in Southeast Asia (30–40 cases/$10^5$ inhabitants) and low incidence in Western countries (fewer than 5 cases/$10^5$ inhabitants)[13]. Nevertheless, steady increases have been reported[13, 14]. Risk factors for iCCA development include primary sclerosing cholangitis (PSC), biliary duct cysts, hepatolithiasis and parasitic biliary infestation with flukes—an etiology prevalent in Asia and linked to a specific molecular fingerprint[13]. More recently, shared risk factors with HCC have also been identified, such as HBV and HCV—particularly for iCCAs that develop in cirrhotic liver[15].

HCC and iCCA have been considered to be independent tumors that originate from distinct cell populations. However, more recently, some have been recognized as tumor subtypes of a continuum spectrum of diseases. We review the theories behind the cell(s) of origin of liver

cancer, describe emerging molecular classes, link these classes with their etiology and prognosis, and define pathways for future translation.

## CELL(S) OF ORIGIN

Parenchymal (hepatocytes and cholangiocytes) and non-parenchymal cells (fibroblasts, stellate cells, Kupffer cells, and endothelial cells) form the basic hepatic structure (**Figure 2**); the existence of stem cells in adult liver has been heavily debated. Hepatocytes constitute 60%–80% of the total liver mass. Architecturally, these cells are organized in lobules, which can be further divided in functional regions or zones[16]. Liver zonation is particularly relevant for hepatocytes, since it affects their function without altering their phenotype. Hepatocytes are frequently polyploid (4N, 8N, etc.); polyploid cells make up 50% of human liver and 90% of mouse liver[17].

Liver tumors are heterogeneous in morphology, within the same and between different tumors. Some subtypes of HCCs and iCCAs have stem cell features[18-20], such as HCCs with CK19-positive cells[21]. The rare, mixed HCC-CCAs have cells with a phenotype that is intermediate between hepatocytes and cholangiocytes[22]. These observations have led to several hypotheses about liver cancer's cell(s) of origin. Hepatic progenitor cells might generate primary liver tumors because, during liver development, hepatocytes and cholangiocytes each arise from a common progenitor (hepatoblasts). Or, HCC and iCCA might be distinct tumors that originate from mature hepatocytes and cholangiocytes, respectively.

We review findings from recent studies indicating that adult hepatocytes are the source of HCCs, because they can directly degenerate into HCC following sequential genomic insults, de-differentiate into precursor cells that ultimately can transform into HCC cells with markers of progenitor cells, and trans-differentiate into biliary-like cells able to transform into iCCA[23-25] (**Figure 3**). In parallel, progenitor cells may also give rise to HCCs and iCCA with progenitor-like features, whereas adult cholangiocytes, which lack the plasticity and transforming capacity of hepatocytes, can only give rise to iCCAs[26].

*Progenitor cells and hepatocytes in liver regeneration*

Adult stem cells or somatic stem cells are defined as undifferentiated cells with limitless replicative potential. They can differentiate into all or some specialized cell types, and their primary role is to sustain physiological tissue turnover and to direct tissue repair upon different

types of injury[27]. Stem cell compartments have been successfully identified in several adult tissues with fast turnover, including the gastrointestinal tract, skin, and bone marrow. Because of their self-renewal potential and long lifespan, these cells are thought to be more prone to malignant transformation[28, 29] and have been identified as the cell of origin of cancer in several organs, for example skin and intestine[30-32]. Unlike these organs, however, cells of the adult liver have relatively slow turnover (a period of several months), with an average hepatocyte life span ranging from 200 to 300 days[33, 34]. In this context, the contribution of stem cells to liver turnover is unclear (reviewed in ref. [35]).

Hepatocytes are quiescent in normal liver, but their turnover in the liver dramatically accelerates upon hepatic injury or after a significant reduction of liver mass such as following partial hepatectomy. Under these conditions, adult hepatocytes have enormous proliferative potential (capacity to replicate more than 50 times), a feature that has enabled human liver organ donor transplantation as a standard procedure to treat end-state liver disease[33-35]. There is evidence that regeneration after partial hepatectomy relies predominantly on the replication of hepatocytes, rather than stem cells. However, when the replicative capacity of hepatocytes is severely impaired, such as in patients with acute liver failure or chronic hepatitis, epithelial cells with intermediate hepatocyte–cholangiocyte phenotypes emerge and expand[36-38]. These cells, also known as oval cells, are thought to localize to the peri-portal area in the canals of Hering and are considered to be bi-potential progenitors[35, 39] (**Figure 2**). The capacity of these cells to repopulate the damaged liver has been demonstrated in mice with massive liver damage, restoring more than 80% of hepatocytes[40]. Other studies from liver injury models have supported the liver stem cell hypothesis, showing proliferation of duct-like cells, frequently termed the ductular reaction, in the portal zone of the hepatic lobule[41-43]. However, this feature is controversial[44, 45].

Other cells have been implicated in regeneration, repair, and eventually carcinogenesis. HCV-related chronic injury can induce ductular metaplasia and proliferation, with mature hepatocytes trans-differentiating to bi-potential oval cells via epigenetic re-programing[25]. These cells are different from the classical oval cells of the canals of Hering, and may have a more relevant role in hepatocarcinogenesis. However, other sources of precursors have been identified. These include specialized peri-central liver cells, capable of self-renewal in the uninjured liver under the influence of endothelial Wnt signaling[46]. These pericentral cells express the early liver progenitor marker TBX3[47] and are diploid. Hybrid periportal cells express hepatocyte markers,

along with low levels of SOX9 and several bile duct genes, are able to repopulate the healthy and diseased liver mass[48]. It is not clear whether these cell populations are bona fide stem or progenitor cells, or distinct hepatocyte subpopulations, and whether they contribute to tumorigenesis[49].

*Progenitor cells as precursors*

Extensive experimental studies support the hypothesis that progenitor cells might originate liver cancer[50-52] (**Table 1**). For example, mice with genetic alterations that affect the Hippo pathway within the liver expand progenitor-like cells and subsequently develop HCC, iCCA, and mixed HCC-CCA[53-55]. Similarly, liver-specific deletion of the neurofibromatosis type 2 (*NF2*) tumor suppressor gene in developing or adult mice leads to expansion of progenitor cells without affecting differentiated hepatocytes; these mice develop HCC and iCCA[56]. Furthermore, any mouse hepatic cell, including hepatic progenitors, hepatoblasts, and hepatocytes, that express activated oncogenes (such as H-RAS or SV40LT) can undergo transformation to develop into iCCAs or HCCs[57] (**Table 1)**. There could also be subpopulations of stem or progenitor cells in peribiliary glands, located throughout the biliary tree,[58] that give rise to iCCAs[59] or fibrolamellar HCC[60]. Proteins involved in stem cell renewal and cell fate decisions include MET[50], which primarily induces hepatocyte differentiation, and EGFR and Notch, which promote cholangiocyte specification[50, 51]. Activation of Notch in mice leads to development of HCC[61] and iCCA[62]. In addition, mutations in genes encoding isocitrate dehydrogenases, frequently detected in iCCAs[14] but rarely in HCCs, inhibit hepatocyte differentiation and promote oval cell proliferation and biliary transformation following liver injury, in cooperation with *KRAS* mutations[63] (**Table 1**). Similarly, *KRAS* mutations and homozygous *PTEN* deletion in embryonic bi-potential progenitor cells cooperate to induce onset of iCCA[64]. Overall, these experimental studies implicate progenitor cells in the development of the two most common primary liver cancers.

*Adult hepatocytes as cell of origin*

There is significant experimental evidence implicating adult hepatocytes as the cell of origin in liver cancer[48, 65-69]. Studies using fate-tracing systems have observed that HCC does not originate from progenitor cells, but rather from hepatocytes in both hepatotoxin-induced[66, 67] and carcinogen-free (Mdr2 knock-out) models[65, 67] (**Table 1**). In particular, these data indicate that Foxl1+ cells, which express the progenitor markers EPCAM, SOX9, PROM1, do not contribute to HCC tumorigenesis[66], in agreement with other studies showing that biliary cells do not give

rise to HCC[65]. In addition, a recent study has demonstrated that hepatocyte-specific p62 expression promotes c-MYC induction, mTORC1 activation and HCC initiation[70].

Adult hepatocytes, however, have additional properties consistent with a cell that originates cancer. These include their remarkable plasticity, which entails their capacity to de-differentiate into a progenitor state and then can restore the hepatocyte pool[25]. Mature hepatocytes can de-differentiate following loss of the tumor suppressor *TP53*, which yields nestin-positive progenitor-like cells that can expand and generate primary liver cancers after acquiring lineage-specific oncogenic lesions, such as mutations in WNT (in HCC) or Notch (in iCCA)[52] (**Table 1**). In addition, adult hepatocytes can also trans-differentiate into biliary-like cells (**Figure 3**)[24] that can degenerate into iCCA. Upon injury, Notch activation leads to reprogramming of mature hepatocytes into cells that closely resemble biliary epithelial cells,[71] to induce iCCA. Notch can do this on its own[72] or in cooperation with AKT[73] (**Table 1**).

There is evidence that different cell types can serve as the cell of origin for primary liver cancers (**Figure 3, Table 1**). Two complementary features of HCC and ICCAs could account for their phenotypic complexity, their molecular features (pathways of activation, mutations, etc.), and the types of cells that become transformed. For example, development of HCC could involve specific molecular alterations in adult hepatocytes (**Figure 3**), whereas iCCA could originate from cholangiocytes, probably as a result of chronic biliary damage due to liver flukes, PSC, or other insults.

However, adult hepatocytes have also been implicated in the formation of other tumor types. First, tumors with progenitor cell phenotype could derive from progenitor-like cells, either because they are bona fide progenitor cells or following de-differentiation of adult hepatocytes. Transformation of progenitor cells could occur at any stage of hepatic development—from progenitor to adult liver cells—giving rise to tumors of different morphologies, ranging from HCC and iCCA with stem-cell phenotypes to mixed HCC-CCAs (**Figure 3**). Clinical and pathology analyses of mixed HCC-CCAs have indicated an origin from the progenitor cell compartment[5, 14, 74]. Finally, mature hepatocytes can trans-differentiate, generally as a results of viral infection, to biliary-like cells that give rise to iCCA.

Regardless of their cell of origin (mature hepatocytes vs progenitor cells), liver cancers with stem cell features have a more aggressive clinical behavior and worse prognosis than those without stem cell features [19, 21]. However, these are difficult to study due to discrepancies

between mice and humans. Studies are needed to determine under what circumstances adult hepatic cells, rather than progenitor cells, give rise to liver tumors and vice versa, and to fill the gap between findings from animal models (cell fate tracing studies) and studies patients with severe liver injury. It will also be important to determine the dominant reprogramming events involved in tumorigenesis in different species. Studies should explore which cells are most affected by oncogene mutations associated with HCC, such as those in the *TERT* promoter or *CTNNB1*.

**Immune Response**

Regardless of the cell type that becomes transformed to initiate tumorigenesis, the developing tumor also requires a specific microenvironment. This is particularly relevant to liver tumors, 90% of which develop under conditions of chronic inflammation[2]. Factors that contribute to tumor formation include changes in the liver's extracellular matrix, signaling between parenchymal and non-parenchymal cells, and immune dysfunction[75]. An altered immune response is an important factor in carcinogenesis.[76] Cancer cells produce growth and angiogenic factors that promote tumor growth, invasion, and metastasis. iCCAs develop under conditions of chronic inflammation, due to viral infections (in about 20% of cases), chronic fluke infestation, PSC, and hepatolithiasis[13].

Chronic inflammation contributes to liver carcinogenesis through a cycle of cell death and regeneration, leading to production of cell survival and proliferation signals that promote formation of regenerative nodules, dysplasia, and cancer[75]. In this context, the phenotype of liver tumors could depend on interactions between oncogenes and the immune microenvironment. In a recent animal study, different types of liver tumors, such as HCCs, iCCAs, and mixed HCC-iCCA arise via activation of distinct oncogenes, such as AKT1 and CTNNB1, in combination with inflammatory microenvironment conditions, such as carbon tetrachloride or 3,5-diethoxycarbonyl-1,4-dihydrocollidine[77]. Tumors that develop via activation of the same oncogenes can still have different transcriptomes, depending on level of inflammation and features of the microenvironment.

Several mechanisms have linked the immune system with liver cancer development. First, secretion of cytokines by immune cells, such as tumor necrosis factor and IL6, can activate inflammatory signaling pathways in hepatocytes, via JAK–STAT, nuclear factor (NF)-kB, etc., to promote cell proliferation and survival [75]. A model of HCC tumorigenesis has been proposed in

which leukocytes that infiltrate the liver organize into ectopic lymphoid-like structures, providing microniches that contain malignant hepatocytic progenitor cells[78]. The role of the adaptive immune system is becoming apparent from studies of animal models of liver cancer development with non-alcoholic steatohepatitis. Factors that activate inflammation, such NF-κB and insulin receptor, contribute to carcinogenesis in patients with non-alcoholic fatty liver disease[79]. CD8+ and CD4+ T cells and natural killer cells [80,81] also promote oncogenesis.

The interaction between inflammation and iCCA development has been demonstrated in mice in which biliary epithelial cells that express transgenic *AKT* and *YAP* develop only iCCA upon promotion of IL33-mediated biliary tract inflammation[82]. Other inflammatory mediators that contribute to oncogenesis include IL6 and iNOS[83]. IL6 is highly expressed in human CCAs and promotes cell survival in a STAT3-dependent manner[84]. Interestingly, a gene expression signature associated with IL6–STAT3 signaling has been observed in a subset of human iCCAs[85].

Normally, nascent transformed cells are eliminated by both the innate and adaptive immune systems in a process called immune surveillance. Cancer cells express antigens that induce adaptive responses mediated by T cells[86]. In the chronically inflamed liver, immune surveillance prevents proliferation and expansion of cancer cells. Some, but not all, recent reports have warned about an unexpectedly higher incidence of HCC occurrence and recurrence in patients treated with DAAs for HCV infection[8-11]. If confirmed, the potential role of immune system should be explored, because rapid clearance of HCV in patients with chronic infection for years might affect immune surveillance.

*Immune therapies*

The immune microenvironment of HCC is characterized by the co-existence of immune responses leading to both immunogenicity and tolerance[87]. HCCs promote immunotolerance through the release of cytokines such as interleukin 10 (IL10) and transforming growth factor beta (TGFB). The tumors and tumor-infiltrating immune cells also express immune regulators such as programmed cell death protein 1 (PD1), its ligand PDL1, and cytotoxic T-lymphocyte protein 4 (CTLA4), which suppress the anti-tumor immune response, allowing tumor growth and progression[87, 88]. Tumors with immune infiltrates have been associated with longer survival

times and reduced relapse after resection and transplantation[2, 88]. In addition, some HCC cases of spontaneous regression have been linked to immune-mediated mechanisms[2]. These observations establish the rationale for immunotherapy of HCC. Promising responses have been reported with nivolumab, a monoclonal antibody directed against PD1, in a phase 2 trial[89]. It appears that a T-cell response, features of inflammatory cells (PD1, PDL1 expression), number of mutations in tumor cells, and gene expression profiles might predict patient responses[90-93]. Using source separation techniques to dissect gene expression patterns of tumor cells from those of tumor-infiltrating immune cells, an immune-specific subclass has been identified in 25% of HCC patients[94]. This subgroup is characterized by activation of immune cells, expression of PD1 and PDL1, and enrichment in signatures of response to immunotherapies. Furthermore, 2 robust microenvironment-based types have been identified, with either active or exhausted immune responses; these findings could be used to improve design of clinical trials of immunotherapies[94]. Similarly, a subtype of iCCA characterized by high mutation load and increased expression of immune checkpoint genes has been identified that may predict response to immunotherapies[95].

**Classification Based on Molecular Features**

Characterization of molecular subtypes and/or oncogene-addiction loops has generated treatment algorithms for several types of cancer, including breast cancer; these have improved patient outcomes. High-throughput technologies, including single-nucleotide polymorphism arrays, combined with transcriptomic and exome sequencing analyses, have identified molecular subtypes of liver cancer, with distinct oncogene signaling pathways and recurrent mutations[96]. Even though molecular classifications have not affected decision making for patients with liver cancer, correlations between these subclasses and clinical and pathology characteristics (such as risk factors, outcome, etc.) have been proposed.

*Reaching a consensus*

The development of a molecular classification of HCC has paralleled the progress in genomic profiling technologies, and is extensively reviewed elsewhere[97]. Initial studies relied on differences in gene expression patterns determined by microarrays, whereas more recent, sequencing-based studies used mutation signatures to classify HCCs[3]. Unsupervised clustering of gene expression patterns accurately identified 2 major subgroups (reviewed in[2, 97]). These 2 classes, broadly termed proliferation and non-proliferation, correlate with clinical and pathology features, etiology, and patient outcomes[97]. Tumors of the proliferation class are highly

heterogeneous with significant enrichment in signaling pathways related to proliferation, such as insulin like growth factor 1 (IGF1)[98] and mechanistic target of rapamycin (MTOR) or stem cell features, such as Notch[61]. This class also contains most gene expression patterns associated with tumor recurrence[99] and shorter survival time[100] (**Figure 4**). Epigenetic features, such as expression patterns of micro RNAs [101] and DNA methylation patterns,[102] also associate with these subclasses. Chromosomal aberrations are also frequent in HCCs—mostly in chromosomes 1 and 8—which correlate with transcriptome-based subclasses. High-level amplifications have been reported at Chr. 11q13 (at *CCND1* and *FGF19*) and 6p21 (at *VEGFA*); gains in 11q13 are enriched in tumors from the proliferation subclass (**Figure 4**). Tumors in the non-proliferation subclass tend to retain hepatocyte-like features; a subset has activation of the canonical WNT signaling pathway, mostly via mutations in *CTNNB1*[97]. This class contains many less-aggressive, well-differentiated tumors with low levels of alpha fetoprotein, compared to the proliferation class. In parallel, molecular analyses of the adjacent non-tumor tissues have provided valuable insights into mechanisms that promote hepatocarcinogenesis, including aberrant EGF signaling and activation of inflammation pathways (involving NF-kB and IL6)[103, 104].

Next-generation sequencing (NGS) has provided a more detailed picture of molecular alterations in HCCs[105]. This technique can be used to define mutation signatures—recurrent patterns of nucleotide substitutions across different samples. These data provide for an additional layer of stratification beyond transcriptome-based classes. Interestingly, mutation signatures can reflect the exposure history of a tumor. In HCC, specific mutation signatures are associated with environmental exposures such as smoking, alcohol, aflatoxin B1 or aristolochic acid[96] (**Figure 4**). Data from exome sequencing of large patient cohorts have confirmed the most prevalent mutations in HCC pathogenesis[106, 107] (such as the *TERT* promoter, *CTNNB1*, *TP53*, *AXIN1*, *ARID1A*), but also uncovered novel low-frequency events[106, 107] and implicated mutations in non-coding DNAs[108]. In addition, NGS has also provided a map of viral integrations in HCC, mostly related to HBV[109] and more recently for the adeno-associated virus type 2 (AAV2)[110]. Recurrent clonal HBV integrations affect *TERT*, *MLL4* and *CCNE1*, some of which may impact patient survival[109]. AAV2-mediated insertional mutagenesis has been reported in 5% of HCC, mostly in non-cirrhotic patients without a clear etiological factor, predominantly affecting *TERT*, *KMTB2*, *CCNA2* and *CCNE1*[110].

*Obstacles to clinical implementation*

Ultimately, molecular classification of both the tumor and its microenvironment will translate into more effective clinical decision-making and treatment selection. This means optimizing clinical staging systems, both in their ability to predict prognosis and in their capacity to stratify patients based on response to therapies. Despite substantial progress, clinicians cannot yet use molecular information to guide therapy, primarily because there are not yet biomarkers that predict the response to a given therapy, unlike other malignancies. For instance, lung cancer patients with ALK rearrangements have a significantly better response to ALK inhibition with crizotinib than those without ALK rearrangements[111].

There are several reasons that molecular information on tumors is not incorporated into clinical practice. Unlike other solid tumors, the most frequent mutations in HCC are un-targetable, including those in the *TERT* promoter (60%–70%), *TP53* (25%–50%) and *CTNNB1* (25%–30%). Furthermore, the few potentially actionable events uncovered have not yet been evaluated in clinical trials that include biomarkers. Only recently have some potential biomarkers been tested in early clinical trials, such as FGFR4 inhibitors in patients with high level amplifications of Chr11q13, locus of FGF19[112]. Finally, a therapeutic response to blocking an oncogene in a few patients will be lost among many patients who lack a responsive phenotype if the trial has not been enriched to include those patients whose tumors harbor targetable defects. A recent report suggests that TSC2 loss predicts response to the MTOR inhibitor everolimus in different experimental models of HCC[113], yet there is no clear biomarker of treatment response in the phase 3 clinical trial[114]. Indeed, the two systemic therapies that increase survival in HCC patients in first (i.e., sorafenib[115]) and second line (i.e., regorafenib[116]) have been tested in 'all-comers', with no patient enrichment strategy.

Options to improve the current trial strategy could include conducting clinical trials with enriched populations, based on biomarkers that predict response (FGF19[117], MET). Or they could investigate non-mutated de-regulated pathways as targets, such as IGF2[98], or NOTCH[61] signaling. It might also be possible to use alternative anti-tumor approaches, such as immune checkpoint inhibitors, or strategies to antagonize the most prevalent genetic defects found in HCCs, such as mutations in *TERT*, *TP53* and *CTNNB1*.

**Molecular Subclasses of iCCA**

There is no effective treatment for iCCA. Reasons include our poor understanding of its pathogenesis and the lack of studies conducted specifically in patients with iCCA, apart from patients with all biliary tract cancers[13, 14]; this is important because iCCAs are characterized by a distinct set of mutations[14]. iCCAs were only recently recognized as a distinct entity with both its own unique staging system (see the 7th edition of the American Joint Committee on Cancer Staging Manual[118]) and ad hoc practice guidelines [see guidelines of the International Liver Cancer Association (ILCA)[13]]. The classification of iCCA's distinct phenotype has led to the first studies that characterize its genomic landscape in order to generate an initial molecular classification. Other CCA, such as perihiliar (pCCA) and distal (dCCA) are different molecular entities[14].

Two main molecular subtypes of iCCA, proliferation and inflammation, have been proposed based on an integrative analysis of whole-genome expression patterns, chromosomal aberrations, mutation, and alterations in signaling pathways in a large cohort from USA and Europe[85]. The inflammation subclass was characterized by activation of inflammatory pathways, overexpression of cytokines, and STAT3 activation, whereas the proliferation subclass was characterized based on activation of oncogene signaling pathways (KRAS, EGFR, and Notch) and HCC gene expression signatures associated with short survival times and earlier recurrence[85]. While neither of the two subclasses displayed specific broad chromosomal gains and losses, the proliferation subclass is enriched in focal aberrations including DNA amplifications at 11q13.2 (locus of CCND1 and FGF19), and deletions at 14q22 (locus of SAV1). The proliferation class also includes a subtype with stem cell–like features[119], chromosomal instability[85] and mutations in isocitrate dehydrogenase genes[63] (**Figure 5**). Clinical characteristics such as moderate/poor differentiation, intra-neural invasion and poor outcome are significantly more frequent in the proliferation class. Interestingly, there is a genomic resemblance observed between the proliferation subclass of iCCA and HCC subtypes with poor prognosis (i.e. cluster A[19], G3[120]) and stem cell features (S2)[121, 122] (**Figure 5**). This evidence further supports the presence of a common cell of origin in liver cancers.

An independent whole-transcriptomic analysis of a large cohort including iCCA and extrahepatic cholangiocarcinomas also confirmed the existence of the two prognostic CCA molecular subtypes[123]. In this study, two main classes were reported: a poor prognosis group characterized by enrichment of *RAS* mutations, *MET* and *EGFR* overexpression, and a good

prognosis subgroup characterized by signatures of immune response[123]. Genomic overlap between the poor prognosis group[123] and the proliferation subclass was observed[85].

Similar to HCC, NGS studies have generated insights into the molecular subtypes of iCCA[95, 124-131]. The most common genetic aberrations include *FGFR2* gene fusions (~25%) and mutations in *KRAS* (20%), *IDH1/2* (~20%) and chromatin remodeling genes [*ARID1A, BAP1, PBRM1*, ~30%,] (reviewed elsewhere [14]). Besides *IDH1* and IDH2[63], *KRAS*,[85, 123] and *EGFR*[85] mutations, which are enriched in the proliferation subclass, no significant distribution of other molecular aberrations has been reported in any of the iCCA molecular subtypes.

The incidence of iCCA varies significantly among geographic regions. Incidence is highest in Asian countries, where infections with liver flukes such as *Opisthorchis viverrini* and *Clonorchis sinensis* are the major risk factor[13]. In non-endemic regions, other factors, including hepatolithiasis and PSC, likely contribute to risk. Associations between different risk factors and mutations detected in iCCAs were explored in 209 CCAs from Asia and Europe. Mutations in *BAP1* and *IDH1/2* were found in a higher proportion of non-*O viverrini*-related iCCAs (22% vs 3.2%), whereas mutations in *TP53* were found in a higher proportion of tumors from patients with *O viverrini* infection (45.2% vs 7.4%)[124]. Although there have been other studies of large cohorts of patients, it has been difficult to draw conclusions from these due to the different types of neoplasms included (iCCAs, extrahepatic cholangiocarcinomas, and gallbladder tumors)[85, 123].

Importantly, multiple *FGFR2* gene fusions have been discovered in approximately 25% of iCCAs[126, 127, 132-135] (reviewed in ref. [14]); these are used to define tumor subtypes. Tumors with *FGFR2* fusions and *KRAS* mutations[126] or *BAP1* mutations[95] have been reported. No significant differences in patient age or outcome, or tumor differentiation or stage, were found in comparing patients with or without the *FGFR2* fusion. One study reported a higher frequency of HCV infection in patients with *FGFR2* fusions[132], although this finding has not been confirmed[95, 126, 134]. Early-phase clinical trials testing selective FGFR inhibitors in advanced iCCA patients carrying alterations in *FGFR2* are underway (NCT02150967 and NCT01752920) [136, 137]. These studies reported an objective response rate of approximately 15% (a signal of efficacy) and manageable safety profile[136]. The discovery that *FGFR2* fusions can promote development of iCCAs has been quickly translated to clinical trials. Fusions of the FGFR2 gene might be used as biomarker to select patients for treatment with these agents.

**Molecular Features of Uncommon Tumors**

Fibrolamellar HCC (FLC) is a rare primary liver cancer with few treatment options that typically affects children and young adults without background liver disease[60]. FLC is now considered a unique molecular entity within primary liver malignancies[138]. FLC is caused by an approximate 400 kb deletion in chromosome 19 that incorporates the first exon of *DNAJB1* into all but the first exon of *PRKACA*[139]. This fusion is detected in 70%[138] to 100% of FLCs[139]. In addition, exome sequencing and RNA sequencing studies found de-regulation of additional actionable candidates such as *ERBB2* and *AURKA*[140]. Unlike HCC or iCCA, there are no prevalent mutations in more than 10% of patients. Unsupervised clustering of gene expression data identified FLC molecular subclasses in a cohort of 58 FLCs, 2 of them associated with proliferative and inflammatory molecular traits. The same study provided a prognostic 8-gene expression signature[138]. FLC has less chromosomal aberrations compared to HCC or iCCA without recurrent high-level amplifications or deletions.

Hepatoblastoma is the most frequent primary liver tumor in children younger than 5 years old. Like FLC, background liver disease is rare in these patients. WNT signaling plays a major role, with *CTNNB1* mutations (70%) as the most frequently reported molecular event. Integrative analysis of gene expression and chromosomal aberrations in 24 hepatoblastoma samples distinguished 2 classes, with different DNA gains, histological phenotype and clinical outcome[141]. The recent use of patients-derived xenografts (PDX) in hepatoblastoma enabled the identification of novel therapeutic targets such as *NRAS* mutations[142, 143].

Mixed HCC-iCCA is a rare neoplasm accounting for less than 1% of all primary liver cancers[5]. Diagnosis is mainly based on histological examination and requires features of HCC and iCCA. According to the 2010 World Health Organization classification, there is a classical type, characterized by areas of typical HCC and iCCA with a transition area, and a stem cell-feature type[144]. The stem cell-feature type can be further divided into typical, intermediate, and cholangiolocellular carcinoma (CLC) subtypes. Gene expression profiling of small series of HCC-CCA samples indicated that this tumor type might share common characteristics with poorly differentiated HCC and iCCA with stem cell traits[119, 122, 145]. A comprehensive integrative genomic analysis of 18 mixed HCC-CCA subtypes defined 3 molecular types (CLC, stem cell, and classical subtype)[146]. In particular, the CLC subtype, despite being grouped, based on histology, within the stem cell-feature subtype, has a unique molecular profile, with biliary traits,

low levels of chromosome instability, and activation of TGFB and inflammation-related signaling pathtways[146].

**Challenges in tumor heterogeneity**

Liver tumors have a high degree of molecular heterogeneity, not only among patients with the same types of tumors, but also within regions within the same tumor or tissue[147]. Expert recommendations and consensus definitions have been established to better address the extent and role of cancer heterogeneity in oncology research[148].

From a clinical perspective, molecular heterogeneity should be considered within the concepts of trunk and branch, and driver and passenger mutations. Trunk mutations (mutations that occur early in tumorigenesis) can be found in every cancer cell of a tumor [148]. Branch mutations are found in only a subgroup of tumor cells; these are acquired at later stages of tumor development, frequently following treatment, and are associated with resistance. Studies of other malignancies have indicated that trunk mutations promote tumor progression, and are therefore the best targets for therapies. Trunk mutations identified in single-biopsy should be the first targets of molecular therapies [2]. Finally, passenger mutations account for the most common type of mutations, but their relevance to tumor development is marginal. However, they are often used to define a tumors' clonal features and anti-tumor response.

**Prognostic Markers**

Molecular biomarkers have many applications in cancer. The can improve clinical decision making by helping to predict a patient's outcome, select the patients most likely to respond to a specific treatment, and avoid treatments not likely to be effective. Unlike other cancers such as breast[149] and colorectal[150] cancers, HCC has no molecular markers that have been incorporated into clinical management.

As a general rule, clinical practice guidelines include recommendations based on a specified set of metrics, which rate the level of evidence and the strength of recommendations for each statement; the higher the evidence, the stronger the recommendation. For example, sorafenib is recommended for treatment of patients with advanced-stage HCC; a strong recommendation was derived from a prospective, double-blind, randomized, placebo-controlled trial (the highest level of evidence)[151]. These metrics are less clear when evaluating biomarkers for HCC. Initial recommendations for biomarkers were developed mostly to address technical aspects of the

assay such as the Tumor Marker Utility Grading System[152]. This was later improved upon by the REMARK guidelines for reporting prognostic biomarker in oncology[153], which also has addressed issues related to clinical utility. The PROGRESS initiative was the most recent attempt to provide a methodological framework for biomarker research[154].

The highest level of evidence in biomarker research (Level A) comes from randomized trials[155]. A large prospective study of 10,253 women demonstrated that a 21-gene expression signature could identify patients with early-stage breast cancer at high risk for recurrence[156]. Level B evidence is provided by companion studies of biomarkers in randomized clinical trials, such as those reported for HCC in the Sorafenib HCC assessment randomized protocol[157] and everolimus for liver cancer evaluation[114] trials. Neither of these studies identified markers associated with response to either sorafenib or everolimus, but they confirmed the prognostic role of levels of angiopoietin 2 and vascular endothelial growth factor in plasma of patients with advanced-stage HCC[157]. Level C evidence has been provided most frequently for markers of HCC, from studies of archived samples, with extensive validation (reviewed elsewhere)[97]. Most level C studies have evaluated prognostic markers, identifying patients with the proliferation subclass of tumor, either S1 (WNT and TGFB expression), or the S2 (progenitor cell) subtype-[97], with or without amplification of *FGF19* [106]. The S2 subtype expresses many makers of progenitor cell cells and high levels of alpha fetoprotein. Tumors in the S1–S2 subgroups are poorly differentiated and patients have worse outcomes than patients with other HCC subtypes[97]. Level D evidence comes from studies of convenience, in which specimens were collected for unknown reasons and happen to be available for assay.

Gene signatures associated with risk of HCC development may have a more direct application (**Table 2**)[100, 102, 158-161]. For instance, a 186-gene signature in liver tissues was able to predict occurrence of HCC[158] in patients with hepatitis C-related early-stage cirrhosis, and might be used to select patients for trials of chemopreventive agents (**Figure 4, Table 2**). However, chemoprevention studies are a challenge, because it takes a long time to collect data on a low number of events. Studies that included populations of patients at high-risk for HCC may shorten trial time and identify effective agents more quickly. For predictive biomarkers in advanced HCC, ongoing clinical trials include populations selected based on high expression of MET in tumor cells (trial of tivantinib, NCT01755767) or high serum level of alpha fetoprotein (trial of ramucirumab, NCT02435433), both as second-line therapies.

**Molecular markers of iCCA**

Even fewer biomarkers of iCCA have been developed than for HCC; there is only level C data for the efficacy of a biomarker for this tumor type. iCCA is one of the few solid tumors for which no systemic molecular therapy has been approved by the Food and Drug Administration[13]. Surgery is the only potentially curative option, although chemotherapy with gemcitabine plus cisplatin is the standard for patients with advanced-stage iCCA[162]. According to the ILCA practice guidelines, no studies have provided level A or B evidence for a marker of this tumor—some data from low-quality studies have led to weak recommendations[13]. These studies have been retrospective in design, without extensive validation in independent cohorts. Some gene expression and microRNA patterns have been associated with features of iCCA[85, 119, 123], as well as mutations in *KRAS* and *IDH1 or IDH2* and focal deletions at 14q22.1[85, 128, 129] (**Table 2**). Gene expression patterns have been reported to identify an iCCA proliferation subclass of tumor[85], patients with a poor prognosis, [85, 123] and tumors with a stem cell–like subtype[119], who have aggressive disease and worse outcome.

Features of cancer cells in a tumor determine not only the tumor's growth and progression, but also affect the tumor microenvironment (non-cancer cells and supporting stroma)[76]. Some studies associated features of the iCCA microenvironment with patient outcome[123, 163-165]. Two studies identified a stromal signature associated with shorter survival time by combining laser capture microdissection with gene expression profile analysis[123, 165]. Although many studies have validated gene expression signatures associated with patient outcomes, the clinical significance of microRNA signatures is not yet clear, due to the limited number of large comprehensive profiling studies[119, 166-168].

Mutations in oncogenes such as *KRAS* and *BRAF* can be used to determine prognosis for patients with pancreatic or colorectal cancers[169-171]. However, there is controversy over whether these mutations can predict outcomes of patients with iCCA. Some studies have reported significant associations between mutations in *KRAS*[128, 129, 131] or chromatin-remodeling genes (*BAP1*, *PBMR1*, and *ARID1A*)[125, 172] and either shorter disease-free survival or shorter overall survival, respectively. It is not clear whether mutations in *IDH1* or *IDH2* associate with overall survival [125, 129, 173].

Most biomarker assays use cells or tissue collected by invasive biopsy procedures or from resection specimens, but these specimens are rarely collected from unresectable or metastatic

iCCAs—the tumors of most patients in clinical trials for iCCA. Strategies should be developed to assess circulating markers (called a liquid biopsy), from tumor cells and cell-free nucleic acids in blood[174]. Detection of molecular signatures or aberrations in the blood could significantly facilitate implementation of prognostic markers. Liquid biopsies might also be used to monitor response to treatment, especially in clinical trials of selective inhibitors of IDH1 and IDH2 (NCT02073994, NCT02273739) and FGFR2 (NCT02150967)[14, 136].

**Future Directions**

In the last decade, there have been tremendous advances in our understanding of the cellular and molecular complexity of liver cancers. Sophisticated functional studies and NGS-based research studies have provided information about the cells that form liver tumors and the proteins and pathways involved in hepatocarcinogenesis. These studies support a model of multiple cells of tumor origin. Most of the studies, using in vivo lineage tracing models, revealed an unexpected plasticity of mature hepatocytes[24, 25, 44, 52, 72] that could be involved in their transformation into cancer cells. Hepatic progenitor cells might also become transformed to give rise to different subtypes of HCC, iCCA, and mixed HCC-iCCA with progenitor markers.

Studies of humanized mice to mimic the onset of liver cancers in multiple settings may help address existing challenges. In addition, cell- and lineage-tracing models should improve so that they recapitulate the severity of liver injury observed in humans, and must clarify if hepatic progenitor cells can replenish the liver mass after marked liver injury, as demonstrated in zebrafish models[175]. Finally, linking the main genomic hits at pre-neoplastic and early stages of human cancer development with the cell of origin is also an area where further studies are required.

Identifying the cells that give rise to liver tumors and elucidating the different classes of tumors, based on their molecular features, could lead to new approaches to treatment and prognosis. Our knowledge of the molecular mechanisms of HCC and iCCA pathogenesis has not yet been translated into clinical tools, because few studies have linked specific molecular classes of tumors or aberrations to responses to therapy. It is important to incorporate biomarker studies into trials, as in the trials of patients with iCCAs with FGFR2 aberrations[136]. The successes of immune therapies in different solid tumors, including HCC,[176] indicate the need to focus greater attention on the hepatic tumor microenvironment and interactions between the tumor and the immune response; we also need to identify markers of response to these therapies. Learning

more about the effects of tumor heterogeneity, and the use of liquid biopsies to determine the molecular and genetic features of patients' tumors, could overcome barriers to personalized treatment of liver cancer[174].

## FIGURE LEGENDS

**Figure 1: Mortality trends of patients with different malignancies in the USA from 1990through 2009** (reprinted from ref [3]). Changes in cancer mortality, among tumor types, in the US. Mortality from liver and bile duct cancers is increasing more rapidly than any other cancer, in men and women. Data obtained from the 2013 American Association for Cancer Research Cancer Progress Report.

**Figure 2: Structure of the liver lobule and location of candidate cell of origin of liver cancer. I**ntrahepatic organization of the liver lobule and the localization of hepatic cells that form liver tumors. Different types of cancer (i.e. HCC, iCCA, mixed HCC-iCCA) can originate in the liver depending on the transformation event and the cell type undergoing neoplastic transformation. Hepatic stem or progenitor cells are believed to reside within the most terminal branches of the biliary tree (referred to as Canals of Hering). Upon injury hepatocytes can undergo reversible ductal metaplasia and de-differentiate into hepatocyte-derived progenitor-like cells.

**Figure 3: Multiple cells of origin of primary liver cancers.** HCC and iCCA can develop via transformation of mature hepatocytes and cholangiocytes, respectively. There is evidence that hepatic progenitor cells (HPCs), their intermediate states, or de-differentiated hepatocytes can form liver tumors with progenitor-like features, including mixed HCC-CCA, such as cholangiolocellular carcinoma (CLC). Mature hepatocytes can be also re-programmed into cells that closely resemble biliary epithelial cells and contribute to development of iCCA.

**Figure 4: Molecular biomarkers of HCC.** Genetic features and gene expression of the tumor or its surrounding microenvironment correlate with clinical and pathology features, etiology, and patient outcomes. The proliferation subclass is characterized by proliferation signaling pathways, which involve proteins such as MTOR, Notch, and TGFB and several gene expression signatures associated with poor outcomes of patients (such as the G3 and 5-gene signatures)[97]. The non-proliferation subclass is characterized by mutations in CTNNB1, the S3 gene expression signature, and the classical WNT signaling pathway. Specific mutations associated with etiology (smoking and aflatoxin B exposure) have also been described[96]. An expression pattern of 186 genes in the liver tissue surrounding the HCC can identify patients HCC at higher risk for tumor recurrence after resection[103].

**Figure 5: Main characteristics of subclasses of iCCA.** Analyses of gene expression patterns have shown that iCCAs can be assigned to the proliferation or inflammation subclasses. The proliferation class is characterized by chromosome instability, activation of pathways related to cell cycle progression, mutations in oncogenes and shorter survival times of patients. Tumors of the inflammation subclass have gene expression patterns associated with activation of an immune response and less aggressive clinical behavior. FGFR2 fusion events are evenly distributed between these subclasses.

**ACKNOWLEDGMENTS**

The authors would like to thank Jill Gregory for her contribution in the design work of the figures.

## REFERENCES


1. Murray CJ, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2197-223.
2. Llovet JM, Zucman-Rossi J, Pikarsky E, et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2016;2:16018.
3. Llovet JM, Villanueva A, Lachenmayer A, et al. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. Nat Rev Clin Oncol 2015;12:436.
4. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095-128.
5. Bosman FT, Carneiro Ft, World Health Organization., et al. WHO classification of tumours of the digestive system. Lyon: IARC, 2010.
6. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
7. Singal AG, El-Serag HB. Hepatocellular Carcinoma From Epidemiology to Prevention: Translating Knowledge into Practice. Clin Gastroenterol Hepatol 2015;13:2140-51.
8. Llovet JM, Villanueva A. Effect of HCV clearance with direct-acting antiviral agents on HCC. Nat Rev Gastroenterol Hepatol 2016 (in press).
9. **Reig M, Marino Z**, Perello C, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol 2016;65:719-26.
10. **Conti F, Buonfiglioli F**, Scuteri A, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol 2016;65:727-33.
11. Cheung MC, Walker AJ, Hudson BE, et al. Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. J Hepatol 2016;65:741-7.
12. Chuang SC, La Vecchia C, Boffetta P. Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. Cancer Lett 2009;286:9-14.
13. Bridgewater J, Galle PR, Khan SA, et al. Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. J Hepatol 2014;60:1268-89.
14. Moeini A, Sia D, Bardeesy N, et al. Molecular Pathogenesis and Targeted Therapies for Intrahepatic Cholangiocarcinoma. Clin Cancer Res 2016;22:291-300.
15. Palmer WC, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. J Hepatol 2012;57:69-76.
16. Stanger BZ. Cellular homeostasis and repair in the mammalian liver. Annu Rev Physiol 2015;77:179-200.
17. Knouse KA, Wu J, Whittaker CA, et al. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. Proc Natl Acad Sci U S A 2014;111:13409-14.
18. Roskams TA, Libbrecht L, Desmet VJ. Progenitor cells in diseased human liver. Semin Liver Dis 2003;23:385-96.
19. Lee JS, Heo J, Libbrecht L, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med 2006;12:410-6.
20. Wang M, Xiao J, Jiang J, et al. CD133 and ALDH may be the molecular markers of cholangiocarcinoma stem cells. Int J Cancer 2011;128:1996-7.
21. Roskams T. Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. Oncogene 2006;25:3818-22.

22. Singh S, Chakraborty S, Bonthu N, et al. Combined hepatocellular cholangiocarcinoma: a case report and review of literature. Dig Dis Sci 2013;58:2114-23.
23. Tanimizu N, Nakamura Y, Ichinohe N, et al. Hepatic biliary epithelial cells acquire epithelial integrity but lose plasticity to differentiate into hepatocytes in vitro during development. J Cell Sci 2013;126:5239-46.
24. Chen Y, Wong PP, Sjeklocha L, et al. Mature hepatocytes exhibit unexpected plasticity by direct dedifferentiation into liver progenitor cells in culture. Hepatology 2012;55:563-74.
25. Tarlow BD, Pelz C, Naugler WE, et al. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. Cell Stem Cell 2014;15:605-18.
26. Guest RV, Boulter L, Kendall TJ, et al. Cell lineage tracing reveals a biliary origin of intrahepatic cholangiocarcinoma. Cancer Res 2014;74:1005-10.
27. Tang DG. Understanding cancer stem cell heterogeneity and plasticity. Cell Res 2012;22:457-72.
28. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 2003;3:895-902.
29. Arwert EN, Hoste E, Watt FM. Epithelial stem cells, wound healing and cancer. Nat Rev Cancer 2012;12:170-80.
30. **Barker N, Ridgway RA**, van Es JH, et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 2009;457:608-11.
31. Zhu L, Gibson P, Currle DS, et al. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. Nature 2009;457:603-7.
32. **Lapouge G, Youssef KK,** Vokaer B, et al. Identifying the cellular origin of squamous skin tumors. Proc Natl Acad Sci U S A 2011;108:7431-6.
33. Duncan AW, Dorrell C, Grompe M. Stem cells and liver regeneration. Gastroenterology 2009;137:466-81.
34. Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech Dev 2003;120:117-30.
35. Miyajima A, Tanaka M, Itoh T. Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. Cell Stem Cell 2014;14:561-74.
36. Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology 2004;39:1477-87.
37. Roskams TA, Theise ND, Balabaud C, et al. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. Hepatology 2004;39:1739-45.
38. Turanyi E, Dezso K, Csomor J, et al. Immunohistochemical classification of ductular reactions in human liver. Histopathology 2010;57:607-14.
39. Peng N, Li L, Cai X, et al. Liver stem/progenitor cells in the canals of Hering: cellular origin of hepatocellular carcinoma with bile duct tumor thrombi? Stem Cell Rev 2010;6:579-84.
40. Lu WY, Bird TG, Boulter L, et al. Hepatic progenitor cells of biliary origin with liver repopulation capacity. Nat Cell Biol 2015;17:971-83.
41. Furuyama K, Kawaguchi Y, Akiyama H, et al. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nat Genet 2011;43:34-41.
42. Espanol-Suner R, Carpentier R, Van Hul N, et al. Liver progenitor cells yield functional hepatocytes in response to chronic liver injury in mice. Gastroenterology 2012;143:1564-1575 e7.
43. Petersen BE, Grossbard B, Hatch H, et al. Mouse A6-positive hepatic oval cells also express several hematopoietic stem cell markers. Hepatology 2003;37:632-40.

44. **Yanger K, Knigin D,** Zong Y, et al. Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. Cell Stem Cell 2014;15:340-9.
45. Schaub JR, Malato Y, Gormond C, et al. Evidence against a stem cell origin of new hepatocytes in a common mouse model of chronic liver injury. Cell Rep 2014;8:933-9.
46. Wang B, Zhao L, Fish M, et al. Self-renewing diploid Axin2(+) cells fuel homeostatic renewal of the liver. Nature 2015;524:180-5.
47. Suzuki A, Sekiya S, Buscher D, et al. Tbx3 controls the fate of hepatic progenitor cells in liver development by suppressing p19ARF expression. Development 2008;135:1589-95.
48. Font-Burgada J, Shalapour S, Ramaswamy S, et al. Hybrid Periportal Hepatocytes Regenerate the Injured Liver without Giving Rise to Cancer. Cell 2015;162:766-79.
49. Zaret KS. Regenerative biology: Maintaining liver mass. Nature 2015;524:165-6.
50. Kitade M, Factor VM, Andersen JB, et al. Specific fate decisions in adult hepatic progenitor cells driven by MET and EGFR signaling. Genes Dev 2013;27:1706-17.
51. Chiba T, Zheng YW, Kita K, et al. Enhanced self-renewal capability in hepatic stem/progenitor cells drives cancer initiation. Gastroenterology 2007;133:937-50.
52. Tschaharganeh DF, Xue W, Calvisi DF, et al. p53-dependent Nestin regulation links tumor suppression to cellular plasticity in liver cancer. Cell 2014;158:579-92.
53. Fitamant J, Kottakis F, Benhamouche S, et al. YAP Inhibition Restores Hepatocyte Differentiation in Advanced HCC, Leading to Tumor Regression. Cell Rep 2015.
54. Lu L, Li Y, Kim SM, et al. Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver. Proc Natl Acad Sci U S A 2010;107:1437-42.
55. **Lee KP, Lee JH,** Kim TS, et al. The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. Proc Natl Acad Sci U S A 2010;107:8248-53.
56. **Benhamouche S, Curto M**, Saotome I, et al. Nf2/Merlin controls progenitor homeostasis and tumorigenesis in the liver. Genes Dev 2010;24:1718-30.
57. Holczbauer A, Factor VM, Andersen JB, et al. Modeling pathogenesis of primary liver cancer in lineage-specific mouse cell types. Gastroenterology 2013;145:221-31.
58. Cardinale V, Wang Y, Carpino G, et al. The biliary tree--a reservoir of multipotent stem cells. Nat Rev Gastroenterol Hepatol 2012;9:231-40.
59. Cardinale V, Wang Y, Carpino G, et al. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. Hepatology 2011;54:2159-72.
60. Torbenson M. Fibrolamellar carcinoma: 2012 update. Scientifica (Cairo) 2012;2012:743790.
61. **Villanueva A, Alsinet C, Yanger K**, et al. Notch signaling is activated in human hepatocellular carcinoma and induces tumor formation in mice. Gastroenterology 2012;143:1660-1669 e7.
62. **Zender S, Nickeleit I, Wuestefeld T**, et al. A critical role for notch signaling in the formation of cholangiocellular carcinomas. Cancer Cell 2013;23:784-95.
63. **Saha SK, Parachoniak CA,** Ghanta KS, et al. Mutant IDH inhibits HNF-4alpha to block hepatocyte differentiation and promote biliary cancer. Nature 2014;513:110-4.
64. **Ikenoue T, Terakado Y**, Nakagawa H, et al. A novel mouse model of intrahepatic cholangiocarcinoma induced by liver-specific Kras activation and Pten deletion. Sci Rep 2016;6:23899.
65. Mu X, Espanol-Suner R, Mederacke I, et al. Hepatocellular carcinoma originates from hepatocytes and not from the progenitor/biliary compartment. J Clin Invest 2015;125:3891-903.
66. **Shin S, Wangensteen KJ, Teta-Bissett M,** et al. Genetic Lineage Tracing Analysis of the Cell of Origin of Hepatotoxin-Induced Liver Tumors in Mice. Hepatology 2016.

67. Jors S, Jeliazkova P, Ringelhan M, et al. Lineage fate of ductular reactions in liver injury and carcinogenesis. J Clin Invest 2015;125:2445-57.
68. **He G, Dhar D, Nakagawa H**, et al. Identification of liver cancer progenitors whose malignant progression depends on autocrine IL-6 signaling. Cell 2013;155:384-96.
69. Marquardt JU. Deconvolution of the cellular origin in hepatocellular carcinoma: Hepatocytes take the center stage. Hepatology 2016;64:1020-3.
70. Umemura A, He F, Taniguchi K, et al. p62, Upregulated during Preneoplasia, Induces Hepatocellular Carcinogenesis by Maintaining Survival of Stressed HCC-Initiating Cells. Cancer Cell 2016.
71. **Yanger K, Zong Y,** Maggs LR, et al. Robust cellular reprogramming occurs spontaneously during liver regeneration. Genes Dev 2013;27:719-24.
72. Sekiya S, Suzuki A. Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. J Clin Invest 2012;122:3914-8.
73. Fan B, Malato Y, Calvisi DF, et al. Cholangiocarcinomas can originate from hepatocytes in mice. J Clin Invest 2012;122:2911-5.
74. Komuta M, Spee B, Vander Borght S, et al. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology 2008;47:1544-56.
75. **Hernandez-Gea V, Toffanin S**, Friedman SL, et al. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. Gastroenterology 2013;144:512-27.
76. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
77. Matter MS, Marquardt JU, Andersen JB, et al. Oncogenic driver genes and the inflammatory microenvironment dictate liver tumor phenotype. Hepatology 2016;63:1888-99.
78. Finkin S, Yuan D, Stein I, et al. Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. Nat Immunol 2015;16:1235-44.
79. **Lade A, Noon LA,** Friedman SL. Contributions of metabolic dysregulation and inflammation to nonalcoholic steatohepatitis, hepatic fibrosis, and cancer. Curr Opin Oncol 2014;26:100-7.
80. Wolf MJ, Adili A, Piotrowitz K, et al. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. Cancer Cell 2014;26:549-64.
81. Ma C, Kesarwala AH, Eggert T, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. Nature 2016;531:253-7.
82. Yamada D, Rizvi S, Razumilava N, et al. IL-33 facilitates oncogene-induced cholangiocarcinoma in mice by an interleukin-6-sensitive mechanism. Hepatology 2015;61:1627-42.
83. Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology 2013;145:1215-29.
84. Isomoto H, Kobayashi S, Werneburg NW, et al. Interleukin 6 upregulates myeloid cell leukemia-1 expression through a STAT3 pathway in cholangiocarcinoma cells. Hepatology 2005;42:1329-38.
85. Sia D, Hoshida Y, Villanueva A, et al. Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. Gastroenterology 2013;144:829-40.
86. Kroemer G, Senovilla L, Galluzzi L, et al. Natural and therapy-induced immunosurveillance in breast cancer. Nat Med 2015;21:1128-38.
87. Prieto J, Melero I, Sangro B. Immunological landscape and immunotherapy of hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2015;12:681-700.

88. Greten TF, Wang XW, Korangy F. Current concepts of immune based treatments for patients with HCC: from basic science to novel treatment approaches. Gut 2015;64:842-8.
89. El-Khoueiry AB, Melero I, Crocenzi T, et al. Phase I/II safety and antitumor activity of nivolumab in patients with advanced hepatocellular carcinoma (HCC): CA209-040. J Clin Oncol 33, 2015 (suppl; abstr LBA101) 2015.
90. Ji RR, Chasalow SD, Wang L, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 2012;61:1019-31.
91. Bald T, Landsberg J, Lopez-Ramos D, et al. Immune cell-poor melanomas benefit from PD-1 blockade after targeted type I IFN activation. Cancer Discov 2014;4:674-87.
92. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189-99.
93. Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. J Clin Oncol 2015;33:1974-82.
94. Sia D, Jiao Y, Martinez-Quetglas I, et al. Characterization of the immune class of hepatocellular carcinoma 10th ILCA Annual Conference. 9-11 September 2016. Vancouver, Canada (abstract O-007).
95. **Nakamura H, Arai Y, Totoki Y**, et al. Genomic spectra of biliary tract cancer. Nat Genet 2015;47:1003-10.
96. Schulze K, Nault JC, Villanueva A. Genetic profiling of hepatocellular carcinoma using next-generation sequencing. J Hepatol 2016.
97. Zucman-Rossi J, Villanueva A, Nault JC, et al. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. Gastroenterology 2015;149:1226-1239 e4.
98. Tovar V, Alsinet C, Villanueva A, et al. IGF activation in a molecular subclass of hepatocellular carcinoma and pre-clinical efficacy of IGF-1R blockage. J Hepatol 2010;52:550-9.
99. Villanueva A, Hoshida Y, Battiston C, et al. Combining clinical, pathology, and gene expression data to predict recurrence of hepatocellular carcinoma. Gastroenterology 2011;140:1501-12 e2.
100. **Nault JC, De Reynies A,** Villanueva A, et al. A hepatocellular carcinoma 5-gene score associated with survival of patients after liver resection. Gastroenterology 2013;145:176-87.
101. Toffanin S, Hoshida Y, Lachenmayer A, et al. MicroRNA-based classification of hepatocellular carcinoma and oncogenic role of miR-517a. Gastroenterology 2011;140:1618-28 e16.
102. **Villanueva A, Portela A**, Sayols S, et al. DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma. Hepatology 2015;61:1945-56.
103. Hoshida Y, Villanueva A, Kobayashi M, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med 2008;359:1995-2004.
104. Fuchs BC, **Hoshida Y, Fujii T**, et al. Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. Hepatology 2014;59:1577-90.
105. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415-21.
106. **Schulze K, Imbeaud S, Letouze E**, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet 2015;47:505-11.
107. **Totoki Y, Tatsuno K, Covington KR,** et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. Nat Genet 2014;46:1267-73.

108. **Fujimoto A, Furuta M, Totoki Y,** et al. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. Nat Genet 2016;48:500-9.
109. **Sung WK, Zheng H, Li S,** et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet 2012;44:765-9.
110. **Nault JC, Datta S, Imbeaud S**, et al. Recurrent AAV2-related insertional mutagenesis in human hepatocellular carcinomas. Nat Genet 2015;47:1187-93.
111. **Shaw AT, Kim DW,** Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med 2013;368:2385-94.
112. Hagel M, Miduturu C, Sheets M, et al. First Selective Small Molecule Inhibitor of FGFR4 for the Treatment of Hepatocellular Carcinomas with an Activated FGFR4 Signaling Pathway. Cancer Discov 2015;5:424-37.
113. **Huynh H, Hao HX**, Chan SL, et al. Loss of Tuberous Sclerosis Complex 2 (TSC2) Is Frequent in Hepatocellular Carcinoma and Predicts Response to mTORC1 Inhibitor Everolimus. Mol Cancer Ther 2015;14:1224-35.
114. Zhu AX, Chen D, He W, et al. Integrative biomarker analyses indicate etiological variations in hepatocellular carcinoma. J Hepatol 2016;65:296-304.
115. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-90.
116. Phase III Trial of Regorafenib in Patients with Unresectable Liver Cancer Meets Primary Endpoint of Improving Overall Survival. PRESS RELEASE. http://www.prnewswire.com/news-releases/phase-iii-trial-of-regorafenib-in-patients-with-unresectable-liver-cancer-meets-primary-endpoint-of-improving-overall-survival-300262856.html 2016.
117. Sawey ET, Chanrion M, Cai C, et al. Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by Oncogenomic screening. Cancer Cell 2011;19:347-58.
118. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17:1471-4.
119. Oishi N, Kumar MR, Roessler S, et al. Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. Hepatology 2012;56:1792-803.
120. **Boyault S, Rickman DS, de Reynies A**, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 2007;45:42-52.
121. Hoshida Y, Nijman SM, Kobayashi M, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res 2009;69:7385-92.
122. Woo HG, Lee JH, Yoon JH, et al. Identification of a cholangiocarcinoma-like gene expression trait in hepatocellular carcinoma. Cancer Res 2010;70:3034-41.
123. Andersen JB, Thorgeirsson SS. Genomic decoding of intrahepatic cholangiocarcinoma reveals therapeutic opportunities. Gastroenterology 2013;144:687-90.
124. **Chan-On W, Nairismagi ML, Ong CK,** et al. Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. Nat Genet 2013;45:1474-8.
125. **Jiao Y, Pawlik TM, Anders RA**, et al. Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas. Nat Genet 2013;45:1470-3.
126. Sia D, Losic B, Moeini A, et al. Massive parallel sequencing uncovers actionable FGFR2-PPHLN1 fusion and ARAF mutations in intrahepatic cholangiocarcinoma. Nat Commun 2015;6:6087.

127. Ross JS, Wang K, Gay L, et al. New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. Oncologist 2014;19:235-42.
128. **Simbolo M, Fassan M, Ruzzenente A**, et al. Multigene mutational profiling of cholangiocarcinomas identifies actionable molecular subgroups. Oncotarget 2014;5:2839-52.
129. **Fujimoto A, Furuta M,** Shiraishi Y, et al. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. Nat Commun 2015;6:6120.
130. **Zou S, Li J, Zhou H**, et al. Mutational landscape of intrahepatic cholangiocarcinoma. Nat Commun 2014;5:5696.
131. Churi CR, Shroff R, Wang Y, et al. Mutation profiling in cholangiocarcinoma: prognostic and therapeutic implications. PLoS One 2014;9:e115383.
132. **Arai Y, Totoki Y, Hosoda F,** et al. Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. Hepatology 2014;59:1427-34.
133. **Borad MJ, Champion MD, Egan JB**, et al. Integrated genomic characterization reveals novel, therapeutically relevant drug targets in FGFR and EGFR pathways in sporadic intrahepatic cholangiocarcinoma. PLoS Genet 2014;10:e1004135.
134. Graham RP, Barr Fritcher EG, Pestova E, et al. Fibroblast growth factor receptor 2 translocations in intrahepatic cholangiocarcinoma. Hum Pathol 2014;45:1630-8.
135. Wu YM, Su F, Kalyana-Sundaram S, et al. Identification of targetable FGFR gene fusions in diverse cancers. Cancer Discov 2013;3:636-47.
136. Javle M, Shroff R, Zhu A, et al. A phase 2 study of BGJ398 in patients (pts) with advanced or metastatic FGFR-altered cholangiocarcinoma (CCA) who failed or are intolerant to platinum-based chemotherapy. Clin Oncol 2016;34.
137. Mazzaferro V, Shaib W, Rimassa L, et al. ARQ 087, an oral Pan-Fibroblast Growth Factor Receptor (FGFR) inhibitor, in patients with advanced and/or metastatic intrahepatic cholangiocarcinoma (iCCA). https://globenewswire.com/news-release/2016/06/30/852679/0/en/ArQule-Presents-Preliminary-Clinical-Data-for-ARQ-087-Demonstrating-Evidence-of-Anticancer-Activity-in-Intrahepatic-Cholangiocarcinoma-at-the-ESMO-18th-World-Congress-on-Gastrointe.html 2016.
138. Cornella H, Alsinet C, Sayols S, et al. Unique genomic profile of fibrolamellar hepatocellular carcinoma. Gastroenterology 2015;148:806-18 e10.
139. **Honeyman JN, Simon EP, Robine N**, et al. Detection of a recurrent DNAJB1-PRKACA chimeric transcript in fibrolamellar hepatocellular carcinoma. Science 2014;343:1010-4.
140. Simon EP, Freije CA, Farber BA, et al. Transcriptomic characterization of fibrolamellar hepatocellular carcinoma. Proc Natl Acad Sci U S A 2015;112:E5916-25.
141. **Cairo S, Armengol C,** De Reynies A, et al. Hepatic stem-like phenotype and interplay of Wnt/beta-catenin and Myc signaling in aggressive childhood liver cancer. Cancer Cell 2008;14:471-84.
142. Nicolle D, Fabre M, Simon-Coma M, et al. Patient-derived xenografts from pediatric liver cancer predict tumor recurrence and advise clinical management. Hepatology 2016.
143. Bissig-Choisat B, Kettlun-Leyton C, Legras XD, et al. Novel patient-derived xenograft and cell line models for therapeutic testing of pediatric liver cancer. J Hepatol 2016;65:325-33.
144. Theise ND, Nakashima O, Park YN. Combined hepatocellular-cholangiocarcinoma. In: osman FT, Carneiro F H, RH TN, eds. WHO classification of tumours of the digestive system. 4th ed. Lyon: IARC; 2010:225–227.

145. Coulouarn C, Cavard C, Rubbia-Brandt L, et al. Combined hepatocellular-cholangiocarcinomas exhibit progenitor features and activation of Wnt and TGFbeta signaling pathways. Carcinogenesis 2012;33:1791-6.
146. Moeini A, Sia D, Zhang Z, et al. Mixed hepatocellular-cholangiocarcinoma tumors: stem-cell subtype and cholangiolocellular carcinoma are unique molecular entities. Hepatology 2015;62, Number 1 (SUPPL): 342A.
147. **Gerlinger M, Rowan AJ, Horswell S**, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883-92.
148. Alizadeh AA, Aranda V, Bardelli A, et al. Toward understanding and exploiting tumor heterogeneity. Nat Med 2015;21:846-53.
149. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817-26.
150. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 2009;27:2091-6.
151. European Association For The Study Of The L, European Organisation For R, Treatment Of C. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2012;56:908-43.
152. Hayes DF, Bast RC, Desch CE, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1456-66.
153. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005;97:1180-4.
154. Hemingway H, Croft P, Perel P, et al. Prognosis research strategy (PROGRESS) 1: a framework for researching clinical outcomes. BMJ 2013;346:e5595.
155. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 2009;101:1446-52.
156. Sparano JA, Gray RJ, Makower DF, et al. Prospective Validation of a 21-Gene Expression Assay in Breast Cancer. N Engl J Med 2015;373:2005-14.
157. Llovet JM, Pena CE, Lathia CD, et al. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. Clin Cancer Res 2012;18:2290-300.
158. Hoshida Y, Villanueva A, Sangiovanni A, et al. Prognostic gene expression signature for patients with hepatitis C-related early-stage cirrhosis. Gastroenterology 2013;144:1024-30.
159. Ji J, Shi J, Budhu A, et al. MicroRNA expression, survival, and response to interferon in liver cancer. N Engl J Med 2009;361:1437-47.
160. Yamashita T, Forgues M, Wang W, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res 2008;68:1451-61.
161. **Budhu A, Jia HL**, Forgues M, et al. Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology 2008;47:897-907.
162. **Valle J, Wasan H,** Palmer DH, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med 2010;362:1273-81.
163. Gu MJ, Choi JH. Epithelial-mesenchymal transition phenotypes are associated with patient survival in intrahepatic cholangiocarcinoma. J Clin Pathol 2014;67:229-34.
164. **Huang XY, Zhang C, Cai JB,** et al. Comprehensive multiple molecular profile of epithelial mesenchymal transition in intrahepatic cholangiocarcinoma patients. PLoS One 2014;9:e96860.
165. Sulpice L, Rayar M, Desille M, et al. Molecular profiling of stroma identifies osteopontin as an independent predictor of poor prognosis in intrahepatic cholangiocarcinoma. Hepatology 2013;58:1992-2000.

166. Plieskatt J, Rinaldi G, Feng Y, et al. A microRNA profile associated with Opisthorchis viverrini-induced cholangiocarcinoma in tissue and plasma. BMC Cancer 2015;15:309.
167. Li L, Masica D, Ishida M, et al. Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. Hepatology 2014;60:896-907.
168. **Zhang MY, Li SH**, Huang GL, et al. Identification of a novel microRNA signature associated with intrahepatic cholangiocarcinoma (ICC) patient prognosis. BMC Cancer 2015;15:64.
169. Ryan DP, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. N Engl J Med 2014;371:2140-1.
170. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. N Engl J Med 2009;361:98-9.
171. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 2005;65:6063-9.
172. **Ruzzenente A, Fassan M,** Conci S, et al. Cholangiocarcinoma Heterogeneity Revealed by Multigene Mutational Profiling: Clinical and Prognostic Relevance in Surgically Resected Patients. Ann Surg Oncol 2016;23:1699-707.
173. **Wang P, Dong Q,** Zhang C, et al. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. Oncogene 2013;32:3091-100.
174. Labgaa I, Villanueva A. Liquid biopsy in liver cancer. Discov Med 2015;19:263-73.
175. He J, Lu H, Zou Q, et al. Regeneration of liver after extreme hepatocyte loss occurs mainly via biliary transdifferentiation in zebrafish. Gastroenterology 2014;146:789-800 e8.
176. Sangro B, Park JW, De la Cruz CM, et al. A randomized, multicenter, phase 3 study of nivolumab vs sorafenib as first-line treatment in patients (pts) with advanced hepatocellular carcinoma (HCC): CheckMate-459. J Clin Oncol 34, 2016 (suppl; abstr TPS4147).

Notes: Author names in bold designate shared co-first authorship

Table

**Table 1.** Experimental models supporting the different hypotheses regarding the cell of origin in primary liver cancers

| Hypothesis tested | Method | Animal models | Altered pathway | Tumor Type | Proposed cell of origin | Reference |
|---|---|---|---|---|---|---|
| *HPC/oval cells as cell of origin* | Clonal analyses | - | BMI1, WNT | mixed HCC-iCCA | HPCs | 51 |
| | GEMM | Sav1fl/fl, mst1fl/fl, and mst2fl/fl | HIPPO | HCC, iCCA | oval cells | 54 |
| | GEMM | WW45flox/floxAlbumin-Cre | HIPPO | mixed HCC-iCCA | oval cells | 55 |
| | Multilineage differentiation | - | H-Ras, SV40LT | HCC, iCCA | HPC, hepatoblasts, hepatocytes | 57 |
| | GEMM | Alb-*Cre*;*Nf2*$^{lox/lox}$ | NF2 | HCC, iCCA, mixed | oval cells | 56 |
| | GEMM | NotchIC::AlbCre | NOTCH | iCCA | HPCs | 62 |
| | GEMM | Alb-Cre;LSL-IDH2R172K;LSL-KrasG12D | IDH, KRAS | iCCA | hepatoblasts | 63 |
| | GEMM | LSL-KrasG12D -Ptenflox | KRAS, PTEN | iCCA | hepatoblasts, cholangiocytes | 64 |
| *Hepatocytes as cell of origin* | GEMM | AlfpCre$^{+}$Trp53$^{\Delta 2\text{-}10/\Delta 2\text{-}10}$ | TP53, WNT | HCC | hepatocytes-derived HPC | 52 |
| | GEMM | AlfpCre$^{+}$Trp53$^{\Delta 2\text{-}10/\Delta 2\text{-}10}$ | TP53, NOTCH | iCCA | hepatocytes-derived HPC | 52 |
| | GEMM | (Mst1/Mst2 DKO, YAP KO, DKO Yap+/−, and tTKO | HIPPO | HCC | hepatocytes-derived HPC/oval cells | 53 |
| | Multilineage differentiation | - | H-Ras, SV40LT | HCC, iCCA | HPC, hepatoblasts, hepatocytes | 57 |
| | lineage-tracing system | liver injury (BDL, DDC, MDA, CDE); GEMM (Mdr2–/–) | MDR | HCC | hepatocytes | 67 |
| | lineage-tracing system | liver injury (DEN); GEMM (Mdr2–/–; PTEN-/-) | MDR, PTEN | HCC | hepatocytes | 65 |
| | lineage-tracing system | DEN + TAA | - | HCC, HPA | hepatocytes | 66 |
| | GEMM | Tsc1/Sqstm1Δhep and Sqstm1Δhep/MUP | p62 | HCC | hepatocytes | 70 |
| | lineage-tracing system | Alb-CreERT2;R26RlacZ/+ and CK19-CreERT2;R26RlacZ/+ | NOTCH | iCCA | hepatocytes | 72 |
| | lineage-tracing system | hydrodinamic tail injection | NOTCH, AKT | iCCA | hepatocytes-derived biliary-like cells | 73 |
| *Cholangiocytes as cell of origin* | lineage-tracing system | Liver injury (TAA) and CK19CreER$^{T}$eYFPp53$^{f/f}$ | TP53 | iCCA | cholangiocytes | 26 |
| | GEMM | LSL-KrasG12D -Ptenflox | KRAS, PTEN | iCCA | hepatoblasts, cholangiocytes | 64 |

**Abbreviations:** HPC, Hepatic progenitor cells; GEMM: genetically engineered mouse model; HCC, hepatocellular carcinoma; HPA, hepatocellular adenoma; iCCA, intrahepatic cholangiocarcinoma; mixed HCC-iCCA, mixed hepatocellular-cholangiocarcinoma; KO, knock-out; DKO, double knock-out; TKO, triple knock-out; DEN, N-nitrosodiethylamine; TAA, Thioacetamide; BDL, bile duct ligation; MDA, methylene dianiline; CDE, choline-deficient ethionine-supplemented; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine.

**Table 2.** Prognostic signatures in primary liver cancer

| Signature | Cohort (etiology) | Number of patients tested Training/Validation set | Clinical endpoint | REMARK recommendations | Level of evidence (Simon et al. JNCI 2009) | Reference |
|---|---|---|---|---|---|---|
| **Hepatocellular Carcinoma** | | | | | | |
| *mRNA-based* | | | | | | |
| 5-gene signature | HCC (Alcohol, HCV, HBV) | 189/434 | survival | OK | C | 100 |
| EpCAM-signature | HCC (HBV) | 40/238 | survival | OK | C | 160 |
| 186-gene signature (adjacent tissue) | HCC (HCV) | 82/441 | survival, HCC development | OK | C | 103, 158 |
| *miRNA based* | | | | | | |
| 20-miRNA signature | HCC (HBV) | 131/110 | venous metastasis, survival | OK | C | 161 |
| Down-regulation miR-26a | HCC (HBV) | 241/214 | survival | OK | C | 159 |
| *DNA methylation* | | | | | | |
| 36 CpG DNA methylation signature | HCC (HCV) | 221/83 | survival | OK | C | 102 |
| *Protein markers (Plasma)* | | | | | | |
| Ang2, VEGF | HCC (HCV) | 491 | survival | OK | B | 157 |
| | | | | | | |
| **Cholangiocarcinoma** | | | | | | |
| *mRNA-based* | | | | | | |
| poor prognosis/proliferation class | iCCA | 119/104 | survival and recurrence | further validation required | C | 85 |
| poor prognosis group | iCCA and eCCA | 52/52 | survival and recurrence | further validation required | C | 123 |
| Stem cell-like subtype | iCCA and mixed HCC-CCA | 23/68 | survival | further validation required | C | 119 |

**Abbreviations:** HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; mixed HCC-iCCA, mixed hepatocellular-cholangiocarcinoma; eCCA, extrahepatic cholangiocarcinoma.

Figure 1

Click here to download Figure Figure 1.tif

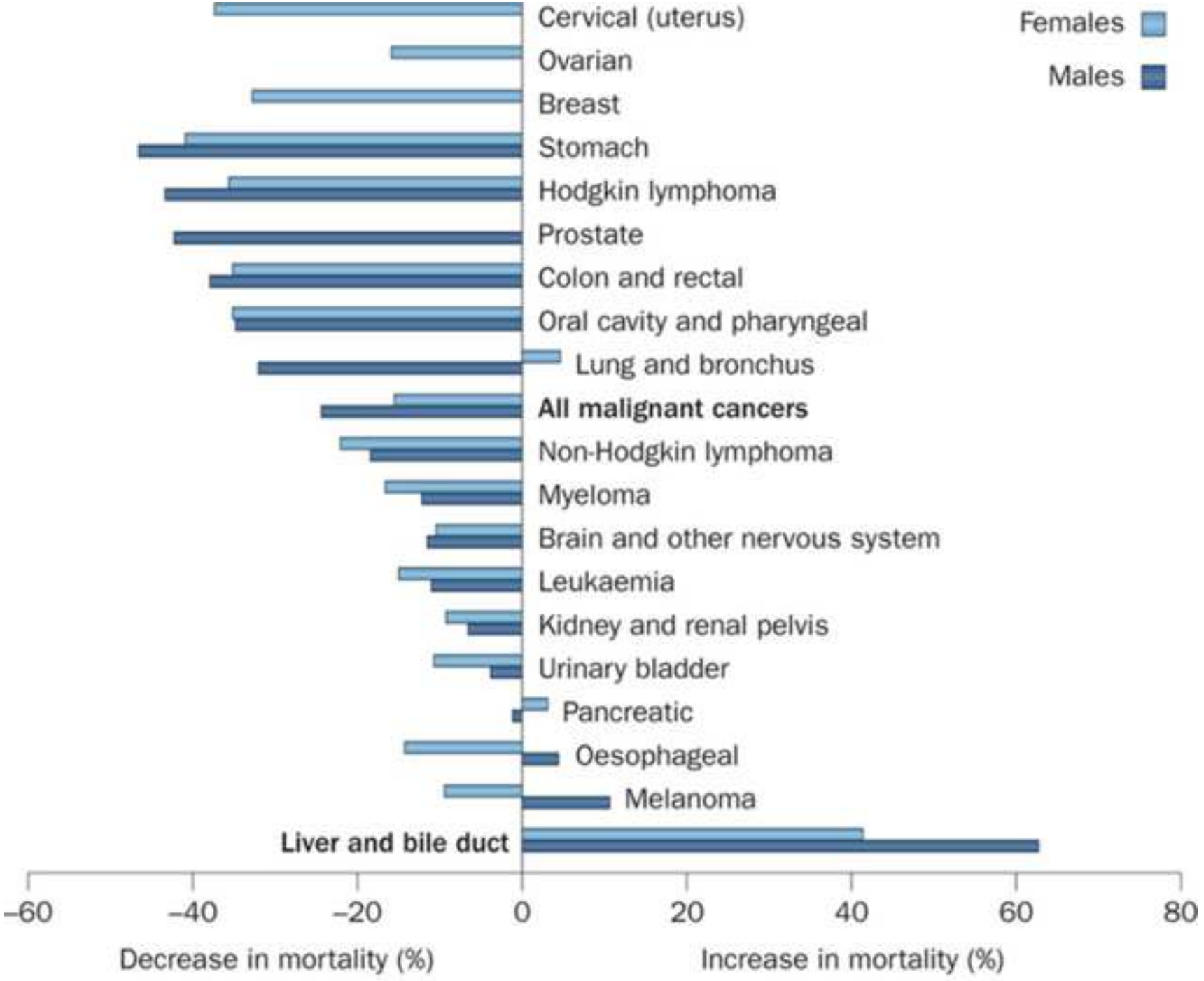

Figure 2

Click here to download Figure Figure 2_new.tif

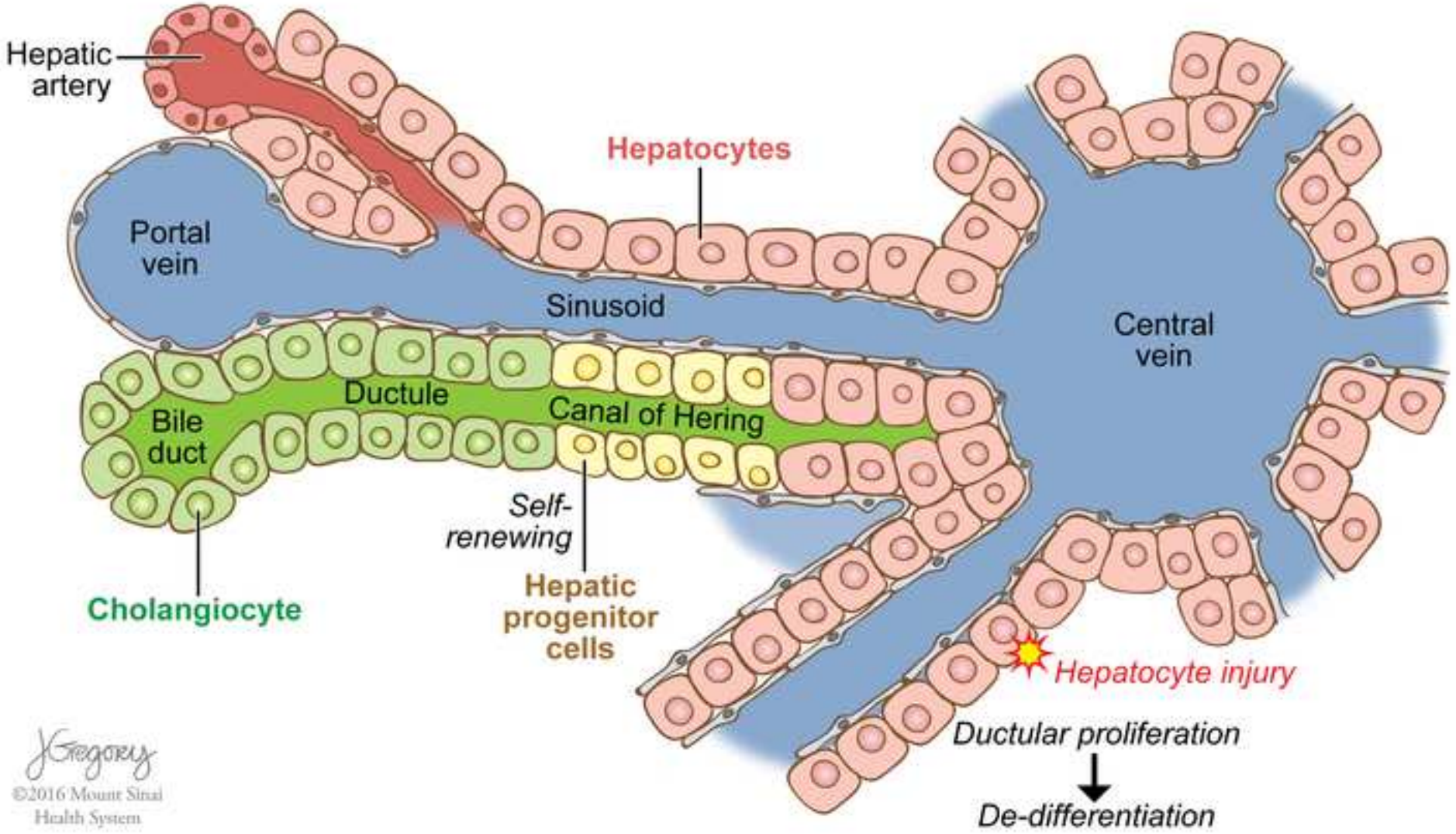

Figure 3

Click here to download Figure Figure 3.tif

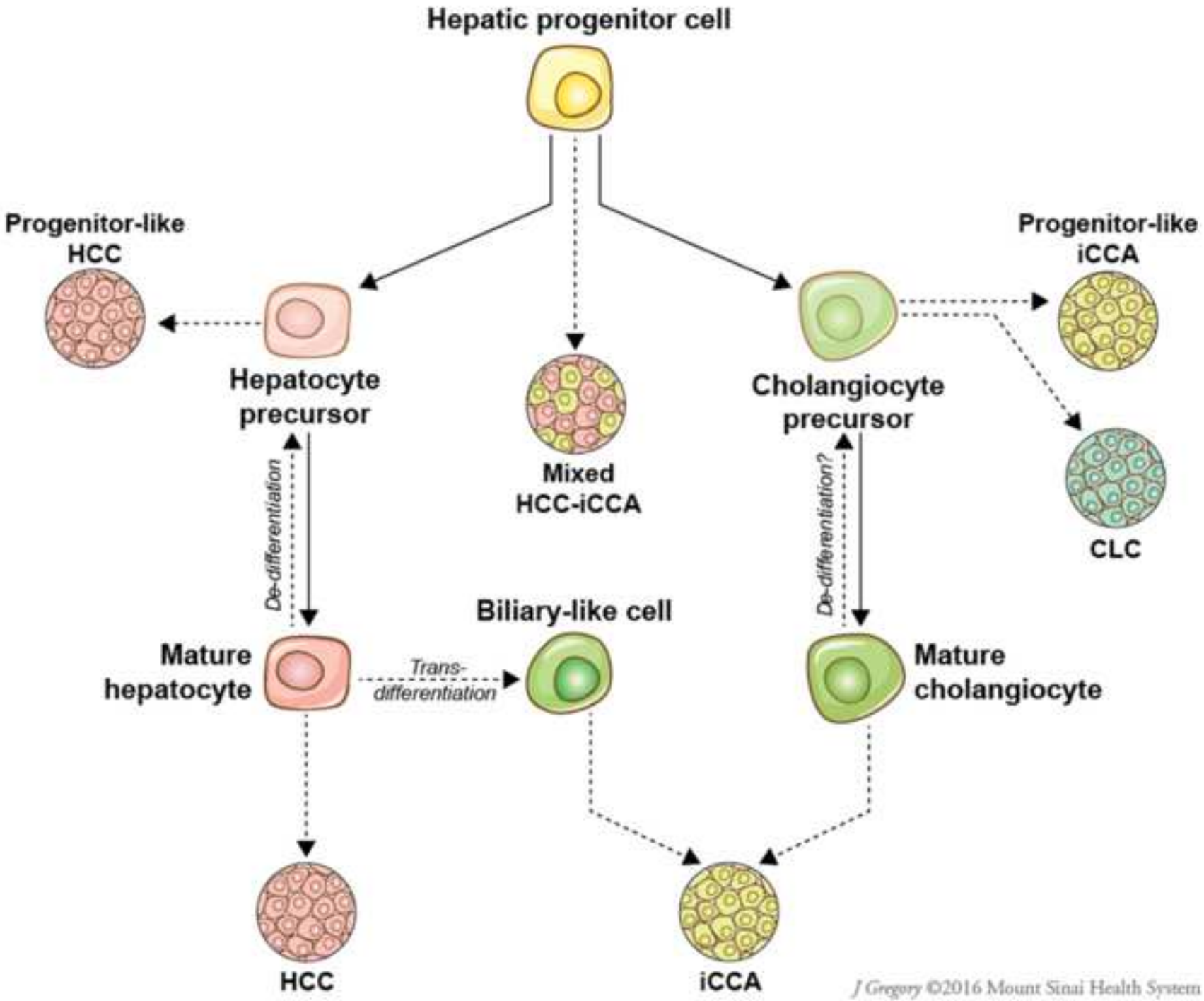

Figure 4

Click here to download Figure Figure 4.tif

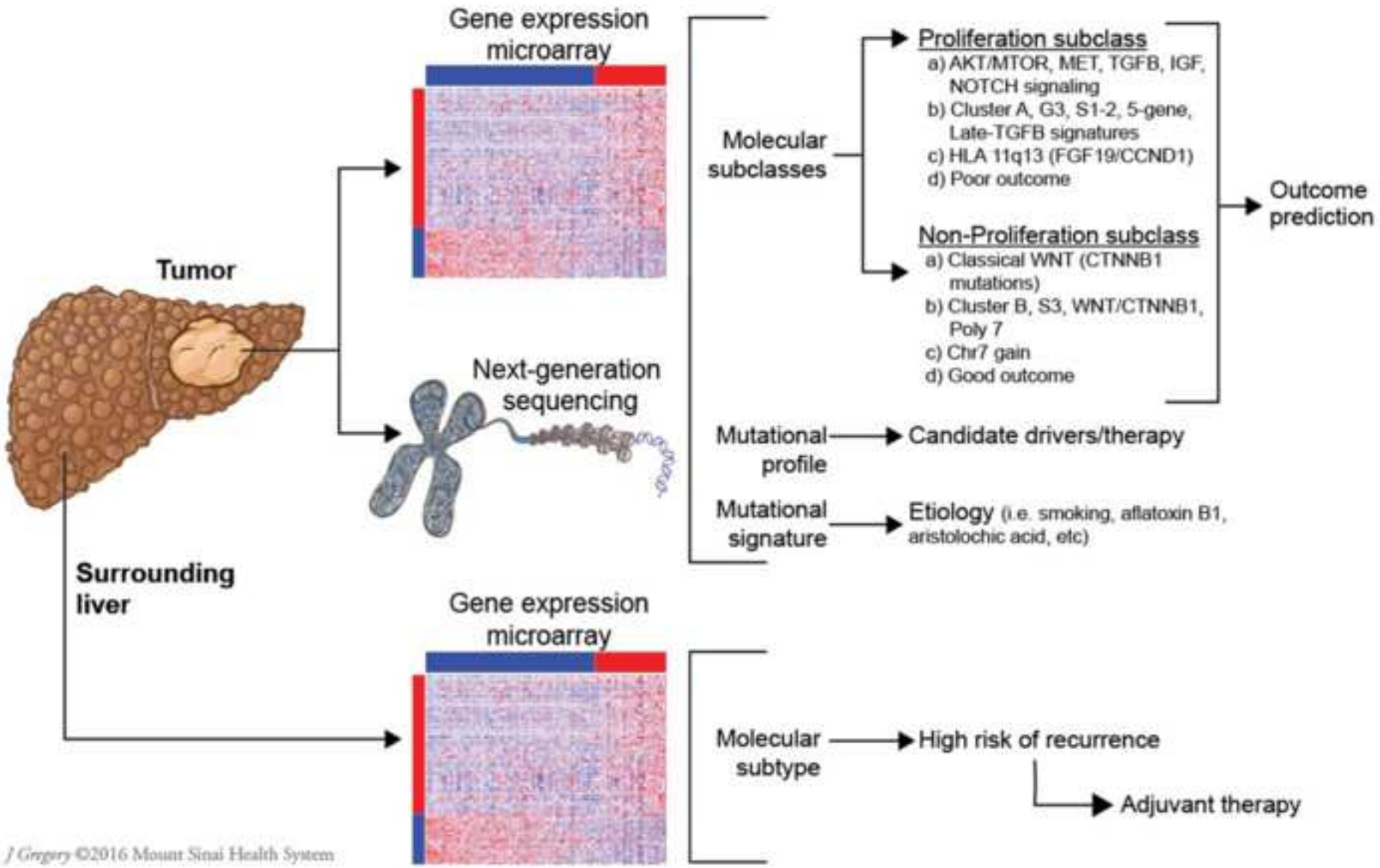

Figure 5

Click here to download Figure Figure 5.tif